UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE FARMÁCIA
TRABALHO DE CONCLUSÃO DE CURSO EM FARMÁCIA

Antibiofilm activity of essential oils in *Candida* spp .: a literature review

Thayna Da Silva Vargas

Porto Alegre, 2021

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE FARMÁCIA
TRABALHO DE CONCLUSÃO DE CURSO EM FARMÁCIA

Antibiofilm activity of essential oils in *Candida* spp.: a literature review

Thayna Da Silva Vargas

Profa Dra. Adelina Mezzari Orientadora
M.a Farmacêutica Simone Krause Ferrão Coorientadora

Porto Alegre, 2021

Agradecimentos

Agradeço, primeiramente, a Deus e as pessoas mais importantes da minha vida, meus pais Adriana e André e meus irmãos, Thiago e Júlia. Obrigada por todo apoio durante esta trajetória, por toda luta e por todos os conselhos e amor que vocês me deram nestes anos. Obrigada por nunca me deixarem faltar nada e por sempre estarem do meu lado, principalmente, nos meus dias ruins e de cansaço e por me ajudarem a fazer a melhor escolha em todas as vezes que pensei em desistir. Agradeço a minha avó Dejanira que sempre me aconselhou em tudo na vida com sua sabedoria e sempre esteve ao meu lado e minha avó Maria Santa, que mesmo de longe, acompanhou grande parte da minha trajetória.
Sou muito grata e amo vocês, família!

Agradeço, ao meu namorado Denis Erdmann, que esteve presente desde o início desta trajetória, e que sempre me apoiou e me deu forças para seguir. Obrigada por ser meu companheiro nesses longos seis anos e por sempre tentar me fazer sorrir, mesmo nos meus piores momentos.

Agradeço, a minha amiga Andrielli, companheira de profissão, que está do meu lado muito antes da faculdade e que por vezes, mesmo distante, me ajudou e apoiou na realização deste sonho. Obrigada por entender minhas faltas e por sempre estar presente.

Agradeço as minhas amigas e colegas Camilla, Larissa e Stéffanie que estiveram presentes desde a primeira semana do curso. Por todos os momentos compartilhados, por todos os trabalhos realizados, por todo sofrimento coletivo e acima de tudo por todos conselhos e pela amizade que construímos. Vocês fizeram toda diferença na realização deste sonho e é um prazer imenso ter vocês ao meu lado neste momento.

Agradeço, a minha orientadora Adelina Mezzari por toda dedicação e apoio durante esses anos de aprendizado, desde a iniciação científica, monitoria acadêmica e agora no TCC. Por ser sempre muito atenciosa e prestativa e por estar sempre presente para ensinar, incentivar e ajudar. És uma inspiração para mim.

Agradeço, a minha coorientadora Simone Krause Ferrão por estar sempre presente e disponível, por sua paciência, pois nem sempre é fácil lidar com nossas dúvidas, e por nos ajudar com sua sabedoria e experiência no meio científico.

Por fim, agradeço a todos que de alguma forma cruzaram meu caminho e fizeram parte desta trajetória.

Muito obrigada!

Este trabalho foi elaborado na estrutura de artigo conforme as normas do "**Journal of Innovations in Pharmaceutical and Biological Sciences"** apresentadas em anexo.

Antibiofilm activity of essential oils in *Candida* spp .: a literature review

Thayna da Silva Vargas[1], Simone Krause Ferrão[1], Adelina Mezzari[1*.]

*** Corresponding Author:**

Dr. Adelina Mezzari

University of Rio Grande do Sul, Faculty of Pharmacy (UFRGS)

Ipiranga Avenue, 2752 - Azenha

ZIP Code: 90610-000 Porto

Alegre, RS, Brazil

mezzari@ufrgs.br

(+55) 51**33082105**

[1] Department ofAnalysis, FacultyofPharmacy, Federal Universityof Rio Grande do Sul (UFRGS), Porto Alegre, Rio Grande do Sul (RS), Brazil

ABSTRACT: Species of the genus *Candida*, despite part of the normal human microbiota, can cause important fungal infections ranging from superficial clinical manifestations to fatal invasive lesions. Most of these infections are related to the ability of *Candida* spp. To form biofilm, which results in increased resistance to antifungal agents, such as ketoconazole, amphotericin b and fluconazole, hindering proper treatment. The increase in these infections together with increased resistance to antifungal drugs has made it necessary to seek new therapeutic alternatives. Among the new alternatives is the search for essential oils of plants that have antibiofilm properties. A search was performed in the Science direct, Scopus, PubMed and Scielo databases. From this search, a total of 39 essential oils were found, related to antibiofilm activity in front of *Candida* species, among them are lemon grass, eucalyptus, cinnamon and tea tree oils. The data found in this review demonstrated the antibiofilm activity of most essential oils, suggesting that they can be used as new treatment alternatives and reinforcing the need for further studies to prove their efficacy.

Keywords: *Candida*, essential oils, antibiofilm, virulence, candidiasis.

INTRODUCTION

Candidiasis is one of the main causes of opportunistic fungal infections. The factors that imply the increased occurrence of candidiasis are immunosuppressive therapies, invasive surgical procedures and the use of broad-spectrum antimicrobials [1,2]. *Candida* species infections range from mucosal colonization to invasive lesions that can be fatal [1]. *Candida albicans* is the most frequent fungal species and cause superficial, cutaneous, subcutaneous and systemic infections [3]. However, other species known as "nonalbicans", including *Candida tropicalis, C. glabrata, C. parapsilosis* and others have been isolated regularly [4].

Candida species are found both in the environment and in the normal flora of humans, so their presence in the organism will not always indicate fungal infection. They are causatous agents that can reach high mortality rates, especially in immunocompromised individuals, intensive care patients, post-surgical units and neoplasms [3,5]. *Candida* species can colonize and cause diseases in various sites of the body such as the skin, oral cavity, gastrointestinal tract, genital, vascular system and others [5,6].

The pathogenesis of candidiasis depends on the health of the host, as well as the virulence factors that these species express in certain situations. Virulence factors include germ tube formation, adhesin production, adhesions, biofilm formation and hydrolytic enzyme production [3,6].

The expression of virulence factors may vary depending on the infecting species, geographic origin, type of infection, location and stage of infection, and host reaction. Knowledge of these factors is considered an important tool to understand the pathogenesis of fungal diseases caused by *Candida* spp. and to assist in the development and discovery of new therapeutic targets [7].

Most diseases caused by *Candida* species are related to biofilm formation [3]. Biofilm production varies according to *Candida* species and consists of a pathogenetic factor that plays an important role in tissue adhering and colonization by *Candida* spp. [8]. The biofilm corresponds to a group of microorganisms embedded in an extracellular matrix that form a three-dimensional structure on biotic and abiotic surfaces [6,9]. Microbial biofilms are the

main cause of hospital infections and the source of many diseases that become recurrent and persistent. In addition to biofilms being genetically resistant to some antifungals, including amphotericin B and fluconazole, studies show that biofilms of *Candida* species considerably reduce sensitivity to different antifungals, hindering their treatment [3,4,6].

The increased resistance of these pathogens to conventional antifungals as well as their side effects, such as nausea, vomiting, rash and others, encouraged the search for new therapeutic alternatives. There has been a growing interest in the study of secondary metabolites of plants as new therapeutic alternatives, especially essential oils, as they have antibacterial and antifungal properties [10,11].

Essential oils (OEs) are natural compounds constitued of a complex mixture of volatile compounds extracted from medicinal and aromatic plants with promising biological properties [10,12]. They have wide application in popular medicine, aromatization, food preservation and fragrance industries. In recent years, studies have shown that essential oils and their constituents have antimicrobial properties against some bacteria and fungi. However, there are still few studies on the action of essential oils against the biofilm formed by *Candida* spp. [13,10].

In view of the need for new therapeutic alternatives for the treatment of infections caused by *Candida* spp. and, the use of essential oils from plants with antibiofilm activity appears as a promising therapeutic alternative. Thus, the objective of the present study was to conduct a literature review on the antibiofilm activity of some essential oils against *Candida* spp.

A literature review was carried out in the online databases Science direct, Scopus, PubMed and Scielo. Scientific articles related to essential oils from plants with antibiofilm activity against *Candida* spp were searched.The keywords used were Candida spp. AND oil AND antibiofilm for the last 10 years (2010 to 2020).

In this research 279 articles were found in Science direct, 48 in Scopus, 40 in PubMed and 3 articles in Scielo. These articles were analyzed in their entirety to verify the relevance for the purpose of this review. After reading and excluding articles not truly related to the purpose of this study, 31 articles of oils were found associated with the antibiofilm activity of *Candida albicans, C. tropicalis, C. krusei, C. dublinienses, C. glabrata, C. parapsilosis, C. guillermondii, C. rugosa, C. lusitaniae, C. membranafaciens C. famata, C. boidinii, C. pelliculosa e C. colliculosa* (Table 1).

1.Essential oils with antibiofilm activity for *Candida* spp.

In the last 10 years, few studies have been done on the effectiveness of essential oils in inhibiting the biofilm formation of *Candida* species. The main results obtained from the antibiofilm activity of essential oils found in *Candida* species are listed below.

Essential oil of *Anthemis stiparum*

Elkhalifa e*t al.*analyzed the antibiofilm activity of essential oil and methanolic extract of *Anthemis stiparum* on *Candida albicans* and other microorganisms. The essential oil of *Anthemis stiparum* showed moderate antibiofilm activity against *C. albicans* ATCC 10239 in the MIC (50 μL / mL).The methanolic extract demonstrated greater antibiofilm activity against *C. albicans*, inducing 80.02% biofilm inhibition when used tested by the MIC [14].

Essential oil of *Aeollanthus cucullathus, Aeollanthus heliotropioides* and *Plectranthus glandulosus*

Mback*et al*. evaluated the antibiofilm activity of the essential oils of *Aeollanthus cucullathus, Aeollanthus heliotropioides* and *Plectranthus glandulosus* against the species of *Candida albicans* and *C. glabrata*. The results obtained demonstrated that there was inhibition of the mature biofilm at the tested concentrations (MIC/2; MIC/4; MIC/6 e MIC/10), 0,06mg / mL, 0,5 mg / mL e 3 mg / mL, respectively. The best result was obtained by the essential oil of *A. helitropioides* followed by the essential oil of *P. glandulosus* and *A. cucullathus* [15].

Essential oil of *Melaleuca alternifolia* (tea tree)

Francisconi *et al*. tested and demonstrated antibiofilm activity of tea tree oil and its main component terpinen-4-ol against two clinical strains of *Candida albicans.* These strains, identified by molecular typing as genotypes A and B, were isolated from diabetic patients with chronic periodontitis. Nystatin tests using the reference strains *C. albicans* ATCC 90028 and SC 5314 were also compared to the results. Mouthwash simulations containing the oil, terpinen-4-ol and nystatin were also performed. The researchers also observed that at concentrations of 17.92 mg / mL for tea tree oil and 8.86 mg / mL of terpinen-4-ol, both were effective against the biofilm formed in the samples when compared to treatment with nystatin. In addition, it was possible to observe that there was less antibiofilm effect of the oil and its main component against the strain of *Candida albicans* genotype A [16].

Souza*et al*. tested the antibiofilm activity of tea tree essential oil and nanoparticles of tea tree oil to reduce problems due to the low solubility and high volatility that this oil presents. Both were tested for action on biofilms formed by*C. albicans, C. glabrata, C. tropicalis, C. parapsilosis* and *C. membranafaciens.* The essential oil of tea tree showed a significant reduction in the biomass of the biofilm formed when compared to the positive control in all tested strains. The best result was obtained against *C. glabrata*, both for oil and for nanoparticles. It was also observed that at the same concentration, nanoparticles show a greater reduction in biofilm formation compared to *Candida* species [17].

Essential oil of *Cymbopogon* sp.

Khan and Ahmad evaluated the antibiofilm activity of essential oil of *Cymbopogon citrates* (lemongrass) against *Candida albicans* at concentrations of 1/2 MIC and 1/4 Mic and obtained a reduction of 11,46 to 24,67% [18]. In the study of Sahalet al.the antibiofilm activity of lemon grass oil in three strains of *Candida tropicalis.* The results showed inhibition in the formation of biofilms in the three strains of *Candida tropicalis* tested at concentrations of 0, 2, 4 and 8% (w/w) [19].Ngo-Mback et al.tested the antibiofilm activity of lemongrass essential oil in front of *Candida glabrata*. The oil was fractionated by silica gel chromatography in order to find the most active compounds. The results showed that the combination of the F7F10 fractions of lemongrass oil inhibited the formation of *Candidaglabrata* biofilm in the dose-dependent form. A 30% decrease in biofilm formation was observed when compared to the control at a MIC/10 concentration [21].

Taweechaisupapong et al. tested the antibiofilm activity of lemon grass oil in biofilm formation and against preformed biofilm of *Candida dubliniensis*. At the same time, the same tests were also performed for nystatin. The results revealed that the inhibiting effect of lemongrass and nystatin oil is related to doses. Lemongrass oil was tested at concentrations

between 0.11 and 27.6 mg/mL and exhibited 40 to 99% inhibition in biofilm formation. Nystatin at concentrations between 4 and 512 μg/mL and exhibited 20-93% inhibition in biofilm formation. Regarding antibiofilm activity in relation to the preformed biofilm of *Candida dubliniensis*, both lemongrass oil and nystatin were less efficient, in the same concentrations [13]. In another study by the same author was evaluated the antibiofilm activity of lemongrass essential oil against *Candida albicans* and *C. krusei*, The results revealed that the inhibiting effect of oils on biofilm formation and against preformed biofilm was dose-related.The essential oil of lemongrass showed inhibition in the formation of biofilm in *candida* spp isolates, being higher for *Candida krusei* than in *Candida albicans*. In addition, it was observed that lemongrass oil and its main constituents were able to reduce the adhesion of *candida* cells used [21]. Da Silva et al. evaluated the antibiofilm activity of lemongrass essential oil in front of*Candida albicans* biofilm simulating mouthwash applications to evaluate the best action of the oil as to inhibition of formation, biofilm removal or in both situations. This study aimed at a further development of mouthwash based on lemongrass oil. The results showed that there was inhibition in the formation of the biofilm of *Candida albicans*, however, there was no effect on the removal of the mature biofilm by the strains used [22].

Cymbopogon flexuosus essential oil (Indian lemongrass) was evaluated in the study developed by da Silva Gundel et al.This study verifies the antibiofilm activity of *C. flexuosus* (Indian lemongrass) free and nanoemulsion in front of the *Candida albicans* biofilm. and other micro-organisms of interest. As a result, it was observed that the formation of the biofilm of *C. albicans* was inhibited at concentrations of 1.22 mg/mL and 2.56 mg/mL for free oil and at concentrations of 0.28 mg/mL and 0.58 mg/mL for nanoemulsion This result indicates that the nanoemulsion containing the oil has higher antibiofilm activity than the free oil against *C. albicans*. Further studies should be conducted to prove its effectiveness [23].

Cymbopogon winterianus(citronella) essential oil showed antibiofilm activity against *Candida albicans* isolates at concentrations of 5 and 10 MIC. The best results were in the inhibition of biofilm formation. For the biofilm of *C. albicans*, the lowest microbial counts were obtained in citronella oil concentration 10 MIC. The commercial mouthwashes tested showed lower antibiofilm activity in relation to citronella essential oil [24].

Essential oil of *Thymus capitatus* (thyme)

Essid et al. analyzed the antibiofilm activity of the essential oil *Thymus capitatus* and their combined effect with amphotericin B and nystatin. The results showed that the essential oil of *T. capitatus* significantly inhibited the formation of the biofilm of *C. albicans*. The thyme essential oil at ½ MIC (62.5μg/mL) the inhibition in biofilm formation of *C. albicans* was 80.6%, In comparison, at 1/2 MIC, essential oils had a higher inhibiting effect than amphotericin B and nystatin. It was also observed that the combination of the essential oil of *T. capitatus* with amphotericin B or nystatin showed a significant increase in antibiofilm activity. Both oils showed great eradication activity of preformed biofilm at concentrations twice as high as inhibition in biofilm formation. In addition, the combination of essential oils with antifungal was used in an in vitro biofilm model of *C. albicans* associated with a venous catheter. The combinations showed almost complete rupture of the biofilm on the catheter surface, demonstrating synergistic effect between the oils and antifungal agents tested [25]. In another study with thyme oil, total inhibition of biofilm development was observed for *C. tropicalis, C. pelliculosa* and *C. albican*s strains in polypropylene and glass. Only a few

strains of *C. colliculosa* and *C. parapsilosi*s were resistant to thyme oil at the concentrations used [26]

Jafri; Ahmad aluated the antibiofilm activity of thymus vulgaris essential oil and its main component, thymol, in *Candida albicans* and *C. tropicalis* isolates. These were evaluated for biofilm inhibition and eradication of preformed biofilm at different concentrations, including sub-inhibition concentrations. The variable level of attenuation of the biofilms of *C. albicans* and *C. tropicalis* obtained in the presence of *T. vulgaris* and timol indicated that there was inhibition of the biofilm, preventing adhesion and its development. [27].

Alves et al. evaluated the ability of the essential oils of *Thymus carnosus and T. camphoratus* to decrease the mass and viability of the *Candida albicans* biofilm. As a result, both oils were effective at concentrations close to their MIC, and the essential oil of *T. carnosus* was slightly more active in reducing the viability of the biofilm [28].

Essential oil of *Syzygium aromaticum* (clove)

Rajkowska et al. reported the antibiofilm activity of clove essential oils in different strains of*C.albicans, C. krusei, C. lusitaniae, C. rugosa, C. tropicalis, C. parapsilosis, C. famata, C. boidinii, C. pelliculosa and C. colliculosa.* The results showed that the essential clove oil at MIC resulted in the total inhibition of the biofilm formation of the strains of *C. lusitaniae, C. krusei, C. tropicalis, C. rugosa* and *C albicans* on glass surface and polypropylene (PP). On polyethylene terophalate (PET) surface, clove oil completely inhibited the biofilm formation of the strains of *C. lusitaniae, C. boidinii, C. tropicalis, C. rugosa,* and *C. albicans.* Only three isolates, one strain of *C. krusei, C. parapsilosis* and *C. pelliculosa,* showed resistance to clove oil in the tested materials. Biofilms not completely inhibited by clove oil were significantly reduced in the materials analyzed [26]. In the study by Khan and Ahmad,clove oil at the concentration of 1/2 MIC inhibited 50% of *Candida albicans* biofilm formation [18].

Essential oil of *Juniperus virginiana* (cedar)

Manoharanet al. evaluated the antibiofilm activity of the essential oil of cedar leaves and their main components against *Candida albicans*. The result shows that the essential oil of the cedar leaf inhibited, in a dose-dependent manner, the formation of the biofilm of *C. albicans* in 87% at a concentration of 0.01%. The components examined at concentrations of 0.01% significantly inhibited the formation of the biofilm. In addition, the metabolic activity of biofilm cells and their adherence were reduced [29].

Essential oil of *Eucalyptus globulus* (eucalyptus)

Quatrin et al. analyzed the antibiofilm activity of eucalyptus essential oil by comparing the activity of free oil and the nanoemulsion containing the oil against isolates of *Candida albicans, C. tropicalis* and *C. glabrata*. The results obtained proved the antibiofilm activity of eucalyptus oil. In the comparison of the forms presented, it was observed that there were no significant difference between the activity of eucalyptus essential oil and their nanoemulsion against *C. albicans, C. tropicalis* and *C. glabrata*, since the variations found in the tests were the same between the species [30].

Essential oil of *Gaultheria procumbens* L. (wintergreen**)**

Nikolic et al. tested the antibiofilm activity of the essential oil of *Gaultheria procumbens* L. against the biofilm of *Candida albicans* and *Streptococcus mutans*. For this, they used the essential oil of *Gautheria procumbens* L., Hexoral® and Tebodont®, (mouthwashes). It was observed that Hexoral® and Gaultheria procumbens oil showed good antibiofilm activity, and Hexoral ® most effective. It is suggested that the antibiofilm activity of mouthwashes is due to the presence of its components, especially methyl salicylate, which is the main component of essential oil of *Gaultheria procumbens* L. Further studies are needed to prove such antibiofilm activity [31].

Essential oil of *Laurus nobilis* (bay)

Peixoto et al. analyzed the inhibition of adhesion, biofilm formation and reduction of mature biofilm of *Candida albicans* by bay essential oil. The results showed that essential oil of *L. nobilis* inhibited the initial adhesion of *C. albicans* at 1000μg/mL (2 MIC,). At the tested concentrations (MIC, 2 MIC and 4 MIC), the essential oil inhibited the formation of the biofilm. In addition, when the oil was applied for 1 min every 8h, for 24h and 48h, a decrease in mature biofilm of *Candida albicans* was observed. There was no significant difference when compared to nystatin. This indicates that essential oil may be a treatment alternative for *Candida* spp infections however, more studies should be carried out [10]

Essential oil of *Mentha* sp.

Pazarci and Tutar analyzed the antibiofilm activity of *Mentha longifolia* (wild mint) against *Candida albicans* and others biofilm-producing microorganisms *in vitro* implant surfaces. The surfaces tested were titanium and stainless steel, which showed no variation in biofilm growth. The results obtained showed good inhibition in the formation of biofilm, mainly of *C. albicans* which was susceptible to *M. longifolia* oil. In the highest concentrations of oil, a better eradication of the biofilm was observed, which may demonstrate that the antibiofilm activity is related to the dose of the applied oil. In addition, differences were observed in relation to the eradication of biofilm on titanium and stainless-steel surfaces at the different concentrations of essential oil used [32].

Saharkhiz et al. evaluated the antibiofilm activity of essential oil of *Mentha piperita* L. (peppermint) against *Candida albicans* e *C. dubliniensis*. Peppermint essential oil completely inhibited the biofilm formation of *C. albicans* and *C. dubliniensis* in a dosedependent form at concentrations of 1 μL/mL and 2 μL/mL, respectively [33].

Essential oil of *Pelargonium graveolens* (geranium)

Giongo et al. evaluated the antibiofilm activity of the essential oil of *Pelargonium graveolens* and its nanoemulsion against *Candida* species. The geranium essential oil and its nanoemulsion inhibited the formation of the biofilm of *C. albicans, C. tropicalis, C. glabrata* and *C. krusei.* The nanoemulsion containing the oil presented better antibiofilm activity for *C. albicans* and *C. krusei* when compared to the action of the essential oil. Against *C. glabrata* and *C. tropicalis* was not difference, both, the nanoemulsion and the essential oil have a similar antibiofilm activity. When tested on catheters containing biofilm formed from the *Candida* species, the nanoemulsion containing the oil significantly increased activity in

all species, when compared to the control of biofilm formation. In addition, in the presence of oil and nanoemulsion, a reduction in protein was observed, which is used as a measure of biofilm biomass, in plates and catheters, confirming their antibiofilm activity in the *Candida* species used [34].

Essential oil of *Cinnamomum* sp.

Banu et al. tested the inhibition of biofilm formation and the rupture of preformed biofilm of the Essential Oils of *Cinnamomum tamala* (Indian cassia) and Cinnamomum camphora (Camphor) against *Candida albicans, C. tropicalis* and *C. glabrata*. All essential oils tested were able to inhibit the biofilm formation of the Candida species used and significantly reduced preformed biofilms of *C. albicans, C. tropicalis* and *C. glabrata* in a range of 5567%. In addition, it was observed that essential oils reduced cell viability in preformed biofilms [12]. In other study Essid et al. analyzed the antibiofilm activity of the essential oils of *Cinnamomum verum* and their combined effect with amphotericin B and nystatin. The results showed that the essential oils of *C. verum* significantly inhibited the formation of the biofilm of *C. albicans*. At ½ MIC (31.25 μg/mL) inhibition in biofilm formation of *C. albicans* was 85.57%. The combination of *C. verum* with amphotericin B and nystatin showed no significant increase in inhibition [25].

Essential oil of *Pogostemon heyneanus* (patchouli)

Banu et al. verified the antibiolme activity of the essential oil of *Pogostemon heyneanus* (patchouli). All essential oils tested were able to inhibit the biofilm of the *Candida* species used. Regarding the preformed biofilm, all three reduced 60% in the biofilm formation of *C. albicans, C. tropicalis* and *C. glabrata*. In addition, it was observed that essential essentials reduced cell viability in preformed biofilms [12].

Essential oil of de *Salvia* sp

Al-Bakri; Othman and Afifi tested the antifungal activity of seven salvia species *(Salvia ceratophylla L., S. dominica* L., *S. hierosolymitana* Boiss., *S. indica* L., *S. syriaca* L., *S. fruticosa* Mill. [syn.*S. triloba* L.] e *S. verbenaca* L. in *Candida albicans* and other microorganisms. For biofilm inhibition, the essential oil of *S. triloba* L. The essential oil showed good prevention and control of biofilm, presenting anti-adhesion and antibiofilm activities [35].

Essential oil of *Satureja* sp.

Motamedi et al. tested the antibiofilm activity of the essential oil of *Satureja macrosiphon* against *Candida albicans* and *C. dubliniensis*. The essential oil showed effective antibiofilm activity against *C. albicans* and *C. dubliniensis* at concentrations of 4 and 8 μg/mL, respectively. It was observed that inhibition was dose-dependent [36].
Sharifzadeh, khosravi and Ahmadian tested the antibiofilm activity of the essential oil of *Satureja hortensis* L. in preformed biofilms of *Candida albicans* isolates from oral lesions of HIV+ patients. the inhibiting effect of essential oil is related to the dose used. At concentrations of 4800, 3200, 2400 and 1600 μg/mL, biofilm formation was reduced on average 87.1%, 73.6%, 69.4% and 67%, respectively. At concentrations below the reduction rate was less than 50%. The decrease in metabolic activity of mature biofilms of *C. albicans* ranged from 7.1 to 80%. The morphology of the biofilm of *C. albicans*, presented morpho

structural changes, shrinkage in the cell membranes of sessis cells and the destruction of the three-dimensional structure of the biofilm[37].

Essential oil of *Thymbra capitata*

Palmeira de Oliveira et al evaluated the antibiofilm activity of *Thymbra capitata* essential oil in 15 strains of *Candida* spp. (*Candida albicans, C. glabrata, C. tropicalis, C. guilliermondii* and *C. parapsilosis*). The essential oil of *Thymbra capitata* showed an inhibitative effect against candida spp. biofilms. For *C. albicans* and other tested species, the inhibiting effect of biofilm biomass was significant at 2x MIC (0.64 μL/mL).*C. glabrata* was the most resistant to the biomass reduction of the biofilm being less than 50%. However, in relation to the metabolic activity of *C. glabrata* biofilm, a reduction effect of more than 80% was observed at the same concentration, demonstrating that the remaining cells were metabolically inactive or dead. *C. glabrata*, the essential oil when used in *C. albicans* had a higher inhibiting effect for biomass reduction than for metabolism activity. In short, biofilm biomass and metabolic activity were reduced by up to 50% in all species in the MIC value and the most significant effects were observed at 2x MIC [38].

Essential oil of *Zingiber officinale* Roscoe *var rubrum* (red ginger)

Rinanda et al. analyzed the antibiofilm activity of the essential oil of red ginger, whose main components are monoterpenes, on *candida albicans* isolates for inhibition and degradation of biofilm. The results showed that at the lowest concentration (0.125%) the essential oil has already demonstrated activity in the degradation of the biofilm and this increased with the increase of its concentration. For inhibition in the biofilm formation of *C. albicans* the concentration of red ginger oil found was 0.5%, thus demonstrating that this essential oil showed significant degradation activity and inhibition of *C. albicans* biofilm [39].

Essential oil of *Ziziphora tenuior, Lavandula angustifólia* (lavander) and *Cuminum cyminum* (cumin)

Dolatabadi, Salari and Mahboubi evaluated the antibiofilm activity of the essential oils of *Ziziphora tenuior, Lavandula angustifólia* and *Cuminum cyminum* against *Candida albicans* isolates of vaginal secretion. The antibiofilm activityof the oils was related to the dose of the applied oil, that is, the highest concentrations used showed greater elimination of the biofilm. The essential oils of *C. cyminume L. angustifolia* showed the greatest elimination effects of *C. albicans* biofilm, followed by the essential oil of *Z. tenuior* [40].

CONCLUSION

The search for therapeutic alternatives for the treatment of infections caused by *Candida* species is extremely important, especially when biofilm is formed, resulting in increased resistance to conventional antifungals. The data found in this literature review demonstrated antibiofilm activity of essential oils, including tea tre oil that showed good antibiofilm activity for Candida species and lemongrass oil that considerably inhibited the biofilm formation of *Candida albicans,* suggesting that they can be used as treatment alternatives. Further studies are needed before its use in the therapeutic clinic.

REFERENCES

1. KRISHNASAMY, L.; RUBINI, D.; SENTHILGANESH, J. Gene Reports Phylogenetic characterization of bio fi lm forming multidrug resistant Candida albicans and Non albicans Candida causing vulvovaginal candidiasis. **Gene Reports**, v. 19, p. 100644, 2020.

2. SADEGHI, G. et al. Emergence of non- Candida albicans species : Epidemiology , phylogeny and fluconazole susceptibility profile. **Journal de Mycologie Medicale**, v. 28, n. 1, p. 51–58, 2018.

3. MARAK, M. B.; DHANASHREE, B. Antifungal susceptibility and biofilm production of candida spp. Isolated from clinical samples. **International Journal of Microbiology**, v. 2018, p. 6–11, 2018.

4. SEDDIKI, S. M. L. et al. Infectivités fongiques des cathéters implantés dues à Candida sp. Formation des biofilms et résistance. **Journal de Mycologie Medicale**, v. 25, n. 2, p. 130–135, 2015.

5. SILVA, S. et al. Candida glabrata, Candida parapsilosis and Candida tropicalis: Biology, epidemiology, pathogenicity and antifungal resistance. **FEMS Microbiology Reviews**, v. 36, n. 2, p. 288–305, 2012.

6. PRISTOV, K. E.; GHANNOUM, M. A. Resistance of Candida to azoles and echinocandins worldwide. **Clinical Microbiology and Infection**, v. 25, n. 7, p. 792–798, 2019.

7. DEORUKHKAR, S. C.; SAINI, S.; MATHEW, S. Virulence factors contributing to pathogenicity of candida tropicalis and its antifungal susceptibility profile. **International Journal of Microbiology**, v. 2014, p. 1-6, 2014.

8. ARSLAN, S. et al. Genotypes and virulence factors of Candida species isolated from oral cavities of patients with type 2 diabetes mellitus. **Turkish Journal of Medical Sciences**, v. 46, n. 1, p. 18–27, 2016.

9. ALVARENGA, T. A. et al. In Vitro and In Vivo Anti-Candida spp. Activity of Plant-Derived Products.**Plants**, n. November, 2019.

10. PEIXOTO, L. R. et al. Antifungal activity, mode of action and anti-biofilm effects of Laurus nobilis Linnaeus essential oil against Candida spp. **Archives of Oral Biology**, v. 73, p. 179–185, 2017.

11. BASSYOUNI, R. H. et al. Fennel oil: A promising antifungal agent against biofilm forming fluconazole resistant Candida albicans causing vulvovaginal candidiasis. **Journal of Herbal Medicine**. v. 15, p. 100227, 2019.

12. FARISA BANU, S. et al. Effects of patchouli and cinnamon essential oils on biofilm and hyphae formation by Candida species. **Journal de Mycologie Medicale**, v. 28, n. 2, p. 332–339, 2018.

13. TAWEECHAISUPAPONG, S. et al. Antibiofilm activity and post antifungal effect of lemongrass oil on clinical Candida dubliniensis isolate. **South African Journal of Botany**, v. 78, p. 37–43, 2012.

14. ELKHALIFA, A. et al. Microbial Pathogenesis antibio fi lm activities of essential oil and methanolic extract of Anthemisstiparum subsp. sabulicola(Pomel)Oberpr. **Microbial Pthogenesis**,v. 119, p.233–240, 2018.

15. MBACK M. N. L. N. et al. Biocatalysis and Agricultural Biotechnology An investigation of chemical composition and antimicrobial activity of essential oils extracted from Aeollanthus and Plectranthus species. **Biocatalysis and Agricultural Biotechnology**, v. 22, p. 101412, 2019.

16. FRANCISCONI, R. S. et al. Antibiofilm efficacy of tea tree oil and of its main component terpinen-4-ol against Candida albicans. **Brazilian oral research**,v. 34, n. 1, p. 050, 2020.

17. SOUZA, M. E. et al. Melaleuca alternifolia nanoparticles against Candida species biofilms. **Microbial Pathogenesis**,v. 104, n. 1, p.125–132, 2017.

18. KHAN, M. S. A; AHMAD, I. Biofilm inhibition by Cymbopogon citrates and Syzygium aromaticum essential oils in the strains of Candida albicans. **Journal of Ethnopharmacology**, v. 14, n 3, p. 1-14, 2019.

19. SAHAL, G. et al. Antifungal and biofilm inhibitory effect of Cymbopogon citratus(lemongrass) essential oil on biofilm forming by Candida tropicalis isolates; an in vitro study. **Journal of Ethnopharmacology**,v. 246, p. 112188, 2020.

20. NGO-MBACK, M. N. L. et al. Anticandidal and synergistic effect of essential oil fractions from three aromatic plants used in Cameroon. **Journal de MycologieMedicale**, v. 30, p. 100940, 2020.

21. TAWEECHAISUPAPONG, S. Inhibitory effect of lemongrass oil and its major constituents on Candida biofilm and germ tube formation. **South African Journal of Botany**, v.81, p. 95–102, 2012.

22. DA SILVA, N. B. et al. Anti-biofilm and hemolytic effects of cymbopogon citratus (dc) stapf essential oil. **Pesquisa Brasileira em Odontopediatria e Clinica Integrada**, v. 19, n. 1, p. 1–10, 2019.

23. DA SILVA GUNDEL, S. et al. Nanoemulsions containing Cymbopogon flexuosus essential oil: Development, characterization, stability study and evaluation of antimicrobial and antibiofilm activities. **Microbial Pathogenesis**,v. 118, p. 268–276, 2018.

24. GUANDALINI CUNHA, B. et al. Cytotoxicity and antimicrobial effects of citronella oil (Cymbopogon nardus) and commercial mouthwashes on S. aureus and C. albicans biofilms in prosthetic materials. **Archives of Oral Biology**,v. 109, n. September, p.10577, 2020.

25. ESSID, R. et al. Combined effect of Thymus capitatus and Cinnamomumverum essential oils with conventional drugs against Candida albicans biofilm formation and

elucidation of the molecular mechanism of action. **Industrial Crops and Products**, v. 140, n. May, p.111720, 2019.

26. RAJKOWSKA, K.; NOWICKA-KRAWCZYK, P.; KUNICKA-STYCZYNSKA, A. Effect of clove and thyme essential oils on Candida biofilm formation and the oil distribution in yeast cells. **Molecules**, v. 24, n. 10, 2019.

27. JAFRI, H.; AHMAD, I. Thymus vulgaris essential oil and thymol inhibit biofilms and interact synergistically with antifungal drugs against drug resistant strains of Candida albicans and Candida tropicalis. **Journal de Mycologie Medicale**, v. 30, n. 1, p. 100911, 2020.

28. ALVES, M. et al. Unveiling the antifungal potential of two iberian thyme essential oils: Effect on C. albicans germ tube and preformed biofilms. **Frontiers in Pharmacology**,v. 10, p.1–11, 2019.

29. MANOHARAN, R. K.; LEE, J. H.; LEE, J. Antibiofilm and antihyphal activities of cedar leaf essential oil, camphor, and fenchone derivatives against Candida albicans. **Frontiers in Microbiology**, v. 8, n. AUG, p. 1–12, 2017.

30. QUATRIN, P. M. et al. Antimicrobial and antibiofilm activities of nanoemulsions containing Eucalyptus globulus oil against Pseudomonas aeruginosa and Candida spp. **Microbial Pathogenesis**, v. 112, p. 230–242, 2017.

31. NIKOLIĆ, M. et al. Chemical composition and biological activity of Gaultheria procumbens L. essential oil. **Industrial Crops and Products**, v. 49, p. 561–567, 2013.

32. PAZARCI, O.; TUTAR, U.; KILINC, S. Investigation of the antibiofilm effects of mentha longifolia essential oil on titanium and stainless steel orthopedic implant surfaces. **Eurasian Journal of Medicine**, v. 51, n. 2, p. 128–132, 2019.

33. SAHARKHIZ, M. J. et al. Chemical Composition, Antifungal and Antibiofilm Activities of the Essential Oil of Mentha piperita L .**ISRN Pharmaceutics, v.** 2012; p. 1– 6, 2012.

34. GIONGO, J. L. et al. Anti-Candida activity assessment of Pelargonium graveolens oil free and nanoemulsion in biofilm formation in hospital medical supplies. **Microbial Pathogenesis**, v. 100, p. 170–178, 2016.

35. AL-BAKRI, A. G.; OTHMAN, G.; AFIFI, F. U. Determination of the antibiofilm, antiadhesive, and anti-MRSA activities of seven Salvia species. **Pharmacognosy Magazine**,v. 6, n. 24, p. 264–270, 2010.

36. MOTAMEDI, M. et al. Chemical compositions and antifungal activities of Satureja macrosiphon against Candida and Aspergillus species. **Current Medical Mycology**, v. 5, n. 4, p. 20–25, 2019.

37. SHARIFZADEH, A.; KHOSRAVI, A. R.; AHMADIAN, S. Chemical composition and antifungal activity of Saturejahortensis L. essentiall oil against planktonic and biofilm growth of Candida albicans isolates from buccal lesions of HIV+ individuals. **Microbial Pathogenesis**,v. 96, n. 1, p.1–9, 2016.

38. PALMEIRA-DE-OLIVEIRA, A. et al. The anti-Candida activity of Thymbracapitata essential oil: Effect upon pre-formed biofilm. **Journal of Ethnopharmacology**,v. 140, n. 2, p. 379–383, 2012.

39. RINANDA, T.; ISNANDA, R. P.; ZULFITRI. Chemical analysis of red ginger (Zingiber officinale Roscoe var rubrum) Essential oil and its anti-biofilm activity against candida albicans. **Natural Product Communications**, v. 13, n. 12, p. 1587–1590, 2018.

40. DOLATABADI, S.; SALARI, Z.; MAHBOUBI, M. Antifungal effects of Ziziphoratenuior, Lavandula angustifolia, Cuminumcyminum essential oils against clinical isolates of Candida albicans from women suffering from vulvovaginal candidiasis. **Infectio**, v. 23, n. 3, p.222–226

TABLES

Table 1. Essential oils studied for antibiofilm activity against *Candida* species.

Oils found	Cited Candida species													
Aeollanthhus cucullathus	C1				C5									
Aeollanthus heliotropioides	C1				C5									
Anthemis stiparum	C1													
Cedrela fissilis	C1													
Cinnamomum camphora	C1	C2			C5									
Cinnamomum tamala	C1	C2			C5									
Cinnamomum verum	C1	C2			C5									
Citrus hystrix	C1		C3											
Citrus x limon		C2												
Cymbopogon citratus	C1	C2	C3	C4										
Cymbopogon flexuosus	C1													
Cymbopogon winterianus	C1		C3											
Cuminum cyminum	C1	C2												
Eucalypthos globulus	C1	C2			C5									
Gaultheria procumbens L.	C1													
Hedychium coccineum	C1													
Longifolia mint	C1													
Lavandula augustifolia	C1													
Laurus nobilis	C1	C2	C3		C5									
Mint x piperita L.	C1	C2	C3	C4	C5	C6								
Melaleuca alternifólia	C1	C2			C5	C6								
Ocimum basil	C1		C3											
Ocimum tenuiflorum	C1		C3											
Pelargonium graveolens	C1	C2	C3		C5									
Plectranthus glandulosus	C1				C5									
Pogostemon heyneanus	C1	C2			C5									
Sage triloba L.	C1													
Satureja macrosiphon	C1			C4										
Satureja hortensis L.	C1													
Syzygium aromaticum	C1	C2	C3			C6		C8	C9	C10	C11	C12	C13	C14
Thymbra capitata	C1	C2			C5	C6	C7							
Thymus capitatus	C1													
Thymus vulgaris	C1	C2	C3			C6		C8	C9	C10	C11	C12	C13	C14
Turmeric longa L.	C1		C3											
Zingiber cassumunar	C1		C3											
Zingiber officinale	C1		C3											
Ziziphora Tenuior	C1													

C1 - *Candida albicans;* C2 - *Candida tropicalis;* C3 - *Candia krusei*; C4 - *Candida dubliniensis;* C5 – *Candida glabrata*; C6 – *Candida parapsilosis*; C7 – *Candida guillermondii*; C8 – *Candida rugosa*; C9 – *Candida lusitaniae*; C10 – *Candid afamata*; C11 – *Candida boidinii*; C12 – *Candida pelliculosa*; C13 – *Candida colliculosa*; C14 – *Candida membranafaciens*.

ANEXO I: Normas da revista Instructions for authors – "JournalofInnovations in PharmaceuticalandBiologicalSciences"

Manuscript Preparation: The manuscript should be typed single-spaced on A4 (8.5" × 11") paper size with 1 inch margins on all sides. Times New Roman font 12 should be used. Manuscript should be arranged in the following order: Title, Abstract, Keywords, Introduction, Materials and Methods, Results, Discussion, Conclusion, Acknowledgement, References, Figures and Tables.

Title Page: The title page should contain a clear, concise and informative title of the article followed by the names and affiliations of the authors. The affiliation should comprise the department, institution, city, and state (or nation) and should be typed as a footnote to the author's name. The Corresponding Author must indicate his or her complete mailing address, office/cellular phone number, fax number, and email address at the lower left of the Title Page.

Abstract: The abstract should start on a new page after the title page and should not be more than 250 words and should contain objectives, material and methods, Results and Conclusions. Reviews and mini reviews also require an abstract

Keywords: Following the abstract, provide a maximum of 5-6 keywords, which reflect the content of the study.

Introduction: This should be brief and indicates aim of the study and the essential back ground information. The introduction should not be an extensive literature review although it should provide sufficient background information for the reader to understand and evaluate the results of the present study without referring to previous publications on the same topic.

Material and methods: Please provide concise but complete information about the material and the analytical, statistical and experimental procedures used. This part should be as clear as possible to enable other scientists to repeat the research presented. In case of animal/human experiments or clinical trials authors must give the details of ethical approval.

Result and Discussion: Data acquired from the research with appropriate statistical analysis described in the methods section should be included in this section. In this part, the same data/ information given in a table must not be repeated in a figure, or vice versa. Tables and Figures should be self explanatory and it is not acceptable to repeat extensively the numerals from tables into text and give lengthy and unnecessary explanations of the Tables and Figures. Discussion should relate the results to current understanding of the scientific problems being investigated in the field.

Conclusion: A short, paragraph summarizing the most important finding(s) of the research is required.

Acknowledgement: All acknowledgments (if any) should be included at the very end of the paper before the references and may include supporting grants, presentations, and so forth.

References: Should be numbered consecutively in the order in which they are first mentioned in the text (not in alphabetic order). Indicate references by number(s) in square brackets [Reference no] in line with the text References cited only in tables or figure

legends should be numbered in accordance with the sequence established by the first identification in the text of the particular table or figure.

Journal Articles: Shashi A, Jain SK and Pandey M: In-vitro evaluation of antilthiatic activity of seeds of Dolichos biflorus and roots of Asparagus racemosus . International Journal of Plant Sciences 2008; 1:67-71.

Tables & Figures: Tables and figures should not be embedded in the text, but should be included at the end of the manuscript on separate pages. Tables should be created with a word processor and cited consecutively in the text. Number tables and figures consecutively in accordance with their appearance in the text. Place footnotes to tables below the table and give proper heading on table.

Abbreviations: Standard abbreviations should be used throughout the manuscript. Use of nonstandard abbreviations can be confusing to readers.