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Review article

Potential additive or synergistic effect of the essential oils of *Eucalyptus citriodora*, *Eucalyptus camaldulensis* and *Eucalyptus globulus* and their interactions with antifungal agents to evaluate anti-*Candida* spp. activity: A literature review

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Keywords: Candida; Eucalyptus; antifungals; essential oils; synergism; additive effect.

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Abstract

The genus *Candida* covers a diversity of species responsible for causing important fungal infections in individuals. *Candida* species are among the most frequent pathogens in hospital infections considered severe. The increasing resistance to antifungal drugs is one of the factors that promote prospecting for new therapeutic agents. Essential oils have shown promising results by inhibiting or preventing fungal growth.
A literature review was performed in the online databases PubMed, Scielo, Scopus, LILACS, CAPES periodicals and ScienceDirect, with the aim of verifying the anti-candid activity and possible interactions with antifungals of essential oils of *Eucalyptus citriodora, Eucalyptus camaldulensis* and *Eucalyptus globulus.* The main constituents of these essential oils are citronellal, 1,8-cineole (eucalyptol), have anti-*Candida* activity and have a potential additive or synergistic effect when combined with antifungals. The MIC range of *E. citriodora* essential oil for different *Candida* species was 0.02 µg/mL to 5 µg/mL. The range of the inhibition zone of the essential oil of *E. camaldulensis* against the different species of *Candida* was 18 – 23 mm for the leaves of the plant and 12 - 20 mm for the fruits. The MIC for the essential oil of *E. globulus* was 1000 µg/mL, while in combination with an antifungal, the value was 32 times lower, thus presenting an additive effect with ICIF of 1.031. They also have a potential additive or synergistic effect with antimicrobials. However, further studies are still needed to consolidate knowledge about these species for their use in the therapeutic clinic in infections caused by *Candida* spp.

Introduction

Candida is a yeast present in the normal microbiota of humans. It is present in the oral cavity, genital tract, perianal region and gastrointestinal tract. The presence of *Candida* does not necessarily imply infection. However, when there is an imbalance in defense mechanisms or anatomical barriers, especially in individuals with immunosuppression, it may become pathogenic. Some factors may contribute to the development of the disease, such as malnutrition,

obesity, diabetes, pregnancy, antibiotic therapy, chemotherapy, corticosteroid use, neoplasms and other debilitating diseases [1, 2].

Candidiasis is an opportunistic mycosis caused by *Candida* spp. with varied clinical manifestations, which may cause superficial, cutaneous, subcutaneous and systemic infections. Acute and chronic infections manifest with lesions in the mouth, pharynx, skin, nails, bronchopulmonary system, intestinal, perianal, and occasionally, endocarditis, meningitis, fungemia or infections elsewhere [1, 2].

Treatment of candidiasis is done according to the clinical manifestations of the patient and the severity of the disease. In superficial candidiasis, imidazole derivatives such as clotrimazole, miconazole, ketoconazole, oxyconazole, terconazole and polyenics such as nystatin are used. In more extensive cases, systemic drugs such as itraconazole or fluconazole are administered. In systemic candidiasis, amphotericin B is also one of the drugs chosen, usually in association with 5 fluorocytosine or other drugs such as triazole derivatives, fluconazole and itraconazole [3, 4].

According to the Center for Diseases Control and Prevention (CDC) some *Candida* species have demonstrated resistance to commonly used antifungal drugs. About 7% of all *Candida* spp. infected blood samples tested at CDC are fluconazole resistant. In addition, the resistance to the class of echinocandins by *Candida glabrata* has become worrisome due to the fact that it also presents high rates of resistance to fluconazole. This limits the therapeutic options for patients infected by this species. Another species that has been resistant to antifungals is *Candida auris*. About 90% of *C. auris* samples in the USA were resistant to fluconazole and 30% resistant to amphotericin B [5]. Other studies report intrinsic resistance to antifungals in species of *Candida. C. krusei*, intrinsically resistant to fluconazole and some other *Candida* species have intrinsic resistance to amphotericin B [6, 7].

Thus, prospecting for new therapeutic agents for the treatment of candidiasis is increasingly important. Natural products have been used in practice for many years to control and cure diseases. Currently, plant essential oils have been studied by several researchers around the world and have shown promise. Several studies have shown that some essential oils may present antifungal activity in their constituents, isolated or not, and may also have a synergistic effect when in use with antimicrobial drugs. A synergistic effect between the two compounds can reduce the dose to be used and thus reduce the toxicity and possible side effects of both compounds, representing an alternative to the resistance of microorganisms [8, 9].

Several essential oils are reported in the literature with antifungal activity. The species of the genus *Eucalyptus* have a smaller number of studies when compared to other plant genera. In this sense, this review aims to verify the studies conducted with the essential oils of *Eucalyptus citriodora*, *Eucalyptus camaldulensis* and *Eucalyptus globulus* for chemical composition, anti-*Candida* spp. activity and possible pharmacological interactions with antifungals used in therapeutic clinic.

Methodology

This study constitutes a review of the anti-*Candida* spp. activity by the essential oils of *Eucalyptus citriodora*, *Eucalyptus camaldulensis* and *Eucalyptus globulus* and the possible interactions with antifungals. The databases of the Scientific Electronic Online Library (SciELO), National Library of Medicine (PubMed), Scopus, LILACS, CAPES periodicals and Science Direct were used for the research. The searches were carried out from May to August 2020. The inclusion criteria were scientific articles addressing the proposed theme published between 2015 and 2020.

The first search was conducted with the combinations of words "antifungal AND oils AND *Candida* AND synergism" to list the essential oils being studied in the last five years. After that, a second survey was conducted to search for data on *Eucalyptus* species, using the combinations of words "antifungal AND oils AND *Candida* AND synergism AND *Eucalyptus camaldulensis*; antifungal AND oils AND *Candida* AND synergism AND *Eucalyptus citriodora*; antifungal AND oils AND *Candida* AND synergism AND *Eucalyptus globulus*".

The chemical composition of *E. citriodora* and *E. camaldulensis* was researched under the same conditions, using the combinations of words "*Eucalyptus citriodora* AND composition; *Eucalyptus camaldulensis* AND composition; *Eucalyptus globulus* AND composition; *Eucalyptus citriodora* AND oil essential; *Eucalyptus camaldulensis* AND oil essential; *Eucalyptus globulus* oil essential". In this search, there were no criteria of choice for the selection of articles in relation to the regions of cultivation, seasons or extraction methods of the species of interest.

The anti-*Candida* spp. activity of the two species of *E. citriodora*, *E. camaldulensis* and *E. globulus* was researched in the databases maintaining the five-year filter, using the combinations of words "*Eucalyptus citriodora* AND *Candida*; *Eucalyptus camaldulensis* AND *Candida*; *Eucalyptus globulus* AND *Candida* ".

The evaluation of the interactions of essential oils and their main constituents of both species of *E. citriodora* and *E. camaldulensis* with antifungals against *Candida* spp. was performed using the combinations of words "checkerboard AND *Eucalyptus citriodora;* checkerboard AND *Eucalyptus camaldulensis*; checkerboard AND *Eucalyptus globulus*; checkerboard AND citronellal; checkerboard AND 1,8 cineole; checkerboard AND eucalyptol".

Discussion

Several plants are used in the therapeutic routine for the treatment of different diseases. The essential oils of some

plants, alone or in combination, are being studied to prove their activities against different pathogens. The genus *Candida* spp. includes species responsible for causing severe infections and, in addition, some species have been presented to resistance to commonly used antifungals. Thus, the present study evaluates the anti-*Candida* spp. activity by the essential oils of *Eucalyptus citriodora*, *Eucalyptus camaldulensis* and *Eucalyptus globulus*, as well as the possible interactions with antifungals, in order to contribute to the therapeutic arsenal the infections caused by *Candida* spp. The first search generated a total of 496 newspapers.

Duplicate and tripiclata newspaper were excluded. The others were selected for title and abstract reading to verify the relevance of the study. After reading the titles and abstracts it was possible to list the species that are being studied in the last 5 years. Each species listed was searched in the databases to analyze, in numbers, the relevance of each species. *Thymus vulgaris* appears at the top of the list, being cited in 143 articles. The *Origanum vulgare* is the second most researched, being cited in 108 articles. *Citrus limonum* was cited in 86 articles.

The plant species most used for the evaluation of anti-*Candida* activity are the essential oils of *Thymus vulgaris, Origanum vulgare* and *Citrus limonum*. The species *Eucalyptus camaldulensis* was cited in 26 articles and *Eucalyptus citriodora* was mentioned in 18 articles.

1. Essential oils with anti-*Candida* spp. activity

In recent years, the antifungal activity of several plant species has been studied. The most researched essential oils against *Candida* spp. are thyme *(Thymus vulgaris)*, oregano (*Origanum vulgare*), lemon (*Citrus limonum*), clove (*Syzygium aromaticum*), melaleuca (*Melaleuca alternifolia*), cinnamon (*Cinnamomum verum*), mint (*Mentha piperita*) and others.

Gukwa K *et al.* [10] reported fungistatic and fungicide activity in relation to *C. albicans* and *C. glabrata* for the essential oils of *Thymus vulgaris*, *Citrus limonum*, *Pelargonium graveolens*, *Cinnamomum cassia*, *Ocimum basilicum* and *Eugenia caryophyllus*. The best activity was observed for essential oil of *Cinnamomum cassia*.

Mandras N *et al.* [11] demonstrated that the essential oils *of Thymus vulgaris* (thyme), *Foeniculum vulgare* (fennel), *Eugenia caryophyllata* (carnation), *Pinus sylvestris* (pine), *Salvia officinalis* (sage), Melissa *officinalis* (lemon grass) and *Lavandula vera* (lavender) have good activity against *Candida* sp. The higher activity of thyme and pine essential oils may be related to its main components, carvacrol, thymol and α-pinene, which have proven antifungal action.

Cardoso NNR *et al.* [12] reported the antifungal activity of the essential oil of *Ocimum basilicum* and its main components against *Candida albicans*. The best results were presented by the geraniol component.

Bhat V et al. [13] proved that *Origanum vulgare* essential oil has high anti-*Candida* spp. properties against oral clinical isolates. *O. vulgare* showed much lesser MIC/MFC as compared to fluconazole indicating that the herb can be effective even in a lower dose of MIC/MFC. *O. vulgare* showed much lower MIC/MFC values compared with fluconazole indicating that essential oil may be effective even at a lower dose of MIC/MFC. The main component of *O. vulgare* is the carvacrol, which has proven antifungal activity.

2. Pharmacological interactions of antifungals against *Candida* spp.

Recent studies have shown that essential oils extracted from plants can have a synergistic effect when associated with an antimicrobial agent. Different authors report synergism between essential oil constituents and antifungal drugs. De Castro RD *et al.* [14] showed that the thymol has a fungicide effect on the species *Candida* and also has a synergistic effect with nystatin. Gukwa K *et al.* [10] observed that the essential oils of *Pelargonium graveolens* and *Cinnamomum cassia* showed synergistic activity with amphotericin B against *C. albicans* and *C. glabrata*. Cardoso NNR *et al.* [12] reported synergistic activity of geraniol and linalool, essential oil components of *Ocimum basilicum*, with fluconazole, especially against fluconazole-resistant *C. albicans* strain.

3. *Eucalyptus citriodora, Eucalyptus camaldulensis* and *Eucalyptus globulus*

The genus *Eucalyptus* contains more than 800 species and is one of the most used plants in the world. Different parts of the plant are used in various areas such as in the pulp and paper industry, food industry, dentistry and medicine. More than 300 species of this genus contain volatile oils in their leaves, being used as fragrance elements in household and cosmetic products such as soaps, detergents, lotions and perfumes. They are also used as flavor elements in food and beverages. In addition, in clinical practice *Eucalyptus* is used to prevent and treat human diseases due to its antimicrobial, anti-inflammatory and antioxidant properties. *Eucalyptus* essential oil is present in different formulations, such as syrups, tablets and ointments. It is widely used as expectant for cough, in burns, as muscle relaxant and analgesic. Many researchers have reported the chemical composition, antioxidant and antimicrobial activities of *Eucalyptus* species. In most species, essential oil is composed mainly of 1.8 cineole (or eucalyptol). However, the geographical distribution and variation of species greatly affect these properties, which requires extensive studies to explore the potential of this plant [8,15].

3.1 Chemical constitution

The chemical composition of essential oils may vary in some situations, for example, with the region of cultivation, season and extraction method. However, the species have some characteristic constituents, which are repeated in different studies, as shown in Table 1. Citronellal and 1.8-cineole or eucalyptol have a concentration range between

22,30% to 78,15% and 5,90% to 89,90%, respectively, indicating that they are the most present constituents in *E. citriodora, E. camaldulensis* and *E. globulus* [15-25]. The studies selected for the preparation of the table did not take into account the regions, seasons or extraction methods.

Citronellal is an aldehyde and is the main component of *Cymbopogon* essential oils, *Eucalyptus citriodora*, and *Leptospermum petersonii*. Morcia C *et al.* [26] observed that citronellal was effective in reducing the in vitro growth of three species of *Fusarium*. Feyaerts AF *et al.* [27] tested 37 phytoconstituents and citronellal showed higher activity, especially against *Candida glabrata*. Tsai ML *et al.* [28] proved that citronellal exhibits antimicrobial effect against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans*. Wu Y *et al.* [29] observed that citronellal has antifungal activity against *Penicillium digitatum* and, in addition, suggested that the constituent damages the cell membrane of the fungus, increases extracellular conductivity and the release of cellular constituents.

1,8-cineole (eucalyptol) is a cyclic ether and has been reported as the main constituent of many essential oils such as *Eucalyptus camaldulensis*, *E. globulus*, *Cinnamomum longepaniculatum*, *Rosmarinus officinalis*, *Psidium pohlianum*, *P. guyanensis* and *Salvia libanotica* [30]. Sun WB *et al.* [31] reported that *Eucalyptus* showed inhibition of mycelial growth for *Fusarium oxysporum*, *F. solani* and *Cylindrocarpon destrutans*. Lee EH *et al.* [32] demonstrated that *Eucalyptus* performs anti-inflammatory function, inhibiting inflammatory signaling pathways induced by *Propionibacterium acnes*. Noumi E *et al.* [33] observed that 1,8-cineol attenuated the expression of virulence factors controlled by Quorum Sensing tested (violacein pigment production, production of elastase, protease and motility) in a dose-dependent manner for *Pseudomonas aeruginosa* and *Chromobacterium violaceum*. Şimşek M and Duman R [34] reported that cineol increased the antimicrobial activity of chlorhexidine gluconate against *Staphylococcus aureus*, *S. aureus* resistant to methicillin, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis* and *Candida albicans*.

Salem et al. [35] investigated the effect of the phenological stage on the yield and chemical composition of essential oils extracted from parts of *Eucalyptus globulus*. The composition analysis showed two different chemotypes depending on the growth stage which were characterized as 1,8-cineole (13.23%) at vegetative stage and p-cymene at full flowering (32.19%) and fructification (37.82%) stages. A predominance of monoterpene hydrocarbons (72.84%) during the fructification stage was detected with p-cymene (12.58%–37.82%) and α-pinene (10.41%–13.39%) as the determinants of this class. the essential oil of E*ucalyptus globulus* was active against different bacterial strains, especially during the full flowering stages (MIC = 2 mg/mL) against *Bacillus cereus* and *Enterococcus faecalis*. The fruiting stage was more sensitive for *Candida albicans* than for bacteria.

In general, many studies point out that the constituents of essential oils, isolated or in combination, can exert antimicrobial activity. Among the mechanisms of action mentioned, the most accepted is that the constituents cause a rupture in the plasma membrane, affecting the proton pump and causing damage in the flow of electrons and imbalance of active transport through the membrane, besides inhibiting mitochondrial respiration of bacteria and fungi [36].

3.2 Anti-*Candida* spp. activity

The antimicrobial activity of *E. citriodora* has been proven to be a diversity of bacterial and fungal species. Bacterial species reported are *Staphylococcus aureus*, *Methicillin-resistant S. aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Haemophilus influenzae*, *Acinetobacter baumannii*, *Bacillus subtilis*, *B. cereus*, *Escherichia coli*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Agrobacterium tumefaciens*, *Dickeya solani*, *Pectobacterium atrosepticum* and *P. carotovorum*. Among the fungal species reported are *Aspergillus niger*, *A. flavus*, *A. ochraceus*, *Fusarium oxysporum*, *Penicillium funiculosum*, *P. ochrochloron*, *Rhizoctonia solani* and *Rhizopus solani* [15, 16, 37].

The activity of *E. camaldulensis* has been reported against bacteria and fungi. Among the bacterial species reported are *Staphylococcus aureus*, resistant to methicillin, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Escherichia coli* and *Acinetobacter baumannii*. Fungal species are *Aspergillus niger*, *Fusarium oxysporum*, *F. solani*, *F. verticillioides*, *F. proliferatum*, *F. subglutinans* and *Rhizopus solani*. Antiparasitic activity in *Trichomonas vaginalis* and *Plasmodium berghei* was also reported, as well as antiviral activity in the A/H1N1 virus [38-41].

Candida spp. is responsible for causing different types of infections, including those considered severe. In this sense, the evaluation of the anti-*Candida* spp. activity of the species of *E. citriodora, E. camaldulensis* and *E. globulus* is extremely important. For this, articles from the last five years were selected that prove the anti-*Candida* spp. activity of these species.

The activity of *E. citriodora ag*ainst *Candida species* was reported by Paosen S *et al.* [45], Salem MZM *et al.* [21] and Cavalcanti AL *et al.* [46] using the broth microdilution method. The relationship of *Candida* species tested with the minimum inhibition concentration (MIC) ranges, as well as the minimum fungicide concentration (MFC) ranges are found in table 2.

The activity of *E. camaldulensis* against two species of *Candida* was proven by Dogan G *et al.* [47] using the disc-diffusion method, using increasing concentrations (10, 20 and 30 μg/mL) of the essential oil extracted from the leaves and fruits of *E. camaldulensis*. The relationship of *Candida* species tested with the inhibition zone ranges is found in table 3.

The essential oil activity of *E. globulus* was analyzed using the disk-diffusion method at a concentration of 5 µL/disk. The essential oil presented a strong antifungal potential with inhibition zone of up to 25 mm. The result of the oil inhibition zone was higher in relation to the inhibition zone of the antifungals tested, Amphotericin B and Fluoroscytosine, 20 mm and 18 mm, at concentrations of 10 µg/disc and 20 µg/disc, respectively. The MIC was equivalent to 1 mg/mL. In addition, the essential oil of *E. globulus* was more sensitive to *Candida albicans* than to the bacteria also evaluated, *Staphylococcus aureus,* Methicillin-resistant *Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae* and *Salmonella enteridis* [35]

Thus, the essential oils of *E. citriodora, E. Camaldulensis* and *E. globulus* present activity against different pathogens. However, further studies on anti-*Candida* spp. activity are still needed in order to obtain more consistent data, especially with *E. camaldulensis*, which only found a single study during this research.

Table 1. Chemical composition of *E. citriodora*, *E. camaldulensis* and *E. globulus* .

Species	Constituents	Concentration range
Eucalyptus citriodora	citronellal	22.30% - 78.15%
	citronellol	5.55% - 20.00%
	citronellol acetate	1.33% - 12.30%
	isopulegol	1.12% - 7.60%
	a-pineno	1.15% - 3.60%
	eucalyptol	2.00% - 2.50%
Eucalyptus camaldulensis	*1*.8-cineole	5.90% - 62.70%
	p-cymene	6.70% - 35.70%
	terpinene	10.70% - 22.04%
	a-pinene	3.03% - 15.60%
	terpinen-4-ol	2.00% - 5.30%
	a-terpineol	2.85% - 4.40%
Eucalyptus globulus	*1*.8-cineole	13.23 – 89.80%
	a-pinene	2.00 -16.06%
	Aromadendrene	0.57 -19.70%
	o-Cymene	0.50 – 2.35%
	D-Limonene	0.30 – 2.59%
	Camphene	0.19 – 2.43%

Table 2. Anti-*Candida* spp. activity of *E. citriodora* essential oil.

	Candida spp.	MIC	MFC RANGE
E. citriodora	*C. albicans*	0.02 µg/mL – 0.25 mg/mL	0.09 µg/mL – 0.52 mg/mL
	C. krusei	5 µg/mL	5 µg/mL
	C. tropicalis	2.5 µg/mL	10 µg/mL

Table 3. Anti-*Candida* spp. activity of *E. camaldulensis* essential oil.

		Inhibition Zone	
	Candida spp.	Leaves	Fruits
E. camaldulensis	*C. tropicalis*	18 - 22 mm	12 - 18 mm
	C. glabrata	19 - 23 mm	13 - 20 mm

3.3 Pharmacological interactions of essential oils and their phytoconstituents with antifungals against *Candida* spp.

Knezevic P *et al.* [42] determined the antimicrobial potential of two essential oils of *E. camaldulensis* against multiple clinical isolates resistant to *Acinetobacter baumanii* drugs. Antibacterial activity and synergistic effect was observed between essential oils and all antibiotics tested, ciprofloxacin, gentamicin and polymicin B. The detected MICs for the *E. camaldulensis* essential oils were in range from 0,5 to 2 µl/mL. The synergistic interaction was confirmed by time-kill curves for *E. camaldulensis* essential oil and polymyxin B combination which reduced bacterial count under detection limit very fast, after 6 h of incubation.

Silva PDC *et al.* [48] determined through *checkerboard* methodology the effect of citronellal combination with amphotercin B and ketoconazole against *Candida albicans*. The results show that the association of citronellal with ketoconazole has an additive effect against one of the strains of *C. albicans* (ICIF de 0,625) and indifferent to the other strain. While the combined activity of citronellal with amphotericin B showed an indifferent effect for all strains tested.

Şimşek M and Duman R [34] investigated the efficacy of 1,8-cineole (eucalyptol), the main constituent of *E.*

camaldulensis, in the antimicrobial effect of chlorhexidine against some microorganisms using the *checkerboard* method. Synergistic activity has been demonstrated between chlorhexidine and 1,8-cineol against *Staphylococcus aureus*, methicillin-resistant *S. aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis* and *Candida albicans*. Indifferent interactions for these compounds were demonstrated against *Pseudomonas aeruginosa*. The MIC values chlorhexidine was 2 mg/L and in combination with 1.8-cineole it was 0.5 mg/L for *C. albicans*.

Silva D *et al.* [49] evaluated the behavior of positive and negative enantiomers of β-citronellol, constituent of *E. citriodora*, in *Candida albicans* and *C. tropicalis strains* involved in candidemia. Association studies have been conducted with amphotericin B using the *checkerboard* method. The two isomers showed synergistic and indifferent effect against *C. albicans* resistant to amphotericin B and *C. tropicalis*, respectively. An additive effect was also observed for the negative enantiomer against *C. albicans*. In addition, a mechanism of action assay revealed that β-citronellol isomers exhibited action on the fungal membrane of *Candida* spp. R-(+)-β-citronellol and S-(−)-β-citronellol presented a MIC 50% of 64 µg/mL and a MFC 50% of 256 µg/mL for *C. albicans* strains. For *C. tropicalis*, the isomers exhibited a MIC 50% of 256 µg/mL and a MFC 50% of 1024 µg/mL.

Tonon CC *et al.* [50] evaluated the antifungal activity of terpinen-4-ol associated with nystatin in biofilms of single and mixed species formed by *Candida albicans* and *Candida tropicalis*. The minimum inhibitory concentration and minimum concentration fungicide of terpinen-4-ol and nystatin were determined by the broth microdilution method, together with their synergistic activity (checkerboard method). An additive effect was observed in some concentrations of terpinen-4-ol and nystatin and also a synergistic effect at concentrations of 1.06 mg/mL of terpinen-4-ol and 0.00012 mg/mL of nystatin against *Candida albicans*. An additive effect was observed at concentrations of 1.06 mg/mL of terpinen-4-ol and 0.0003 mg/mL of nystatin against *C. tropicalis*. Terpinen-4-ol was able to reduce nystatin MIC 128 times for *C. albicans* and 64 times for *C.tropicalis*.

Salem N *et al.* [35] evaluated the synergistic effect of *E. globulus* essential oil with conventional antimicrobials against nine pathogenic bacteria and *Candida albicans*. The results of the combination of amphotericin B with the oil showed an additive effect with ICIF of 1.031. This combination showed a potential antifungal effect, the MIC value of the was 32 times lower than the oil MIC against *Candida albicans* (MIC = 1000 µg/mL).

During the research for the present study, no article specifically addressed the synergistic or additive effect of *E. citriodora* or *E. camaldulensis* in association with antifungals against *Candida* spp. However, studies were found on the association of the main constituents of *E. citriodora* and *E. camaldulensis* and antifungals commonly used in clinical practice. Thus, the anti-*Candida* activity of essential oils has been the subject of several studies, however the evaluation of the interactions of these oils with antifungal agents are still scarce requiring greater knowledge about this as well as clarifying which mechanism of action is active.

Conclusions

Eucalyptus citriodora was effective against *Candida albicans*, *C. tropicalis* and *C. krusei*. Its main constituent, citronellal, was effective against *C. albicans* and *C. glabrata* and, an additive effect was observed in combination with ketoconazole against *C. albicans*. *Eucalyptus camaldulensis* was effective against *C. tropicalis* and *C. glabrata*. Its main constituent, 1,8-cineole (eucalyptol), was effective against *C. albicans* and synergic effect it was observed in combination with chlorhexidine against the same species. *Eucalyptus globulus* was effective against *C. Albicans* and also presented a adictive effect with amphotericin B.

The essential oils of *E. citriodora*, *E. camaldulensis* and *E. globulus*, as well as their main constituents, exhibit proven anti-*Candida* spp. activity. They also have a potential additive or synergistic effect with antimicrobials. However, further studies are still needed to consolidate the information generated in the present study, so that it can actually contribute to the therapeutic arsenal in infections caused by *Candida* spp.

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.

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