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CURATIVE EVALUATION OF MEMALEUCA ALTERNIFOLIA OIL IN ORAL CANDIDIASIS IN IMMUNOSUPPRESSED PATIENTS

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ABSTRACT

The current research aims to verify, measure and test the microbicidal efficacy of Melaleuca essential oil *in vitro* against the pathogen *Candida albicans*. The study is experimental research, developed at MicroLAB/UFCG and BM-Biolam, evaluating the essential oil of *Melaleuca alternifolia* (tea tree) against pathogens *in vitro* in Saboraud dextrose agar - ASD, subjecting them to variation factors, incubated in a BOD type incubator at 36±1°C, according to the quantitative inoculum of the microorganism. For the *Candida albicans*, the MIC (Minimum Inhibitory Concentration) of 16 µL g-1 (EC50%) in CA-I to up to 128 $\mu L \cdot g^{-1}$ (EC100%) in CA-II of the isolated TTO demonstrated activity equivalent to *Cet*, reaching its maximum efficacy at the EC100% of 240 $\mu L \cdot g^{-1}$ in CA-III when associated. Regarding the remissive effect (ERD), the probability of survival - PS in 50% (IC_{50}) in CA-I was 19.1% (A), in CA-II with the index of 35.7% (C) and in CA-III at the value close to 44.4% (E). In this context, when evaluating the antibiograms against the taxa, it is possible to suggest the use of TTO in the preparation of mouthwashes with a value of $[\sigma_{ca}] \leq 128$ $\mu L \cdot g^{-1}$ and $D \geq 16$ mm, attesting to the viability of including the essential oil as a phytotherapeutic agent as a complementary alternative route in clinical dental treatments caused by *Candida albicans* that trigger oral candidiasis, being effective against the pathogen described, eliminating it in a time interval of $T \leq 8$ days when associated.

Keywords: Candida Albicans. Melaleuca Alternifolia. Candidiasis. Efficacy. Inhibitory Concentration 50.

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INTRODUCTION

Candidiasis, produced by yeasts of various species, belongs to the genus *Candida* and presents a wide variety of clinical conditions. Naturally, these fungi are part of the microbiota of the mouth, gastrointestinal system, skin and vagina, and only produce infections in the mucosa in the presence of predisposing factors, by opportunistic fungi. In general, candidiasis has its origin endogenously and can be attributed to one of two causes: imbalance of the accompanying microbiota or diseases and processes that influence the immune response, such as prolonged treatment with corticosteroids, extreme diseases, acquired immunodeficiency syndrome, among others[1].

Candidiasis manifests as diseases of the oral and genital mucosal layers, skin and nails, and recurrent infections may occur and, less frequently, present as invasive candidiasis in immunocompromised individuals, with very high morbidity and mortality[1]. The genus *Candida* is the main group of yeasts that cause opportunistic infections in humans and comprises approximately 200 distinct species, approximately 10% of which are associated with infections. In healthy individuals, *Candida* colonizes mainly the mucosal surfaces of the gastrointestinal and urogenital tracts, without showing any symptoms of the disease[2].

Yeasts of the genus *Candida* are integral to the normal microbiota of healthy humans. However, because they are opportunistic microorganisms, factors such as decreased immunity can facilitate their invasion and dissemination in the human host. *Candida albicans* is the most commonly isolated species, however, other non-albicans species of *Candida*, such as *Candida parapsilosis, Candida tropicalis, Candida glabrata* and *Candida krusei*, have been isolated from patients, and some of these species have drawn attention due to their frequent resistance to some antifungals[3].

Microorganisms of the genus *Candida* are present in the microbiota of the reproductive and gastrointestinal mucosa, living harmoniously in about 50-70% of healthy individuals. However, this genus is formed by opportunistic microorganisms, mainly affecting immunosuppressed patients or those undergoing treatment with broad-spectrum antimicrobials. Such conditions make these microorganisms important agents of infection, such as Candidiasis, which can be superficial or invasive[4].

Candidiasis is an infection whose origin is intrinsically endogenous and occurs as a consequence of the disruption of the parasite-host balance, triggered by changes in the tissue barrier and in the autochthonous microbiota or by the impairment of natural and

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immunological defenses. Some factors correlated to the host have been related to facilitating the development of fungal infections in the oral cavity, including diabetes mellitus, *Cushing's* syndrome, malignancies, immunosuppressive conditions, smoking, and use of dental prostheses[5].

Oral candidiasis, as it presents clinically, can be treated with topical and systemic antifungals. The treatment of oral candidiasis uses nystatin suspension as the drug of choice, while other antifungals, such as fluconazole and itraconazole, are indicated when topical treatment is inefficient. In severe cases of oropharyngeal candidiasis, voriconazole, amphotericin B, or the echinocandins – caspofungin, micafungin, and anidulafungin[5] are used.

The study focused on the clear intention of verifying, as well as accurately testing and measuring the microbicidal efficacy of Melaleuca essential oil *in vitro*, alone or associated with an elective drug, against the pathogen *Candida albicans*, which causes painful and very uncomfortable forms of oral candidiasis, such as oral canker sores.

METHODOLOGY

The study is an experimental research, initially developed at MicroLAB/UFCG and complemented at the BM-Biolam laboratory, of an eminently evaluative character of the essential oil of *Melaleuca alternifolia* (melaleuca) 100% pure against the pathogen already described *in vitro* on Saboraud Dextrose agar – ASD respectively, submitting the means of bacterial cultures agent with proven antibiotic action, a remissive effect was observed as a measurement of the halo, the probability of survival of the pathogen (PS) and death in loco in the plates strictly controlled via variation factors, incubated in a BOD type incubator at 36±1°C, as the case may be.

SELECTIVE MEDIUM AND SPECIFIC CULTURE

Before being collected and inoculated, the samples of fungal microorganisms were pricked in a Petri dish with the culture medium previously prepared in stock with strict monoclonal control of the species studied, with Sabouraud Dextrose agar agar being the nutritional medium of choice for *Candida albicans* prepared in accordance with the concentration of the inoculum.

Before the execution of the above tests, three systematic schemes were defined to measure the concentrations applied to each microorganism, determining both the minimum

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and the maximum molar concentration, as well as aspects of the culture, such as its microscopic and macroscopic appearance, in addition to the odor and growth with hyphae formation characteristic of the species, in order to make the precise determination of the inhibitory concentrations [σ_{ca}], as shown in Table 1 below for *C. albicans* and its subgroups labeled CA-I, CA-II and CA-III.

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

Candida albicans – ATCC 64124				
		IC50%	IC100%	
Cet	*Cet*	24*	48	CA-I
TTO	TTO	16	32	
Cet+TTO	*Cet*+TTO	30	60	CA-II
TTO	TTO	64	128	
Cet+TTO	*Cet*+TTO	120	240	CA-III
Cet	*Cet*	256	512**	

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

The use of Sabouraud Agar was justified by the fact that it is a selective culture medium for fungi, thus nullifying the possibility of growth of another microorganism in addition. Therefore, when *C. albicans* colonies are cultivated in Sabouraud agar medium at 37°±1°C, noting a considerable growth in 24 to 48 hours, and in a macroscopic way, it is possible to notice colonies with opaque or shiny appearances with creamy texture and color ranging from white to creamy, with regular or irregular edges and leaven odor[7].

Sabouraud agar is a medium intended for the qualitative cultivation of fungi (filaments and yeasts), pathogenic and non-pathogenic. The addition of ketoconazole makes this medium more selective, inhibiting the growth of most bacteria and some saprophytic fungi. Its hydrogen potential – pH favors the growth of dermatophytes and inhibits certain species of bacteria of clinical interest. The peptones existing in the culture medium are sources of excellent nitrogenous compounds for the development of fungi, thus being relevant in these cases.

Dextrose provides an energy source for the development of microorganisms. The high concentration of dextrose provides an advantage for the development of fungi (stable by osmosis) whereas most bacteria do not tolerate the high concentration of sugar. The Triple Sugar Iron Agar Medium (TSI) determines the ability of a microorganism to hydrolyze glucose, sucrose and lactose incorporated into a "base medium" with acid production with or without gas[8].

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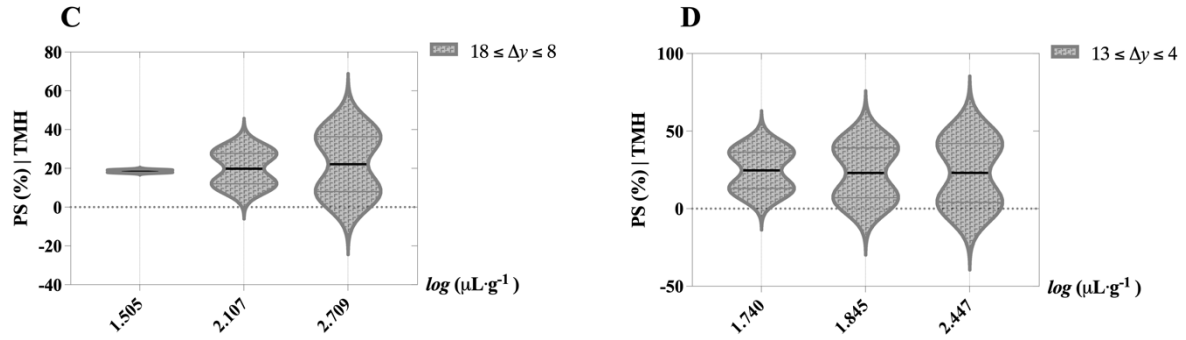
Therefore, Figure 1C illustrates the analysis of the same variables applied to groups CA-I, CA-II and CA-III, however, at concentrations of 32 μL·g^{-1} (TTO), 128 μL·g^{-1} (TTO) and 512 μL·g^{-1} (*Cet*) regarding the aspect of effective inhibitory concentration (IC100) and, in Figure 1D, at concentrations of 48 μL·g^{-1} (*Cet*), 60 μL·g^{-1} (*Cet*+TTO) and 240 μL·g^{-1} (*Cet*+TTO) of the same groups, each of them is parameterized by the numerical value of its unknown (Δy) measured in day regression, similar to the previous graph, as shown in Figure 1 below.

Figure 1: Ratio of PS (%) by TMH in the strains of Candida albicans (CA-I), (CA-II) and (CA-III) by *log* of inhibitor concentrations in (C) and (D), in the samples (IC100), with expression of the regressive interval in days (Δy).



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

Over the years, the usefulness of a selective and differential means for the primary isolation of *Candida* spp. species has been observed. In 1953, for example, Nickerson developed a medium following a study of sulfite reduction by *Candida* species. In 1958, Pagano added triphenyltetrazolium chloride to Sabouraud Dextrose agar to differentiate *C. albicans* from other yeasts. From the addition of chromogenic substrates to the culture medium, it was identified that the colonies of *C. albicans, C. tropicalis, and C. krusei* produce different colors, facilitating the direct detection of these yeast species in the isolation plate[9].

RESULTS

STATISTICAL ANALYSIS OF THE RESULTS

Thus, the mean of each column containing data on TTO (essential oil) and *Cet* (ketoconazole), both applied separately, was compared to the mean of the column (*Cet*+TTO – associated) with maintenance of control groups for the test subgroups in CA-I, CA-II and CA-III. Regarding the purpose of quality control, it is possible to verify whether the

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materials used in the technique provide and present performance compatible with what is expected. Thus, the results that present any deviation must be carefully analyzed and, while the verification is carried out, it is indicated that the medium should not be used until the final validation of the product[8].

Such statistical analyses were necessary because, in a certain context, the microorganism was a fungus, in which the drug of clinical choice (ECP) adopted was ketoconazole, and in another, a bacterium, for which the appropriate drug of clinical choice was penicillin. Different microorganisms require different treatments from the clinical perspective of therapeutic treatments. Based on this assumption, studying microorganisms (fungi and bacteria) depends on obtaining a large number of identical microorganisms (pure culture), which are obtained in the laboratory through isolation from a mixed population.

In this regard, sterile culture media must be prepared and preserved in sterile conditions. When introduced into the sterile environment as inoculum, it is necessary to adopt technical care so that there is no external (environmental) contamination[7]. The tests were also corrected by performing multiple comparisons using hypothetical statistical tests according to the Dunnet method, with 95% of the confidentiality interval ($p < 0.05$).

The statistical data related to the CA-I group test proved to be statistically significant, with $p < 0.0001$ for a set *n* of 18 samples and frequency (f) equal to 5.667 without assumption of sphericity. The assumption of sphericity was not demonstrated in this group, which is relevant, since the samples of the analyzed populations did not maintain identical standard deviations among themselves, according to the description already presented.

Statistical data related to the test of the CA-II group were obtained, as in the CA-I subgroup, and were also statistically significant with $p < 0.0001$ for a set *n* of 18 samples and frequency (f) equal to 6.999 without assumption of sphericity. This reproduction of similar data corroborates the technical rigidity of the tests performed and the quality standards followed by international protocols, and this statistical reproduction of aspects from the physiology of the fungus converges to another CA-III subgroup, since the samples of the analyzed populations did not maintain similar standard deviations from each other.

From the previous investigative methods, and after exposure to higher levels of concentration, corresponding to 50% more F relative to E (CA-III), it is evident the significant reduction in the regressive effect in days (ERD) in the colonial units, in the order of 3 days (*Cet*+TTO) and 5 days (*Cet)*, corroborating the expectation that the increase in

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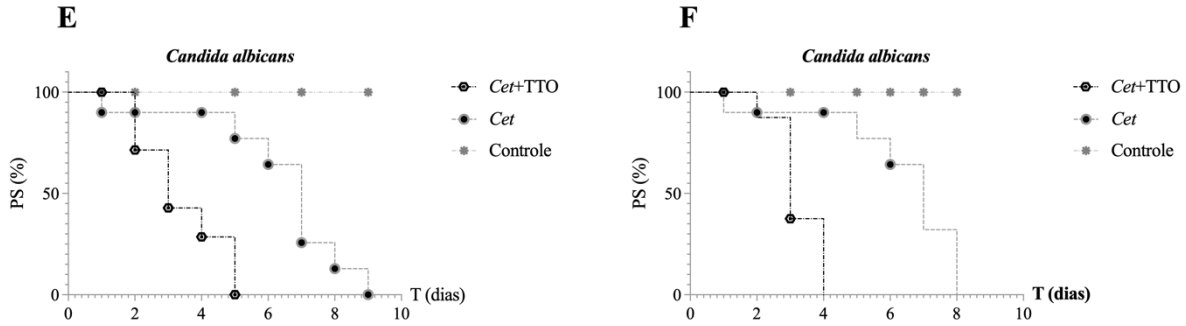
the synergistic dose would result more quickly in cell death, demonstrating a recurrent trend in subsequent evaluations.

When pharmacological interactions occurred resulting from the positive synergism between the herbal medicine and the clinical drug, it was demonstrated stable interactivity between both, good lipid solubility of the TTO oil in ketoconazole with complement of distilled water and previous homogenization and subsequent use before the *albicans* specimen, reducing the number of days of exposure by about 31%. This is reflected in the treatment time for patients, and it is desirable to minimize health expenses and reduce uncomfortable episodes caused by the "canker sores" in patients, whose remission is illustrated in Figure 2 below.

Figure 2: Analysis of the cross-referencing effect by the action of *Cet*+TTO and TTO against the taxon *Candida albicans* with expression of the percentage of survival (SP) in samples evaluated *in vitro* under the effects of CI_{50} and CI_{100}.



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

The statistical data related to the CA-III group assay proved to be statistically significant with $p < 0.0001$ for a set n of 18 samples and frequency (f) equal to 1.617 with no assumption of sphericity. The assumption of sphericity, as in CA-I and CA-II, was not demonstrated in this group, since the samples of the analyzed populations also did not maintain identical standard deviations, which is desirable because, if the tirated concentrations on the crops were different, different results are expected regarding the variables of time (T), growth velocity (CV) and probability of survival (PS%).

Similar to the tests of the previous hypothesis, another group of strains (E and F) of the CA-III subgroup were prepared and readings were taken in Petri dishes, exposed to ketoconazole (*Cet)* associated with TTO and, in another set of samples, only to ketoconazole, at concentrations of 120 $\mu L \cdot g^{-1}$ and 256 $\mu L \cdot g^{-1}$ respectively, equivalent to the 50% CI, according to the CA-III culture (Figure 3E) and in another group, at concentrations

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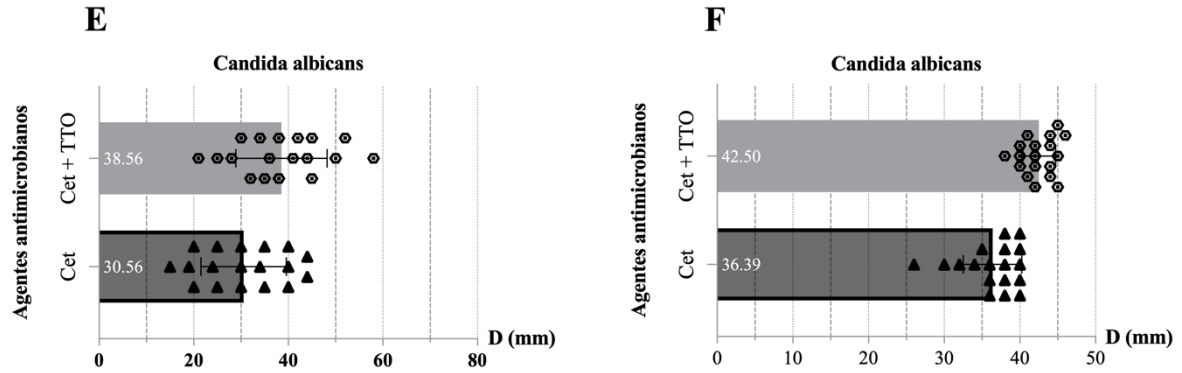
of 240 μL·g^{-1} and 512 μL·g^{-1}, respectively, equivalent to the 100% CI evident in the CA-II crop (Figure 3F). This was providential for the comparative effect between ketoconazole applied alone and, in other samples, associated with TTO, to measure its antifungal potential, illustrated in Figure 3.

Figure 3: Means for the Mean Halo Size (TMH) versus the taxon *Candida albicans* in samples randomized to 36 (E) and 36 (F) 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.

In continuity actions, similarly to the previous careful analyses, exposure only to Cet was designated, which contained *Candida albicans*, in the so-called CA-III group, as an example, and in them half of the maximum concentration of the phytopharmaceutical was added, that is, 256 μL·g^{-1} with a halo diameter close to 24.7 mm and, in the maximum concentration, the approximate value of 36.39 mm, that is, 512 μL·g^{-1} (Figure 4E). On the other hand, regarding the drug *Cet*+TTO, more intense results were obtained, compared to the previous ones, emphasizing that the suppression of the colonies provided halos with a mean diameter close to 39.5 mm at half the maximum concentration of 120 μL·g^{-1}and a mean halo diameter greater than 41 mm, at a concentration of 240 μL·g^{-1} (Figure 4F). The diameters verified macroscopically are exemplified in Figure 4 below.

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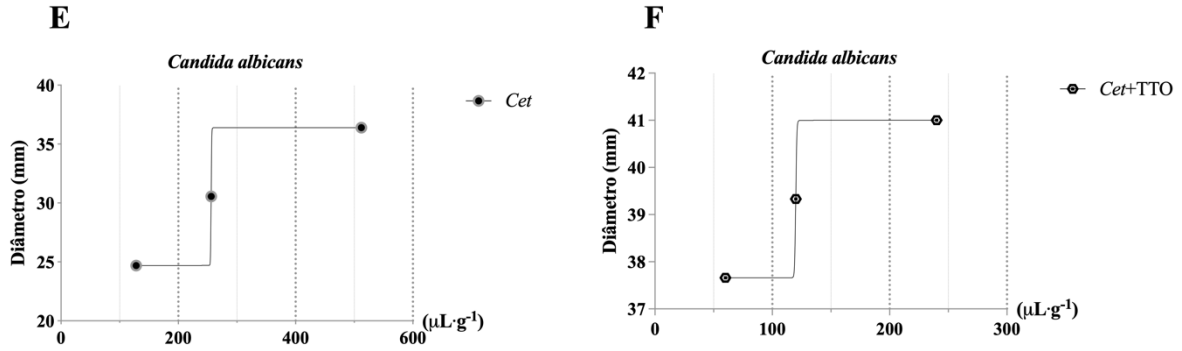
Figure 4: Analysis of the inhibitory effect by the action of Cet and *Cet*+TTO, respectively, against the taxon *Candida albicans* with expression of 50% CI and 100% CI in (E) and (F), in each case, in samples evaluated *in vitro*.



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

GROWTH OF THE COLONIES

Therefore, at the dose at 50% of molar concentration more in B relative to A, it is already notorious that there is a significant reduction in the regressive effect in days (ERD) in the colonial units evaluated, in the order of 4 days (*Cet*) and 3 days (TTO), corroborating the expectation that the increase in the dose would result more quickly in the death of the organisms. This confirms the harmful action of the herbal medicine similar to the drug-clinical used as a parameter against the pathogen, but a stabilization of the inhibitory action is perceived in excessively high percentages (CA-I subgroup).

Reproducing in the CA-II subgroup at higher concentration levels, corresponding to 50% more in D relative to C (Figure 2), a significant reduction in the regressive effect in days (ERD) is observed in the colonial units, in the order of 3 days (*Cet*+TTO) and 4 days (TTO), corroborating the expectation that the increase in the dose would result more quickly in the death of the organisms. This ratifies its fungicidal action with intensity in the CA-II subgroup, since this group has molar concentrations [σ_{ca}]≥16 µL·g^{-1}and halo with diameter ≥ 15 mm.

The previous examples should expose the CA-III subgroup to higher concentration levels, corresponding to 50% more in F relative to E (CA-III), demonstrating the evident significant reduction in the regressive effect in days (ERD) in the colonial units, in the order of 1 day (Cet+TTO) and 1 day (Cet), corroborating the previous expectations, without any deviation from the parameters, in mathematical reasoning logic, demonstrating a recurrent trend in subsequent evaluations even more evident in the CA-III subgroup.

When pharmacological interactions occurred resulting from the positive synergism between the herbal medicine and the pharmaco-clinical, it was demonstrated stable

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physicochemical interactivity, relative solubility by the partition solubility coefficient between the phytopharmaceutical and ketoconazole, for the albicans specimen (CA), reducing the number of days by about 28%. It is seen that this is desirable, because the faster the etiological agent of oral candidiasis is eliminated, the less discomfort will be there, determined by local pain or during chewing.

ANALYSIS OF THE ANTIBIOGRAM

After the time required for the growth of the fungal culture, readings were taken in the Petri dishes (E and F) of the CA-I subgroup (Figure 2) to measure the mean diameter of the inhibitory halos, since they were exposed to ketoconazole (*Cet*) and TTO, at concentrations of 24 $\mu L \cdot g^{-1}$ and 16 $\mu L \cdot g^{-1}$, respectively, equivalent to the 50% CI, submitted to CA-I and ketoconazole (Cet) and TTO cultures, at concentrations of 48 $\mu L \cdot g^{-1}$ e 32 $\mu L \cdot g^{-1}$, respectively, equivalent to the 100% CI evident in the CA-II culture. The desired potential effect on the dose-response relationship in the results was proven, as evidenced by the increase in the mean diameter of the halos.

After the previous evidence, readings were also performed in another group of Petri dishes (E and F) of the CA-II subgroup (Figure 3) to measure the mean diameter of the inhibitory halos, this time exposed to ketoconazole (Cet) associated with TTO and, in others, only to TTO, at concentrations of 30 $\mu L \cdot g^{-1}$ and 64 $\mu L \cdot g^{-1}$, respectively, equivalent to 50% CI, according to the CA-I crop and in another group, at concentrations of 60 $\mu L \cdot g^{-1}$ and 128 $\mu L \cdot g^{-1}$, respectively, equivalent to the 100% CI evident in the CA-II crop. This methodology was instrumental in establishing a comparison between the microbicidal efficacy of TTO applied alone and, in other samples, associated with ketoconazole, both in the condition of fungal mycelial growth antagonists.

Similar to the tests of the previous hypothesis, the concentration of both ketoconazole and that pharmacologically associated with TTO was increased in order to assess whether there would be optimization in the enlargement of halos. Thus, another group of strains (E and F) of the CA-III subgroup (Figure 4) were prepared and readings were taken in Petri dishes, exposed to ketoconazole (Cet) associated with TTO and, in another set of samples, only to ketoconazole. At concentrations of 120 $\mu L \cdot g^{-1}$ and 256 $\mu L \cdot g^{-1}$, respectively, equivalent to 50% CI, according to culture CA-III and in another group, at concentrations of 240 $\mu L \cdot g^{-1}$ and 512 $\mu L \cdot g^{-1}$, respectively, equivalent to the 100% CI evident in culture CA-II. This was providential for a comparative effect between ketoconazole

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applied alone and, in other samples, associated with TTO, to measure its antifungal potential.

The concentration $f(x)$ as a function of the diameter of the halo $f(y)$, with exposure only to TTO under the taxon Candida albicans, in the so-called CA-I group, in which half of the maximum concentration of the phytopharmaceutical was added, i.e., 16 $\mu L \cdot g^{-1}$ with a minimum halo diameter of 12.49 mm and, at the maximum concentration, the approximate value of 19.17 mm, that is, 32 $\mu L \cdot g^{-1}$. On the other hand, regarding the drug Cet, more intense results were obtained, emphasizing that the suppression of the colonies provided halos with a mean diameter of close to 24 mm at half the maximum concentration of 24 $\mu L \cdot g^{-1}$and a larger halo diameter of around 36.39 mm, at a concentration of 48 $\mu L \cdot g^{-1}$ (Figure 4).

DISCUSSION

In daily clinical analyses, it is common for prescribing agents to commonly encounter obstacles to establishing a reasonable relationship between the drug dose and the intensity of the desired therapeutic response. Not to mention that side effects or adverse reactions that could aggravate the patient's clinical condition need to be avoided, but that, in this context, any undesirable effects in this regard were successfully ruled out due to the protocols adopted.

As drug-resistant microorganisms emerge, alternative treatments have been chosen, among them, the use of essential oils and plant extracts end up emerging as sources of natural medicines. Plant extracts have been shown to be efficient antimicrobials and antifungals, including against the formation of *Candida albicans* biofilm. Regarding mouthwashes, it was observed that these are important tools in the control of the microbiota, complementing the results obtained through mechanical hygiene measures and allowing the gathering, in their formulations, several effective antimicrobial agents to minimize or eliminate dental biofilm. Plant extracts, for example, have their potential curative effects, especially antimicrobial and antifungal[10].

Based on the study developed by[11], the treatment of oral candidiasis can be based on systemic antifungals such as fluconazole, itraconazole, ketoconazole, posoconazole, amphotericin B. In the case of topical treatment, the most viable treatment alternative is nystatin, oral solution (100,000 IU), miconazole, chlorimazole, each with its specific dosage depending on the degree of infection. Each of these treatments can have several specific

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adverse effects, such as: nausea, vomiting, liver damage, after long periods of use. The presence of other *Candida* species in oral candidosis, such as *C. dubliniensis, C. parapsilosis, C. glabrata, C. tropicalis, C. Krusei,* may be partially associated with resistance to treatment with conventional antifungals in some patients. In addition, high rates of infection recurrence are observed, resulting in resistance to the antifungal or the patient's non-adherence to treatment.

Both forms are undesirable to patients and when they emerge, they bring with them consequences greater than the pharmacological benefit and, in this expectation, the aim was to quantify values at the threshold of action with the observance of actions potentially harmful to the individual. All the graphs below were constructed using the formula $\ddot{Y} = \alpha_{Bottom} + (\beta_{Top} - \alpha_{Bottom})/(1+10^{(\chi \cdot Log\ IC50)})$, adapted to the competitive Binding method, calculated by the One Site – Fit *log* IC50 of the non-linearized regressive type aided by the GraphPad Prism software.

This step was conducted by the researcher to conjecture, through robust scientific evidence, the way in which the preparation of a sample of mouthwash (oral mouthwash) should be proposed, on a scale that does not result in a scaly lesion of the oral mucosa, does not harm the enamel and does not reach the dentin of the teeth. Therefore, this required during the pharmacologist's analysis precautionary measures in all pharmacopoeia manipulations used and adequate dosage of the essential oil, these parameters being determinant to avoid unwanted cellular toxicity.

The components commonly present in oral mouthwashes are liquid, fluid or viscous agents with binders, surfactants and humectants and, thus, have potential desquamative action of the epidermal mucosa of the oral tract. The main function of using mouthwash is to help remove debris and fungus adhered to the epidermis of oral gingival tissue or present in the lateral cavities inside the mouth. However, it is still possible to observe important secondary functions in mouthwashes, such as promoting breath freshening and tooth whitening[12].

Mouthwashes, on the other hand, consist of a mixture of the constituents in water or alcohol, are stable and have an acceptable taste, require the addition of flavoring, coloring, preservatives and surfactant (anionic detergents cannot be formulated with cationic antiseptic such as cetylperidine chloride or chlorhexidine). Mouthwashes with alcohol are used to stabilize some components to grant greater longevity to the products, and

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mouthwashes with a high concentration of alcohol are marketed by the manufacturing companies, reaching a concentration of 26% in some cases[13].

Oral mouthwashes as antiseptics are necessary as chemical compounds, and can be natural or modified, originated with the objective of inhibiting microorganisms that cause oral and systemic conditions. These antiseptics should be fast-acting, since their importance is present in their easy targeted access and they are difficult to clean, that is, areas that are not covered by the brushing bristles, should be prescribed by a dental professional[14].

CONCLUSION

Through rigorous analyses, the in vitro efficacy of Melaleuca essential oil against the specimen *Candida albicans* was verified, attesting to the bioactive therapeutic efficacy of the essential oil on the pathogen expressively demonstrated, acted as a potent antifungal inhibitor agent inhibiting its biological growth, when subjected to increasing exposures of the oil, either in an isolated state or when associated with ketoconazole, effectively inhibiting mycelial growth. With each additive increment of the essential oil, the inhibitory response became greater, evidenced by the enlargement of the inhibitory halo as well as by the significant regression in the growth velocity (CV) of the microorganisms *in vitro* and by the number of days (T) respectively.

Thus, it is concluded that the inclusion of the essential oil in toothpastes enables the development of promising pharmaceutical formulas, as happened with mouthwashes derived from the same essential oil, since it has fungicidal properties of clinical interest and by the industry that earns millions of reais annually with these products. The only caveat is that this pharmaceutical formulation is indicated for young people over the age of 12 and for adults and the elderly who enjoy full health and whose formulation is used for up to 60 days (validity) and it is shaken before use, yawned in the mouth and sprinkled outside.

Therefore, this work does not make the relevance of oral mouthwashes on the market unfeasible, but provides an alternative complementary herbal route in dental clinical treatments, facilitated by access to the oil, by the application of the phytopharmaceutical by the patient (user) himself and low financial cost for the execution of the project. Thus, it can be suggested the use of TTO in the elaboration of mouthwashes with maximum values of $[\sigma_{ca}] \leq 128\ \mu L \cdot g^{-1}$, obtaining diameters greater than $D \geq 16$ mm, attesting to the feasibility of

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including the essential oil as an effective herbal agent until total remission of the pathogen less than T≤ 8 days of treatment.

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