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Revisión / Review

Antimicrobial activity of limonene: Integrative review

[Actividad antimicrobiana del limoneno: Revisión integrativa]

Adyelle Dantas Ribeiro, Maria Novélia Amaral Cardoso, Julliana Cariry Palhano Freire, Ernani Canuto Figueirêdo Júnior, Mariana Mélani Alexandrino Costa, Priscilla Guimarães Silva, Daliana Queiroga de Castro Gomes, Edja Maria Melo de Brito & Jozinete Vieira Pereira

Department of Dentistry, Universidade Estadual da Paraíba, Campina Grande, Brazil

Reviewed by:
Arnaldo Luis Bandoni
Universidad de Buenos Aires
Argentina

José L. Martínez
Universidad de Santiago de Chile
Chile

Correspondence:
Adyelle Dantas **RIBEIRO**
adriana@cpqba.unicamp.br

Section Review

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Abstract: Limonene is the main component of citrus essential oils, and can reach a concentration of up to 96%. Popularly known for its potential therapeutic effects on the body, among these we point out its broad antimicrobial activity against various types of pathogens. Therefore, the purpose of this study was to address the antimicrobial and antifungal properties of limonene compared to microorganisms of interest in dentistry, based on a bibliometric study and literature review. The following databases were analyzed: PubMed, Google Scholar, SciELO and Science Direct, from which ten articles published between 2011-2021 were selected. Most of results indicate a satisfactory antimicrobial potential, besides providing important data and perspectives regarding the indication and clinical use, in addition to innovative therapeutic modalities for diseases that affect the oral cavity, such as tooth decay, periodontal disease and candidosis.

Keywords: Limonene; Anti-infectious; Dentistry; Phytotherapy; Medicinal plants

Resumen: El limoneno es el componente principal de los aceites esenciales cítricos, y puede alcanzar una concentración de hasta el 96%. Popularmente conocido por sus potenciales efectos terapéuticos en el organismo, entre ellos se destacan su amplia actividad antimicrobiana frente a diversos tipos de patógenos. Por lo tanto, el objetivo de este estudio fue abordar las propiedades antimicrobianas y antifúngicas del limoneno en comparación con microorganismos de interés en la odontología, a partir de un estudio bibliométrico y una revisión bibliográfica. Se analizaron las siguientes bases de datos: PubMed, Google Scholar, SciELO y Science Direct, de las cuales se seleccionaron diez artículos publicados entre 2011-2021. La mayoría de los resultados indican un potencial antimicrobiano satisfactorio, además de proporcionar datos y perspectivas importantes con respecto a la indicación y el uso clínico, así como modalidades terapéuticas innovadoras para enfermedades que afectan la cavidad oral, como caries, enfermedad periodontal y candidosis.

Palabras clave: Limoneno; Antiinfecciosos; Odontología; Fitoterapia; Plantas medicinales

INTRODUCTION

Products from nature, especially from vegetables, have been used since early times by man for the treatment and recovery of health (Kingston, 2011). The habit of resorting to plants is due to the beneficial effects of the secondary metabolites that are present in them, mainly due to their antimicrobial properties (Cunha *et al*., 2016).

In the present times, even after the technological development of the pharmaceutical industry, natural products are promising sources for the discovery of new drugs (Kingston, 2011; Hotwani *et al*., 2014; Da Silva & Aquino, 2018). In addition, lately, the loss of the curative effects of many synthetic active ingredients has been observed due to the emergence of increasingly resistant microorganisms (Dias, 2010; WHO, 2014). This situation has restarted the interest of researcher to intensively search new antimicrobial substances from various sources, including medicinal plants (Bansod; Rai, 2008; Da Silva & Aquino, 2018).

Essential oils (EO) are odoriferous, lipophilic and volatile compounds that are part of the secondary metabolism of plants. They have a broad spectrum of biological activities and perform a potential antimicrobial activity against bacteria, fungi and yeasts (Lang & Buchbauer, 2011). In nature, they act in the defense mechanism against predators, and at the same time attract pollinating insects, so ensuring their survival and evolution (Silva *et al*., 2019; Borges & Amorim, 2020).

Limonene is one of the most studied types of essential oils lately, found in more than 300 species of different plants (Jongedijk *et al*., 2016), and is the main component of citrus essential oils (Zahi *et al*., 2015; Simas *et al*., 2015), where it represents from 30% to 97% of its composition (Simas *et al*., 2015). Limonene is represented by the chemical formula $C_{10}H_{16}$, belongs to the terpene family, and is an unsaturated cyclic monoterpene that can occur in two optical forms (d-limonene and l-limonene) (Jongedijk *et al*., 2016), while d-limonene is one of the most important cyclic monoterpenes (Zahi *et al*., 2015). In recent years the use of limonene has been increasing significantly in several areas, such as in the food industry, green chemistry, pharmaceutical, and also as a safer pesticide (Cirimina *et al*., 2014).

In medicine, limonene plays different roles, such as therapeutic effects against cancer (Zhang *et al*., 2014; Miller *et al*., 2011), infections (Astani & Schnitzer, 2014), diabetes (Murali *et al*., 2013), inflammation (Hirota *et al*., 2010), allergy and asthma (Hirota *et al*., 2012). Its biological activities include antioxidant (Eddin *et al*., 2021), antiarrhythmic (Nascimento *et al*., 2019) and chemopreventive properties, especially against breast cancer (Miller *et al*., 2011; Sun, 2007).

Its therapeutic effect is well known in the literature for the potential antimicrobial activity against various pathogenic microorganisms, such as *Streptococcus mutans* (Lemes *et al*., 2018; Bezerra *et al*., 2013), *Streptococcus salivaris*, *Streptococcus oralis* (Bezerra *et al*., 2013), *Escherichia coli* (Gallegos-Flores *et al*., 2019; Salehi *et al*., 2021), *Pseudomona* spp, *Salmonella* spp, (Gallegos-Flores *et al*., 2019), *Staphylococcus aureus* (Gallegos-Flores *et al*., 2019; Salehi *et al*., 2021; Han *et al*., 2021), *Candida albicans* (Omran *et al*., 2011; D'arrigo *et al*., 2019), *Streptococcus sobrinus* (Liu *et al*., 2020), and *Enterococcus* (Salehi *et al*., 2021). Since limonene shows a broad spectrum of antimicrobial action, especially for these microorganisms, which are of great importance in dental clinics, there was great efficacy expectation of its application as a dental therapy.

Therefore, considering the exposed above and the importance of the topic, this study aims to address the antimicrobial and antifungal properties of limonene, based on a bibliometric study and literature review.

LITERATURE REVIEW

General characteristics of limonene

This compound is obtained mainly from citrus fruits by extracting the essential oils present in the peels (pericarps), which later undergo a purification process to finally obtain the pure limonene (Silvestres & Pauletti, 2018).

There are several methods for obtaining essential oils. According to Koch *et al*. (2015) and Azambuja (2017), the most used method to extract EO from citrus fruits is cold pressing, and the steam-drag distillation process is also widely used (Simas *et al*., 2015).

This is a colorless, oily liquid with citrus odor, characteristic of citrus fruits. Regarding its solubility, limonene is non-polar (insoluble in water or with very low solubility in aqueous environment), and on the other hand, it has affinity with fats, so confirming its lipophilic property (Cirimina *et al*., 2014).

Application of limonene in several areas

The application of limonene has increased significantly in the last decade. It has a pleasant citrus odor, and because of this, has broad use in industries as aroma and fragrance additive in perfumes, soaps, foods, chewing gums and beverages (Ciriminna *et al*., 2014). In addition, the FDA (Food and Drug Administration) has recognized the limonene compound as Generally Recognized as Safe Substances (GRAS), and therefore, has released its use as a flavoring agent for the food industry and other areas (Ciriminna *et al*., 2014).

The chemical structure of limonene terpene is very important because it is unstable and passive to chemical modifications (Wilbon *et al*., 2013), providing it several possibilities of application (Jongedijk *et al*., 2016). In recent years, limonene has acquired a critical importance due to its demand as a biodegradable solvent, and as a sustainable fuel. In addition, it also has applications as an aromatic component and is widely used in the synthesis of new compounds (Silva *et al*. 2019).

Regarding health in general, limonene has shown its therapeutic potential in several situations. Miller *et al*. (2011), after collecting scientific evidence, concluded that there is a possibility of cancer treatment, especially against breast cancer, since limonene, when extensively distributed in human breast tissue promoted the reduction of cyclin D1 expression in breast tumor that can lead to cell cycle interruption and cell proliferation reduction. In addition, they observed that after oral ingestion, most compounds are deposited in adipose tissues due to the lipophilic character of this compound.

Muralli *et al*. (2013), in his study, treated diabetic rats with limonene and observed that the compound has the ability to potentiate insulin secretion. And Nascimento *et al*. (2019), also in a study with rats, observed that limonene promotes bradycardia, that is, it shows potential antiarrhythmic activity.

Antimicrobial activity and limonene mechanism of action

The increasing microbial resistance to drugs has led to a greater attention from researcher for the search of new antimicrobial agents, and with this, limonene has become a target of interest. Its antimicrobial potential has been evaluated in many studies against a broad spectrum of pathogenic microorganisms (Omran *et al*., 2011; Pinto *et al*., 2013; Pinto *et al*., 2017; D'Arrigo *et al*., 2019; Gallegos-Flores *et al*., 2019; Liu *et al*., 2020).

In a study conducted by Souza *et al.* (2010), it was found that limonene showed antibacterial activity against Gram-positive and Gram-negative bacteria, *Streptococcus aureus* and *Pseudomonas aeruginosa*, respectively. However, *S. aureus* was more sensitive to limonene when compared to the Gram-negative microorganism and this fact was justified by the more complex structure of *P. aeruginosa* (Nazzaro, 2013), which has an efflux system capable of removing antimicrobial compounds from the intracellular medium to the extracellular medium (ANVISA, 2007; Radulovic *et al*., 2013).

The mechanism of action of essential oils in antimicrobial activity is associated with the presence of certain compounds that can change the permeability of the microorganisms cell membrane and/or inhibit important enzymes for their growth and survival (Bakkali *et al*., 2008; Oliveira *et al*., 2016). Several studies have proven the potential effect of limonene in inhibiting the microorganisms growth, but their way of acting has not been fully clarified yet (Bezerra *et al*., 2013). It is suggested that the antimicrobial activity of limonene is associated with its lipophilic behavior capable of denaturating proteins and lipid layers, changing the properties and function of the cell wall, and leading to the loss of intracellular components and eventual cell death (Hernandes *et al*., 2014).

Use in dentistry

Tooth decay is one of the most prevalent human diseases worldwide (Kassebaum *et al*., 2015), mainly caused by bacteria of *Streptococcus mutans* species due to their acidic and acidogenic characteristics (Bezerra *et al*., 2013; Subramenium *et al*., 2015; Tardugno *et al*., 2017). Chlorhexidine is considered the most effective method against these microorganisms (Bezerra *et al*., 2013), however, its use is becoming reduced because it has undesirable effects, such as dental staining, taste change and bacterial resistance (Freires *et al*., 2010; Zhang *et al*., 2019). Therefore, there is a growing interest in the use of natural products (Liu *et al*., 2020), because they bring more advantages and safety (Bezerra *et al*., 2013).

Researches on limonene application in Dentistry are increasing and obtaining positive results. Liu *et al*. (2020) and Sun *et al*. (2018), in their studies, observed the inhibitory action of limonene against the virulence of cariogenic bacteria. In addition, limonene shows the ability to inhibit the proliferation of *Streptococcus sobrinus*, prevent tooth decay and stop its progression (Liu *et al*., 2020). Ma *et al*. (2020), has found in his study that limonene promotes the replacement of Ca and P on the demineralized surfaces of the tooth, in addition to protecting dentin collagen and inhibiting its hydrolysis, so enabling the prevention of root decay. Salehi *et al*. (2021), also reported that limonene has action against *Enterococcus*, a species that despite being a commensal in the oral microbiota, is commonly associated with persistent periapical infections and failures of endodontic treatment (Coelho *et al*., 2020).

Oral Candidiasis is another condition which treatment was investigated using limonene, given that it is a very frequent pathology in patients seeking dental offices, and an infection mainly caused by fungi of the genus *Candida*, especially *Candida albicans* (Vila *et al*., 2020). Its treatment is based on the use of conventional antifungals (Shapiro *et al*., 2011). However, the use of these drugs has been limited due to the presence of resistant strains, cases where limonene at certain concentrations is effective in inhibiting these microorganisms (Thakre *et al*., 2018; Muñoz *et al*., 2020).

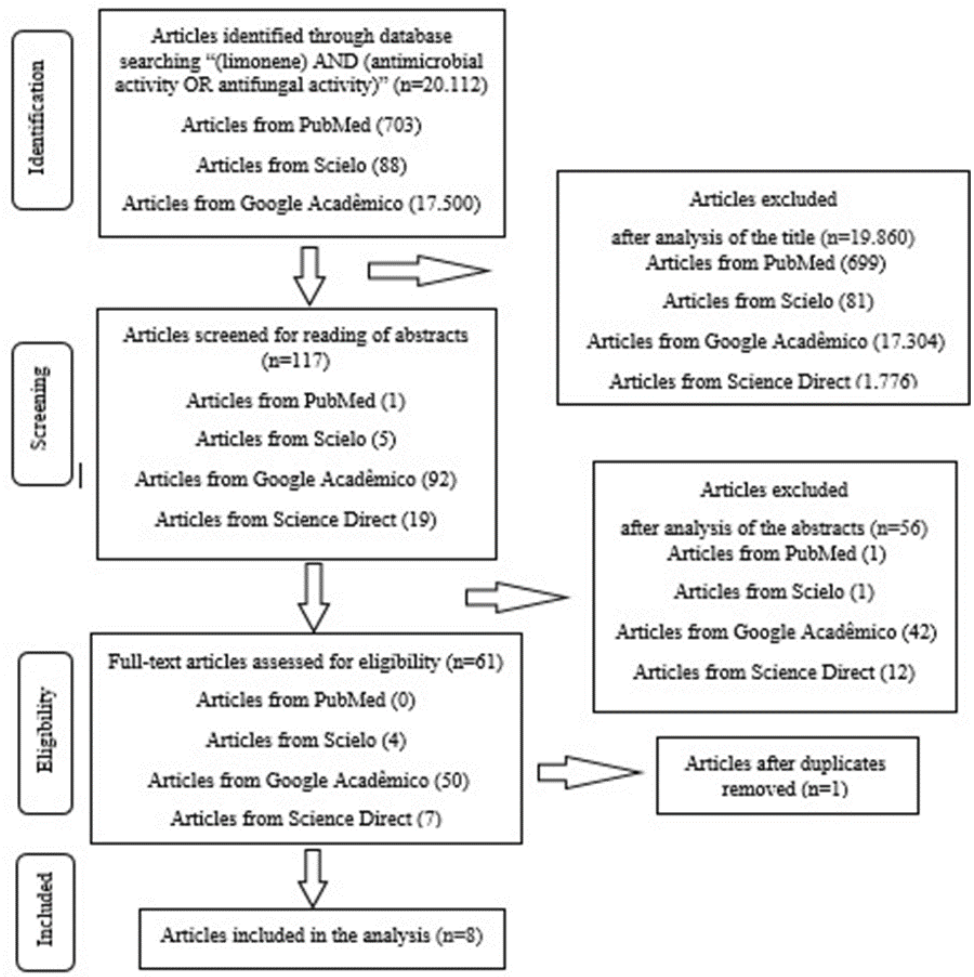

Figure No. 1
Research data flowchart (Source: Own authorship)

MATERIALS AND METHODS

This is an integrative literature review study of research articles that discuss the use of limonene in antimicrobial and/or antifungal activity trials.

For the study, databases such as the National Library of Medicine of the National Institutes of Health (PubMed) and Science Direct, Scielo and Google Scholar were searched using the following descriptors “Limonene”, “antimicrobial activity”, and “antifungal activity”, in addition to the Boolean operators “AND” and “OR” arranged as follows: “Limonene” AND (antimicrobial activity OR antifungal activity)”.

Articles published between 2011 and 2021 were selected, with no language restriction. Searches in the PubMed, Science Direct, Scielo and Google Scholar databases recovered, respectively 785; 2,011; 92 and 19,360 articles, and we selected by title, respectively, 4; 45; 7 and 196 articles, as shown in Figure No. 1.

After this stage, the abstracts were read to determine if they were related to the proposed topic, and so perform the studies refinement. The criteria for selecting the articles were: articles that perform limonene trials against microorganisms of interest to dentistry, which texts were later read in full. After the removal of duplicates, at the end, a total of 10 articles were selected for the bibliometric analysis and literature review.

RESULTS

Several studies using limonene, evaluating its antibacterial and antifungal activity, have been published in recent literature. Therefore, based on the above considerations, the studies selected in the inclusion criteria are shown in Table No. 1.

Table No. 1
Antimicrobial activity of limonene against oral pathogens

Limonene Type	Microorganisms	Antimicrobial Susceptibility Test	Results	Control Antibiotic	References
D-limonene	*C. Albicans*(a) *C. glabrata* (b) *C. parapsilosis*(c)	MIC	125–250 62.50 31.25–62.50	(a) Voriconazole: >16; Fluconazole: >16; Caspofungin: 0,015 (b) Voriconazole: 0,015-0,031; Fluconazole: 8-8; Caspofungin: 0,015-0,031 (c) Voriconazole: 0,015-0,031; Fluconazole: 0,5-1; Caspofungin: 0,5-0,025	D’Arrigo *et al*., 2019
Limonene	*Escherichia coli* (a) *Staphylococcus aureus* (b) *Streptococcus* spp (c)	Inhibition halos in millimeters (Paper disc)	(a) 0.75: 0 ± 0 / 0.45: 0.5 ± 0 / 0.15: 2.0 ± 1.4 / 0.075 2.0 ± 1.4 / 0.05 1.5 ± 0.7 (b) 1.5 ± 0.7 / 4.0 ± 3.0 / 3.0 ± 2.8 / 0.5 ± 0 / 4.0 ± 2.8 (c) 0 ± 0 / 0 ± 0 / 0 ± 0 / 0 ± 0 / 0 ± 0	(a) Ceftibuten: 0.75: 10.2 ± 0,3 / 0.45: 10.5 ± 0.7/ 0.15: 10.0 ± 0/ 0.075 9.0 ± 0/ 0.05 7.5 ± 0.7 Cefalexin: 0.75: 5.5 ± 0,7 / 0.45: 5.0 ± 1.4/ 0.15: 0 ± 0/ 0.075 0 ± 0/ 0.05 0 ± 0 (b) Ceftibuten: 0.75: 13.0 ± 0 / 0.45: 13.0 ± 0/ 0.15: 12.0 ± 0/ 0.075 9.0 ± 1.4/ 0.05 9.0 ± 0 Cefalexin: 0.75: 7.0 ± 0 / 0.45: 5.5 ± 0.7/ 0.15: 5.0 ± 1.4/ 0.075 4.5 ± 0.7/ 0.05 4.0 ± 1.4 Ceftibuten: 0.75: 0 ± 0 / 0.45: 0 ± 0 / 0.15: 0 ± 0/ 0.075 0 ± 0/ 0.05 0 ± 0 Cefalexin: 0.75: 5.0 ± 0 / 0.45: 4.0 ± 0/ 0.15: 4.5 ±	Gallegos-Flores *et al*., 2019

				0.7/ 0.075 2.5 ± 1.4/ 0.05 0 ± 0	
Limonene	*Streptococcus sobrinus*	MIC	21000 µg/mL	Chlorhexidine 0,2%	Liu *et al.*, 2020
Limonene	*Candida albicans* ATCC 10231 (a) *Candida albicans* D5 (b) *Candida albicans* M1 (c) *Candida dubliniensis* CD1(d) *Candida tropicalis* ATCC 13803 (e) *Candida krusei* ATCC 6258 (f) *Candida glabrata* D10R (g) *Candida parapsilosis* ATCC 90018 (h) *Aspergillus niger* ATCC 16404 (i) *A. fumigatus* ATCC 46645 (j) *A. flavus* F44 (k)	MIC/MLC	(a) 0.64-1.25/ 1.25–2.5 (b) 0.64/ 0.64 (c) 0.64/ 0.64 (d) 0.64/ 0.64 (e) 2.5/ 2.5 (f) 0.64/ 0.64 (g) 2.5/ 2.5 (h) 1.25-2.5/1.25-2.5 (i) 5/20 (j) 5/ 5-10 (k) 5–10/ 10	(a) Fluconazole: 1/>128 Amphotericin B: N.T./N.T. (b) Fluconazole: 64/>128 Amphotericin B: N.T./N.T. (c) Fluconazole: 2/>128 Amphotericin B: N.T./N.T. (d) Fluconazole: 1/>128 Amphotericin B: N.T./N.T. (e) Fluconazole: 4/>128 Amphotericin B: N.T./N.T. (f) Fluconazole: 64/64-128 Amphotericin B: N.T./N.T. (g) Fluconazole: 8/8 Amphotericin B: N.T./N.T. (h) Fluconazole: <1/<1 Amphotericin B: N.T./N.T. (i) Fluconazole: N.T./N.T. Amphotericin B: 1-2/4 (j) Fluconazole: N.T./N.T. Amphotericin B: 2/4 (k) Fluconazole: N.T./N.T. Amphotericin B: 2/8	Pinto *et al.*, 2013
R limonene and S limonene	*Candida albicans* *Aspergillus niger* *Aspergillus* sp.	Solid medium (tube) 1 Well diffusion 2 Diffusion on paper disc 3	1) R limonene: 10 µL = 1 ± 0 / 5 ± 1,41 / 5 ± 0; 20 µL = 3 ± 1.41 / 5.5 ± 2.12 / 5 ± 1.41; 30 µL = 4 ± 1.41 / 7.5 ± 3.54 / 9 ± 1.41; S limonene: 10 µL = 3.5 ± 2.12 / 13 ± 0 / 3.5 ± 0.71; 20 µL = 7 ± 1.41 / 13 ±	-	Omran *et al.*, 2011

			0 / 4 ± 1.41; 30 µL = 9.5 ± 3.54 / 15 ± 0 / 5.5 ± 0.71 2) R limonene: 10 µL = 3.5 ± 2.12b / 0 ± 0 / 0 ± 0; 20 µL = 3.5 ± 2.12 / 0 ± 0 / 0 ± 0; 30 µL = 3.5 ± 2.12 / 1.5 ± 0.71 / 0.5 ± 0.71; S limonene: 10 µL = 7 ± 2.83 / 2 ± 0 / 2 ± 0; 20 µL = 9.5 ± 3.54 / 3.5 ± 0.71 / 3.5 ± 0.71; 30 µL = 13 ± 2,836.5 ± 0.71 / 5 ± 1.41 3) R limonene: 10 µL = 1 ± 2.12b / 2 ± 0; 20 µL = 1.5 ± 0.71; 0 ± 0; 4 ± 0; 30 µL = 3.5 ± 0.71 / 0.5 ± 0.71 / 6 ± 0; S limonene: 10 µL = 3.5 ± 0.71 / 6 ± 0 / 5 ± 0; 20 µL = 5 ± 1.41 / 6 ± 0; 30 µL = 7 ± 1.41 / 9.5 ± 2..12 / 7.5 ± 0.71		
(R) - (+) - Limonene	*Candida albicans* ATCC 10231 (a)	MIC/MLC	0.32/ 0.32	(a) Fluconazole: 1/>128 Amphotericin B: -./-	Pinto *et al*., 2017
	C. krusei ATCC 6258 (b)		0.16/ 0.16	(b) Fluconazole: 64/64-128 Amphotericin B: -/-	
	C. tropicalis ATCC 13803 (c)		0.64/ 0.64	(c) Fluconazole: 4/>128 Amphotericin B: -/-	
	C. parapsilosis ATCC 90018 (d)		0.64/ 0.64	(d) Fluconazole: 1/1-2 Amphotericin B: -/-	
	C. albicans D5 (e)		0.16/ 0.16	(e) Fluconazole: 64/>128 Amphotericin B: -/-	
	C. albicans M1 (f)		0.64/ 0.64	(f) Fluconazole: 2/128 Amphotericin B: -/-	
	C. dubliniensis CD1 (g)		0.16/ 0.16	(g) Fluconazole: 1/>128 Amphotericin B: -/-	

	C. glabrata D10R (h) *Aspergillus flavus* F44 (i) *A. fumigatus* ATCC 46645 (j) *A. niger* ATCC 16404 (k)		0.32/ 0.32–0.64 0.32–0.64/ 0.64 0.32/ 0.32 0.32/ 0.64	(h) Fluconazole: 32/32 Amphotericin B: -/- (i) Fluconazole: -/- Amphotericin B: 2/8 (j) Fluconazole: -/- Amphotericin B: 2/4 (k) Fluconazole: -/- Amphotericin B: 1-2/4	
Limonene	*Streptococcus pyogenes Streptococcus mutans Streptococcus mitis.*	MIC	400 µg mL-1 (83%) – *S. pyogenes* SF370 400 µg mL-1 (75% - 95%) - All MO	DMSO	Subramenium *et al.*, 2015
(R) - (+) - Limonene	*Candida albicans* (37 strains)	MIC	5 Mm/5mM	Fluconazole <0,41mM/<0,83Mm	Thakre *et al.*, 2018
Limonene	*Escherichia coli Staphylococcus aureus Enterococcus* *Escherichia coli Staphylococcus aureus Enterococcus*	MIC/MLC Inhibition Zone	26.25/26.25 52.5/210 52.5/420 12.5: 15.5 ± 0.5 / 6.25: 14.0 ± 0 / 3.12: 10.5 ± 0.5 12.5: 14.0 ± 0 / 6.25: 12.5 ± 0.5 12.5: 13.0 ± 0 / 6.25: 12.0 ± 0	Meropenem: 16 µg/mL Penicillin: 32 µg/mL	Salehi *et al.*, 2021
Limonene	*Staphylococcus aureus*	MIC	20000 µL/mL	2% ethanol	Han *et al.*, 2021

DISCUSSION

The mechanism of action of limonene is not yet fully established yet, however, authors such as Di Pasqua *et al*. (2006), and Hernandes *et al*. (2014), reported that the effect of these compounds occurs due to their lipophilic aspect, causing changes or damage to the composition of fatty acids in the outer membrane of bacteria and increased permeability that causes losses of adenosine triphosphate acid, ion leakage and, therefore, cell lysis.

Oral candidiasis is a frequent infection, with a high incidence mainly in immunocompromised individuals, such as patients with diabetes, prematurity, transplant recipients, HIV/AIDS and oral cancer patients undergoing radiotherapy and/or head and neck chemotherapy (Zhang *et al*., 2016). In addition, it was observed in the literature that limonene showed antifungal potential against different species of these pathogens: *Candida albicans* (Pinto *et al*., 2013; Pinto *et al*., 2017; Thakre *et al*., 2018; D'Arrigo *et al*., 2019), *Candida glabrata*, *Candida parapsilosis* (Pinto *et al*., 2013; Pinto *et al*., 2017; D'Arrigo *et al*., 2019), in addition to *Candida dubilienses*, *Candida tropicalis* and *Candida Krusei* (Pinto *et al*., 2013; Pinto *et al*., 2017), showing an antagonistic effect when administered with Fluconazole, Voriconazole and Caspofungin. And this interaction may result in greater efficacy, better antifungal effects due to its mechanism of action on the membrane, and contribute to the reduction of effective doses, so reducing the likelihood of adverse effects (D'Arrigo *et al*., 2019).

However, Pinto *et al*. (2017), in his research where he reported the antifungal activity of limonene against *Candida* spp., *Cryptoccocus neoformans*, *Malassezia furfur, Aspergillus* spp. and several dermatophytes, he found a synergism between limonene and fluconazole. Therefore, it is important to take into account the complex composition of essential oils, which makes it quite difficult to predict the mode of interaction, especially because pharmacokinetic profiles are not elucidated.

The results found by Thakre *et al*. (2018), suggest that limonene inhibits the growth of *C. albicans* by gene overexpression and causing damage to the cell membrane induced by oxidative stress, which lead to DNA damages, resulting in cell cycle modulation and induction of apoptosis through nucleolar stress and the metacaspase-dependent route. Limonene showed excellent anti-*Candida* potential against planktonic forms (yeast), morphogenesis (hyphal), significant inhibition of biofilm adherence and growth, showing fungicide activity against *Candida albicans*.

Several researches point to the presence of yeasts (Baumgartner *et al*., 2000; Siqueira & Rôças, 2004; Brook, 2006; Lu *et al*., 2012) and filamentous fungi (Gomes *et al*., 2010) in endodontic infections and in the maxillary sinus in cases of chronic sinusitis. Gomes *et al*. (2010), in his study, observed the presence of *Aspergillus niger*, *Aspergillus versicolor* and *Aspergillus fumigatus* also in root canals with endodontic treatment and periapical lesion. Therefore, based on the results found by Pinto *et al*. (2013, 2017), limonene can be used in endodontic treatments due to its action against *Aspergillus spp*. Consequently, Salehi *et al*. (2021), found antimicrobial potential against *Enterococcus*, another species of pathogens also commonly associated with defective endodontic treatments.

Regarding the importance of the microbiota present in the oral cavity, bacterial infections involve periodontal diseases and decayed lesions, where bacteria such as *S. mutans* and *S. sobrinus* are found (Zheng, 2015). Limonene showed potent inhibiting effects against targets of cariogenic virulence, such as bacterial acidogenicity, aciduricity, and glucan synthesis (Zhang *et al*., 2010; Liu *et al*., 2013; Sun *et al*., 2018), which were observed in the study of Liu *et al*., (2020), Moreover, limonene significantly reduced the virulence of *Streptococcus sobrinus* without significantly interfering in the balance of the saliva microbiota. As observed in other studies against these microorganisms, limonene had an antibacterial effect similar to the antibiotic Cefalexin (Gallegos-Flores *et al*., 2019), and also showed anti-biofilm effect possibly by inhibiting bacterial adhesion to surfaces (Subramenium *et al*., 2015).

In view of this, antibacterial and antifungal effects on these pathogenic microorganisms reinforce the promising medicinal effect of this essential oil, indicating possible applications in the treatment of dental disorders, such as tooth decay, endodontic infections and oral candidiasis. All this, combined with its broad spectrum of action against microorganisms and the growing microbial resistance that has been emerging, makes limonene an option that can be widely explored in researches focused on phytotherapy, considering it as a promising alternative for the development of herbal medicines with antimicrobial potential.

CONCLUSIONS

In view of the results, it is perceived that limonene is a promising alternative for the development of herbal drugs with antifungal and antibacterial potential for dental indication. Therefore, more *in vivo* and *in vitro* researches are required to address this topic, given the great pharmacological potential of this compound associated with biological effects.

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