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| ORIGINAL

Antifungal analysis of incorporation of the essential oil of *Cymbopogon citratus* (D.C.) Stapf into polymethyl methacrylate

Análise antifúngica da incorporação do óleo essencial de Cymbopogon citratus (d.c.) Stapf em polimetacrilato de metila

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ABSTRACT

Objective: Evaluated the antifungal effect of the incorporation of different concentrations of the essential oil *Cymbopogon citratus* (capim santo), into polymethylmethacrylate (PMMA) against Candida albicans. **Methods**: Fifty specimens were fabricated and divided into five groups: Group 1, PMMA + 10% essential oil (n=10); Group 2, PMMA + 15% essential oil (n=10); Group 3, PMMA + 20% essential oil (n=10); Group 4, PMMA + 25% essential oil (n=10); Group 5, PMMA (n=10). PMMA powder was mixed with the monomer and the mixture was placed in disc-shaped cavities measuring 15 mm in diameter, 2 mm thick. To evaluate the antifungal activity of the experimental specimens, the standard strain of Candida albicans was tested. After incubation, the colony count of each plate was performed using a digital colony counter, obtaining the number of colony forming units (CFU) and the Kruskal-Wallis test was applied. **Results**: There was statistically significant difference in the CFU count of Candida albicans as a consequence of the addition of *Cymbopogon citratus* essential oil to PMMA ($p < 0.001$) and values were significantly higher in comparison with those of all the other groups, when the essential oil was incorporated as incorporated into the PMMA in the concentration of 20%. In the other concentrations, no difference in values was observed in comparison with the Control Group without essential oil of *Cymbopogon citratus*. **Conclusion**: The acrylic resin with the essential oil incorporated into it in different concentrations provided no effect against development of the genus Candida.

Indexing terms: Cymbopogon. Dental prosthesis. Oral candidiasis.

RESUMO

***Objetivo**: Avaliar o efeito antifúngico da incorporação de diferentes concentrações do óleo essencial Cymbopogon citratus (capim santo), em polimetilmetacrilato (PMMA) contra Candida albicans. **Métodos**: Cinquenta corpos de prova foram confeccionados e divididos em cinco grupos: Grupo 1, PMMA + 10% de óleo essencial (n=10); Grupo 2, PMMA + 15% de óleo essencial (n=10); Grupo 3, PMMA + 20% de óleo essencial (n=10); Grupo 4, PMMA + 25% de óleo essencial (n=10); Grupo 5, PMMA (n=10). O pó de PMMA*

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foi misturado ao monômero e a mistura foi colocada em cavidades em forma de disco medindo 15 mm de diâmetro por 2 mm de espessura. Para avaliar a atividade antifúngica dos espécimes experimentais, foi testada a cepa padrão de Candida albicans. Após a incubação, foi realizada a contagem de colônias de cada placa por meio de um contador digital de colônias, obtendo-se o número de unidades formadoras de colônias (UFC) e para isso foi aplicado o teste de Kruskal-Wallis. ***Resultados****: Houve diferença estatisticamente significativa na contagem de UFC de Candida albicans como consequência da adição do óleo essencial de Cymbopogon citratus ao PMMA (p < 0,001) e os valores foram significativamente maiores em comparação com todos os outros grupos, quando o essencial óleo foi incorporado como incorporado ao PMMA na concentração de 20%. Nas demais concentrações, não houve diferença nos valores em relação ao Grupo Controle sem óleo essencial de Cymbopogon citratus.* ***Conclusão****: A resina acrílica com o óleo essencial incorporado a ela em diferentes concentrações não apresentou efeito contra o desenvolvimento do gênero Candida.*

Termos de indexação: *Cymbopogon. Prótese dentária. Candidíase bucal.*

INTRODUCTION

In Brazil, population aging has been particularly rapid, with the elderly increasing from 11% of the economically active population in 2005 to 49% by 2050. Among the main changes in oral conditions found in the elderly are tooth loss and edentulism. In the epidemiological survey of oral health conducted in Brazil in 2010, denominated SB Brasil, the results relative to the use and need for dentures showed that in the age group from 65 to 74 years, only 23.5% of people did not wear some type of dental prosthesis in the maxillary arch and 46.1% did not use dentures in the mandibular arch. Furthermore, in the age group from 35 to 44 years, only 31.2% did not need any type of dental prosthesis [1].

Dentures are made of acrylic resin, methyl polymethacrylate that can undergo deformations and changes in its properties, thereby causing a decrease in their longevity. Moreover they are capable of being a reservoir for microorganisms present in the oral cavity. The internal surface of the dental prosthesis is rough, and not only local factors such as poor hygiene, local trauma, loss of tissue integrity, but also systemic factors such as malnutrition, diabetes mellitus, human immunodeficiency virus infection and xerostomia, contribute to the proliferation of Candida albicans, and to adhesion of this pathogen to removable dentures in 60% of patients who wear them [2].

The development of denture stomatitis is influenced by the denture base material, among other factors. A denture placed in the oral cavity leads to changes in environmental conditions, preventing the mechanical cleaning effect of the tongue and salivary flow, and stimulating the formation and deposit of biofilms on both the dentures and adjacent mucosa [2].

Oral candidiasis, also known as creamy stomatitis or popularly called thrush, is characterized by the appearance of isolated or groups of white plaques adhered to the mucosa. They have a membranous appearance and are sometimes surrounded by an erythematous halo. In denture wearers, it is called denture stomatitis, atrophic candidiasis, or denture-related stomatitis [3]. The treatment of candidiasis can be performed topically or systemically, and topical treatment is represented by the use of polyene antifungal agents, nystatin and Amphotericin B, however, it can have side effects such as nausea, headache, gastrointestinal disorders [4]. From this perspective, the use of natural products for the maintenance of health has been shown to be a feasible alternative, as these products are not only low cost and easily accepted by the population, but rarely have side effects, if correctly used, and have shown the possibility of action at several sites, with diversified activities [5].

In this context, essential oils are outstanding. They are volatile, water-insoluble substances contained in many plant organs. Moreover, they are related to several functions necessary for plant survival and play a fundamental role in the defense against microorganisms. The liposoluble nature of essential oils and their constituents allows their interaction with cellular structures that consist of lipids, resulting in increased membrane permeability. This can cause electrolyte imbalance and cell death [6]. This mechanism of action allows the oils to show activity biological activity, including antiviral activity, such as the essential oil of Rosmarinus officinalis [7], antifungal activity, such as the essential oil of *Cymbopogon citratus* [8] and the essential oil of cinnamaldehyde [9]. Moreover, they have antibacterial activity, such as that of the essential oil of ginger [10].

Lemongrass, *Cymbopogon citratus* (D.C.) Stapf, belonging to the Poaceae family, is an aromatic plant cultivated for the commercial production of essential oil, of which the majority of constituents are generally the citral monoterpenes (isomeric mixture of neral and geranial types) and the myrceno [11]. In Dentistry, the essential oil of *Cymbopogon citratus* has been shown to have antibacterial properties against Staphylococcus spp., Streptococcus mutans [12] and antifungal properties against several Candida species [12-16]. The antimicrobial properties of the essential oil of *Cymbopogon citratus* can be attributed to two main components: alpha-citral (geranial) and beta-citral (neral), aromatic compounds responsible for the citric characteristic of the oil [17]. However, up to now, it is not known whether the properties of the essential oil of *Cymbopogon citratus* are maintained if different concentrations of it are incorporated into acrylic resin.

The objective of the present research was to evaluate the incorporation of different concentrations of the essential oil *Cymbopogon citratus* into polymethylmethacrylate (PMMA) as an antifungal agent for the purpose of guaranteeing the production of a non-toxic antifungal compound for the bases of dental prosthesis and that it has action against Candida Albicans. The hypothesis tested was that there would be no difference in the colony forming unit counts per milliliter (CFU/mL) of Candida Albicans against the incorporation of different concentrations of the essential oil of *Cymbopogon citratus* into PMMA acrylic resin.

METHODS

Ethical aspects

The present study was exempted from submission to the Research Ethics Committee of São Leopoldo Mandic Dental School because it consisted of exclusively laboratory research, without the involvement of human beings, according to Protocol No. 2021-0170.

Description of essential oil

The essential oil selected was from the plant species *Cymbopogon citratus* (D.C.), Stapf (Lemon Grass) of the Peace family. The essential oil of *Cymbopogon citratus* was obtained from a reference company in the sale of essential oils and essences (Quinari, Ponta Grossa, Paraná, Brazil), submitted to chemical analysis and was later prepared for assessment of the Minimum Inhibitory Concentration (MIC).

Determination of the minimum inhibitory concentration

The Minimum Inhibitory Concentration (MIC) was performed in accordance with the reference method for Broth Dilution Tests to determine the sensitivity of yeasts to antifungal therapy.

Thus, 96-well U-bottom plates were used, in which 100 µL of the microbial inoculum added to the broth was adjusted to obtain a cell concentration of 2.5 x 103 CFU/mL and was added to the wells. Right after this, 100 µL of *Cymbopogon citratus*, were added, which was evaluated at concentrations ranging from 1,000 to 15.62 µg/mL against strains of the Candida genus. The plates were incubated in an oven at 35oC for 24h. Subsequently, the results were visually read by observing the formation of fungal cell clusters at the bottom of the wells. Then 50 µL of TTC dye (2,3,5 - triphenyltetrazolium chloride) were placed in each well of the plate, to confirm the presence of viable microorganisms, and the plate was again placed in the oven at 35oC. for 24h. In the second readout, wells containing live microorganisms that were stained red were considered. The test was performed in triplicate and the lowest concentration of the product capable of visibly inhibiting the growth of microorganisms was considered the MIC.

Preparation of thermally activated acrylic resin (PMMA) samples

Initially, ten cylindrical aluminum test specimens 15 mm in diameter and thickness of 2 mm were fabricated. The solid aluminum test specimens were included in a glass muffle (VIPI-STG, Vipi, São Paulo, Brazil).

The technique for inclusion of samples was similar to that used for the inclusion of a Complete Denture. The internal walls of the muffle and countermuffle base were coated with Vaseline, then the muffle base was filled with type III stone plaster, by mixing 150 g of plaster with 45 mL of water, and then spatulated under vibration of a muffle vibrator (Biotron®, Santa Rita do Sapucaí, MG, Brazil), up to the edge of the muffle base. A plaster spatula was used to perform surface finishing to obtain a uniform, smooth finish. The aluminum test specimens were distributed among the samples thus obtained with a distance of 1 cm between them by applying light pressure and waiting for 30 minutes for setting to occur.

Subsequently, the aluminum test specimen and plaster component were insulated with solid Vaseline, using a soft #14 brush. Laboratory silicone putty (Zetalabor, Zhermack, Badia Polestine, Italy) was manipulated with the universal catalyst (Zetalabor, Zhermack, Badia Polestine, Italy). Silicone was distributed on the aluminum test specimens and plaster surface for the purpose of molding them. Immediately after this, type III stone plaster (Vigodent S.A. Indústria e Comércio, Rio de Janeiro, Rio de Janeiro, Brazil) was manipulated using a proportion of 200 g plaster to 60 mL water, and placed in the countermuffle, filling it completely, under the action of a muffle vibrator for 3 minutes.

After 30 minutes, the muffle was separated from the countermuffle, and the ten aluminum test specimens were removed. Then, a soft brush was used to isolate the plaster with ISOCRIL acrylic resin insulator (VIPI Indústria Comércio, Exportação e Importação de Produtos Odontológicas, LTDA, Pirassununga, São Paulo, Brazil).

The essential oil of Cymbopogom citratus was incorporated into the acrylic resin polymer (PMMA) forming a homogeneous mass and then the monomer was added. The test specimens were then divided into five groups (n=10): Group 1 - PMMA + 10% essential oil; Group 2 - PMMA + 15% essential oil; Group 3 - PMMA + 20% essential oil; Group 4 - PMMA + 25% essential oil; Group 5 - PMMA (Control Group).

In the case of the Control Group, the colorless PMMA, thermopolymerizable by microwave energy (VipiWave, VIPI, Indústria, Comércio Exportação e Importação de Produtos Odontológicas Ltda, Piratinga, SP, Brazil), was manipulated according to the manufacturer's instructions. The proportion 6.5 mL of liquid/14 g of powder was used and was mixed in a glass jar with a lid, by using a No. 36 spatula (Golgran ®, São Caetano do Sul, São Paulo, Brazil). The acrylic resin powder was weighed on an analytical balance with a precision of 0.0001g (Ax 200 Shimadzu, Kyoto, Japan), while the monomer was added by using a graduated pipette (1/100 mL) coupled to a pipetting device (Cralplast, Cotia/SP - Brazil).

After manipulation, a waiting time was observed to allow the acrylic resin in all groups to reach the plastic phase before introducing it into the spaces created by the aluminum replicas. The muffle was closed and taken to a hydraulic press, slowly placed under pressure for 20 minutes, until 1 ton was attained. The muffle screws were tightened, one hour was waited for starting polymerization. The acrylic resin was polymerized in a microwave oven, together with a glass receptacle containing 120 mL of water.

The cycle used for microwave processing (Perfect 800W, Panasonic, Japan) was the type recommended by the manufacturer, with an initial 20 minutes at a power of 2w, followed by 5 minutes at a power of 6w. After the polymerization cycle, the muffle was removed from the microwave oven and placed on the bench for a period of 24 h, to allow slow cooling to room temperature to occur.

The samples were removed from the muffles, and excess resin was removed with a MaxiCut drill (Maillefer SA, Ballaigues, Switzerland), mounted on an electric motor (LB -100- Beltec). Subsequently, the specimens underwent the final finishing process, using abrasive paper of decreasing granulation 150, 240, 600, 1200 and finally polishing with a disc soaked with aluminum paste, all mounted on a sanding device at 100 RPM under cooling, to simulate the procedure of fabricating the dental prosthesis.

All specimens were submitted to an ultrasonic bath (BioNash STP, Bio Art, São Paulo, Brazil) with distilled water for 15 minutes, for the purpose of removing polishing debris. Afterwards, they were gently dried with absorbent paper and stored in individual flasks.

Antifungal evaluation

To evaluate the antifungal activity of the experimental specimens the standard strain of Candida albicans ATCC 1023 was tested. The Candida strains used were recovered from the cell bank of the Research Laboratory of the University of São Leopoldo Mandic and the culture medium used was Sabouraud Dextrose Agar. The fungal suspension was obtained from colonies isolated on agar plates and inoculated into YPD (yeast extract peptone dextrose) broth medium and incubated at 37°C for 24 h.

To observe growth of the microorganism, the test tubes were shaken in a mechanical shaker for 30 s and the optical densities of the fungal suspensions were analyzed by spectrophotometry, for the purpose of standardizing them at a concentration of 106 cells/mL.

The Candida albicans biofilm was formed in 12-well cell culture microplates. Initially, each previously sterilized acrylic resin specimen was placed in each well of the plate and then 2 mL of fungal cell suspension (107 cells/mL) would be added to each corresponding well.

With the culture plate previously prepared with the suspension, the specimens were incubated at 37°C for 90 minutes in an orbital shaker at 75 rotations per minute (rpm) for the purpose of promoting adhesion of the microorganism to the specimen surfaces.

After the adhesion phase, the specimens were transferred to new wells and the non-adherent or weakly adherent cells were removed by careful washing that would be performed twice with 0.1M Phosphate Buffer (PBS) solution. To promote biofilm growth and maturation, 2 ml of YPD broth were added to each well and then the plates were incubated at 37°C for 48 h in an orbital shaker at 75 rpm under aerobic conditions.

After the incubation period, the plates were removed, and the wells were gently washed with the PBS solution. After washing, the specimens were transferred to test tubes containing 4.5mL of sterile saline solution (0.85%) and shaken for 20 minutes under ultrasound to detach cell clusters from the biofilm. The resulting suspension containing the biofilm cells that had been detached was vortexed and seeded in Petri dishes with Saboraud Dextrose Agar medium with 5μg/mL Chloramphenicol. Afterwards they were incubated at 37°C for 48 h under aerobic conditions.

After incubation, colony counts of each plate were performed using a digital colony counter. After obtaining this value in the culture media, the number of colony forming units (CFU) was expressed on a logarithmic scale (CFU+1) / mL.

Statistical analysis

Descriptive analysis of minimal inhibitory concentration data was performed. Considering that the data relative to colony forming unit counts of Candida albicans did not meet the normality and homogeneity of variance requirements, the Kruskal-Wallis test was used to investigate the effect of incorporating different concentrations of esssential oil of *Cymbopogon citratus* into PMMA. For multiple comparisons, the Dunn test was used.

The statistical calculation were performed with the aid of the SPSS 23 program (SPSS INC. Chicago, IL, USA) adopting a level of significance of 5%.

RESULTS

Minimum inhibitory concentration

Table 1 shows a summary of the data on the minimum inhibitory concentration of essential oil of *Cymbopogon citratus* in relation to Candida albicans, from which the minimum inhibitory concentration was observed to be higher than 0.0156% and lower than or equal to 0.0313%.

Table 1. Mean values and standard deviation of the median of colony forming units of *Candida albicans*, according to the concentration of essential oil of *Cymbopogon citratus* incorporated into PMMA.

Concentration of essential oil of *Cymbopogon citratus*	Mean (Standard Deviation)	Median
10%	$4.97x10^4$ ($3.84x10^4$)	$5.13x10^4$ A
15%	$4.97x10^4$ ($5.72x10^4$)	$1.04x10^5$ A
20%	$7.33x10^5$ (1.64x105)	7;35x10^5 B
25%	$6.06x10^4$ ($3.78x10^4$)	$5.47x10^4$ A
Without incorporation	$1.15x10^5$ ($1.09x10^5$)	9,67x10^4 A

Legend: Groups of which median values are followed by different letters differed significantly from each other.

Colony forming unit counts

Two samples from the group incorporated with 20% essential oil of *Cymbopogon citratus* were excluded from the analysis due to external contamination. For the same reason, a sample of the group with the addition of 25% essential oil of *Cymbopogon citratus* was also discarded.

The Kruskal-Wallis test showed that there was a statistically significant difference in the count of de Candida albicans colony forming units depending on the concentration of essential oil of *Cymbopogon citratus* that was incorporated into PMMA ($p < 0.001$). The Dunn test identified that the number of colony forming units was significantly higher in comparison with all the other groups when PMMA was incorporated with 20% essential oil of *Cymbopogon citratus* (figure 1). The groups in which 10%, 15% or 25% were added did not differ significantly from each other; the number of colony forming units of Candida albicans showed no difference in comparison with the Control Group without essential oil of *Cymbopogon citratus*.

Table 2 shows a summary of the data on the minimum inhibitory concentration of essential oil of *Cymbopogon citratus* in relation to Candida albicans, from which the minimum inhibitory concentration was observed to be higher than 0.0156% and lower than or equal to 0.0313%.

DISCUSSION

In Brazil, *Cymbopogon citratus* is known by several popular names: holy grass, lemon grass, citronella grass, among others [18]. The main component of essential oil of *Cymbopogon citratus* is citral, an aldehyde formed by the monoterpene geranial in its cis- and trans- configurations, in addition to other photocompounds, such as myrcene, sulcatone, and aromatic compounds. The plant can perform several pharmacological activities, as it has antiamoebic, antibacterial, anti diarrheal, antifungal and anti-inflammatory properties [12,19-22]. Domingues et al. [16] have cited that terpene compounds found in essential oil of *Cymbopogon citratus* interact with lipid compounds in the cell structure,

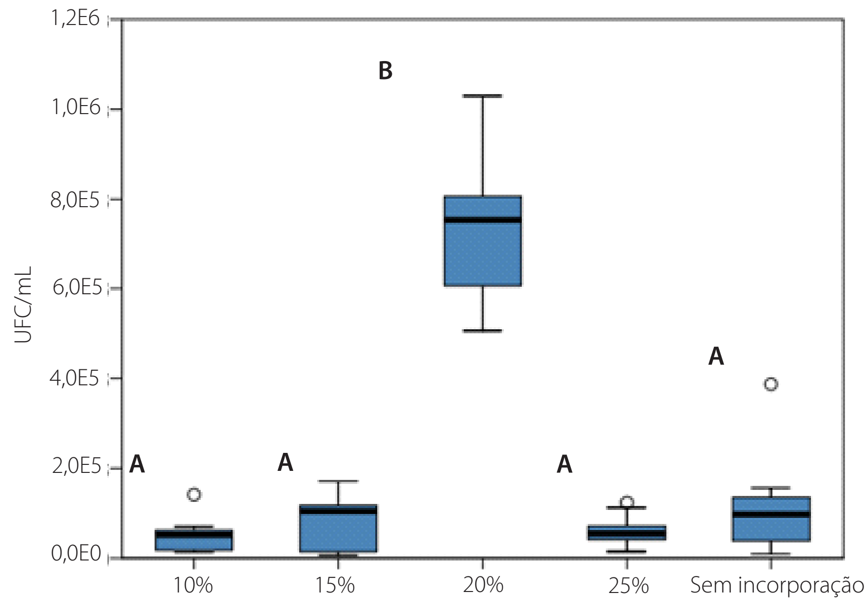

Figure 1. Box diagram of the values of colony forming units of Candida albicans, according to the concentration of essential oil of Cymbopogon citratus incorporated into PMMA.

Legend: Treatments/groups indicated with the same capital letters did not differ significantly from each other.

Table 2. Mean values and standard deviation of the difference in absorbance in comparison with the blank, according to the concentration of essential oil of *Cymbopogon citratus*.

Concentration of essential oil of *Cymbopogon citratus*	Mean	Standard Deviation
0.5000%	0.004	0.002
0.2500%	0.007	0.002
0.1250%	0.006	0.003
0.0625%	0.004	0.002
0.0313%	0.005	0.003
0.0156%	0.034	0.005
0.0078%	0.073	0.025
0.0040%	0.107	0.027
0.0020%	0.108	0.037
0.0010%	0.096	0.037
0.0005%	0.093	0.025
0.0002%	0.116	0.020

thus interfering with the biosynthesis of the cell wall of fungal cells. However, there may be variations in the amounts of the components extracted, as they result from to the difference in the place and periods of collection for the purpose of extracting the essential oil [23].

Addition of essential oil to the acrylic resin commonly used for denture bases can promote antifungal properties that are beneficial to patients who make use of this type of rehabilitation. In this study, the essential oil of *Cymbopogon citratus* was incorporated into the acrylic resin in different concentrations. The null hypothesis that there would be no difference in the colony forming unit counts per milliliter (CFU/mL) of Candida albicans with the incorporation of different concentrations of essential oil of *Cymbopogon citratus* into PMMA acrylic resin was rejected, because among

the concentrations tested, the 20% had a statistically higher CFU/mL count when compared with the other groups, which did not differ from each other.

Thus, in the present study, there was no antifungal effect when the essential oil of *Cymbopogon citratus* was incorporated into the acrylic resin since the growth of Candida albicans occurred and the counts were similar to those of the Control Group, without the essential oil. In a similar manner, Marra et al. [24] evaluated the antimicrobial activity of an acrylic resin combined with an antimicrobial polymer poly (2-tert-butylaminoethyl) methacrylate (PTBAEMA) and demontrated that it showed significant antimicrobial activity against the biofilm of Streptococcus aureus and Streptococcus mutans but was ineffective against the biofilm of Candida albicans. Differently from bacteria, the fungus has a cell wall composed of approximately 80 to 90% carbohydrate. Furthermore, the microfibrillar polymers (b-glucans and chitin) represent the structural components of the wall, giving rise to a rigid skeleton that confers strong physical properties on the cell [25]. Wady et al. [26] incorporated silver nanoparticles (AgNPs) into an acrylic resin denture base and observed no effect on adherence and biofilm formation of Candida albicans. The findings of this study corroborate those of Domingues et al. [16], Marra et al. [24] and Wady et al. [26] and since the fact that there was fungal proliferation, this allows the supposition that the rigid skeleton of Candida albicans protected it against the modified resin and did not allow the antimicrobial agent to displace the Ca2+ and/or Mg2+ ions from the outer cell wall.

Several authors have studied the antifungal activity of the essential oil [12,27,28] in vitro, corroborating the finding of this study. Sousa et al. [9] conducted an in vitro evaluation of the antifungal activity of PMMA modified by the essential oil of cinnamaldehyde in concentrations of 12% and 24%. The concentration of 24% showed antifungal potential and was considered a promising therapeutic method for the prevention of candidosis. In the present study, however, the essential oil in *Cymbopogon citratus* showed no antifungal activity. Due to the temperature inside the muffle during the polymerization process, it may perhaps have caused the evaporation of the essential oil. Moreover, the concentration of 20%, an intermediate concentration among the others tested, showed a higher CFU/mL count than that of the other concentrations.

Using different methodologies, other researches have had results that differed from those of the present research. Mat-Rani et al. [27], for example, in an in vitro study, demonstrated the effectiveness of essential oil of *Cymbopogon citratus* ranging from 0.31% to 5%in the elimination of biofilm of Candida albicans on discs made of silicone, simulating dental prostheses. In a similar study [28], the antifungal effect of cinnamon and lemongrass essential oils at a concentration of 5% was demonstrated, applied to polymerized acrylic resin discs that contained a pre-established biofilm of Candida ssp. In a similar manner, essential oil of *Cymbopogon citratus* was shown to demonstrate fungistatic activity on biofilm of Candida Albicans formed on acrylic resin specimens [12]. The antifungal efficacy in these studies was evaluated with the specimens that had previously been polymerized, differently from the present study, in which the essential oil was incorporated into the acrylic resin powder before polymerization occurred. The majority of essential oils exert their antimicrobial activity through changes in the cell wall structure of the microorganism, by increasing the permeability of the cytoplasmic membrane and promoting the deterioration of processes that are essential to cell survival [29]. In the case of the present study, it is believed that the essential oil molecules may have been trapped inside the polymer or perhaps as a result of the temperature inside the muffle during the polymerization process, it may have caused the essential oil to evaporate.

Santos et al. [8] evaluated the antimicrobial activity of essential oil of *Cymbopogon citratus* on Candida albicans and verified that the MIC was 1250 and 630 μg mL-1-1. Silva et al. [22] found values for MIC that were about 100 times lower than those reported by Santos et al. [8]. In this study, the values found for MIC were higher than 0.0156% and lower than or equal to 0.0313%. In spite of the MIC being so low, we chose the concentration based on studies by Marra et al. [24], who evaluated the antimicrobial effect of acrylic resin combined with an antimicrobial polymer (PTBAEMA) at concentrations of 10% and 25%, but there was no antifungal effect. In a similar manner, Sousa et al. [9], who evaluated the antifungal activity of acrylic resin modified with essential oil of cinnamaldehyde in concentrations close to 12% and 24% and showed that 24% had strong antifungal potential. In this study, we used percentages of essential oil of *Cymbopogon citratus*, similar to those used in the above-mentioned studies.

Further studies must be conducted to determine the antimicrobial activity and cytotoxicity of this association of essential oil of *Cymbopogon citratus* with acrylic resin after its polymerization, in the form of solution or varnish, and evaluate possible changes in the physicochemical properties of the acrylic resin, such as flexural strength, surface roughness, Vickers hardness and color stability.

Within the limitations of this study, unfavorable results were detected when the essential oil was incorporated into the liquid of acrylic resin with the purpose of forming a product with antifungal activity. Perhaps if it were used as a type of "varnish" material, coating the samples and not being incorporated into the acrylic resin, the results could be different from those observed in the present study.

CONCLUSION

The essential oil incorporated into the composition of acrylic resin provided no antifungal effect against development of the Candida species.

Collaborators

RC Santos, writing- first essay. MGC Santos, Writing proofreading. TFO Carneiro, methodology. FLB Amaral, project administration

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