The antimicrobial activity of essentials oils of thyme, oregano, copaiba, tea tree, and frankincense against Enterococcus faecalis

Análise da atividade antimicrobiana dos óleos essenciais de tomilho, orégano, copaíba, melaleuca, e olíbano contra Enterococcus faecalis

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ABSTRACT
Oral diseases are among the problems that most affect the quality of life of people in the world. Traditional methods for drug treatment of these diseases are almost always expensive and, in addition, they cause side effects and can promote an increase in bacterial resistance. Thus, the demand for plant-derived products, such as herbal, floral and essential oils, has been growing every day, favoring the development of new therapeutic products that can replace or complement traditional therapeutic treatment. Among the natural products that have been gaining space, essential oils (EOs) stand out. Therefore, the aim of this study was to evaluate the role of thyme, oregano, copaiba, tea tree and frankincense EOs in inhibiting the growth of Enterococcus faecalis bacteria. The oil diffusion technique in agar was performed to evaluate the efficiency of the EOs, at different concentrations: 25%, 50%, 75% and 100%. The EOs that showed the greatest inhibition halo were, in decreasing order: Thyme Oil (5.0 mm), Tea tree Oil (4.70 mm) and Oregano Oil (3.75mm) in the concentration of 100%, with results similar to 0.12% chlorhexidine (positive control), whose inhibition halo was 3 mm. Copaiba and
Frankincense oils were not efficient to inhibit bacterial growth at concentrations of 25, 50 and 75%. The results obtained in this research demonstrated that thyme, tea tree and oregano oils inhibited the growth of Enterococcus faecalis bacteria.

Keywords: Oral health, Essential oils, Microbiology, Enterococcus faecalis.

INTRODUCTION

Oral diseases are among the diseases that most affect the quality of life of people in the world. It is estimated that approximately 3.5 billion people around the world suffer from pain and discomfort caused by oral diseases (GBD, 2018). Unfortunately, oral diseases are strongly associated with the socioeconomic status of individuals and mostly affect disadvantaged populations (Peres, Macpherson, Weyant, Daly, Venturelli & Mathur, 2019) and traditional methods for drug treatment of these conditions are almost always expensive and most are not covered by public health services, in addition to causing unpleasant side effects, including diarrhea and vomiting, and can promote increased bacterial resistance of the body decreasing the therapeutic effect (Palombo, 2011; Weiss, Freeman, Heslin & Barrett, 2018). Therefore, the demand for plant-derived products, such as herbal, floral and essential oils, has been growing every day, favoring the development of new therapeutic products that can replace or complement traditional treatment (Silva, Abebe, Souza, Duarte, Machado & Matos, 2003; Palombo, 2011).
Among the natural products that have been gaining ground in recent years, the Essential Oils (EOs) stand out, which, despite their apparent novelty, have been used as curative therapeutic methods, in well-being care and also in food preparation since ancient times. Today, approximately 3000 OEs extracted from leaves, legumes, and grains are known. Naturally, these aromatic compounds have as their main function the protection of the plant, in addition to pollination, attracting animals (Ferraz, 2015). From a pharmacological point of view, many of them have antibacterial, antifungal, and antiviral activity. (Rehman, Ahmad, Kazmi & Raza, 2007; Guleria, Tiku, Koul, Gupta, Singh & Razdan, 2013).

EOs are natural, volatile compounds, characterized in that they are obtained from plants by different methods, such as hydrodistillation, steam distillation, or through pressing, in the case of citrus oils, which allows for a wide variety of uses including therapeutic benefits. It’s incredible chemical complexity began to be unveiled in recent decades, due to the substantial advance in laboratory techniques for identifying substances, which allowed the identification of chemical compounds present in EOs, for example, thymol, carvacrol, and monoterpen alcohol, which have a strong anti-inflammatory action, antimicrobial and analgesic (Rocha, 2013; Ferraz, 2015).

In Brazil, a country with an intensely diversified flora, many OEs can be extracted, including thyme, oregano, melaleuca, frankincense, and copaiba OEs. Thyme essential oil is obtained from the leaf of the *Thymus linearis* Benth plant and has an antimicrobial and antiseptic function (Rocha, 2013). As it is composed of thymol, a substance that confers antifungal, bactericidal, and anthelmintic activity, this oil has proven activity as a broad-spectrum anti-infective agent (Grossman, 2005). The essential oil of oregano, which is extracted from the leaves of the herb *Origanum vulgare* L., has analgesic, anti-inflammatory, anti-infective and antiseptic action (Axe, 2021) mainly due to monoterpen alcohols and phenols (Grossman, 2005).

The *Melaleuca alternifolia* plant originates Tea Tree Oil, or Melaleuca Oil, which, mainly composed of monoterpen alcohol, has high medicinal power with antibacterial, antifungal, antiviral, anti-inflammatory and analgesic action (Ferraz, 2015). Known a "blood cleanser", frankincense essential oil, extracted from the resin of the trunk of the tree of the genus *Boswellia carterii*, has an anti-inflammatory, antimicrobial, antioxidant function, in addition to being widely used in religious and spiritual environments (Xiao, Suhail, Yang, Cao, Fung, Postier, Woolley, Young, Zhang & Lin, 2012). Another EO is white copaiba that is extracted from the resin of the trunk of the *Copaifera Officinallis*...
tree, native to the tropical region. It is an oil widely used in the skin region because it has anti-inflammatory, analgesic, and healing functions (Pieri, Mussi & Moreira, 2009).

Interestingly, despite the medicinal diversity of action of these oils, little is known about their applicability for Dental purposes. A few studies report the use of white copaiba oil as a substitute for eugenol for the formulation of endodontic types of cement for root canal fillings (Pieri, Mussi & Moreira, 2009). Other studies have investigated the action of different OEs against different oral microorganisms, however, with controversial results (Tampieri, Galuppi, Macchioni, Carelle, Falconi, Cioni & Morelli, 2005; Silva, Silva, Higino, Pereira & Carvalho, 2007; Silva, Guterres, Weisheimer & Schapoval, 2008). In fact, there are numerous oral microorganisms that cause different pathologies, perhaps because of this the difficulty in establishing the real effectiveness of each EO on oral bacteria. Oral bacteria such as Fusobacterium, Streptococcus, Prevotella, Eubacterium, Actinomyces, Campylobacter, Propionibacterium, Porphyromonas and Peptostreptococcus are responsible for most cases of oral pathologies (Tampieri, Galuppi, Macchioni, Carelle, Falconi, Cioni & Morelli, 2005; 2002) but, among the bacteria mentioned, the facultative anaerobic bacterium Enterococcus faecalis is perhaps the most resistant against antibiotics (Molander, 1998). Given the above, the aim of this study was to evaluate the role of EOs of thyme, oregano, copaiba, melaleuca and frankincense in inhibiting the growth of Enterococcus faecalis bacteria.

2 METHODOLOGY

2.2 ESSENTIAL OILS SELECTION AND PREPARATION

For this study, five commercially available OEs were acquired from the natural cosmetics store in Guarapuava, Paraná, Brazil, and the scientific names of each oil were obtained from the Integrated Taxonomic Information System (http://itis.gov). The oils are i. Thyme Oil (TO) (Thymus linearis Benth), ii. Oregano Oil (OO) (Origanum vulgare L.), iii. Copaiba Oil (CO) (Copaifera Officinalis), iv. Tea tree oil (TTO) (Melaleuca alternifólia) e v. Frankincense Oil (FO) (Boswellia carterii). Prior to the beginning of the experiment, the EOs were subjected to dilutions in an extender vehicle (grapeseed oil) in order to test them at different concentrations: 25% (1.75ml of the extender to 0.25ml of the test oil), 50% (1ml of extender for 1ml of test oil), 75% (0.5ml of extender for 1.5ml of test oil) and 100% (pure test oil). All solutions obtained were homogenized in vortex (Phoenix Lab) and placed in Eppendorf flasks that were kept in an aseptic place.
until the moment of use. For positive control, 0.12% chlorhexidine gluconate solution (CLX) was used.

2.2 AGAR DIFFUSION TEST

For the agar diffusion test, triplicate tests were performed for each oil. Fifteen Petri dishes were used in which 20 mL of Müller-Hinton agar culture medium were dispensed. Into this medium, strains of Enterococcus faecalis (ATCC29212) were obtained from the collection of the Laboratory of Microbiology at Universidade Positivo (Curitiba, Paraná) were inoculated. Colonies of bacteria already grown in growth medium plates were transferred to Petri dishes with the aid of a seeding loop into test tubes with 5ml of sterile saline solution, which were vortexed (Phoenix Lab) until the homogeneity of the medium. The inoculated solution was adjusted to a turbidity reading according to a 0.5 McFarland scale, which corresponds to a bacterial concentration of approximately 1.5 x 10⁸ CFU/mL.

A sterile Swab was immersed in the standardized solution and seeding was performed in streaks until the entire surface of the Müller-Hinton agar was covered. Next, paper discs made with filter paper were placed on the culture medium. Eighty-five discs with 5 mm in diameter, 05 for each plate, were sterilized and soaked for 60 seconds in the EOs described above. The discs were carefully placed on the culture medium with the help of forceps, equidistantly as determined on the rear face of the Petri dish and under aseptic conditions. The distance between each disc was standardized at 2.5 cm. As a positive control, a paper disk immersed in 0.12% chlorhexidine was used.

After sowing and placing the discs, the inoculated plates were sealed with 3M transparent adhesive tape and kept at room temperature for 30 minutes, to allow diffusion of substances before microbial development. Then, the plates were incubated in an oven at 37º C for 48 hours. At the end of this period, the diameter of the bacterial growth inhibition halos around the paper disks promoted by the tested oil was measured and recorded in millimeters by means of a transparent millimeter ruler. For this purpose, two measurements perpendicular to each other were used, and the average of their sizes was obtained. The measurements were performed in triplicate by three previously trained researchers (B.M.B.S., F.A, and M.M.J.).
3 RESULTS

The results obtained in this study are shown in table 1 and figure 1. According to the analysis performed, the EOs that showed greater growth inhibition of Enterococcus faecalis were, in descending order: thyme oil (5.0 mm), oil of tea tree (4.70 mm) and oregano oil (3.75 mm) at 100% concentration, with results similar to 0.12% chlorhexidine, whose inhibition halo was 3 mm. Copaiba and frankincense oils were not efficient to inhibit bacterial growth at concentrations of 25, 50 and 75%.

Table 1 – Description of the results obtained for each oil tested. The images show the halo of inhibition produced by each oil.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Thyme Oil (TO) Inhibition Halo mm</th>
<th>Oregano Oil (OO) Inhibition Halo mm</th>
<th>Copaiba Oil (CO) Inhibition Halo mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>25%</td>
<td>0.60</td>
<td>25%</td>
<td>25%</td>
</tr>
<tr>
<td>50%</td>
<td>2.80</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>75%</td>
<td>2.60</td>
<td>75%</td>
<td>75%</td>
</tr>
<tr>
<td>100%</td>
<td>5.00</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 1 – Description of the results obtained for each oil tested. The images show the halo of inhibition produced by each oil.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Tea tree oil (TTO) Inhibition Halo mm</th>
<th>Frankincense Oil (FO) Inhibition Halo mm</th>
<th>Chlorhexidine Oil (CO) Inhibition Halo mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>25%</td>
<td>0.00</td>
<td>25%</td>
<td>0.12%</td>
</tr>
<tr>
<td>50%</td>
<td>0.80</td>
<td>50%</td>
<td>0.00</td>
</tr>
<tr>
<td>75%</td>
<td>1.40</td>
<td>75%</td>
<td>0.00</td>
</tr>
<tr>
<td>100%</td>
<td>4.70</td>
<td>100%</td>
<td>3.00</td>
</tr>
</tbody>
</table>

Note: symbol means there was no inhibition.
Source: Authors

It is interesting to mention that the oils of copaiba and frankincense only showed some inhibition when they were used pure, that is, in a concentration of 100%. Oregano oil, on the other hand, at lower concentrations (25 and 50%) showed a very high efficiency in inhibiting bacterial growth, therefore, due to the surface tension of oils in general, this oil is perhaps the most suitable for use.
Figure 1 – Graph representing the inhibitory capacity of each EO in millimeters, according to their respective concentrations

4 DISCUSSION

Essential oils are metabolites produced by roots, trunks, leaves and other parts of plants and have specific functions such as protecting plants against insects, fungi, bacteria and other organisms (Bakkali, Averbeck, Averbeck & Idaomar, 2008). This broad spectrum of activities against different microorganisms is due to the fact that EOs have numerous chemical compounds, including phenols, esters, and aldehydes that, alone or together, have strong biocidal activity (Burt, 2004). Due to these properties, pharmaceutical interest in EOs has increased substantially in recent decades, whether in the medical field, for the production of medicines or in the manufacture of cosmetic chemical solvents (Silva, Abebe, Souza, Duarte, Machado & Matos, 2003; Williams & Barry, 2004; Adorjan & Buchbaue, 2010).

Research involving the application of OEs in Dentistry has also been gaining ground and the results are very promising (Palombo, 2011; Sampaio, Oliveira & Oliveira Filho, 2021). In this sense, this laboratory study used the oil diffusion in agar technique to evaluate the efficiency of five essential oils: Thyme, Oregano, Copaiba, Tea Tree and Frankincense, in inhibiting the growth of bacteria Enterococcus faecalis and the results obtained allow us to make some considerations about the efficiency of the tested oils. The first consideration concerns the microorganism chosen for this study, the Enterococcus faecalis bacteria. This bacterium is classified as a facultative anaerobe, very resistant to antibiotics and with a very large capacity to penetrate and survive as a monoculture in oral microenvironments such as root canals and dentinal tubules, even when deprived of
small nutrients (Molander, 1998; Casella, Leonardi; Melai, Fratini & Pisteli, 2013), therefore, this bacterium was chosen for the tests performed in this research.

The second point that needs to be explained and discussed is the reason for choosing the tested EOs. All of them have, to a greater or lesser degree, antimicrobial and anti-inflammatory activity (Rocha, 2013; Grossman, 2005; Pieri, Mussi & Moreira, 2009; Xiao, Suhail, Yang, Cao, Fung, Postier, Woolley, Young, Zhang & Lin, 2012; Ferraz, 2015; Axe, 2021), two essential characteristics for the treatment of oral diseases. In this study, three oils, Thyme, Tea tree and Oregano, when used without any dilution, that is, pure, demonstrated to inhibit bacterial growth in a similar way to Chlorhexidine, a compound used as the gold standard in the disinfection of the oral cavity. Additionally, the oil of oregano also showed great efficiency at concentrations of 25 and 50%.

In common, Thyme and Oregano oils have thymol as one of the main components. Thymol is a hydrophobic molecule, with alkaline characteristics, which has a high affinity with the non-polar portion of lipids present in bacterial cell membranes, breaking the stability of the cytoplasmic membrane by reducing the membrane potential. This event would cause the extravasation of intracellular proteins and consequent bacterial death. Furthermore, thymol decisively affects the pumping of protons through bacterial ion channels, significantly decreasing the mitochondrial activity of bacteria, thus causing a blockage in the synthesis of ATP, that is, it blocks the mitochondrial activity of bacteria, thus exhibiting a bacteriostatic effect (Sakkas & Papapodoulou, 2017; Tariq, Wani, Rasool, Shafi, Bhat, Prabhakar, Shalla & Rather, 2019). Other compounds present in these oils that have biocidal activity are carvacrol and eugenol. Carvacrol is a thymol isomer that has been shown to be a very promising molecule in the treatment of fungal and bacterial infections (Bakkali, Averbeck, Averbeck & Idaomar, 2008; Veras, Rodrigues, Botelho, Menezes, Coutinho & Costa, 2013; Özkan & Erdoğan, 2011). Eugenol, a derivative of guaiacol, is perhaps one of the chemical compounds most used in dentistry as it is active against oral bacteria that cause caries and periodontal disease (Oliveira, Gobira, Guimarães, Bastista, Barreto & Souza, 2007; Cai & Wu, 1996). Both exert a strong action on the bacterial cell membrane, breaking it.

The main constituents of Tea tree oil are terpinen-4-ol, 1,8-cineol, α-terpinene, γ-terpinene, α-pinene, β-pinene, α-terpineol, ρ-cimene and sesquiterpene alcohols, which represent approximately 90% of the oil (Brophy, Vavies, Southwell, Stiff & Williams, 1989). The action of these compounds has already been evaluated in different bacteria, including E. coli, Salmonella typhimurium and Pseudomonas, and their mechanism of
action involves the inhibition of bacterial biofilm formation, alteration of permeability and cell membrane morphology (Tariq, Wani, Rasool, Shafi, Bhat, Prabhakar, Shalla & Rather, 2019). This same oil had already been tested by Sinha, Vasudeva, Jaiswal, Garg, Tyagi & Singh (2015) and had its efficiency proven against *Enterococcus faecalis*. These same authors suggested that the plant extract of this oil could be an alternative to replacing sodium hypochlorite as an irrigation solution for the root canal system, however they emphasized that additional studies would need to be carried out to test this oil in clinical and pre-clinical trials for other parameters such as toxicity and inhibition of biofilm formation.

Interestingly, Tea tree oil has already been tested against *Candida albicans* fungus, a pathogen that causes fungal infections quite frequently in the oral cavity. Hammer, Carson & Riley (2004) demonstrated that the compounds present in this oil caused important morphological changes in the cell membrane of *Candida albicans*, results similar to those reported by Tariq, Wani, Rasool, Shafi, Bhat, Prabhakar, Shalla & Rather (2019) but which were obtained from the analysis of the morphology of *E. coli* bacteria, *Salmonella typhimurium* and *Pseudomonas*. The other two oils tested, Copaiba oil and Frankincense oil, under the conditions designed for the experiment carried out in this study, were not efficient in inhibiting the growth of the bacteria. However, these two oils have already shown efficiency against fungi and bacteria in previous studies (Diefenbach, Muniz, Oballe & Rösing, 2018). A recently published study on frankincense oil revealed a possible mechanism for inhibiting the growth of SARS-CoV-2 by alpha-Boswellic and beta-Boswellic acids, the two main components of the *Boswellia carterii* plant (Kadhim, Salman, Zarzoor & Kadhum, 2021).

Finally, despite the limitations of this study, the data collected allow us to conclude that thyme, tea tree and oregano oils, as they inhibited the growth of an extremely resistant bacteria, are promising in the treatment of oral diseases and other studies deserve to be carried out for test the toxicity of these oils in models involving cell culture. The next step would be the investigation of their clinical efficiency.

**5 CONCLUSION**

The results obtained in this research demonstrated that thyme, tea tree and oregano oils inhibited the growth of *Enterococcus faecalis* bacteria.
REFERENCES


