EVALUATION IN VITRO OF ANTIMICROBIAL ACTIVITY OF BASIL EXTRACT (*Ocimum basilicum L.*) ON AN ACRYLIC SURFACE OF REMOVABLE ORTHODONTIC APPLIANCES

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ABSTRACT

AIM: The aim of this study was to evaluate in vitro the antimicrobial activity of Ocimum basilicum L. (basil) extract in S. Muttans biofilm colonized in specimens confectioned in the same acrylic used for removable orthodontics appliances. **MATERIAL AND METHODS:** To perform this work, 42 specimens were confectioned (sterile spherical acrylic disks) and immersed in pure extract and in serial dilution of extract (1:2 until 1:10) during 24, 48 and 72 hours. For each time of exposition, a disintegration of bacterial film by sonication in saline and posterior seed in agar, for colony count, were carried out. ATCC strains of S. muttans were selected, and 2% chlorhexidine solution was used as inhibition control. **RESULTS:** The results showed, by quantitative analysis, that basil extract has antibacterial activity in S. muttans biofilm, when used in pure state or in dilution until 1:4. **CONCLUSION:** Besides, it was possible observe the more increase the incubation time, independent the dilution, the higher degradation of extract.

KEYWORDS

Biofilm. Biomaterial. Vegetable extracts.

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INTRODUCTION

Orthodontic appliances may change the oral microbiota, and produce physical, chemical and biological changes in it[1], mainly by the introduction of new retentive areas which facilitate the micro-organisms colony. Removable orthodontic appliances favor a fast microbial colonization due to the hydrophobicity and high content of surface free energy in acrylic resin (polymethylmethacrylate)[2].

The control of bacterial biofilm within the several dentistry specialties is very important, because points both to the prevention and the treatment of diseases. One of the ways to decrease the plaque accumulation on the acrylic of removable orthodontic appliances is their cleaning and sanitation, by abrasive methods or disinfectant solutions. However, Suga et al (2005)[2] proved that the chemical method is more efficient when compared to the mechanical one for removable appliances confectioned in acrylic resin.

Among chemical agents for bacterial biofilm control, Chlorhexidine digluconate is considered the Golden standard due to its good antimicrobial effects and its high substantivity[3]. However, Chlorhexidine digluconate may bring side effects, like stains on teeth, on restorations, prosthesis, desquamation of oral mucosa, reduction of taste sensitivity and formation of supragingival calculus before its prolonged use[4]. In this context, it seems to have great value the discovery of a chemical agent less aggressive to the oral cavity that can be used routinely, no side effects which can damage the patient's health.

Herbal antibacterial and antifungal potential has been searched against several oral pathogens[5-8] and demonstrated effective results. The use of these products based on medicinal plants is very interesting by the economical viewpoint, because they become products with less time for production, and consequently they are more accessible for population[9].

Based on the previously exposed, this work had as aim to evaluate *in vitro* the antibacterial action of basil extract (*Ocimum basilicum L.*) before the bacterial plaque present in acrylic resin specimens, simulating the removable orthodontic appliance.

MATERIAL AND METHODS

For this study, the extract of *Ocimum basilicum L.* (compounding pharmacy-SANTA PAULA-Araraquara-SP) Araraquara-SP, Brazil) was used in pure state, saline at 1:2, 1:4, 1:6, 1:8, 1:10, and 2% Chlorhexidine digluconate (compounding pharmacy-SANTA PAULA-Araraquara-SP) Araraquara-SP, Brazil).

The strains of *S. muttan* pattern used in this work were gently donated by Prof. Dr. Clovis Wesley de Oliveira, of Department of Morphology and Parasitology of Federal University of São Carlos-UFSCar, Brazil. The strains were conserved in nutrient Agar, and they were reactivated in the moment of use.

From a silicone rubber mold ASB-10 (Polipox – Indústria e Comércio Ltda. – Cesário Lange, SP, Brazil) disks with 3mm diameter by 1mm thickness, as 42 specimens were confectioned using self-polymerizable acrylic resin (VIPI - Indústria, Comércio, Importação e Exportação de produtos Odontológicos Ltda, Pirassununga, São Paulo, Brazil) following the manufacturer recommendations.

After the complete polymerization of the acrylic resin, the disks were washed, dry and sterilized in 70% alcohol, where they were immersed during half hour. Next, they were dry again in biological cabinet and aseptically stored in sterile test tubes.

For evaluation of antibacterial activity of extracts, the disk agar diffusion method was used, based on the technique described by Bauer *et al*. (1966)[10], and adaptations by Bertini *et al*. (2005)[11] and Lima *et al*. (2006)[12]. A culture middle Agar base Tryptic Soy Broth (TSB) (HIMEDIA®) 6g and D-glucose 15g, specific for *S. mutans*, according with manufacturer instructions, was used, and next the *Streptococcus muttans* was introduced in the TSB during 24 at 35-37 ^{0}C, after reactivation of frozen strain. To obtain biomass, the culture middle was centrifuged at 1500 rpm during 15 min. Supernatant and re-suspended biomass 10 ml BPS were removed. Bacterial inoculation was the representative of Mac Farland scale at 0.5, what correspond to 10^8 UFC/mL for micro-organism in study.

In order to simulate the bacterial biofilm adhered to the appliance, it was induced, for the duplicated micro-organism and for each agent, the formation of bacterial microfilm in the specimens in a period of 24, 48, 72 hours in polystyrene plaques with 24 orifices.

In biological cabinet, an aliquot of 500µL of bacterial suspension at 0.5 Mac Farland scale was added for each micro-organism in study, and a specimen sterilized and incubated in the different time periods inside a bacteriological incubator at 35-37^{0}C. This procedure was repeated for each solution and control test group as follows: 1. line (A): 1 mL TSB broth supplemented with 0.25% glucose + *Streptococcus muttans* cells (100 µL) + basil in different concentrations: pure (1), 1:2 (2), 1:4 (3), 1:6 (4), 1:8 (5), 1:10 (6): period: 24h; 2. line (B): 1 mL TSB broth supplemented with 0.25% glucose + *Streptococcus muttans* cells (100 µL) – positive control; 3. line (C): 1 mL TSB broth supplemented with 0.25% glucose + *Streptococcus muttans* cells (100 µL) + 2% Chlorhexidine – positive control; 4. line (D): 1 mL TSB broth supplemented with 0.25% glucose + *Streptococcus muttans* cells (100 µL) + basil in different concentrations: pure (1), 1:2 (2), 1:4 (3), 1:6 (4), 1:8 (5), 1:10 (6): period: 48h.

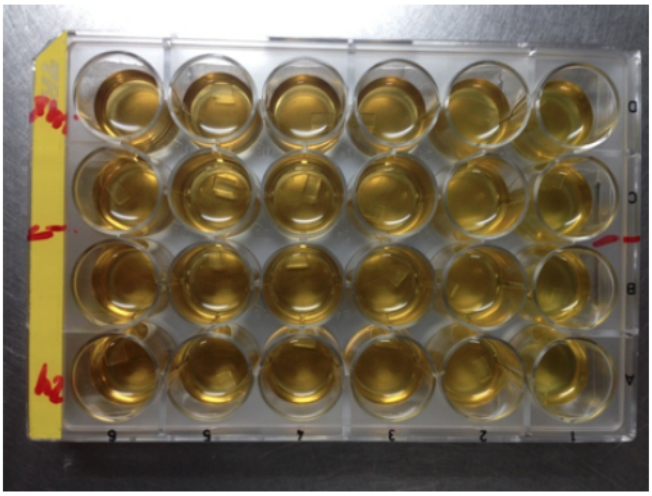
Another plaque in similar way was used; however, during 72h of incubation.

After the periods of incubation (24, 48 and 72 hours), the specimens were removed with use of a sterilized tweezers, washed in saline (PBS buffer solution) to withdraw non adhered bacteria.

After the washing process, the specimens regarded to the micro-organism testes were placed in conical plastic tubes 15mL (Falcon) containing saline 2.0 mL (PBS) and submitted to mild sonication during 8 minutes at 80W (Figure 1), following fast homogenization (30 seconds) in vortex. After the bath, a sample of 1.0 mL was obtained from each tube, and transferred for plastic tubes for micro-centrifugation type *eppendorf*. The plastic tubes for micro-centrifugation type *eppendorf* were centrifuged during 10 minutes at 9500g, and the biomass re-suspended in 1.0 mL of sterile saline. After these procedures, serial dilutions (10^{-1} – 10^{-6}) were carried out for each bacteria recovered from the specimens surface.

To obtain the appropriate value of the number of colonies, the sample to be counted always has to be diluted. To perform serial dilutions, the bacteria must be in liquid middle. For extract dilution, 10 µL of bacterial suspension was seed in triple on the Petri agar TSA (tryptic soy agar). For the result, we pipette in triple the biofilm disaggregated of sonication to visualize the *S. muttans* bacteria.

Figure 1. Specimens in different solutions and incubation periods.



The number of bacteria per mL in the original culture was calculated by the multiplication of the number of colonies counted by the dilution factor: number of cells per mL = number of colonies x dilution factor (quantitative analysis method).

RESULTS

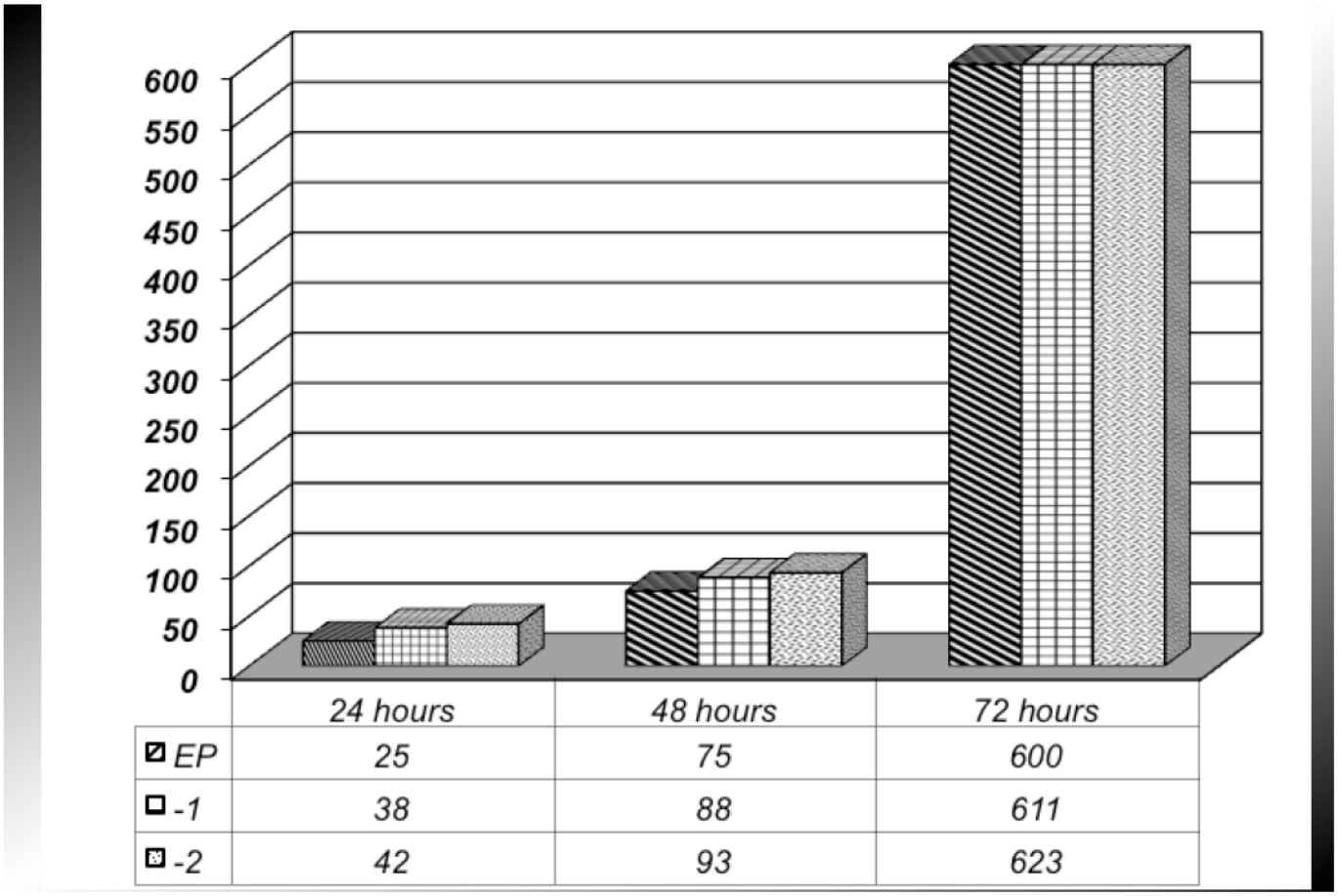
As expected, the positive control group did not demonstrate inhibition of bacterial growth, and in the negative control (2% chlorhexidine) the inhibition of growth was total. Regarding to the basil extract, the most effective inhibition was the action of the pure extract, decreasing according to the dilution.

The Minimum Inhibitory Concentration found was 1:4, because dilution at 1:6 and 1:8 demonstrated similar results to the positive control group. The basil action was better in biofilms of 24 hours of development, independent of the dilution degree (Table 1 and Graph 1).

Table 1. Minimum Inhibitory Concentration of *Ocimum basilicum* (basil) extract in *S. muttans* biofilm on acrylic surface.

Biofilm time	Inhibition of growth with (UFC) Extract concentration (mg/mL)						
	Control +	Control (-)	EP	1:2	1:4	1:6	1:8
24 hours	10^8	0	25	75	600	$>10^8$	$>10^8$
48 hours	10^8	0	38	88	611	$>10^8$	$>10^8$
72 hours	10^8	0	42	93	623	$>10^8$	$>10^8$

Graph 1. *Ocimum basilicum L.* (basil) action of pure and diluted extract. EP: pure extract; dil -1: dilution 1:2; dil -2: dilution 1:4.



DISCUSSION

This study evaluated the use of basil extract diluted in saline in order to verify the utilization of herbal products with any chemical addition on the efficiency of anti-bacterial in buccal bacteria. This purpose meets the World Health Organization, which stimulates the study and the use of regional herbs as medicine, as a way to reduce costs of public health programs, mainly in countries in development[13], because it could enable the home use of herbs, evidently after scientific prove for human use.

Another factor that stimulated this study was how benefit would be found a substitution to Chlorhexidine digluconate,

which despite to be considered golden standard of antimicrobial agents[3] produces side effects as strains on teeth, on restorations, on prosthesis and tongue, desquamation of oral mucosa, reduction of the sensitivity of taste and formation of supragingival calculus when its use is prolonged[4].

Herbal action against *S. muttans* is successfully reported in the literature: Drumond *et al.,* in 2004[14], evaluated antibacterial action of several herbs and reported its effectiveness in products from Mallow; Vasconcelos *et al*., in 2006[6], evaluated anti-microbial effect of *P. Granatum* gel against *S. mutans* bacterial strains, suggesting the use of the herb in the control of adherence of these micro-organisms in the buccal cavity; and Melo et al. (2006)[15] proved antimicrobial activity of *Anacardium occidentale L*. extract of the stem bark, against de *S. mitis, S. muttans* and *S. Sanguis* cultures.

Still with the aim to eliminate the *S. muttans*, Soares *et al*., in 2008[7], tested the crude hydro alcoholic extract of *barbatimão* over *S. muttans* standart strains, and the results demonstrated antibacterial activity in the extract. Bezerra *et al*., in 2013[16], also reported great potential of several herbs, such as citronellol, linalool, thymol, and D-limonene against *S. Muttans*, detaching thymol and citronellol.

Ocimum basilicum L., known as basil, presents several applications, revealing great herbal potential, with antimicrobial action in different areas of study[18-21]. However, regarding to the evaluation of specific bacterial potential of basil against micro-organisms in the oral cavity, few works can be mentioned among them: Rao et al. (2011)[22] who proved that *S. muttans* is sensitive to the basil essential oil, and Yosephine *et al*. (2013)[8] , who proved antibacterial effect of solutions base basil essential oil. Despite the use of basil essential oil, the studies mentioned above corroborate with the results found in this work. However, it is important highlight that this study proved, that after 24 hours, the action is widely reduced, suggesting degradation both of diluted extract in saline and the pure extract interfere in its bacterial action.

Suga et al (2005)[2], aware of the need of bacterial control in patients using removable orthodontic appliances, verified the kind of polishing, if chemical or mechanical, performed in the internal face of appliances would not influence the adhesion of *S. mutans*, and that chemical action is more effective than the mechanical to remove the same bacteria, suggesting the association of both techniques to improve the antimicrobial action on the acrylic.

Based on this study, acrylic samples used were not submitted to the chemical polishing and even removal by mechanical brushing, but only the chemical action of basil extract.

Before the limitations of *in vitro* study, it is important detach that findings might not correspond to the real behavior of products tested *in vivo*, once they were not exposed to the same condition of the oral cavity. However, laboratorial works, like this one developed, are necessary to provide subsidies for clinical tests. Thereunto, the research must be continuous in order to establish a protocol of use to be applied *in vivo*.

CONCLUSION

Based on the methods used, it is possible assert that: 1. *Ocimum basilicum L*. (basil) extract has anti-bacterial activity in the *Streptococcus muttans* biofilm; 2. ideal CIM of *Ocimum basilicum L*. (basil) found was the pure extract or in dilution until 1:4, other dilutions demonstrated themselves ineffective; 3. the time exposition of the extract leads to its degradation.

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