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STUDY OF BIOACTIVITY OF MELALEUCA ALTERNIFOLIA ESSENTIAL OIL IN CARIES REMISSION AND IT USE TO PROMOTE ORAL HEALTH

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ABSTRACT

The study aims to verify, measure and test the microbicidal efficacy of Melaleuca essential oil *in vitro* against the pathogen *Streptococcus mutans* (ATCC 25175). Methodologically, the study was experimental research, developed at MicroLAB/UFCG and BM-Biolam, evaluating the essential oil of *Melaleuca alternifolia* (melaleuca) against the pathogen already described *in vitro* in Muller-Hinton agar – AMH respectively, subjecting the samples to variation factors when incubated in a BOD type incubator at approximately 26±1°C. In all *in vitro* tests performed, a considerable increase in the Mean Halo Size – MHT was demonstrated with a concomitant reduction in the number of days for remission on the pathogen. Regarding the remissive effect (ERD) of the Probability of Survival – PS, percentage indices of PS at 50% (IC_{50}) were obtained in SM-I of 25% (G), in SM-II of 21.4% (I) and in SM-III of 30% (K). At the 100% level (IC_{100}) the average values increased in SM-I of 25% (H), in SM-II of 30% (J) and in SM-III of 50% (L). In this context, when evaluating the antibiograms against the taxon, it becomes viable to suggest the use of the essential oil – TTO in the preparation of dentifrices at the common concentration of $[\sigma_{sm}] \leq 120\ \mu L \cdot g^{-1}$ with $D \geq 54{,}4$ mm, isolated or associated with FEC, and the value of $[\sigma_{sm}]$ should be adjusted to each case.

Keywords: Caries. In Vitro Techniques. Dentifrices. Melaleuca Alternifolia. Inhibitory Concentration 50.

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INTRODUCTION

There are more than 700 species of microorganisms in the oral cavity, which associated with favorable factors related to the environment and type of surface, increases the possibility of biofilm formation. Because it is considered a complex and highly dynamic structure as a result of bacterial colonization, the biofilm is formed especially by bacteria of the genus *Streptococcus*, so that *Streptococcus mutans* is the main primary pathogen in the etiology of caries and *Lactobacillus acidophilus* that contributes to the evolution of carious lesions established in dental tissues[1].

Regarding oral infections, the colonization of oral microbial occurs mostly on the surfaces of the teeth, leading to the formation of biofilm (dental plaque). The board is a complicated and active ecosystem. The biofilm present on the surfaces of the teeth has the ability to cause dental caries and the supra and subgingival biofilms can induce periodontal problems. Dysbiosis of the oral microbiota is at the root of two important oral pathologies: dental caries and periodontal disease, which have different characteristics. Caries is responsible for the destruction of the hard tissues of the teeth, while periodontitis is associated with a destructive immuno-inflammatory reaction that leads to the progressive and irreversible destruction of the periodontium and, consequently, of the tooth[2].

Dental caries is a diet-dependent biofilm-dependent disease that develops through the interaction of certain factors, such as the presence of susceptible teeth on which microbial biofilms are formed. This disease affects more than 2 billion people worldwide[3]. Dental caries is an important public health problem in Brazil, affecting mainly the poor classes. It is a chronic disease that results from the mineral dissolution of dental tissues resulting from the production of acids by bacteria when they metabolize carbohydrates, especially sucrose, from the diet[4].

The development of caries lesions in dental tissues involves a dynamic biological process, where the acids produced by the fermentation of dietary carbohydrates affect the integrity of these tissues, altering the physicochemical balance between the tooth surface and the external environment, favoring the loss of minerals. From the constant acidification of the oral environment, the proliferation of acid-tolerant and acidogenic microorganisms is observed, which is a selective process that results in the disruption of microbial homeostasis of dental biofilm[3].

The treatment of the disease during the early stages or called early treatment, ends up being more effective when the mechanical and chemical removal of the biofilm is

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performed, with the use of scraping instruments. The general practitioner cannot refrain from complementing this clinical treatment with phytotherapy if the objective is to quickly eliminate the pathogen and avoid damage to anatomically adjacent dental tissues. However, as periodontitis progresses, treatment through surgery and the use of antibiotics becomes necessary[5].

The use of antibiotic therapy in dentistry, since endodontic infections are polymicrobial, and predominantly caused by strict gram-negative anaerobes, being in some cases asymptomatic and others associated with severe infections. In this sense, the use of antibiotics as an adjuvant to the treatment of these infections becomes an important alternative when clinically indicated. Thus, the choice of the prescribed antibiotic, when necessary, should be made based on laboratory data, patient health, age, history of allergy, absorption, drug distribution capacity, in addition to being based on the professional's up-to-date knowledge of endodontic microbiology[6].

The aim of this study was to verify, test and measure the molar concentrations of pure oil, oil associated with the elective drug and the elective drug alone, in order to determine at which concentrations maximum therapeutic efficiency was obtained, considering parameters such as: pathogen growth rate (CV), remission time (T) and concentration [σ_{sm}] in order to establish optimized therapeutic regimens resulting from the specimen Streptococcus mutans, among others clinical manifestations related to it.

METHODOLOGY

In this analysis, for streptococcal prokaryotes with plate culture, the nutrient substrate was prepared in flasks with a volumetric capacity of 100 mL, and about 25 mL of nutrient broth was distributed in each of the 107 Petri dishes, all with uniform diameters of 90 mm. Before this stage, the vials were previously taken to a boiling water bath to obtain liquefaction of the medium, but without boiling it. The nutrient solutions presented previously defined formulations in which the bacterial pathogen *Streptococcus mutans* Clarke (ATCC 25175) was cultured, which were subdivided into groups SM-I, SM-II and SM-III, as shown in Table 1.

Table 1: Analytical scheme describing the methodology adopted for analysis in samples with 50% (CI50) and 100% (CI100) containing penicillin and its distribution in groups.

Streptococcus mutans – ATCC 25175				
		IC50%	IC100%	
Pen	*Pen*	55*	110	SM-I

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TTO	TTO	40	80	
Pen+TTO	*Pen*+TTO	70	140	SM-II
TTO	TTO	60	120	
Pen+TTO	*Pen*+TTO	280	560	SM-III
Pen	*Pen*	240	480**	

*: [σ_{sm}] Minimum molar concentration; **: [σ_{sm}] Maximum molar concentration.

It should be noted that with a single flask it was possible to fill 4 Petri dishes of 90 mm in diameter and that the described formulation can be adjusted and/or supplemented, as needed, to meet performance criteria. The standard bacterial culture strains, each with colonial beings of the same morphological type, were numerically identified, isolated and maintained from 18 to 24 hours at 35±1°C. During the experiment, no contamination or growth of another species was observed in the primary culture, ensuring the exclusive bacterial monoculture of the pathogen studied.

According to Diniz (2018), microorganisms form mixed populations, that is, several types of microorganisms belong to the same habitat. On the other hand, the development of microbiology, as well as all laboratory procedures for diagnosis, depends on obtaining microbial biomass in the form of pure populations, which when developed/grown in culture media are called pure or "axenic" cultures. After sowing the microorganisms, their probability of survival (PS) was evaluated by the logarithm of the inhibitor concentration at [$\mu L \cdot g^{-1}$] at minimum and maximum concentration.

That said, it was necessary to elaborate subgroups in distinction of concentrations, but with the same amount of inoculum in SM-I, SM-II and SM-III, at concentrations of 40 $\mu L \cdot g^{-1}$ (TTO), 60 $\mu L \cdot g^{-1}$ (TTO) e 240 $\mu L \cdot g^{-1}$ (*Pen*) in Figure 1E and as for the aspect of the effective inhibitory concentration (IC50), in Figure 1F, at concentrations of 55 $\mu L \cdot g^{-1}$ (*Pen*), 70 $\mu L \cdot g^{-1}$ (*Pen*+TTO) e 280 $\mu L \cdot g^{-1}$ (*Pen*+TTO) of the same groups, in the numerical examination of the unknown (Δy) measured in regression of days (cross-reference effect), as can be inferred below, where TMH represents the mean size of the observed inhibitory halo, exemplified in Figure 1.

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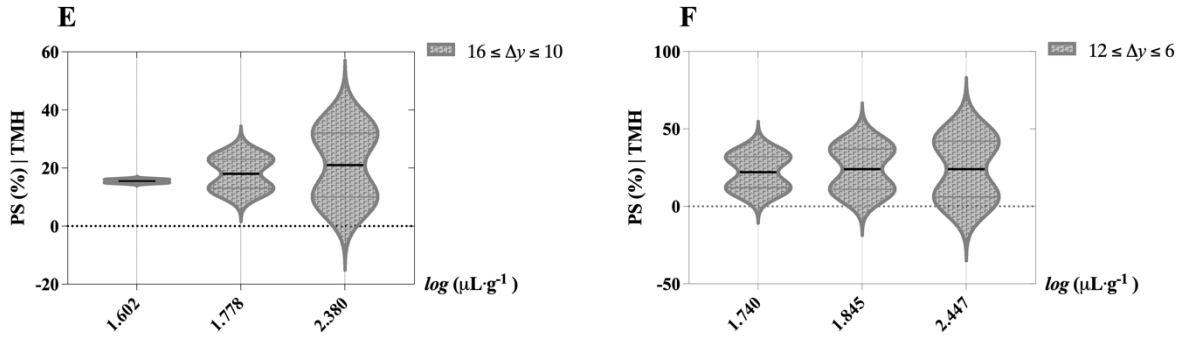
Figure 1: Ratio of PS (%) by TMH in the strains of *Streptococcus mutans* (SM-I), (SM-II) and (SM-III) by *log* of inhibitor concentrations in (E) and (F), in the samples (IC50), with expression of the regressive interval in days (Δy).



Source: Microbiology Laboratory, MicroLAB (UAO/CSTR/UFCG, 2021), adapted by the author via GraphPad.

Regarding the prokaryotes of the genus Mutans, samples of the bacterial were cultured in specific test tubes, which were previously sterilized in an autoclave, then coated in aluminum foil and placed in the bacteriological oven at a temperature of 26±1°C until it remained stable in order to obtain the ideal growth of this microorganism. Only the amount of tubes used on the day of the experiment with the handle previously flambéed and carefully seeded in a bacteriological chamber were removed from these wrappers.

According to the study developed by8, the strains were seeded by the depletion technique in a Petri dish containing Muller Hinton Agar (AMH) medium. The seeded plates were then incubated at 37º±1°C for a period of 24 hours. From the cultures, five colonies of similar morphology were chosen and transferred to 4 mL of sterile saline solution (NaC*l* – 0.9%), adjusting the inoculums by comparing the turbidity of the McFarland scale to 0.5. The resulting suspension had approximately a concentration of 1,5 x 10^8 UFC·mL^{-1} with sui generis turbidity obtained in a centrifuge at 2000 rpm.

In some cases, the disk diffusion (DD) method is more reliable than the determination of MIC (maximum inhibitory concentration). Taking as an example the case of detection of penicillinase-producing strains of *S. aureus*, the diameter of the inhibition zone combined with the zone edge test based on the European Committee for Antimicrobial Susceptibility Testing (EUCAST) is the most sensitive and specific phenotypic method. However, the bacterial culture must be observed every day and the average diameter of the halo must be measured with the help of a millimeter ruler and magnifying glass to ensure the accuracy of the measurements taken.

A petri dish refers to a type of container with a flattened, cylindrical shape, made of plastic or glass, consisting of two parts: the base and the lid. There are models composed

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of rings that secure the lid to the base, so that several plates can be stacked, without them sliding and causing accidents during use. They are used with culture media, since they allow a more detailed and safe observation of microorganisms[9].

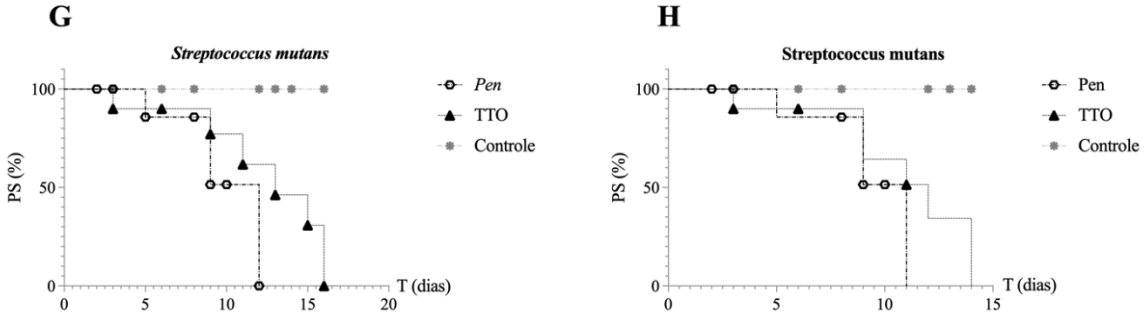
According to the findings of the study carried out by[10], the microorganisms (*Staphylococcus aureus*-NEWP0038, *Enterococcus faecalis* – NEWP 0012, *Escherichia coli* – ATCC 2592 and *Candida albicans* – NEWP 0031) were prepared and standardized with poured suspensions (0.4 ml) and spread in culture medium suitable for the microbial type, using a sterile Drigalski loop, which was slightly slid with the suspension over the Petri dish in three different directions, then the plate dried at room temperature (37º±1°C) for 5 minutes so that the inoculum was absorbed by the agar.

RESULTS

The statistical data related to the test of the SM-I group, applied to the others, proved to be statistically significant with $p < 0.0001$ for a set *n* of 18 samples and frequency (*f*) equal to 4.466 without assumption of sphericity. The assumption of sphericity, as before, was not demonstrated in this group, since the samples of the analyzed populations did not maintain identical standard deviations among themselves.

Furthermore, adopting the investigative method in the previous perspective and, after being exposed to higher levels of antimicrobial drug concentration, corresponding to 50% more in H relative to G (Figure 2), a significant reduction in the regressive effect in days (ERD) resulting from the cell death of microorganisms, in the order of 1 day (*Pen*) and 2 days (TTO), is noticeable. corresponding to previous expectations for the *mutans* (SM) specimen.

Figure 2: Analysis of the remissive effect of the action of *Pen* and TTO against the taxon *Streptococcus mutans* with expression of the percentage of survival (SP) in samples evaluated in vitro under the effect of 50% (CI_{50}) and 100% (CI_{100}).



Source: Microbiology Laboratory, MicroLAB (UAO/CSTR/UFCG, 2021), adapted by the author via GraphPad.0).

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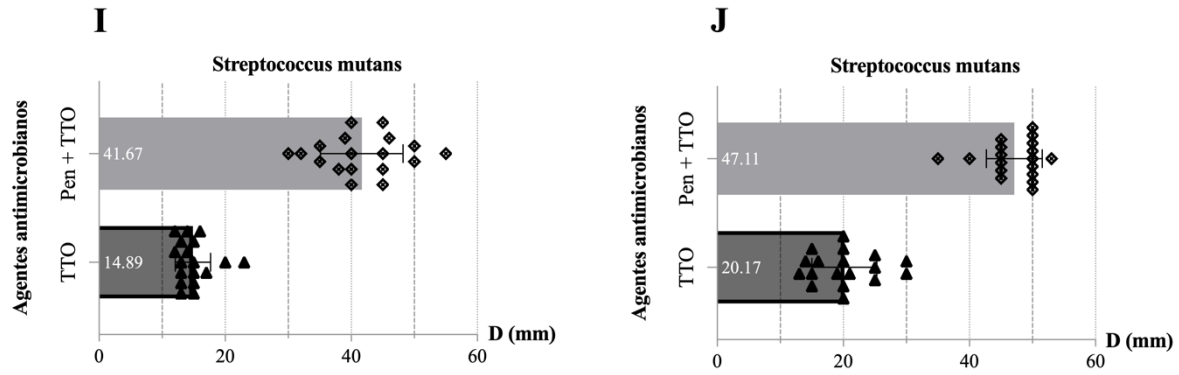
The statistical data related to the SM-II group test proved to be statistically significant, with $p < 0.0001$ for a set *n* of 18 samples and frequency (*ƒ*) equal to 4.760 without assumption of sphericity. The assumption of sphericity, as before, was not demonstrated in this group, since the samples of the analyzed populations did not maintain identical standard deviations among themselves.

Adopting different schemes in molar concentrations, the aim was to test the microbicidal action of TTO against the etiological agent that primarily causes caries, the previous hypothesis maintained the same reasoning regarding the occurrence of halos in plaques. Thus, readings were applied to groups SM-I, SM-II and SM-III, and readings were taken in Petri dishes (I and J) exposed, this time, to penicillin (*Pen*) associated with TTO and, in another set of samples, only to TTO, at concentrations of 70 $\mu L \cdot g^{-1}$ e 60 $\mu L \cdot g^{-1}$ respectively, equivalent to 50% CI, according to the SM-II culture (Figure 3I) and in another group, at concentrations of 140 $\mu L \cdot g^{-1}$ e 120 $\mu L \cdot g^{-1}$, respectively, equivalent to 100%CI evident in SM-II culture (Figure 3J). The average values of the halos are described below. The average values titrated with reagent by Petri dishes are described below, both for pure oil and in synergistic action.

Figure 3: Means for the Mean Halo Size (TMH) compared to the taxon *Streptococcus mutans* in samples randomized to 36 (I) and 36 (J) units, respectively, relative to the reading with 50% (CI_{50}) and 100% (CI_{100}) of the inhibitory agent.



Source: Microbiology Laboratory, MicroLAB (UAO/CSTR/UFCG, 2021), adapted by the author via GraphPad.

The statistical data related to the SM-III group assay proved to be statistically significant with $p < 0.0001$ for a set *n* of 18 samples and frequency (*ƒ*) equal to 6.878 without assumption of sphericity. The assumption of sphericity, as before, was not demonstrated in this group, since the samples of the analyzed populations did not maintain identical standard deviations among themselves.

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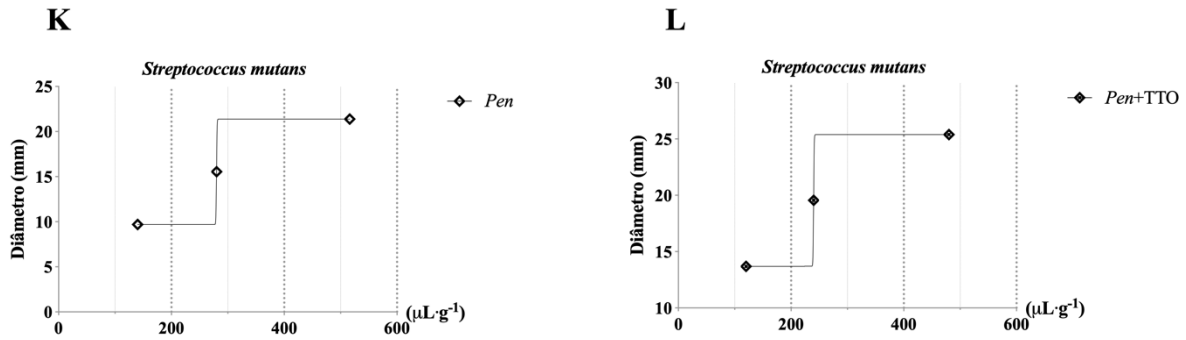
In a systematic experimental continuation, similar to the previous analyses, exposures were assigned only to *Pen*, in the so-called SM-III group, in which half of the maximum concentration of the phytopharmaceutical was added, i.e., 240 $\mu L \cdot g^{-1}$, obtaining a halo diameter close to 9.7 mm, and at the maximum concentration, the approximate value of 21.39 mm, i.e., 480 $\mu L \cdot g^{-1}$ (Figure 4K). On the other hand, regarding the drug *Pen*+TTO, more intense results were obtained, emphasizing that the suppression of the colonies provided halos with a mean diameter close to 13.7 mm at half the maximum concentration of 280 $\mu L \cdot g^{-1}$ and a mean halo diameter of 25.39 mm, at a concentration of 560 $\mu L \cdot g^{-1}$ (Figure 4L).

Figure 4: Analysis of the inhibitory effect by the action of *Pen* and *Pen*+TTO, respectively, against the taxon *Streptococcus mutans* with expression of 50% CI and 100% CI in (K) and (L), in each case, in samples evaluated *in vitro*.



Source: Microbiology Laboratory, MicroLAB (UAO/CSTR/UFCG, 2021), adapted by the author via GraphPad.

Thus, it is possible to ensure the maintenance of the repetitive pattern inserted in each group within the limits of common reliability among all statistical tests performed and to presume the dose/prescription at reliable levels, obtaining effective speed in bacterial remission.

GROWTH OF THE COLONIES

Furthermore, preserving the investigative method for the other bacterial groups, after being exposed to higher levels of antimicrobial drug concentration, corresponding to 50% more in H relative to G (SM-II Group). The significant reduction in the regressive effect in days (ERD) resulting from the cell death of the microorganisms is noticeable, in all cases of the order of 1 day (*Pen*) and 2 days (TTO), by means of twice the titrated dose on the surface, after growth at sowing. It was proven that molar augmentation resulted in greater

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bacterial remission up to values with specific limits already postulated in Table 1 of this study.

The beneficial association of TTO with penicillin was demonstrated by the good dispensability in the medium and satisfactory solubility due to the approximate partition coefficient between both, after exposure to higher levels of concentration of the antimicrobial drug, corresponding to 50% more than the subsequent group, with a significant reduction in the regressive effect in days (ERD) resulting from the cell death of the microorganisms, of the order of 5 days (*Pen*+TTO) and 3 days (TTO), again refuting any doubt as to their possible association.

With the increase in the use of broad-spectrum antibiotics, the proliferation of opportunistic fungi is favored by promoting a marked decrease in the oral microbiota. However, it is noted that some biofilm bacteria have the ability to inhibit the action of these fungi by competition, some of them related to the development of dental caries, such as *S. mutans*, which has the ability to secrete trans-2-denoic acid signaling molecules that stimulate peptides, capable of inhibiting the transition from yeast to the hyphal state of *C. albicans*[11] studied in other scientific literature.

Due to the growing increase in cases of clinical resistance to antibacterial treatments, there is a need to introduce new options for eminently preventive therapeutic regimens, as well as herbal medicines. In this sense, dental professionals indicate the use of oral dentifrices (toothpaste) as an aid to the mechanical treatment of caries removal or even as a preventive measure, as it is effective in minimizing or completely eliminating dental biofilm[12].

ANALYSIS OF THE ANTIBIOGRAM

Adopting a systematic scientific methodology aimed at testing the microbicidal action of TTO against another microorganism, the bacterium *S. mutans*, the previous hypothesis maintained the same reasoning regarding the occurrence of halos in plaques. Thus, other groups of bacterial strains were studied and readings were taken in Petri dishes (G and H) exposed, this time to penicillin (*Pen*) and, in another set of samples, associated with TTO, at concentrations of 55 $\mu L \cdot g^{-1}$ and 40 $\mu L \cdot g^{-1}$, respectively, equivalent to 50% CI, according to the SM-I culture and in another group, at the concentrations of 110 $\mu L \cdot g^{-1}$ and 80 $\mu L \cdot g^{-1}$, respectively, equivalent to 100% CI.

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Similarly, the precepts already established were adopted, aiming to test the microbicidal action of TTO, and other groups of bacterial strains were studied, and readings were taken in Petri dishes (I and J) exposed, this time, to penicillin (*Pen*) associated with TTO, and in another set of samples, only to TTO, at concentrations of 70 $\mu L \cdot g^{-1}$ and 60 $\mu L \cdot g^{-1}$, respectively, equivalent to 50% CI, according to the SM-II culture and in another group, at the concentrations of 140 $\mu L \cdot g^{-1}$ and 120 $\mu L \cdot g^{-1}$, respectively, equivalent to 100% CI evident in SM-II culture, with successful remission values being acquired in the plates and in the other replicates.

Continuing the reasoning, it is adduced that the confidence interval to administer the TTO would be interposed between 40 $\mu L \cdot g^{-1}$ e 120 $\mu L \cdot g^{-1}$, that is, the dose-response would be included in the mathematical interval $40 \leq [\sigma_{sm}] \leq 120$, where $[\sigma_{sm}]$ It would encompass any acceptable value aiming at its incorporation into pharmaceutical formulations for mild to moderate clinical cases of dental caries in patients in good health. For patients in clinical use of immunosuppressive or chemotherapy drugs, the *Pen*+TTO dosage would be less than $[\sigma_{sm}] \leq 140$ $\mu L \cdot g^{-1}$. It is also extremely important to emphasize that penicillin, applied to oral dentifrice, is restricted to laboratory tests performed exclusively *in vitro*.

The experimental associations performed in the laboratory by means of the method of disk diffusion of the essential oil with penicillin, corroborate its stable pharmacological interaction between the components of the formula and, as the case may be, with a notable inhibitory potentiating action of the bacterial growth of the genus "mutans" addressed in this research. Regarding the side effects that may occur, systemic medication is indicated only in oral infections when there is no response to topical medication. As a result, the extracts proved to be effective, inhibiting the growth of bacteria still in the dental biofilm (clinical pre-test), suggesting the use of plants as an alternative means in dental therapy.

DISCUSSION

It is also extremely important to emphasize that both ketoconazole and penicillin, applied to mouthwash and oral dentifrice, respectively, were restricted to laboratory tests performed *in vitro*. Both are antimicrobial agents that act on microorganisms emanating from different biological kingdoms, serving as a comparative parameter for measuring the microbicidal efficacy of the essential oil and should not be included in these products that are part of oral cavity hygiene. The experimental associations carried out in the laboratory by means of the method of disc diffusion of the essential oil with ketoconazole and

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penicillin, corroborate its stable pharmacological interaction between the components of the formula and, as the case may be, with a notable inhibitory potentiating action on fungal growth, but also bacterial.

Penicillin is the drug of choice, because it is bactericidal, has efficacy against the probable microorganisms present in odontogenic infections and is a drug of low toxicity. However, several hypersensitivity reactions can be identified, which are associated with the use of penicillins, such as skin rashes, from maculopapular forms to exfoliative dermatitis, urticaria; and it is necessary to replace it with another group of drugs[13].

According to the study carried out by14, it is observed that the authors identified that the antimicrobial activity of mouthwashes based on 77 chlorhexidine digluconate – 0.12%, sodium fluoride – 0.05%, chlorhexidine digluconate (Ca) – 0.06%, sodium fluoride – 0.05% and zinc acetate 0.34% (Or), when compared with the ethanol mouthwash based on chlorhexidine gluconate – 0.12% in *Candida albicans* isolates, demonstrated that group Ca showed fungicidal activity on C. albicans similar to the control, but with less fungistatic action, while Or showed only fungistatic action similar to the control in the isolates evaluated.

In this sense, with regard to the treatment of prosthetic stomatitis after adaptation and formation of the biofilm, the use of antimicrobial drugs such as antiseptics, antibacterials or antifungals should be carried out. Regarding the side effects that may occur, systemic medication is indicated only in oral infections when there is no response to the prescribed medication. However, it is worth noting that it has been shown that *Pen*+TTO is less toxic than beta-lactam antibiotics, as they may be associated with hepatotoxicity and nephrotoxicity. Thus, the extracts proved to be effective, inhibiting the growth of dental biofilm bacteria, suggesting the use of plants and encouraging the synthesis of essential oil (TTO) as an alternative means in daily dental therapy[14].

Supporting this discussion[12] explains the relevance of herbal associations, since the treatment carried out with herbal medicines or phytomedicines, that is, pharmaceutical products that are formulated from plants, is based on evidence or science for the use of compounds in the treatment and/or prevention of diseases, where it is possible to use plants or their parts, with known chemical characteristics and that have the proven ability to produce a pharmacological effect on membranes or bacterial nuclear materials, such as RNA or prokaryotic DNA.

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Based on this scenario, herbal medicines are considered to have a wide therapeutic scope and do not present so many drug interactions, electing them to dental practices due to their pharmacokinetic and pharmacodynamic safety, excellent renal clearance rate (excretion) and almost absence of hepatotoxicity, making the risks minimal when ingesting a dose considered toxic or lethal of a drug of plant origin administered orally.

CONCLUSION

Thus, with regard to oral dentifrices, the insertion of the essential oil would compose an effective pharmacochemical formulation route in the treatment and prevention of clinical cases of dental caries, optimizing its antibacterial action, especially against the pathogen *Streptococcus mutans*, enabling its inclusion in toothpastes, gels or toothpastes in therapeutic doses. Its viability was verified with significant suppression already in the first increasing applications of the oil, whether isolated or associated with penicillin, by redeeming the bacterial colonies of the plaques, making its survival unfeasible even in its early stages of development.

These precautions are due to the preventive nature given the scarcity of experiments that attest to the safe interaction of the elements that make up the phytopharmaceutical contained in the essential oil with neoplastic cells. This interaction would be undesirable, as exposed, and could result in clinical complications for individuals in the process of chemotherapy treatment. From this it is inferred the relevance of the dental surgeon's performance in the performance of clinical protocols by the mechanical action of appropriate instruments aimed at removing the biofilm or dental calculus associated with the formulation proposed in this work.

It is concluded that the inclusion of the essential oil in toothpastes up to the concentration of $[\sigma_{sm}] \leq 120\ \mu L \cdot g^{-1}$ ($IC_{100\%}$) with $D \geq 5{,}44$ mm enables the development of promising pharmaceutical formulas, since it has antibiotic properties against the species evaluated in both formulations. It is recommended that it be safe to prescribe in offices to young people over the age of 12 and to adults and the elderly. The prescriptive therapeutic warning rests on the public who have immunity depleted by severe pathological diseases affecting the oral cavity or upper respiratory tract and by those immunosuppressed patients, and even in these cases, a dosage lower than $[\sigma_{sm}] \leq 60\ \mu L \cdot g^{-1}$ ($IC_{50\%}$) in daily dental clinical dosages.

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REFERENCES

1. Contreras, S. C. M. (2022). *Restaurações diretas com materiais bioativos: observações clínicas, in vitro e revisão sistemática* (Tese de Doutorado). Universidade Estadual Paulista (Unesp), São José dos Campos. Disponível em https://repositorio.unesp.br/bitstream/handle/11449/238016/contreras_scm_dr_sjc.pdf?sequence=4&isAllowed=y

2. Morais, D. A. B. (2022). *Prótese dentária e candidíase oral* (Dissertação de Mestrado). Instituto Universitário Egas Moniz. Disponível em https://comum.rcaap.pt/bitstream/10400.26/42642/1/Morais_Daniela_Alexandra_Barroso.pdf

3. Waltermann, E. D. M. (2018). *Candida spp. e cárie dentária: uma revisão sistemática da literatura e meta-análise* (Dissertação de Mestrado). Universidade Federal do Rio Grande do Sul, Porto Alegre. Disponível em https://www.lume.ufrgs.br/handle/10183/238674

4. Sousa, F. S., Lopes, B. C., Costa, E. M., Alves, C. M. C., Queiroz, R. C. S., Tonello, A. S., Ribeiro, C. C. C., & Thomaz, E. B. A. (2021). Persistem iniquidades sociais na distribuição da cárie dentária em adolescentes maranhenses? Contribuições de um estudo de base populacional. *Ciência & Saúde Coletiva, 26*(7), 2625–2634. Disponível em https://www.scielo.br/j/csc/a/npS3dHgCTFcxgv59bGwKRDD/?format=pdf&lang=pt

5. Vasconcelos, M. O. (2019). *Biomembranas contendo nanopartículas poliméricas com lidocaína e cloranfenicol para regeneração tecidual guiada* (Dissertação de Mestrado). Universidade Federal de Goiás, Goiânia. Disponível em https://repositorio.bc.ufg.br/tede/bitstream/tede/9713/5/Dissertação%20-%20Mariana%20Oliveira%20Vasconcelos%20-%202019.pdf

6. Arnaud, R. R., Costa, J. B. R. F., Romão, T. C. M., Santos, M. G. C., Silva, F. V. D., Andrade, K. S., & Silva, L. P. L. (2021). Conhecimento de acadêmicos de Odontologia sobre a prescrição antibiótica em tratamentos endodônticos. *Arch Health Invest., 10*(7), 1195–1200. Disponível em https://archhealthinvestigation.com.br/ArcHI/article/view/5440/7202

7. Matuschek, E., Brown, D. F., & Kahlmeter, G. (2014). Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. *Clinical Microbiology and Infection, 20*(4), 255–266.

Revista ARACÊ
ISSN: 2358-2472

8. Nonato, I. S. (2018). *Efeito antibacteriano do óleo essencial de Mentha piperita L. sobre as bactérias Staphylococcus aureus e Escherichia coli* (Monografia de Graduação). Faculdade Maria Milza, Governador Mangabeira. Disponível em http://famamportal.com.br:8082/jspui/bitstream/123456789/759/1/Isael%20da%20Silva%20Nonato%20TCC.pdf

9. Prolab. (2018). A importância da Placa de Petri para procedimentos com meio de cultura. Disponível em https://www.prolab.com.br/blog/equipamentos-aplicacoes/a-importancia-da-placa-de-petri-para-procedimentos-com-meio-de-cultura/

10. Dutra, M. J., Pizzolatto, G., & Corralo, D. J. (2022). Ação antimicrobiana, in vitro, de antissépticos bucais sobre fungos e bactérias. *FOL - Faculdade de Odontologia de Lins/Unimep, 32*, 3–12. Disponível em https://www.metodista.br/revistas/revistasunimep/index.php/Fol/article/view/41704368/2674

11. Medeiros, M. R. S. (2020). *Uso da terapia fotodinâmica antimicrobiana no tratamento de candidose oral: ensaio clínico, controlado e randomizado* (Dissertação de Mestrado). Universidade Federal do Rio Grande do Norte, Natal. Disponível em https://repositorio.ufrn.br/bitstream/123456789/29288/1/Usoterapiafotodinamica_Medeiros_2020.pdf

12. Chagas, C. F. (2019). *Uso de um enxaguatório bucal com extratos fitoterápicos de Mentha piperita, Pimpinella anisum e Citrus limon no tratamento de candidíase oral: uma avaliação in vivo* (Monografia de Graduação). Universidade de Santa Cruz do Sul, Santa Cruz do Sul. Disponível em https://repositorio.unisc.br/jspui/bitstream/11624/2650/1/Caroline%20Furrati%20Chagas.pdf

13. Prado, L. (2018). Dorothy Hodgkin e seus estudos cristalográficos sobre a estrutura da penicilina. *História da Ciência e Ensino – Construindo Interfaces, 18*, 128–151. Disponível em https://revistas.pucsp.br/index.php/hcensino/article/view/36762/26735

14. Cruzeiro, M. E. S., Cleff, M. B., Blank, D. E., & Meireles, M. C. A. (2022). Controle da formação biofilme por Candida albicans em pacientes usuários de próteses dentárias. *Conjecturas, 22*(11), 1–11. Disponível em http://www.conjecturas.org/index.php/edicoes/article/view/1532/1147