Ciências Odontológicas: Desenvolvendo a Pesquisa Científica e a Inovação Tecnológica 2

Emanuela Carla dos Santos
(Organizadora)

Atena Editora
Ano 2020

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APRESENTAÇÃO

Ao observarmos a evolução da Odontologia ao longo do tempo percebemos que, mesmo sendo uma prática muito antiga, cresceu muito lentamente até alguns anos atrás. As grandes revoluções científicas na área aconteceram nas últimas décadas e, atualmente, a velocidade é tamanha que pode ser difícil manter-se atualizado.

A Atena Editora traz mais este e-book que reúne artigos de diversas áreas de atuação da Odontologia, denotando o desenvolvimento da pesquisa científica juntamente com a inovação tecnológica.

Neste volume, encontram-se publicações atuais e contundentes que expõem o benefício da associação entre Ciências Odontológicas e outras áreas do conhecimento, como ciências exatas e tecnológicas, e como o resultado dessa cooperação auxilia o desenvolvimento da comunidade científica como um todo.

Desejo que você, leitor, tenha um ótimo momento durante a leitura desta obra.

Boa leitura!

Emanuela Carla Dos Santos

SUMÁRIO

CAPÍTULO 4

ANTIFUNGAL EFFECT OF EUGENOL AGAINST STRAINS OF ORAL CAVITY *CANDIDA PARAPSILOSIS* ISOLATED FROM HEALTHY INDIVIDUALS

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ABSTRACT: The objective was to isolate and identify clinical species of *Candida parapsilosis* from the oral cavity in individuals presenting no oral alteration such as oral candidiasis, and then test the sensitivity of these strains to eugenol and nystatin. For this, determination of the Minimum Inhibitory Concentration (MIC), Minimum Fungicide Concentration (MFC), the microbial growth death curve, and inhibition of virulence factors through micromorphology were performed. Seven strains were selected, six of which were of clinical origin from *Candida parapsilosis,* and their ATCC® 22019™. The MIC was determined at 256 µg/mL which in 100% of the strains tested, coincided with the MFC. At the three analyzed concentrations, the micromorphology study demonstrated inhibition of virulence structures such as pseudohyphae and blastoconidia. For the strains studied, the microbial death curve revealed fungicidal effect at 24 hours of experiment. Thus, eugenol is a promising molecule for treatment of oral fungal infections.

KEYWORDS: Eugenol. Phytotherapy. Fungal infections. Candidiasis.

EFEITO ANTIFÚNGICO DO EUGENOL SOBRE CEPAS DE *CANDIDA PARAPSILOSIS* ISOLADAS DA CAVIDADE BUCAL DE INDIVÍDUOS SAUDÁVEIS

RESUMO: Objetivou-se isolar e identificar espécies clínicas de *Candida parapsilosis* da cavidade bucal de indivíduos sem nenhuma alteração bucal, como por exemplo, a candidíase bucal, em seguida foi avaliada a sensibilidade destas cepas frente o eugenol e nistatina. Para tal, foi realizada a determinação da Concentração Inibitória Mínima (CIM); Concentração Fungicida Mínima (CFM) a curva de morte de crescimento microbiano e inibição de fatores de virulência através da micromorfologia. Selecionou-se sete cepas, seis de origem clínica de *Candida parapsilosis* e a sua padrão ATCC® 22019™. A CIM ficou determinada em 256 µg/mL a qual coincidiu com a CFM em 100% das cepas ensaiadas. O estudo da micromorfologia demonstrou inibição das estruturas de virulência como pseudo-hiifas e blastoconídeos nas três concentrações analisadas. A curva de morte microbiana mostrou um efeito fungicida após 24 horas de experimento nas cepas estudadas. Dessa forma, o eugenol constitui uma molécula promissora no tratamento das infecções fúngicas orais.
PALAVRAS - CHAVE: Eugenol. Fitoterapia. Infecções Fúngicas. Candidíase.

INTRODUCTION

Oral candidiasis is a highly prevalent fungal infection, triggered by fungal microorganisms of the genus *Candida* (which comprise about 200 species) though only 15 are of medical interest. In oral candidiasis, the most prevalent species in the onset of the pathology is *Candida albicans* (SHARMA *et al*., 2017). Studies have revealed a significant prevalence of non-albicans species (especially *Candida parapsilosis*) during the course of the pathogenic process and in cases of resistance (PING-FENG *et al*., 2017).

Candida parapsilosis is an ubiquitous microorganism, yet unlike other *Candida* species it is not an exclusively human pathogen (with reports of isolations from sites other than in humans) (NOSEK *et al*., 2009; TROFA *et al*., 2008). The microorganism is considered as important for triggering oral candidiasis, and is the second most reported opportunistic fungal species isolated in blood cultures from various geographical locations (GABLERI *et al*., 2009).

The treatment of fungal infection involves local or systemic medications often in associations (PEMÁN *et al*., 2012). Candidiasis is usually treated with applications of topical agents such as nystatin in suspension (polyenes) or miconazole gel (imidazoles). In recurrent episodes, systemic drug therapy is instituted, in which case fluconazole is the drug prescribed. However, resistance, toxicity, and the unwanted side effects of certain antifungal agents used to treat candidiasis emphasize the need for further research to increase both the efficacy of treatments and minimize patient risk (SARTORATTO *et al*., 2004; DANIEL *et al*., 2009).

Phytotherapy is an alternative therapy to treat oral candidiasis (DANIEL *et al*., 2009). Eugenol, a molecule present in several species of plants (FERREIRA *et al*., 2017) and often a major constituent when extracted from leaves, fruits and stems (CHILUKA *et*

al., 2017) is used in dentistry for the treatment of pulp alterations and tooth restorative procedures (GUENETTe *et al*., 2006).Its properties are reported in the literature: analgesic, antimicrobial, anesthetic, antioxidant, anti-inflammatory, and antiseptic (HIDALGO *et al*., 2009). However, there are no studies in the literature on the anti-fungal's mode of action or on associations with licensed antifungals against clinical strains of *C. parapsilosis*.

The present study isolated and identified clinical species of *Candida parapsilosis* from the oral cavity in systematically healthy individuals. The sensitivity of these strains to eugenol and nystatin (a standard antifungal) was evaluated using Minimum Inhibitory Concentration (MIC), Minimum Fungicidal Concentration (MFC), analysis of mechanisms of action through microbial growth kinetics, and inhibition of virulence factors through micromorphology.

METHODOLOGY

Study design

The collection of biological material was performed in the Recanto do Poço Community, Cabedelo - PB, Brazil. Yeast identification and analyses related to the antifungal activity of the selected natural products were performed at the Research Laboratory for Antibacterial and Antifungal Activity of natural and/or synthetic bioactive products, DCF/CCS/UFPB.

The study was divided into two stages. The first was *ex vivo* with collection of biological material from the individuals' oral cavity, followed by isolation and identification of the *Candida parapsilosis* strains. The second stage was the performance of *in vitro* tests. This study was submitted and approved (CAAE: CAAE: 57435016.4.0000.5188) by the Research Ethics Committee of the Health Sciences Center of the Federal University of Paraíba (CEP-CCS), according to resolution 466/12 of the National Health Council/MS.

Collection, Isolation and Identification of the Candida genus

The collection of biological material, isolation, and identification of the *Candida* species was performed following the criteria established (LOODER, 1970; HOOG and GARRO, 1995; KURTZMANN and FELL, 1998; SIDRIM and ROCHA, 2004.

For collection of biological material, with isolation, and identification of *Candida* species from the individuals, three sterile swabs (INLAB® Confiança, Brazil) were used. Each swab was moistened in sterile saline solution and applied to three different sites in the oral cavity: the patient's hard palate, tongue, and cheek mucosa; with back and forth movements (friction), for 30 seconds, in each anatomical site. The biological material collected was inoculated into disposable 15x90 mm Petri dishes (Dispopetri), containing Sabouraud Dextrose Agar (SDA) (Difco Laboratories Ltda. USA/France), supplemented with 100 μg/mL of chloramphenicol (Sigma Chemical Corporation, St Louis, MO, USA). After 24-48h in a microbiological greenhouse at 35 ± 2ºC, the colonies with aspects of yeast-like

fungi were isolated in CHROMOagar-Candida (Difco Laboratories Ltda.; USA/France). After checking the growth in the plates, the colonies were evaluated as to color and morphotype, and a presumptive identification was performed. To further determine the *Candida* species, germinative tube testing, yeast microculture in Corn Meal Agar, an auxanogram (assimilation of carbohydrates and nitrogen), and carbohydrate fermentation were also performed.

Selection of fungal strains and preparation of inoculum

In accordance with the inclusion criteria established for the research, twenty individuals participated: aged equal to or greater than 18 years old, both genders, not using any type of dental prosthesis, and presenting no oral alterations upon intra-oral clinical evaluation.

Six clinical strains of *C. parapsilosis* from the oral cavity of the participating individuals (LM-1; LM-2; LM-7; LM-70; LM-225; LM-302) were isolated and identified for *in vitro* assays; a standard strain of the American Type Culture Collection (ATCC) 22019 was included.

For the yeast inoculum preparation procedure, isolates were grown in inclined SDA medium at 35 ± 2ºC for 24h (overnight). Suspensions of the microorganisms were prepared in tubes containing 5 mL of sterile 0.9% saline solution (Farmax - Distribuidor Ltda., Amaral, Divinópolis, MG, Brazil), and were stirred for 2 minutes with the aid of a Vortex device (Fanem Ltd., Guarulhos, SP, Brazil).

After stirring, each suspension had its turbidity compared and adjusted to that presented by a barium sulfate suspension in the 0.5 tube; McFarland scale, which corresponded to an inoculum of approximately 10^6 CFU/mL. This suspension was then diluted with distilled water in a proportion of 1:10 resulting in an inoculum containing approximately 10^5 CFU/mL, which was used in the tests (ESPINEL-INGROFF *et al.*, 2002).

Culture media

For the antifungal activity tests, RPMI 1640/with L-glutamine, and without bicarbonate (Sigma-Aldrich®/Stenheim/Germany) and Sabouraud Agar – SDA, and Sabouraud Agar with antibiotic SDAA (Difco®/USA/France) were used. The media were prepared according to the manufacturer's instructions.

Products

The test products used were the phytochemical constituent eugenol (Sigma-Aldrich, São Paulo, SP, Brazil®; Batch: 024), and the standard antifungal agent Nystatin (Sigma-Aldrich, São Paulo, SP, Brazil®; Batch: SLBB5282V)

All products were properly solubilized in dimethyl sulfoxide (DMSO) in proportions of up to 10%, and Tween 80 at 0.02%, being then completed with sterile distilled water (q.s.p. 3 mL) to obtain an emulsion at an initial concentration of 1024 μg/mL.

Screening and determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The screening of the natural products and MIC determination of the selected product were performed using microdilution technique, performed in triplicate, with sterilized "U" bottom microplates containing 96 wells (Kasvi®, Italy) (CLSI., 2010). In each well of the plate, 100 μL of RPMI liquid medium (Sigma-Aldrich®/São Paulo, SP/Brazil) was added in double concentration. Subsequently, 100 μL of the products, also doubly concentrated, were dispensed into the wells of the first row of the plate, which were serially diluted by removal of a 100 µL aliquot from the most concentrated well to the successor well, obtaining concentrations of 32μg/mL to 1024μg/mL for screening, and 2μg/mL to 1024μg/mL for MIC determination, such that in the first line of the plate the highest concentration was found and the lowest concentration in the last line. Finally, 10μL of yeast inoculum was added to each cavity, where each column of the plate marked a specific fungal strain. Viability controls were performed on the fungal strains in liquid medium under the same assay conditions. The plates were sealed and incubated at 35 ± 2ºC for 24-48h. The MIC was defined for the products used in the biological tests as the lowest concentration capable of visually inhibiting fungal growth as verified in the wells, when compared to the control grow

The following values were used as product criteria for determining MIC: 50 to 500 μg/mL = strong/excellent antimicrobial activity; 600 to 1500 μg/mL = moderate activity; above 1500 μg/mL = weak activity or inactive (SADDIQ and KHAYYAT., 2010)

After determining the MIC, 10 μL aliquots of the supernatant from the wells corresponding to each inhibitory concentration and the two immediately higher concentrations (MIC, MICx2 and MICx4) were subcultured in sterile U-bottom microdilution plates with 96 wells (Kasvi, Italy) containing 100 μL RPMI 1640 (Sigma-Aldrich®/Stenheim/Germany) and incubated for 24-48h at 35 ± 2ºC.

Microbial growth was checked visually based on the controls, and the MFC was determined for each strain. The tests were performed in triplicate and the result expressed as the MFC average obtained from the three tests (Borsato et al., 2013)

The MFC/MIC ratio was calculated in order to determine whether the substance presented fungistatic (MFC/MIC ≥ 4) or fungicidal (MFC/MIC < 4) activity (HOLETZ *et al.*, 2002).

Effect of the isolated test products on *C. parapsilosis* micromorphology

To study possible changes in *C. parapsilosis* micromorphology, microculture technique was used in a Petri dish slide (wet chamber) (KLEPSER *et al.*, 1998). A fused agar-cornmeal-Tween 80 culture medium was fractionated into sterile tubes containing the isolated test products in concentrations corresponding to their MICs. A tube of culture medium alone (Control) was included. After homogenization, each culture medium was spread on a glass slide.

Effect of the isolated test products on microbial death kinetics

The interference study on the isolated test products for the fungal strains time of death curves was performed using the KLEPSER *et al*. (1998) methodology with certain improvements. To perform the microbial death kinetics, two strains of *C. parapsilosis* were used, the standard ATCC 22019 and a clinical strain (LM-82). In this test, over 24h, the behavior of minimum inhibitory concentrations of eugenol against the selected yeast strains was observed.

Initially, 100 μL of RPMI 1640 (Sigma-Aldrich®/São Paulo-SP/Brazil) was added to a 96-hole U-shaped microplate using 10 μL of the supernatant from wells corresponding to the inhibitory concentration and the two concentrations (MIC, MICx2 and MICx4) immediately higher, which was then incubated for a period of 24-48 hours at 35 ± 2ºC.

Then the inoculum was plated in a Petri dish (Alamar Tecno Científica LTDA®) containing the Sabouraud Dextrose Agar culture medium (Difco®). A 10 μL aliquot of the inoculum was removed with a calibrated bacterial loop (INLAB Confiança, Brazil) and subsequently sown evenly in the form of striations along the surface of the ASD medium, at the time intervals of 0 h, 2 h, 4 h, 8 h, 12 h and 24 h. The inoculated plates were then incubated at 35 ± 2ºC for 48 hours.

The experiment was performed in triplicate. The curves were constructed by plotting the average colony count ($\log_{10}$CFU/mL) as a function of time (hours). Fungicidal activity was determined when there was a reduction in fungal growth greater than or equal to 3 $\log_{10}$ (≥ 99.9%) from the initial inoculum, and fungistatic activity was determined when there was a reduction in growth of less than 3 $\log_{10}$ (<99.9%) CFU/ml) (LASS-FLÖRL., 2009).

Data analysis

The MIC, MFC, and Association Assay data were analyzed using inferential and descriptive statistics. The microbial growth kinetics curve was plotted by $\log_{10}$CFU/mL as a function of time and concentration. The statistical analysis was performed using Kruskal-Wallis and Dunn tests considering a significance level of 0.05 ($p < 0.05$). The GraphPad Software (GraphPad for Windows, San Diego, CA - USA) was used to generate the results.

RESULTS

During screening of the four products, eugenol presented its best results against the *Candida parapsilosis* LM-70 and ATCC® 22019™ strains. On the other hand, the species tested were resistant to the lots (differing) of *Eugenia uniflora* essential oil (Pitanga). The results are seen below (Table 1).

Essential oils						
Scientific name	**Family**	**Popular name**	**MIC (µg/mL)**		**Density g/cm³**	**Lot**
			A*	**B****		
Eugenia uniflora	Myrtacea	Pitanga	1024	1024	0.905	0717/05209/F Quinare®
Eugenia uniflora	Myrtacea	Pitanga	1024	1024	1.044	5579lmv Sigma Aldrich®
Mentha piperita L.	Lamiaceae	Mint	64	64	0.899	5579lmv Sigma Aldrich®

Phytoconstituent							
Name	**Molecular Formula**	**MIC (µg/mL)**		**Density g/cm³**	**Melting point °C**	**Molar mass g/mol**	**Lot**
		A	**B**				
Eugenol	$C_{10}H_{12}O_2$	32	32	1.06 g/cm³	-7,5	164.2 g/mol	024 Sigma Aldrich®

Table 1: Results of product screening to assess antifungal response. Microdilution technique (1024*µ*g / mL to 32*µ*g/mL). * ATCC 22019 strain ** strain LM-70.

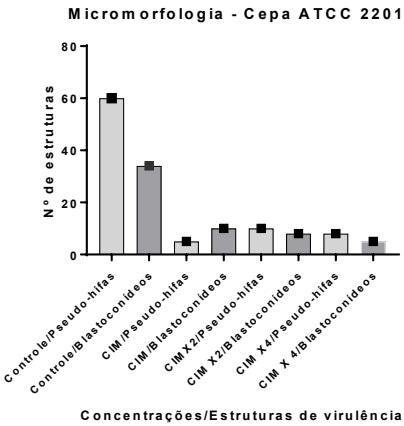
Table 2 below presents the microdilution test results for the six clinical strains and the ATCC® 22019™ standard. The MIC for eugenol against all of the strains tested was 256 µg/mL, whereas for nystatin the species tested were insensitive at 1024 µg/mL. The table also presents minimum fungicidal concentration values.

The micromorphology data are presented as virulence structure averages; performed by counting three slide fields (optical microscopy) for both the control and the studied concentrations (Graphs 1 and 2). Virulence structures remained in evidence, in both the absence and presence of eugenol (Figure 2 - A, B, C, D).

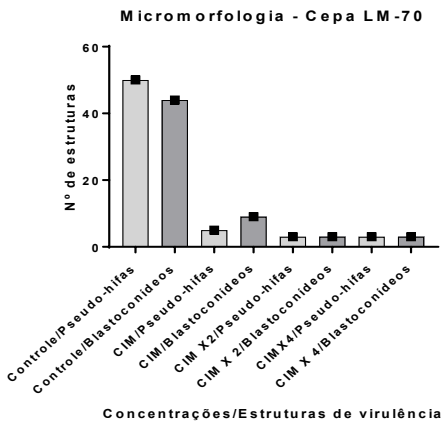
Candida parapsilosis	Eugenol (µg/mL)				Nystatin (µg/mL)	Strain Control **	Medium Control ***
	MIC*	MFC	MFC / MIC	Antifungal activity	MIC**		
ATCC 22019	256	256	1	Fungicidal	+	+	-
LM-1	256	256	1	Fungicidal	+	+	-
LM-2	256	256	1	Fungicidal	+	+	-
LM-7	256	256	1	Fungicidal	+	+	-
LM-70	256	256	1	Fungicidal	+	+	-
LM-225	256	256	1	Fungicidal	+	+	-
LM-302	256	256	1	Fungicidal	+	+	-

Table 2: Results of the MIC and CFM evaluation of eugenol and nystatin on C. parapsilosis MIC * (Eugenol) 100%: 64□g / mL **: There was growth in the largest concentration analyzed

***: No microorganism growth.



Graph 1: Growth/inhibition of pseudo-hyphae and blastoconids in the ATCC 22019 strain in the presence of eugenol at MIC, MICx2 and MICx4 and in its absence (control).



Graph 2: Growth/inhibition of pseudo-hyphae and blastoconids in the clinical strain LM-70 in the presence of eugenol at MIC, MICx2 and MICx4 and in its absence (control).

The graphs in Figure 3 presents $\log_{10}$CFU/mL as a function of exposure time to eugenol (MIC, MICx2 and MICx4) for *C. parapsilosis* ATCC® 22019™, *C. parapsilosis* LM-70, and the control group. The standard strain under the effect of eugenol presented growth reduction at MIC and MICx2 at four hours. The clinical strain exposed to the product presented growth reduction at 2 hours at MIC and MICx2. After 4 hours, no fungal growth was observed.

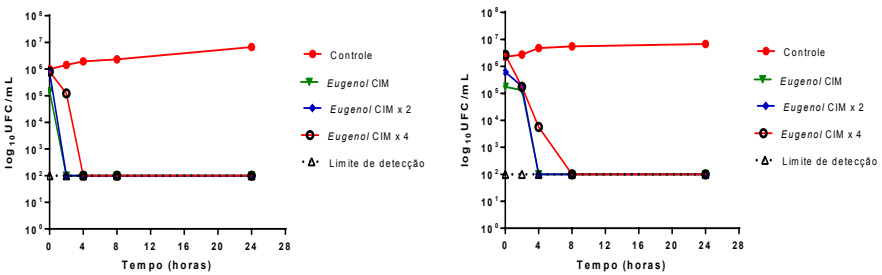


Fig 3: Microbial time-death curve for the antifungal effect of Eugenol (A) ATCC strain 22019 (B) clinical strain LM-70; in MIC, MICx2, MICx4 concentrations at times 0, 2, 4, 8, 12, 24h. Software Prism® Version 7. Time (hours) – Control – Limit of Detection – MIC x 4, MIC x 2, MIC.

DISCUSSION

The incidence of fungal infections caused by *C. parapsilosis* has increased exponentially (TROFA *et al*., 2008; SARTORATTO., 2004). In Europe, there has been a depolarization in the epidemiological indexes of invasive fungal diseases with a significant increase in infections caused by non-albicans species, specifically *C. parapsilosis* (COLOMBO *et al*., 2006).

In Brazil studies are scarce for non-albicans species, especially *C. parapsilosis*. However, the frequency of *C. parapsilosis* has been expressed in a multicenter study between the period 2003 and 2004 in 11 hospitals in nine Brazilian cities, where it was found that *C. parapsilosis* represented the third predominant species (MOHIDDIN *et al*., 2015).

In the oral cavity, wide colonization by albicans and non-albicans species is observable. In our research, a prevalence of 15.3% for *Candida parapsilosis* was diagnosed, characterizing the species as the third most prevalent oral isolate in the participating individuals. This frequency may be justified by *quorum sensing* mechanisms expressed in the species in relation to other microbial communities. The mechanism allows microorganisms to establish relationships, monitor their own population density and regulate gene expression - controlling the formation of biofilms and virulence (signaling pathway) factors. Our data converge with a study by MOHIDDIN *et al.,* (2015); CASTRO AND LIMA (2011), which evaluated the prevalence of albicans and non-albicans species in a sample

composed of 50 children with Down's syndrome, and 50 children constituting the control group. Their results reveal that *Candida parapsilosis* was the third most frequent species found in both groups. *C. parapsilosis* was the type of yeast most found in type II diabetic individuals in research performed by SHARMA *et al*., (2017). These data demonstrate the presence of this species in the oral microbial population, and its emergence as an important pathogen in oral mycoses.

Of the products analyzed during screening, eugenol obtained the best antifungal activity. Yet 100% of the tested strains were resistant to *Eugenia uniflora* esential oil. Similar results were found by Castro and Lima (2011), in which 66.7% of the *Candida albicans* (n = 5) and *Candida tropicalis* (n = 7) strains analyzed, presented resistance to the essential oils of *E. uniflora*, *C. reticulata*, *M. chamomilla*, and *Z. officinale*. The positive effect of eugenol, inhibiting the strains analyzed can be justified by the chemical reaction, (that occurs between the hydroxyl (OH) present in the phytochemical, and components of the fungal cell membrane), and which promotes cell collapse and the death of the yeast (PAULA *et al*., 2014).

In the microdilution test, the antifungal activity concentration of eugenol was 256 µg/mL. Similar results were described by Paula *et al*., (2014), who obtained 90% inhibition at 375 µg/mL for strains of *C. albicans* and *C. tropicalis* when isolated from the oral cavity of positive serum patients. On the other hand, Fontanella *et al.,* (2011) determined an MIC of 620 µg/mL for eugenol in strains of *C. parapsilosis* isolated from dogs, which suggests greater resistance in strains isolated from these animals as compared to the oral isolates tested in this study.

For all tested strains, the MFC coincided with the MIC. According to the parameters established by Saddiq and Khayyat (2010) a substance has a fungistatic activity when the MFC/MIC ratio is ≥ 4, and fungicidal activity when the MFC/MIC ratio is <4. The methodology of Hafidh *et al*. (2011) considers respective ratios of 1: 1 and 2: 1. With a ratio greater than 2: 1, the product is considered both fungicidal and fungistatic. Adopting these methodologies, eugenol presents fungicidal activity. On the other hand, in studies by Marcos Arias *et al*., (2011), where the resistance of the *C. parapsilosis* strains to eugenol was demonstrated, similar MFC values (to those of MIC) were found for *C. albicans*, *C. tropicalis* and *C. glabrata* (50*µ*g/mL). Their findings demonstrated a profile of lower sensitivity for *C. parapsilosis* when facing antifungal substances.

The study of virulence structures in the micromorphology assay demonstrated decreases in pseudohyphae and blastoconidia (Graphs 1 and 2) demonstrate concentration dependent phytochemical activity, inhibiting the growth of the structures (Figures 2 A, B, CD). Virulence factors have the capacity to produce disease and make the fungus more virulent during the infectious process (FREIRE *et al*., 2017). A possible explanation of eugenol's mechanism of action under these factors is that the molecule is supposed to act by inhibiting the MAP-K (Mitogen-Activated Protein - Kinase) signaling pathway, responsible

for activating the Cph1 gene, (which promotes the filamentous phase), as well as the factor CLA4 which is responsible for formation of the hyphae germination tube. In order to clarify and consolidate the mechanism of action, future studies are suggested that focus on elucidating the mechanism of action of eugenol on the MAP-K pathway and in virulence factor inhibition.

In the kinetics test, two strains of *Candida parapsilosis* (ATCC® 22019™, and LM-70) were submitted to the microbial death kinetics test. This experiment entails a count of the number of colony-forming units per mL (CFU/mL) to confirm whether the tested product presents fungicidal or fungistatic action. It analyzes the interaction between the product and the microorganism, characterizing a dynamic relationship between concentration and activity over the evaluated times. Analyzing the data expressed in Figure 2-A, strain observed a decrease of 3 $\log_{10}$CFU/mL at the MIC. At the MICx2 in the first four hours of the test, and from eight hours onwards, no fungal growth was observed. For the clinical strain LM-70, in four hours the product had already killed 99.9% of the viable cells. The product presented fungicidal activity - according to criteria recommended by Klesper *et al.,* (1998) in both microbial death curves.

Under the conditions of this experiment, a nystatin resistance profile was observed in relation to the tested strains, it was not possible to determine the MIC for the antifungal, assuming that the concentration for sensitivity of these strains is greater than 1024 μg/mL. Corroborating this study, Freire *et al* (2017), have reported resistance to nystatin in their studies of *Candida albicans* isolated from the oral cavity of individuals wearing prostheses. This fact might be explained by the general ease of access to antifungals and their disordered use by the population. In addition, pharmaceutical development research is more focused on antibacterials as compared to antifungals, as a consequence the arsenal of antifungal drugs is more limited than antibacterials (SIFUENTES-OSORNI., 2012). Thus, it is imperative to use the available antifungal drugs cautiously, as well as to develop new complementary therapies for treatment of oral fungal infections, as our data confirms.

CONCLUSIONS

The results allow us to conclude that *C. parapsilosis* was prevalent in the oral isolates of the individuals in the studied sample, and can be characterized as an emerging fungus in the development of oral candidiasis. Eugenol presented fungicidal effect on the strains tested, affecting virulence structures, and presenting an inhibitory effect on viable cells in the microbial death curve. Prospects for future studies will focus on *in vitro* tests against phytoconstituent specific target structures in the fungal cell, on cytotoxicity, and on animal models.

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