DOI: 10.56511/JIPBS.2021.v8.i02.pp01-09

Journal of Innovations in Pharmaceutical and Biological Sciences (JIPBS)
ISSN: 2349-2759
Available online at www.jipbs.com

Research article

Evaluation of antifungal activity of essential oils against different *Candida* spp. clinical isolates

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Received on: 16/12/2020, Revised on: 10/01/2021, Accepted on: 12/01/2021, Published on: 01/04/2021.

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Keywords: *Candida* spp., essential oil, antifungal activity.

Vol. 8 (2): 01-09, Apr-Jun, 2021.

Abstract

Candida spp. infections, also called candidiasis or candidosis, vary from mild to invasive and even can be fatal, being oral candidiasis one of the most common fungal infections in humans. Several systemic and local factors can stimulate the development of infections in the oral mucosa. The main treatment for oral candidiasis is the use of antifungals and the nystatin has been the first choice overall. However, with the increase in resistance of *Candida* species by the antifungals used in clinical practice, including the risk of toxicity associated with their use, there is an increase to the interest in researching antifungal activity in natural components. The aim of this study is to evaluate the antifungal activity of essential oils of *Citrus limonum, Eucalyptus globulus, Eucalyptus citriodora, Mentha Piperita and Rosmarinus officinalis* in isolated *Candida* spp. of the patient's oral cavity by disk diffusion test and minimum inhibitory concentration (MIC) determination. The essential oil of *E. globulus* showed the best result in the disc diffusion method, with inhibition zone ranging from 33mm to 65mm. The essential oils of *E. globulus and M. piperita* showed antifungal activity, the MIC values obtained with the essential oil of *E. globulus* ranged from 125 µg/mL to >1000 µg/mL and the MIC values obtained with the essential oil of *M. piperita* ranged 250 µg/mL to >1000 µg/mL.

Introduction

The yeast of the genus *Candida* is one of the main fungal agents that cause human and animal infections, being *C. albicans* the most isolated species. *Candida* spp. are part of the microbiota of different anatomical sites in the human body and usually do not cause any damage, being present in membranes, mucous membranes, skin, gastrointestinal and genital tracts [1, 2].

Infections by *Candida* spp., called candidiasis or candidosis, vary from mild to invasive and even can be fatal [3, 2]. There are several clinical manifestations presented by *Candida* spp., which can be caused by a single species or a mixed infection [4]. One of the most common fungal infections in humans is oral candidiasis [5], referred to as creamy stomatitis and characterized by the presence of white plaques grouped or isolated in the oral mucosa [6].

In the oral flora of healthy humans, the presence of *Candida* spp. it is normal, being present in 45-65% of healthy children and 30-50% of healthy adults [7]. The most commonly isolated species in the oral cavity is *Candida*

albicans. Others such as *C. tropicalis, C. dubliniensis, C. glabrata, C. parapsilosis, C. krusei, C. kefyr* and *C. stellatoidea* have also been isolated [8-10].

The infection development in the oral mucosa can be stimulated by several systemic and local factors, such as xerostomia, use of dentures, use of corticoids, loss of teeth, changes in eating habits, hormonal changes, chemotherapy, leukemia, diabetes mellitus (DM), old age, pregnancy, immunological diseases, among others [8, 9]. In patients infected with HIV virus, oral candidiasis is one of the first clinical symptoms of AIDS, affecting about 90% of these individuals [11, 12].

The main treatment for oral candidiasis is the use of antifungals [13]. The severity and extent of the disease, in addition to possible adverse effects of the drugs, must be considered when choosing the antifungal. There are topical and systemic antifungals [7]. Better responses to treatment are related with early initiation in the use of antifungals [14]. The use of topical nystatin is usually the first choice for oral candidiasis treatment. Miconazole is an antifungal also widely used in topical form [15]. In addition to these, fluconazole, which has a broad spectrum of action, also has good activity in superficial and deep infections [13]. In moderate to severe cases, the recommendation is to use itraconazole, voriconazole, amphotericin B, and echinocandins such as caspofungin, micafungin or anidulafungin, among others [16].

The *C. albicans* species has shown cases of resistance to amphotericin B, nystatin, fluconazole, itraconazole and ketoconazole [4]. The non-*albicans* species are known to have more resistance to the antifungals commonly used in the treatment of candidiasis [17]. *C. glabrata*, naturally, is less susceptible to azole antifungals and has been resistant to echinocandins [18, 19]. *C. krusei* has intrinsic resistance to fluconazole and its resistance to amphotericin B shows signs of increasing [20, 21]. The species *C. lusitaniae* shows intrinsic or acquired resistance against amphotericin B [22, 23].

In view of the increased resistance of *Candida* species by the antifungals used in clinical practice, including the risk of toxicity associated with their use, interest in researching antifungal properties in natural components has been increasing [5, 24].

For a long time, the only therapeutic resource in many communities and ethnic groups was the knowledge about medicinal plants, being used in the treatment of diseases since the dawn of human civilization. [25]. Plants produce an enormous variety of components [24]. Many of them can be used as complementary therapy, as they show antimicrobial activity from the extraction of their essential oils. [26]. Essential oils (EOs) from different plants have pharmacologically active components and a broad spectrum of antimicrobial activity [24]. These essential oils are complex mixtures of organic components. They are volatile, formed naturally as secondary metabolites of plants. EOs can be extracted from leaves, flowers, fruits, stems, buds, roots, seeds and wood [27].

The aim of this study was to evaluate the antifungal activity of essential oils in *Candida* spp. isolates of the oral cavities of a number of patients. The tested essential oils were *Citrus limon* (Lemon; Family *Rutaceae*), *Eucalyptus globulus* (Tasmanian blue gum; Family Myrtaceae), *Eucalyptus citriodora* (Eucalyptus Citriodora or Lemon Eucalyptus; Family *Myrtaceae*), *Mentha piperita* (peppermint; Family *Lamiaceae*), Rosmarinus officinalis (Rosemary; Family *Lamiaceae*), against the strains of *Candida albicans, C. glabrata, C. tropicalis, C. lusitaniae, C. krusei, C. guillermondii* and *C. intermedia.*

Materials and methods

Microorganisms

The microorganisms selected for this study were provided by the Biomicolab Laboratory (Mycology Laboratory of the Federal University of Rio Grande do Sul), all of which were clinical isolates from patients using dental prostheses from the project approved by Research Ethics Committees (CEP - UFRGS) under number 2.236.863.

The fungi used were seven species of the genus *Candida*: *C. albicans* (1 and 2), *C. glabrata* (1 and 2), *C. tropicalis* (1 and 2), *C. lusitaniae* (1 and 2), *C. krusei* (1), *C. guillermondii* (1), and *C. intermedia* (1). All isolates were confirmed by Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) Biotyper 4.0 Microflex Bruker.

All strains were stored in Tryptone Soya Broth (TSB; Himedia®) plus 10% glycerol and frozen at -18°C. At the time of use, the strains were reactivated by inoculating 10 µL of the stock culture in tubes with Sabouraud dextrose agar with chloramphenicol (Kasvi®), and incubated at a temperature of around 30°C for 48 h.

Essential oils

The essential oils of *Rosmarinus officinalis* (Rosemary) lot 190531, *Citrus limon* (Lemon) lot 111224 and *Eucalyptus globulus* (Tasmanian blue gum) lot 120407 were obtained from commercial samples of Floranda Indústria e Comércio de Cosméticos e ProdutosNaturais LTDA ME - São Paulo, Brazil (Bioessência trademark).

The essential oil of *Eucalyptus citriodora* (Eucalyptus Citriodora Lemon Eucalyptus) was obtained from commercial samples from Yanih Produtos Cosméticos e Farmacêuticos Ltda - São José dos Pinhais - Paraná- Brazil (Terra Flor Aromaterapia trademark), CLA lot.

The essential oil of *Mentha piperita* (Peppermint) was obtained from commercial samples of the Phytoterápica brand - Tucuruvi- São Paulo, Brazil, lot PQHP18C2.

The main constituents, found in the literature, of the essential oils used in this study are β-pinene, limonene, γ-terpinene for *Citrus limon* [28, 29]; citronella and citronelol for *Eucalyptus citriodora* [30, 31]; 1,8-cineole, limonene and α-pinene for *Eucalyptus globulus* [28, 31, 32]; menthol,

methyl acetate, menthol, neomenthol, 1,8-cineole and limonene for *Mentha piperita* [28, 33] and 1,8-cineole, camphor, α-pinene, camphene and borneol for *Rosmarinus officinalis* [28, 34, 35].

Evaluation of the antifungal activity of essential oils

The antifungal activity of the essential oils of *Citrus limon* (EOCl), *Eucalyptus citriodora* (EOEc), *Eucalyptus globulus* (EOEg), *Mentha piperita* (EOMp), *Rosmarinus officinalis* (EORo) against strains of *C. albicans, C. glabrata, C. tropicalis, C. lusitaniae, C. krusei, C. guillermondii* e *C. intermedia* was evaluated through disk diffusion tests and Minimum Inhibitory Concentration (MIC) determination.

Disk diffusion test

The disk diffusion test to evaluate antifungal activity was performed as a screening, determining the size of the zones of inhibition produced by the essential oils of *Citrus limon, Eucalyptus globulus*, *Eucalyptus citriodora*, *Mentha Piperita*, *Rosmarinus officinalis* against the strains of *C. albicans, C. glabrata, C. tropicalis, C. lusitaniae, C. krusei, C. guillermondii* e *C. intermedia*.

The fungal suspension of each test strain was prepared in test tubes containing 5 ml of sterile saline. With the aid of a sterile disposable handle, a small amount of inoculum was suspended in the test tube. Using the Bio-Spectrophotometer SP-220, the suspension was adjusted to $1x10^6$ CFU (Colony Forming Units), turbidity corresponding to 0.5 on the McFarland scale. This suspension was spread on sterile Petri dishes containing Sabouraud dextrose agar culture medium with chloramphenicol (Kasvi®).

The test was performed following the protocol described in document M44 of the Clinical and Laboratory Standards Institute, CLSI [36]. Filter paper discs with a diameter of 15mm were used. They contained 15 µL of each essential oil and were deposited on the culture medium already inoculated. The antifungal used in this test was itraconazole in 10 µg discs (Cecon / Brazil). The culture slabs were incubated in the New Lab N1040 oven, at 37ºC for 48 hours. The inhibition zones were measured with the aid of a ruler, registering in millimeters over the course of 24 hours.

The breakpoints used were those recommended by document M44 of the Clinical and Laboratory Standards Institute, in which ≥20 mm inhibition zone means sensitive, 15 mm-19 mm inhibition zone means dose dependent/sensitive intermediate and ≤14 mm inhibition zone means resistant [36].

With the results obtained in this screening test, the oils were selected to perform another test in order to verify their antifungal action.

Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the essential oils *Eucalyptus globulus* and *Mentha piperita* against the strains of *Candida spp.* was performed according to the instructions of the CLSI (Clinical and Laboratory Standards Institute, document M60-2017) and diluted in dimethyl sulfoxide - DMSO (Nuclear®). The reading intervals used for these essential oils ranged from 2000 µg/ml to 3.9 µg/ml [37].

The fungal suspensions were prepared in the same way as in the disk diffusion test, resulting in $1x10^6$ CFU/mL (Colony Forming Units), turbidity corresponding to 0.5 on the McFarland scale. Dilutions were subsequently made, 1:50 in 0.85% sterile saline and then again diluted 1:20 with RPMI 1640 (Sigma Aldrich®), resulting in a final suspension of $1x10^3$ CFU/mL.

The $1x10^3$ CFU/mL suspensions of each *Candida* spp. strain and essential oils were added to 96-well microslabs. For negative control, a well with only RPMI was used and for the positive control, a well with RPMI and fungal suspension. The slabs were incubated at a temperature of around 30ºC for 24 hours. The readings, to visualize the growth, were taken over the course of 24 hours [38].

CIM was defined as the lowest concentration of essential oil in which it was not possible to see any growth when compared to controls. The experiment was carried out in triplicates [4].

The antifungal used in this test was fluconazole, in concentrations ranging from 64 µg/mL to 0.125 µg/mL. To evaluate the results, were used the breakpoints recommended in CLSI for fluconazole [38], for the strains of *C. albicans* and *C. tropicalis* sensitive (S) ≤ 2 µg/mL, dose-dependent sensitive (SDD) 4 µg/mL, and resistant (R)> 8 µg/mL. For strains of sensitive, dose-dependent C. glabrata (SDD) 32 µg/mL, resistant (R)> 64 µg/mL and for resistant C. krusei (R)> 64 µg/mL. The breakpoint for the other strains was (S) ≤ 8 µg/mL, dose-dependent sensitive (SDD) 16-32 µg/mL, resistant (R)> 64 µg/mL [39].

Results and discussion

The overuse of antifungals and the increased resistance by *Candida* species makes it necessary to search for new therapeutic agents for fungal infections caused by these species [40]. In view of this, several studies have shown that essential oils have antifungal activity [41-46, 33] and can become an effective treatment for these infections.

The susceptibility of the strains of *C. albicans, C. glabrata, C. tropicalis, C. lusitaniae, C. krusei, C. guillermondii e C. intermedia* against the oils of EOCl,EOEc, EOEg, EOMp, EORo and itraconazole are shown in table 1 and the best results of inhibition zone, obtained with the essential oil of EOEg are shown in figure 1.

Table 1. Size of inhibition zone (mm) of itraconazole and essential oils against *Candida* spp.

	ITRA	EOCl	EOEc	EOEg	EOMp	EORo
C. albicans (1)	20	15	18	35	15	16
C. albicans (2)	20	20	16	46	15	12
C. glabrata (1)	8	25	19	45	15	10
C. glabrata (2)	10	20	18	33	30	10
C. tropicalis (1)	20	23	18	40	30	27
C. tropicalis (2)	20	20	19	40	68	25
C. lusitaniae (1)	19	35	21	60	9	10
C. lusitaniae (2)	19	32	40	65	10	11
C. intermedia (1)	18	26	30	50	15	14
C. guilhermondii (1)	14	17	19	40	**	15
C. krusei (1)	14	35	38	64	22	9

Note:Itraconazole (ITRA), Citrus limon (EOCl), Eucalyptus citriodora (EOEc), Eucalyptus globulus (EOEg), Mentha piperita (EOMp), Rosmarinus officinalis (EORo).



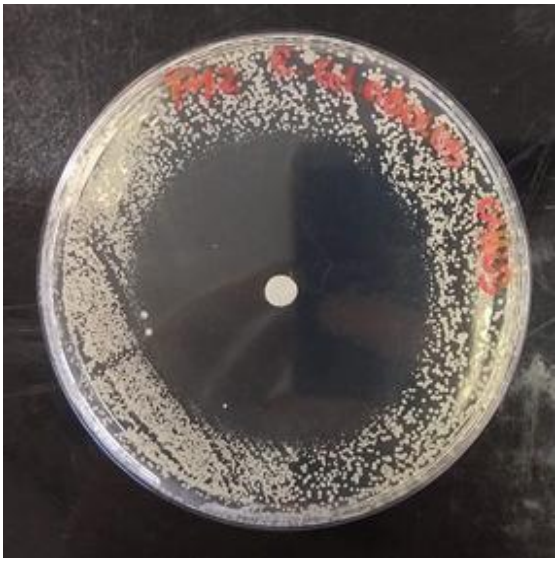
***C. albicans* (1)**



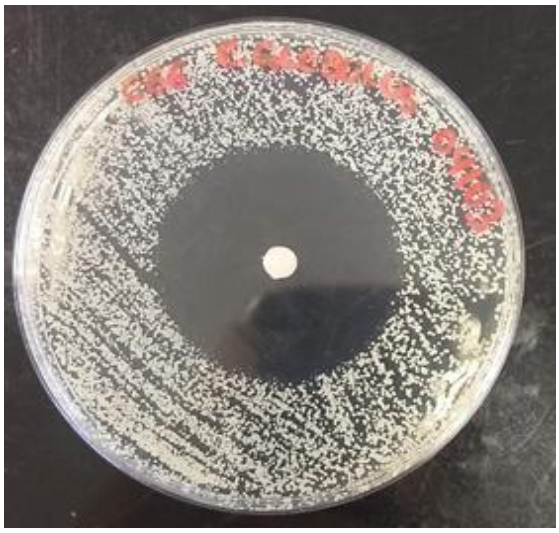
***C. glabrata* (2)**



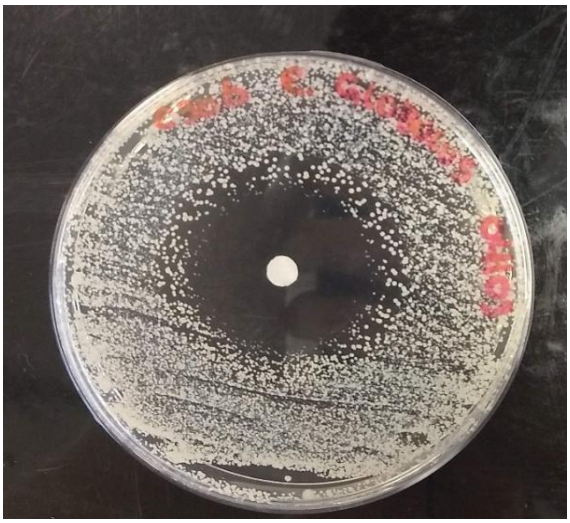
***C. tropicalis* (2)**



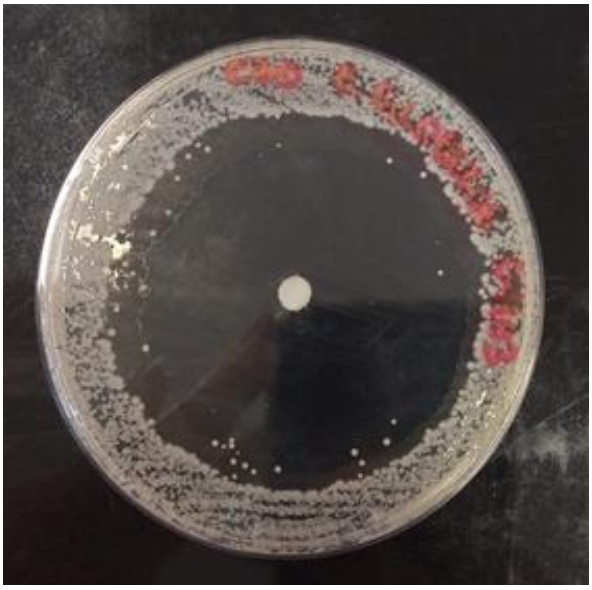
***C. lusitaniae* (2)**



***C. intermedia* (1)**



***C. guilhermondii* (1)**



***C. krusei* (1)**

Figure 1. Inhibition zone by disc diffusion test with the EOEg.

The strains of *C. albicans* and *C. tropicalis* showed a 20mm inhibition zone in the itraconazole disk, thus being sensitive to this antifungal. The strains of *C. glabrata* showed 8mm and 10mm inhibition zones, and the strains of *C. guilhermondii* and *C. krusei*, had, both, a 14mm inhibition zone, being, therefore, resistant to the antifungal. The strains of *C. lusitaniae* had a 19mm inhibition zone. *C. intermedia* had an inhibition zone of 18mm, being classified as intermediate.

The best results in the disk diffusion test were obtained with the essential oil of *Eucalyptus globulus,* followed by the essential oil of *Mentha piperita.* The essential oil of *Eucalyptus globulus* showed a zone of inhibition ranging from 33 mm to 65 mm and the essential oil of *Mentha piperita* showed a zone of inhibition of 68 mm for the strain of *C. tropicalis*. The essential oil of *Citrus limon* showed an inhibition zone ranging 15 mm to 35 mm. The inhibition zone of the essential oil of *Eucalyptus citriodora* was between 18 mm and 40 mm. Finally, the essential oil of *Rosmarinus officinalis* showed inhibition zones ranging from 10 mm to 27 mm.

The oils of *Eucalyptus globulus* and *Mentha Piperita* were selected to verify the minimum inhibitory concentration (MIC) and the results obtained are described in table 2.

Table 2. Minimum Inhibitory Concentration (MIC) (µg/mL) of fluconazole and essential oils of *Eucalyptus globulus* and *Mentha piperita* against *Candida spp.*

	FLU	EOEg	EOMp
C. albicans (1)	0.5	>1000	1000
C. albicans (2)	0.25	250	250
C. glabrata (1)	0.5	500	500
C. glabrata (2)	0.5	250	500
C. tropicalis (1)	2	>1000	>1000
C. tropicalis (2)	64	>1000	>1000
C. lusitaniae (1)	1	125	250
C. lusitaniae (2)	2	1000	500
C. intermedia (1)	4	1000	1000
C. guilhermondii (1)	2	500	500
C. krusei (1)	64	>1000	>1000

Note: Fluconazole (FLU), Eucalyptus globulus (EOEg) and Mentha piperita (EOMp).

The antifungal used in the Minimum Inhibitory Concentration (MIC) test was fluconazole, in concentrations ranging from 64 µg/mL to 0.125 µg/mL. To evaluate the results, were used the breakpoints for fluconazole recommended in CLSI (2017) for the strains of *C. albicans* and *C. tropicalis: sensitive (S) ≤ 2 µg/mL, dose-dependent sensitive (SDD) 4 µg / mL, and resistant (R)> 8 µg/mL. For strains of C. glabrata* : dose-dependent sensitive SDD) ≤ 32 µg/mL, and resistant (R)> 64 µg/mL and for *C. krusei* resistant (R)> 64 µg/mL. The breakpoint for the other strains was (S) ≤ 8 µg/mL, dose-dependent sensitive (SDD) 16-32 µg/mL and resistant (R)> 64 µg/mL (CLSI, 2007).

Observing the values described in table 2 and the breakpoints of fluconazole recommended in CLSI, the two strains of *C. albicans*, the two strains of *C. lusitaniae* and the strain of *C. guilhermondii* tested in this study showed sensitivity to fluconazole. The two strains of *C. glabrata* presented MIC of 0.5 µg/mL, which demonstrates that both are dose-dependent sensitive. For *C. tropicalis* strains, values of 2 µg/mL and 64 µg/mL were found, indicating sensitivity and resistance to fluconazole, respectively. The strain of *C. krusei* presented an MIC of 64 µg/mL, showing resistance to fluconazole.

The MIC values obtained with the essential oil of *Eucalyptus globulus* ranged from 125 µg/mL to >1000 µg/mL, as described in Table 2. The best results were obtained with the strain of *C. lusitaniae*, which presented MIC of 125 µg/mL, and the strain of *C. albicans* (2) and *C. glabrata* (2) that both presented MIC of 250 µg/mL. The strains of *C. glabrata (1) and C. guilhermondii* showed MIC of 500 µg/mL. And values of 1000 µg/mL were found within the strains of *C. lusitaniae and C. intermedia*. The others showed MIC values greater than 1000 µg/mL. Thus, it was observed that the strains used in this study were susceptible to the essential oil of *Eucalyptus globulus*.

The MIC values obtained with the essential oil of *Mentha piperita* ranged from 250 µg/mL to >1000 µg/mL. The best results were obtained with the strains of *C. lusitaniae* (1) and the strain *C. albicans* (2), that showed both MIC of 250 µg/mL, and the two strains of *C. glabrata*, the strain of *C. lusitaniae* (2) and the strain of *C. guilhermondii*, all with MIC of 500 µg/mL. Other strains tested showed MIC greater than or equal to 1000 µg/mL, which demonstrates that the essential oil of *Mentha piperita* is susceptible to 80% of the strains tested in the present study. The data is at Table 2.

There are differences in MIC values found between strains of *Candida spp.*, and also between strains of the same species. This indicates that susceptibility to essential oils can vary. In addition, we can observe that the isolates of *C. glabrata* in the disk diffusion test were resistant to itraconazole and sensitive to dose-dependence against fluconazole, the antifungal used in the minimum inhibitory concentration test, however both isolates, resistant to azoles, showed susceptibility to the two essential oils used in this study.

Our results corroborate data found by other authors who evaluated the antifungal activity of essential oils and the susceptibility to classic antifungals, using the disk diffusion test [41-43]. They described inhibition zone values between 9 and 27mm for itraconazole when tested against *Candida spp.* [41], inhibition zones of 26.7 and 22.2 mm for the essential oils of *Eucalyptus globulus* and *Mentha piperita,* respectively, against clinical isolates of *C. albicans* [42] and inhibition zones of 10 and 30mm for the essential oils of *Mentha piperita*, *Eucalyptus globulus* and *Rosmarinus officinalis* against 40 isolated strains of *C. albicans* [43]. In the present study, for *C. albicans* strains, the inhibition zones obtained with *Eucalyptus globulus* essential oil were 35 mm and 46 mm, With *Mentha piperita* essential oil, the inhibition zone had 15mm. The differences found in the inhibition zone values may be related to the difference in the chemical composition of the essential oils used.

The *Eucalyptus globulus* essential oil showed antimicrobial activity against bacteria, yeasts and filamentous fungi in a work previously described. There, the MIC of *Eucalyptus globulus* essential oil for bacteria and filamentous fungi was in the range of 2.250 to 9000 µg/mL, and 2250 µg/mL for *Candida albicans* strain [44]. We can observe a similarity with the *C. albicans* C10 strain, which presented MIC of 2000 µg/mL, but the same does not apply to the *C. albicans* strain 39 who presented MIC of 250 µg/mL.

Barbosa and collaborators aimed to evaluate the anti-*Candida* activity of the essential oils of *Eucalyptus citriodora* and *Eucalyptus globulus*. Both oils were purchased commercially from the company Terra Flor and the initial oil concentration used in Minimum Inhibitory Concentration (MIC) was 16000 µg/mL. In the study, the strains for reference used were of *Candida rugosa, C. lusitaniae C. glabrata, C. utilis, C. krusei, C. guilliermondii, C. tropicalis, C. albicans*, *C. parapsilosis, C. glabrata* and *C. lusitaniae*. The results of the MIC with the essential oil of *Eucalyptus citriodora* ranged from 125 µg/mL to 500 µg/mL and from 1000 µg/mL to 8000 µg/mL for the essential oil of *Eucalyptus globulus,* results that corroborate with the present study [45].

In another study about anti-candida activity, the way the essential oil of *Mentha piperita* acted and the synergistic action with the antifungal were evaluated. In the study, 3 species of *Candida* spp. were used: *C. albicans, C. tropicalis* and *C. glabrata.* The value found in the MIC, for all strains tested, was 225 µg/mL, in a test performed according to CLSI. In the present study, the MIC for strains of *C. albicans* were 1000 and 250 µg/mL, *C. tropicalis* got >1000 µg/mL and *C. glabrata* were 500 µg/mL. The differences found in the two studies may be related to the compositions of the essential oils used in this study and in Samber's, Menthol, the main constituent of the essential oil of *Mentha piperita* [46], has different percentages in the two oils.

The antifungal and antibacterial activities of the essential oil of *Mentha piperita* L, against standard strains (ATCC) of *C. albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata*, *C. dubliniensis*, *C. parapsilosis* were evaluated by Saharkhiz and collaborators. Also, 35 clinical isolates (sensitive and azole-resistant) were included in the study: *C. albicans*, *C. dubliniensis*, *C. tropicalis*, *C. parapsilosis* e *C. glabrata*. The minimum inhibitory concentration (MIC) was performed according to CLSI M27-A (yeasts) and the oil concentration ranged from 60 to 16.000 µg/mL. For the standard strains, the MIC values ranged from 500 µg/mL to 4000 µg/mL. The best result was found with *C. krusei*, MIC of 500 µg/mL. For clinical isolates sensitive to azoles, the values ranged from 120 µg/mL to 2000 µg/mL, the best result being 120µg/mL for *C. glabrata*. With clinical isolates resistant to azoles, the values ranged from 500 µg/mL to 3000 µg/mL, the best result being 500 µg/mL for *C. dubliniensis* [33].

In the present study, the MIC values for azole-sensitive clinical isolates were 125 µg/mL to > 1000 µg/mL, while the MIC value for all azole-resistant clinical isolates was > 1000 µg/mL. Comparing the MIC results found in the present study and the results found by Saharkhiz and collaborators, it is noticeable the similarities between them [33].

According to the Brazilian Pharmacopeia [28] the essential oil of *Mentha × piperita L,* must contain at least 35.0% of menthol in its chemical constitution, being obtained from the aerial part of the plant, by hydrodistillation. In the study by Desam and collaborators the major constituents of the essential oil of *Mentha × piperita L* were menthol, methyl acetate and menthofuran [56]. Samber and collaborators [46] analyzed the components of the essential oil of *Mentha × piperita L* by GC-MS and the main chemical constituents of the essential oil were carvone and menthol. In the Brazilian Pharmacopoeia [28] the essential oil of *Eucalyptus globulus* must contain at least 70.0% of 1,8- cineole in its chemical composition, being obtained from fresh leaves and terminal branches of the plant by hydrodistillation. The main chemical constituent of the essential oil obtained by hydrodistillation of fresh leaves of *Eucalyptus globulus* was 1,8-cineole, followed by limonene, α-pinene, α-terpineol and globulol [31]. In addition, the main constituents of a commercial sample of essential oil of *Eucalyptus globulus* were1,8- cineole and α-pinene [58].Thus, we can conclude that the main chemical constituents of the essential oils of *Mentha × piperita L* and *Eucalyptus globulus*, are, respectively, menthol and 1,8- cineole.

The main chemical components of essential oils are terpenes and phenolic compounds, and the biological properties of essential oils and their fragrances are due to these compounds [47, 48]. One of the mechanisms for the action of essential oils, because of their lipophilic nature, is the ability to disrupt the fungal cell wall, generating cell death [47, 49]. Other studies suggest that essential oils also inhibit, in the cell of microorganisms, the synthesis of proteins, DNA, RNA and polysaccharides [48].

In general, the expected result in plants that belong to the same family, given the proximity of the chemical components of their essential oils, is a similarity in their inhibitory action [43]. However, even with samples of the same species, essential oils can show a varied chemical composition and this is justified by the difference in geographical location, environmental conditions, the degree of ripeness, existing numerous chemotypes, the conditions and extraction methods used [43, 50, 51]. Furthermore, factors such as the composition of the essential oil, the interaction that occurs between those constituents, which can cause an additive effect, indifferent or antagonistic synergism, and their percentages can also affect an essential oil's antimicrobial property [52]. In view of this, it is necessary, when working with natural products, to observe the differences in the chemical composition of essential oils [53]. The present work did not aim to carry out an analysis of the chemical constituents of the essential elements, but as future perspectives to give continuity to the study.

Conclusion

Considering all of the above, in the disk diffusion test, the essential oil of *Eucalyptus globulus* was the oil with the greatest antifungal activity, followed by the essential oil of *Mentha piperita, Eucalyptus citriodora, Citrus limonum* and *Rosmarinus officinalis*, in order. In minimum inhibitory

concentration (MIC) determination, both *Eucalyptus globulus* and *Mentha piperita* showed antifungal activity.
As a conclusion, essential oils can be an adequate option in the treatment of oral candidiasis. The present work presented an expansion of the studies already reported in the literature on the activity of essential oils against the strains tested here. However, new tests must be carried out, such as the time-kill curve, which allows evaluation of the speed of death of the microorganism by the concentration of the test substance; and hemolytic activity, which assesses the toxicity of the essential oil, so that pharmaceutical formulations can be developed to be used in therapeutic practice.

Conflicts of Interest
All authors have contributed equally for the article. The authors declare no conflict of interest.

Author contributions
All the authors have contributed equally in designing, drafting the manuscript as per the journal submission format. All authors read and approved the final manuscript.

Acknowledgments
Foundation of State Research of Rio Grande do SUL - FAPERGS, National Council for Scientific and Technological Development Organization - CNPq for financial support.

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