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 Mélaní Alexandrino Costa, Priscilla Guimaraêns Silva, Daliana Queiroga de Castro Gomes, Edja Maria Melo de Brito & Jozinete Vieira Pereira

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

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 monoterpenic 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), Pseudomonas 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.,
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., 2014).
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

<table>
<thead>
<tr>
<th>Limonene Type</th>
<th>Microorganisms</th>
<th>Antimicrobial Susceptibility Test</th>
<th>Results</th>
<th>Control Antibiotic</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-limonene</td>
<td>C. Albicans(a)</td>
<td>MIC</td>
<td>125–250</td>
<td>(a) Voriconazole: &gt;16; Fluconazole: &gt;16; Caspofungin: 0,015</td>
<td>D’Arrigo et al., 2019</td>
</tr>
<tr>
<td></td>
<td>C. glabrata (b)</td>
<td></td>
<td>62,50</td>
<td>(b) Voriconazole: 0,015-0,031; Fluconazole: 8-8; Caspofungin: 0,015-0,031</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. parapsilosis(c)</td>
<td></td>
<td>31,25–62,50</td>
<td>(c) Voriconazole: 0,015-0,031; Fluconazole: 0,5-1; Caspofungin: 0,5-0,025</td>
<td></td>
</tr>
<tr>
<td>Limonene</td>
<td>Escherichia coli (a)</td>
<td>Inhibition halos in millimeters (Paper disc)</td>
<td>(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</td>
<td>(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</td>
<td>Gallegos-Flores et al., 2019</td>
</tr>
<tr>
<td></td>
<td>Staphylococcus aureus (b)</td>
<td></td>
<td>0 ± 0 / 0 ± 0 / 0 ± 0</td>
<td>(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</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Streptococcus spp (c)</td>
<td></td>
<td>0 ± 0 / 0 ± 0 / 0 ± 0</td>
<td>(c) Ceftibuten: 0.75: 0 ± 0 / 0.45: 0 ± 0 / 0.15: 0 ± 0 / 0.075 0 ± 0</td>
<td></td>
</tr>
</tbody>
</table>

Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas / 585
<table>
<thead>
<tr>
<th>Limonene</th>
<th>Streptococcus sobrinus</th>
<th>MIC</th>
<th>21000 µg/mL</th>
<th>0.7/ 0.075 2.5 ± 1.4/ 0.05 0 ± 0</th>
<th>Chlorhexidine 0.2%</th>
<th>Liu et al., 2020</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Limonene</strong></td>
<td><strong>Candida albicans</strong> ATCC 10231 (a)</td>
<td><strong>MIC/MLC</strong></td>
<td>(a) 0.64-1.25/ 1.25–2.5</td>
<td>(a) Fluconazole: 1/&gt;128 Amphotericin B: N.T./N.T.</td>
<td></td>
<td>Pinto et al., 2013</td>
</tr>
<tr>
<td></td>
<td><strong>Candida albicans</strong> D5 (b)</td>
<td>(b) 0.64/ 0.64</td>
<td></td>
<td>(b) Fluconazole: 64/&gt;128 Amphotericin B: N.T./N.T.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Candida albicans</strong> M1 (c)</td>
<td>(c) 0.64/ 0.64</td>
<td></td>
<td>(c) Fluconazole: 2/&gt;128 Amphotericin B: N.T./N.T.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Candida dubliniensis</strong> CD1(d)</td>
<td>(d) 0.64/ 0.64</td>
<td></td>
<td>(d) Fluconazole: 1/&gt;128 Amphotericin B: N.T./N.T.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Candida tropicalis</strong> ATCC 13803 (e)</td>
<td>(e) 2.5/ 2.5</td>
<td></td>
<td>(e) Fluconazole: 4/&gt;128 Amphotericin B: N.T./N.T.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Candida krusei</strong> ATCC 6258 (f)</td>
<td>(f) 0.64/ 0.64</td>
<td></td>
<td>(f) Fluconazole: 64/64-128 Amphotericin B: N.T./N.T.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Candida glabrata</strong> D10R (g)</td>
<td>(g) 2.5/ 2.5</td>
<td></td>
<td>(g) Fluconazole: 8/8 Amphotericin B: N.T./N.T.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Candida parapsilosis</strong> ATCC 90018 (h)</td>
<td>(h) 1.25-2.5/1.25-2.5</td>
<td></td>
<td>(h) Fluconazole: &lt;1/&lt;1 Amphotericin B: N.T./N.T.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Aspergillus niger</strong> ATCC 16404 (i)</td>
<td>(i) 5/20</td>
<td></td>
<td>(i) Fluconazole: N.T./N.T. Amphotericin B: 1-2/4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>A. fumigatus</strong> ATCC 46645 (j)</td>
<td>(j) 5/ 5-10</td>
<td></td>
<td>(j) Fluconazole: N.T./N.T. Amphotericin B: 2/4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>A. flavus</strong> F44 (k)</td>
<td>(k) 5–10/ 10</td>
<td></td>
<td>(k) Fluconazole: N.T./N.T. Amphotericin B: 2/8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**R limonene and S limonene**

<table>
<thead>
<tr>
<th><strong>Candida albicans</strong></th>
<th><strong>Aspergillus niger</strong></th>
<th><strong>Aspergillus sp.</strong></th>
<th><strong>Solid medium (tube)</strong> 1</th>
<th><strong>Well diffusion 2</strong></th>
<th><strong>Diffusion on paper disc 3</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>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 ± 0</td>
<td>-</td>
<td>Omran et al., 2011</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(R) - (+) - Limonene</td>
<td>Candida albicans ATCC 10231 (a)</td>
<td>MIC/MLC</td>
<td>(a) Fluconazole: 1/&gt;128 Amphotericin B: -/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>----------------------------------</td>
<td>--------</td>
<td>------------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. krusei ATCC 6258 (b)</td>
<td>0.16/ 0.16</td>
<td>(b) Fluconazole: 64/64-128 Amphotericin B: -/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. tropicalis ATCC 13803 (c)</td>
<td>0.64/ 0.64</td>
<td>(c) Fluconazole: 4/&gt;128 Amphotericin B: -/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. parapsilosis ATCC 90018 (d)</td>
<td>0.64/ 0.64</td>
<td>(d) Fluconazole: 1/1-2 Amphotericin B: -/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. albicans D5 (e)</td>
<td>0.16/ 0.16</td>
<td>(e) Fluconazole: 64/&gt;128 Amphotericin B: -/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. albicans M1 (f)</td>
<td>0.64/ 0.64</td>
<td>(f) Fluconazole: 2/128 Amphotericin B: -/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. dubliniensis CD1 (g)</td>
<td>0.16/ 0.16</td>
<td>(g) Fluconazole: 1/&gt;128 Amphotericin B: -/-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 / 20 μL = 3.5 ± 2.12 / 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 ± 2.83 ± 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

Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas / 587
<table>
<thead>
<tr>
<th></th>
<th></th>
<th>MIC/MLC</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C. glabrata D10R (h)</td>
<td></td>
<td>0.32/ 0.32–0.64</td>
<td></td>
<td>(h) Fluconazole: 32/32 Amphotericin B: +/-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus flavus F44 (i)</td>
<td></td>
<td>0.32–0.64/ 0.64</td>
<td></td>
<td>(i) Fluconazole: +/- Amphotericin B: 2/8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. fumigatus ATCC 46645 (j)</td>
<td></td>
<td>0.32/ 0.32</td>
<td></td>
<td>(j) Fluconazole: +/- Amphotericin B: 2/4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. niger ATCC 16404 (k)</td>
<td></td>
<td>0.32/ 0.64</td>
<td></td>
<td>(k) Fluconazole: +/- Amphotericin B: 1-2/4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**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 dublinienses*, *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., Cryptococcus 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.

REFERENCES
Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas / 589


Hirota R, Nakamura H, Bhatti AS, Ngatu NR, Muzembo BA, Dumaviibhat N, Eitoku M, Sawamura M, Suganuma

Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas / 590


Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas / 591


Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas / 592


