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Original Article

Chemical composition, antimicrobial and antifungal activity of *Lippia Thymoide* essential oil in oral pathogens

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Aim: This study evaluated the chemical composition of *Lippia thymoides* (*Lt*) essential oil and its antimicrobial activity against fungal strains of *Candida albicans* (*Ca*) and Gram-negative bacteria *Prevotella intermedia* (*Pi*) and *Fusobacterium nucleatum* (*Fn*). **Methods:** *Lt* essential oil was obtained by hydrodistillation apparatus with a modified Clevenger extension. The chemical analysis was analyzed by gas phase chromatography and mass spectrometry on Shimadzu QP 2010 plus. Sample sensitivity evaluation was performed by ABHb-inoculum and culture plates were developed with triphenyltetrazolium chloride, also *Fn* and *Pi* samples analysis were in anaerobic environment and *Ca* sample analysis was performed in aerobic environment. The minimum inhibitory concentration (CIM) was determinated by microdilution in eppendorfs tubes. **Results:** The chemical analysis showed that Thymol (59,91%) is the main compound found in *Lt* essential oil, also other antifungal and antimicrobial agents were present γ-terpinene (8.16%), p-cymene (7.29%) and β-caryophyllene (4.49%), Thymol is a central ingredient of many medicinal plants and has a potent fungicidal, bactericidal and antioxidant activity, it has been previously shown to have anti-inflammatory activity against Periodontal Disease (PD) cause can reduces prostanoids, interleukins, leukotrienes levels in periodontium. CIM result *Pi* was 6.5 µg/mL, *Fn* was 1.5 µg/mL and *Ca* was 0.19 µg/mL. **Conclusion:** The antimicrobial activity of *L. thymoides*, through the compound Thymol, has been shown promising potential against gram-negative periodontopathogenic bacteria and fungi whose therapeutic arsenal is still very restricted.

Keywords: Microbiota. Plant extracts. Oils, volatile. *Lippia*. Antifungal agents. Periodontitis.

Introduction

It is well documented that oral microbiome is formed about 700 different microbial species and these interactions might result in a distinct environmental and microbial communities, among the microorganisms we cite bacterias, fungus and viruses, which might coexist in specific and formed organization in oral cavity habitats[1-5]. Which one of these species can colonize a different sub-habitat in our mouth, this discrepant preferences due to anatomy of the region, nutrients sources, oxygen availability, pH range and host immune cells activity, among the colonized sub-habitats we mention: tongue surface and/or dorsum, keratinized and non-keratinized mucosa, cheek, periodontium (supra and subgingival) and teeth[5-7].

In most cases, the organisms presented in microbial communities live in oral cavity normally, harmless and helpful, but under certain conditions such as[8,9]: inadequate oral hygiene, host immunosuppression, risk factors, oral dysbiosis and presence of pathogenic organisms may lead patient to infectious diseases caused by the organisms cited above and the three main oral diseases are: caries, periodontal diseases (PD) and oral candidiasis[10-12]. Oral and periodontal medicine are two dentistry fields, where oral pathologies like PD and oral candidiasis are treated through reducing dental plaque levels, oral hygiene improvement, host immune balance, decrease of pathogenic organisms and risk factors[13,14].

PD is a worldwide oral health problem; it is a multifactorial and poly-microbial disease with host-specificity. PD can be classified mainly in gingivitis and periodontitis, the diagnosis is based upon disease's severity stage and grade, clinical attachment loss, periodontal probe depth, bleeding and/or suppuration on probing, alveolar bone resorption and eventual tooth loss[15,16]. PD's etiopathogenesis is inflammatory and it is resulted to complexes interactions between dental plaque accumulation based on the specific plaque hypothesis and according to this theory the oral microbiome dysbiosis and polymicrobial synergy is caused by specific gram-negative bacterias like *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Treponema denticola*, *Tannerella forsythia*, *Prevotella intermedia* (*Pi*) and *Fusobacterium nucleatum* (*Fn*) and associated with host-immune response decrease, systemic and local risk factors such: HIV, tobacco, diabetes, dental braces and even Alzheimer's disease[17,18].

As the third most common oral disease, Oral candidiasis is clinic diagnosed by the presence of a superficial inflammation on the oral mucosa due to the overgrowth of fungal agents such as *Candida albicans* (*Ca*), *C. tropicalis*, *C. glabrata* and *C. parapsilosis*[4,11].Oral candidiasis clinical presentations include white plaques on tongue, mouth pain and burning, fungal organisms like *Ca* can develop in certain condition and might begin due to systemic or local impairments such as host immune response, organ transplantation, HIV infection, chemotherapy, radiotherapy, elder age, oral prosthesis, poor oral hygiene, tobacco and alcohol use, hyposalivation and sometimes antibiotic drug use[12,19].

PD is an infectious inflammatory disease manifested and aggravated by a subgingival bacterial dysbiosis in dental plaque accumulation[14,15]. PD's treatment, like

gingivitis and early stages of periodontitis, basically consists in supra/ subgingival dental plaque accumulation reduction through non-surgical and mechanical removal known as scaling and root planning (SRP), SRP is well documented as the standard periodontal treatment, although its clinical benefits SRP alone does not always induce in periodontal ecological modification necessary to accomplish and sustain clinical improvements[17]. Therefore, periodontal adjunctive therapies such as systemic antimicrobials and specific mouthwashes have been used to improve clinical and microbiological status and features specially in necrotizing periodontal diseases and severe periodontitis which presents severe clinical conditions and a subgingival microbial ecology bacterias from Socransky's and orange complex[18,20].

As mentioned above, mechanical therapy as SRP besides periodontitis advanced stage and aggressive subgingival bacterias, other limitations might decrease SRP effectiveness, such factors as deeper depths of the periodontal pocket, root anatomy, periodontal instrument design, and operator ability may influence negatively in PD's treatment[20]. Mouthwashes just as chlorhexidine gluconate, sodium fluoride, zinc chloride and saline solution may act as a periodontal adjunctive therapy to control and sometimes even to prevent infections to spread systemically[21,22]. Mouthwashes like chlorhexidine due to great activity against a variety of Gram-positive and Gram-negative bacterias can be used by dental surgeons during SRP sessions to treat severe PD, despite chlorhexidine well-documented and guaranteed effectiveness few complications might appeared during periodontal treatment as chlorhexidine cationic properties, bacterial resistance and strong reactivity[21,22].

Systemic antimicrobial therapy is also used as periodontal adjunctive therapy, antibiotics like metronidazole can be used locally or systemically to treat infections such necrotizing periodontal diseases and severe periodontitis and its effectiveness is influenced by the agent and protocol used[20,23]. According to Teughels et al. (2020)[23] antibiotics associations like amoxicillin and metronidazole has been showed an effective effect in periodontitis therapy. However, despite its positive clinical effects, the enormous and inaccurate use of systemic antimicrobials contributes to bacteria specific-drug-resistant and multidrug resistant and still according to Teughels et al. (2020)[23] periodontopathogens specific-drug-resistant is more frequent due to populations who are more exposure to systemic antimicrobials, like Brazilian population[20,22].

Regarding oral candidiasis treatment when it is caused by *Ca*, it can be carried out through the association between oral hygiene improvement with the use of azole drugs, nystatin and polyenic antibiotics[8,9]. Nystatin is cited as the gold-standard to treat oral candidiasis, nystatin is a polyene antifungal agent and it is widely used due to its effective influence and wide spectrum of pathogenic fungi, especially against *Ca*[21]. Regarding nystatin although is a safe drug, because it is not easily absorbed through skin or mucosae, low toxicity and no reported drug interactions some effects of oral nystatin must be considered such as high risk of developing caries and resistance to polyene antifungals which will decrease oral candidiasis treatment[19].

Medicinal plants (MP) or essential oils (EO) usage as treatment or adjunctive therapy for various pathologies has been a practice since antiquity folk medicine,

although MP and EO have been used for several years by ancient societies[24-28]. In Brazil the scientific and commercial interesting upon natural substances like EO increased in recent years, so naturally the number of studies trying to understand EO medicinal and dental properties such as analgesic, anti-inflammatory, antimicrobial and/or antifungal effects enlarged[24-28]. In dental medicine, oral hygiene products based on EO have been used and one of the substances mainly used is thymol, it has been shown that thymol is an alternative mouthwash due to its potential anti-inflammatory, fungicidal and bactericidal action may serve as medical coadjutant against PD and oral candidiasis[27,28]. The objective of this study was to evaluate the chemical composition of *Lippia thymoides* (*Lt*) essential oil and its antimicrobial and antifungal activities against the fungal strains of *Candida albicans* (*Ca*) and the Gram-negative bacterias *Prevotella intermedia* (*Pi*) and *Fusobacterium nucleatum* (*Fn*).

Materials and Methods

Plant material

Lippia thymoides was collected from Abaetetuba, Brazilian state of Pará, Amazon region, always at the same horary and geographical location 1° 46'15.9 "south latitude and 48° 47'02.2" west longitude, at the end of the first month of each season: April 2017 (autumn), July 2017 (winter) and October 2017 (spring).

Voucher specimens were deposited under the number 213373 at the Herbarium of the Museu Paraense Emílio Goeldi, in Belém, Brazilian state of Pará, Amazon region. The specimen was identified and categorized by a single calibrated botanical researcher, who had previously experience in botanical classification and taxonomy[29,30]. Leaves of a pool of individuals were separated from the stem and air-dried at room temperature, protected from light, until constant weight, and powdered in the cutting mill.

The determination of the residual humidity was carried out in a humidity-determining balance of Marte®, model ID 50, with infrared at the time of extraction[30].

Essential oils extraction

The botanical material was collected manually at 6 o'clock in the morning, transported immediately to the laboratory, then placed on trays, in a greenhouse at 34ºC, with ventilation, for drying for three days, after being crushed in a botanical processor[30,31].

The extraction of the essential oil from the dry leaves of *Lt* was made by hydrodistillation using a modified Clevenger type glass system coupled to a cooling system to maintain the water condensation at 10-15ºC for 3 hours. After extraction the oils were centrifuged for 5 min at 3000 rpm, dehydrated with anhydrous Na_2SO_4 and again centrifuged under the same conditions. The oils were stored in amber glass vials, flame-sealed and conditioned in a refrigerated environment at 5ºC at a concentration of 31.25 µM to immediately use after collection[32,33]. The mass yield in % of the essential oil was on a dry basis according to the equation in Figure 1.

$$T = \frac{V_{oil}}{M_{sample} \; X \; \left(\frac{100-U}{100}\right)}$$

Figure 1. Equation used to determine the mass yield in % of the essential oil on a dry basis.

Chemical analysis

The chemical composition of the essential oils was analyzed by Gas Phase Chromatography and Mass Spectrometry (GC-MS) on Shimadzu QP 2010 plus, self-injector (FIGURE 2): AOC-20i equipped with Rtx-5MS silica capillary column (30m x 0,25 mm; 0,25 mm film thickness) under the following operating conditions: temperature program: 60ºC - 240ºC, with a gradient of 3ºC/min; Injector temperature was 250ºC; the drag gas was helium (linear velocity of 32 cm/s, measured at 100ºC); without flow division (0.1 mL of a 2: 1000 of n-hexane); temperature of the ions source and other parts was 200ºC. The quadrupole filter was used for scanning in the range of 39 to 500 Daltons every second. Ionization was obtained by the electronic impact technique at 70 eV[34-36].

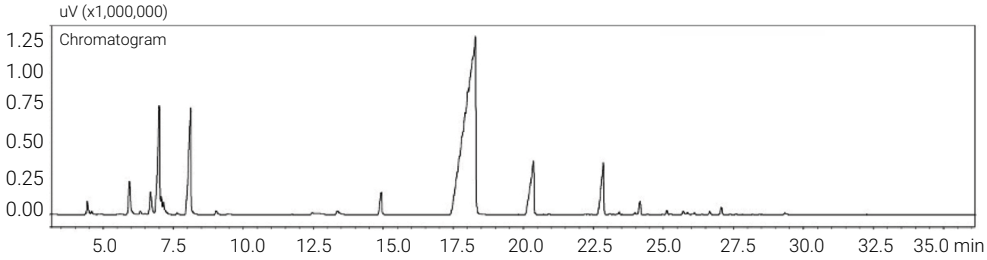

Figure 2. *L. thymoides* chromatographic analysis.

The identification of the volatile components was based on the linear retention index, which was calculated in relation to the retention time of a homologous series of n-alkanes and on the fragmentation, pattern observed in the mass spectra, by comparing them with authentic samples in the libraries the data system and the literature[34-36].

Microbial species and culture conditions

The evaluation of antimicrobial and antifungal activities of *Lt* essential oil against *Fn*, *Pi* and *Ca* strains of were performed by the microdilution method that is based on successive dilutions of the sample in culture containing blood.

The microbiological assays were used with 5 strains of *Ca*-ATCC 90028; 5 strains of *Pi*-ATCC 49046 and 5 strains of *Fn*-ATCC 25586 purchased from the Osvaldo Cruz Foundation (FIOCRUZ), Brazil. Each inoculum was made and developed inde-

pendently according the needs of each microorganism, the bacterial inoculums were obtained from a fresh culture suspension (maximum 48h at 72h) of *Fn* and *Pi*[37]. The fungal inoculum was obtained from a a fresh culture suspension (maximum 24h) of *Ca* in 0.85% saline solution (m/V). The concentration of the microorganism was standardized by comparing turbidity of the inoculum with the MacFarland scale equivalent to a 1.5x108 CFU/mL concentration in a turbidimeter (Grant-Bio-Den-1, model V.1GW)[38].

For the antibiotic control, vancomycin (sigma - Aldrich®) was used as a positive control at the concentration of 16 µg/mL; as negative control the microorganism culture was evaluated in a sterile medium area. The culture media to be used for analysis were the Agar, the Brain and Heart broth (ABHb), respectively, containing polysorbate 80 at 0.5% (m/V) in sheep blood, which will allow bacterial and fungal by growth decreasing interfacial tension neutralizing any disinfectants agents in the agar and defibrinated was also used[39].

As fungal control, nystatin (Nystatin®) N6261- SIGMA-ALDRICHT was used as a positive control at (1 mg/mL) concentration; as negative control the microorganism culture was evaluated in a sterile medium area. The culture media to be used for analysis were the ABHb, containing polysorbate 80 at 0.5% (m/V) and defibrinated sheep blood[40].

Sample sensitivity evaluation

To prepare the plates, 11 ml of ABHb were poured into 15x100 mm plates. On this layer of solidified medium was added 10 mL of ABHb-inoculum containing 106 CFU/mL.

With the two layers uniformly superimposed and already solidified, filter paper was added with approximately 6 and 8 mm diameters and impregnated with 10 µl of essential oil. The samples were placed on the culture and incubated at 35°C / 24h for *Fn* and *Pi*; the *Ca* culture was incubated at 25°C/24h. *Fn* and *Pi* samples analysis were in anaerobic environment (N_2: 80%, H_2: 10%, CO_2: 10%) and *Ca* sample analysis was performed in aerobic environment[22].

After the incubation period, both *Ca*, *Fn* and *Pi* culture plates were developed with triphenyltetrazolium chloride (In the fungal culture plate, it was added to confirm that it was only colonized by *Ca* and in the bacterial culture plate it was added to improve the determination of minimum inhibitory concentrations at 7 mg/ml in bacteriological agar at 1% (m/v) and the results of the halos (mm) were measured using a pachymeter[37,38].

Determination of minimum inhibitory concentrations

The essential oil minimum inhibitory concentrations (MIC) was performed in eppendorfs tubes by microdilution, where a 50 µL aliquot of the essential oil was diluted 1:2 in ABHb with 5% (V/V) and polysorbate 80 defibrinated blood containing 105 CFU/mL to 20 dilutions. Then, as cultures, those aliquots were incubated in triplicate (to increase positive results and prevent experiments failures), *Fn* and *Pi* bacterial strains aliquots were grown in tryptic soy broth and supplemented with hemin (5 mg/ml) and menadione (0.5 mg/ml) 35°C/ 24 hours in anaerobic environment

(N_2: 80%, H_2: 10%, CO_2: 10%)[37]. *Ca* aliquots was performed in a laboratory greenhouse to allow better fungal growth, it contained 105 CFU/mL up to 15 dilutions and incubated at a temperature of 25°C/ 24 hours[38].

After the incubation period the plates were seeded with 40µl of the culture of each dilution in petri dishes (5x50 mm) containing 5% (V/V) defibrinated blood soybean casein agar and then incubated for further 35°C/24h for *Fn* and *Pi*, for *Ca* agar incubated for further 25°C/ 24 hours. The plaques were evaluated for the presence or absence of bacterial and fungal growth, when compared to the negative and positive control groups. MIC were revealed at the last dilution where there was no microbial growth

After incubation, the plates were developed with 1% (m/v) bacteriological broth containing 7mg/ml of triphenyltetrazolium solution. The maintenance of the red color in the medium was interpreted as presence of microbial growth.

Results

Chemical components

The yield of *Lt* oil obtained by hydrodistillation was 0.7%. The chemical composition was determined by GC-MS, where thirty-five volatile constituents were identified, representing 96.7% of the total compounds present in the oil. The main constituents found in the essential oil were Thymol (59,91%) (FIGURE 3); γ-terpinene (8,16%); p-cymene (7, 29%); Thymol acetate (6, 26%) and β-caryophyllene (4, 49%) (Table 1).

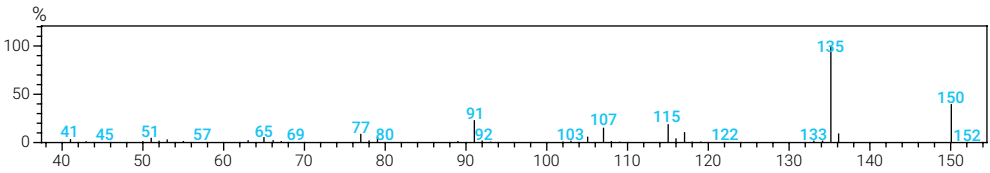

Figure 3. Thymol mass spectrum in *L. thymoides* chromatogram.

Table 1. Chemical composition of *Lippia thymoides* essential oil.

Compounds	%
α-thujene	0.74
α-pinene	0.20
β-pinene	0.09
Myrcene	2.06
α-phellandrene	0.23
δ-3-carene	0.12
α-terpinene	1.58
p-**cymene**	**7.29**
(E)-β-ocimene	0.14

Continue

Continuation	
γ-terpinene	**8.16**
terpinolene	0.30
linalool	0.13
Camphor	0.04
umbellulone	0.21
terpinen-4-ol	0.34
methylthymol	1.43
thymol	**59.91**
thymol acetate	**6.26**
α – copaene	0.02
methyl eugenol	0.05
β-caryophyllene	**4.49**
β-copaene	0.11
trans-α-bergamotene	0.14
γ-elemene	0.14
α- humulene	0.75
allo-aromadendrene	0.06
γ-muurolene	0.24
germacrene D	0.26
γ-amorphene	0.18
δ-amorphene	0.16
trans-β-guiaene	0.05
γ-cadinene	0.20
δ-cadinene	0.42
elemol	0.04
caryophyllene oxide	0.12
Total	**96.7**

[%]percentage of each compound found in the GC-MS

Table 2. Inhibition zone diameter, antimicrobial and antifungal activities of *Lippia thymoides* essential oil.

SAMPLES	*F. nucleatum*	*P. intermedia*	*C. albicans*
Halo (mm)	15	19	38
MIC (μL mL^{-1})	1.6	6.5	0.19
MBC (μL mL^{-1})	2.60	2.44	-
MFC (μL mL^{-1})	-	-	1.30
[1]VCM (μg mL^{-1})	34	28	-
[2]NYS (μg mL^{-1})	-	-	32

[1]Vancomycin; [2]Nystatin; minimum bacterial concentration (MBC) and minimum fungicidal concentration (MFC).

Antimicrobial and antifungal activities

The results showed that *Lt* had a satisfactory bactericidal and antifungal against the 15 strains of *Ca*-ATCC 90028, *Pi*-ATCC 49046 and *Fn*-ATCC 25586 with EO 2% concentration. The inhibition halos were measured and resulted in 15mm (*Fn*), 19mm (*Pi*) and 38mm (*Ca*). As bacterial positive control vancomycin was used and as fungicidal positive control nystatin was used and the respective MIC values were 6.5 µg/mL (*Pi*), 1.5 µg/mL (*Fn*) and 0.19 µg/mL (*Ca*) and the minimum bacterial concentration (MBC) were 2.60 µg/mL (*Pi*) and 2.44 µg/mL (*Fn*) and minimum fungicidal concentration (MFC) was 1.30 µg/mL (*Ca*) and the peak of bactericidal and antifungal action could be observed after 2 hours and maintain during 4 hours.

Discussion

PD and oral candidiasis are two of the three most common oral pathologies in the world, as the aforementioned this pathology is the result of an interaction between microorganisms and an accumulation of dental biofilm and subgingival surface; and non-surgical periodontal mechanical therapy associated with systemic or local anaerobicides and antifungal has been the standard treatment established in the literature for these pathologies[20-22,41,42].

However, this standard protocol is not always effective due to variations of occurrences, being more common cases of bacterial and fungal resistance due to the unreasonable and unrestrained prescription use of antibiotics and fungicides, which leads the periodontist to use more potent antibiotics with bigger adverse effects. In an attempt to resolve such complications and promote more effective and less harmful treatment, different therapeutic alternatives may be tested such as the herbal medicine[25-27,43-45].

The use of phytotherapy is a part of popular medicine, mainly in the Amazon region (northern Brazil). Several studies have shown that medicinal plants sources are abundant in active biological compounds[25-27]. In recent years, the number of studies about medicinal plants has increased and through the results of these studies, we verified that medicinal plants have anti-inflammatory, antimicrobial, fungicidal and adverse effects on its extracts and essential oil which shows the great potential for several therapeutic applications[43-47].

Studies such as Warad et al.[25] (2013) and Mandras et al.[27] (2016) reported excellent therapeutic properties of essential oils in a variety of aerobic, anaerobic and fungal microorganisms. In our study we used *Lt* as the herbal medicine of choice; *Lt* is a native species of Brazilian vegetation, popularly used to treat various pathologies. Thus, due to its ethno-pharmacological potential and innovative; our research was dedicated to studying its chemical composition and the antimicrobial and antifungal properties of the essential oil of this species, looking for a possible auxiliary agent in the periodontal treatment[48-50].

In the present study, we demonstrated that the essential oils extracted from *Lt* maintain an antimicrobial activity against two of the main gram-negative periodontal pathogens, such as *Fn* and *Pi*[47,50]. In addition, it presented satisfactory activity against *Ca*

strain, which we can observe to be the most susceptible strain at MIC <0.19 µL/mL. In contrast, *Pi* was the most resistant strain, with MIC> 6.5 µL/mL. According to Botelho et al.[43] (2016) the use of oral antiseptic containing essential oils associated with non-surgical periodontal therapy can significantly inhibit the levels of *Fn* and *Pi* in the supragingival and subgingival biofilm.

In the study of Sharifzadeh et al.[46] (2018), the authors confirmed in their in vitro study that Thymol has a highly fungicidal action. In their study the authors used different concentrations of Thymol in samples of seropositive patients with candidiasis in the most diverse forms, the authors had the same conclusions that our study obtained that Thymol is a promising fungicide against *Ca*. In relation to the antimicrobial activity, *Lt* extract showed to be sensitive against both bacteria, being more effective against *Fn* corroborating with the results of Arbia et al.[47] (2017). The results of this study are considered promising and innovative because Juiz et al.[45] (2015) analyzed the effects of *Lippia alba* on *Fn* and found that this essential oil had no effect on its samples.

In our study, when we analyze *Lt* extracted essential oil, Thymol (59%) was presented as the main chemical constituent. In the literature Thymol it is already well documented as a viable antifungal agent for oral candidiasis, according with Ahmad et al.[48] (2013) Thymol has amphipathic characteristics which in oral *Ca* can affect the cell membrane structure and its electrostatic surface generating asymmetric tension in the membrane then its rupture, furthermore Thymol might also interfere in mitochondria activities and in the production of viable filamentous forms that are essential to *C. albicans* during oral biofilm formation[28,48-53].

Regarding using Thymol as an alternative to periodontal disease, in the last few years this compound has been used commercially in mouthwashes due to its antimicrobial activity. Since 1879, LISTERINE® is mouthwash used in dentistry as a surgical disinfectant and one of its ingredients is Thymol. Sköld et al.[50] (1998), Antunes et al.[51] (2015) e Vlachojannis et al.[28] (2016) demonstrated Thymol's antimicrobial activity efficiency against aerobic bacterias, such as *Streptococcus mutans*, and anaerobic bacterias, such as *Fusobacterium nucleatum* and yet when associated with Chlorhexidine it decreased prostaglandin E2 levels in gingival crevicular fluid. Contrasting our results of chemical composition Silva et al. (2016)[36] evaluated in their extract of *Lt* that thymol was not present and had as main constituent sesquiterpene β-caryophyllene; such chemical variation may have occurred due to environmental, climatic and regional factors[28,48-53].

Although our study was unable to perform cytotoxic studies and studies about the effectiveness of antimicrobial and antifungal activities through the inhibition zone halo diameter, specifically, it was due to the activity of Thymol or any other components of the sample and such deficiencies occurred due to laboratory limitations, sample and financial. We quote this limit because among the other components of *Lt* we verified the presence of γ-terpinene (8.16%), p-cymene (7.29%) and β-caryophyllene (4.49%) which are also compounds with antimicrobial, antifungal and anti-inflammatory effects in studies such as those by Emiroğlu et al.[52] (2010), Li et al.[53] (2020) and Amankwaah et al.[24] (2020). Despite there were decreasing of *Fn*, *Pi* and *Ca* in our *in vitro* study the oral environment is far more complex due to conditions like temperature, pH, oral hygiene quality, polymicrobial biofilms and mouth humid-

ity so *in vivo* or randomized clinical trials using and testing different EO concentrations and posology is needed to guarantee *Lt* essential oil viability against PD and oral candidiasis[28,48-53].

The antibacterial activity of Thymol is related to its activity on the phospholipids present in the cell membrane, which will increase the permeability of the membrane, destruction of the cell membrane, cytoplasmic leakage, cell lysis, and consequently cell death that's why we assumed by the higher percentage of Thymol this was the main antimicrobial and antifungal agent. Although our study has not been able to perform cytotoxic studies on our samples, some authors state that using essential oils with values above 4.3 mL of Thymol can cause episodes of cytotoxicity. However, De La Chapa et al.[44] (2018) state that Thymol has antimicrobial properties and can be considered as a natural product with low cytotoxicity, even though further studies should be carried out to evaluate the toxicity of the product[28,48-53].

Conclusion

In summary, this study demonstrated that *Lt* has the ability to produce essential oils with biological components such as thymol, which have potential to antifungal and antimicrobial results. This in vitro test evidenced a possible coadjuvant agent for periodontal treatment and further studies are needed. So further research on new medicinal coadjuvants should be developed to better clarify the biological activities of *L. thymoides*.

Acknowledgments

The authors contributed equally to this paper.

Conflict Of Interest

The authors declare that there is no conflict of interest.

Author's Contribution

TMSR and SAFM conceived the original idea and supervised the project and the manuscript; TRBC, EBT and ATV collected the data and wrote the manuscript; RNCJ and EHAA analyzed the statistical methods; MPB, SGS and MSO carried out the laboratory experiment; RRSF conducted the english translation and submission; PCRF carried out the laboratory experiment and wrote the manuscript; ABOF supervised the manuscript and conducted the statistical analyses.

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