IN VITRO EVALUATION OF ANTI-ADHERENCE ACTIVITY OF THE *OCIMUM BASILICUM L.* (BASIL) EXTRACT AND OF THE *CORIANDRUM SATIVUM L.* (CORIANDER) IN ACRYLIC SURFACE OF REMOVABLE ORTHODONTIC APPLIANCES

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ABSTRACT

AIM: The aim of this study is evaluate the antimicrobial activity of the *Ocimum basilicum L.* (basil) extract and of the *Coriandrum sativum L.* (coriander) extract in biofilm of *Streptococcus mutans* colonized in specimens confectioned in acrylic used to confection removable orthodontic appliances. **MATERIAL AND METHODS:** To perform this work, specimens were confectioned (spherical discs in sterile acrylic) which were immersed in extracts pure and in series dilutions (1:2 a 1:128) by 24, 48 and 72 hours. For each time of exposition, the disintegration of bacterial films was performed by sonication in saline solution. **RESULTS:** The results obtained were satisfactory for the extracts studied on the inhibition of biofilm formation. **CONCLUSION:** We conclude that polyphenols present in the extracts interfered on the glucan synthesis engine, possibly inhibiting the enzymes (glucosyl- and fructosyl transferase) which synthesize the extracellular polymers, also acting as antioxidant, and therewith they presented antimicrobial activity.

KEYWORDS

Biofilm. Vegetal extracts. Orthodontic appliances.

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INTRODUCTION

In the last years there was scientific advance involving studies on the medicinal plants in order to obtain new composites with therapeutic properties[1]. The appearance and spread of microorganisms resistant to antimicrobials available in the Market have been reported through decades, what encourages the search for new sources of substances for this purpose, like plants used in the traditional medicine[2,3].

Several microorganisms of dental biofilm are associated to the caries and periodontal diseases. *S. mutans* is one of the most related oral microorganisms. It is believed that it is involved to the initial development of these problems because it is able to colonize teeth[4].

The biofilm formation is seen as a process of growth well regulated, which presents as a result the formation of a complex community of microorganisms. This formation involves some physical, chemical and biological interactions, which results in adherence, colonization and growth[5]. The biofilm might be formed during hours, and because they are tolerant to the microbial agents, they are difficult to remove.

Microorganisms, when associated to biofilms, become more resistant[6]. The safest way to remove this microorganism is the mechanical one; however, the chemical agents are useful[7]. The presence of retentive areas on solid surfaces represent more preferably for colonization by some microorganisms. Therefore, the use of orthodontic appliances and prosthetic devices contribute to the retention of the biofilm and feed[8]. Bacterial cells adhered to the surface of materials are a signal or a target for specific bacterial receptors to be liked[5,6].

Bacterial microfilm control within the several dentistry specialties is very important because it pints both for prevention and treatment of diseases. In order to help the conventional methods of oral hygiene (tooth brushing, dental floss or tape) several chemical agents have been studied, among them the chlorhexidine gluconate. However, the frequent use and for long time of this substance presents some undesirable effects. Therewith, the curative effect of some medicinal plants was noticed out; they are used for thousand years and are base for studies for production of new drugs[9].

Medicinal plants constitute important therapeutic resources for treatment of diseases and serve both as the known *home medicine* and as raw material to elaborate phytotherapy[10].

Microbial activity of vegetable extracts is evaluated through determination of a small quantity of substances necessary to inhibit the growth of microorganism-tests; this value is known as Minimum Inhibitory Concentration (MIC). A very relevant aspect to determine the

MIC of vegetable extracts is the concern regarding to the toxicological, microbiological and legal features related to the natural composites or their combination[11,12].

Owing to the use of plants, many times in an empirical way, this work evaluated the action *in vitro* of anti-adherence action of two vegetable extracts, the basil extract (*Ocimumbasilicum L.*) and the coriander extract (*CoriandrumSativum L.*) which presented great potential of application as antimicrobial agent, as medicinal agent, as flavor in food and also fragrance in pharmaceutical products[13].

MATERIAL AND METHODS

The study was performed um the Microbiology Laboratory of the University Center of Araraquara - Uniara. To evaluate the effect of the antimicrobial agent on the cells of the biofilm.

It was confected 100 specimens in acrylic, equal to the removable orthodontic appliances in the measures of 0.5mm by 0.3mm. They were washed in water and neutral soap, rinsed in sterile distilled water and, next they stay in chlorhexidine 10% for 2 hours. After this period, they were rinsed again in sterile distilled and sterilized under ultraviolet light in biological cabin by a half hour. The specimens were stored aseptically in sterile test tubes.

The *Ocimum basilicum L*. (basil) and *Coriandrum sativum L.* (coriander) extracts were used, and they were obtained from the enterprise Biotae/SP.

In this study, the *S. mutans* (ATCC 25175) standard strains were used. They were assigned by Professor Alessandra N.S. Rastelli, of the Dentistry School of Araraquara - Unesp. The strain was refrigerated and conserved in cryoval with glycerin nutrient broth 40%, and reactivated at the moment of use.

The culture medium used, Tryptic soy agar (TSA) and Tryptic soy broth (TSB) were prepared according to the provider's directions, with 0.25 % glucoses added.

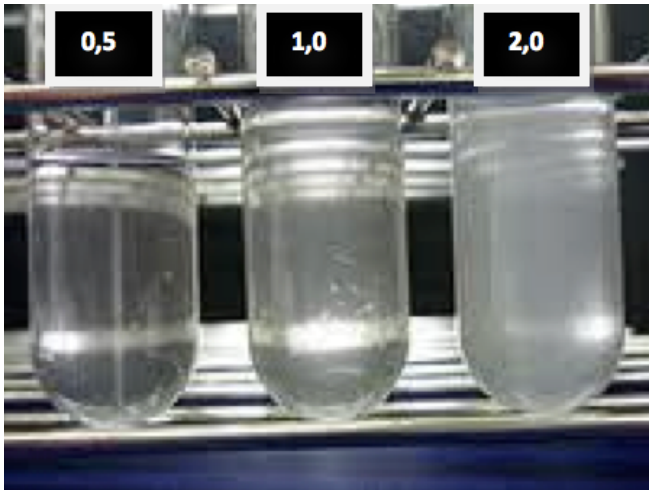
From the cryovals (micro tubes for conservation) the TSA plates were prepared with handle 10µl. The plates TSA were incubated during 24 hours at 35-37°C in microaerophilia. After the incubation period, with a bacteriological needle, thereabout 3 or 4 colonies grown in the TSA plates were transferred to Falcon tubes (15ml) with 5 – 10 ml of TSB.

The tubes were incubated by 16/18 hours (one night) at 35-37 °C.

After the incubation period, the tubes were submitted to centrifugation at by 5 minutes at 2500 rpm. The supernatant was discarded and the biomass obtained was resuspended in 10 ml of TSB in order to obtain a turbidity correspondent to 0.5 in the scale of

Mac Farland, that corresponds to $1.5x10^8$ (Figure 1).

Figure 1. Representation of Mac Farland scale.



A concentrated stock solution was prepared for each pharmacological agent evaluated, for posterior dilution, as illustrated in the Figure 2. On the first line of wells (correspondent to the position vertical 1) in plates of microtiter plates of 96 wells (Sarstedt, Newton, NC, USA), 200µl of stock solution prepared were distributed for each pharmacological agent: lines A, B and C the basin extract and lines D, E and F the coriander agent and the positive and negative controls (the first one the *S. mutans* suspension and the second the TSB pure broth). From the second line of wells in vertical (2), 100µl of TSB with 0.25% glucose were distributed for each well of culture.

Serial dilutions were carried out, transferring 100 µl of the line of wells 2, and consecutively until obtain the concentration expected of the pharmacological agent. At the end of dilution of 100µl of a bacterial suspension prepared $1.5x10^8$ UFC/ml, a specimen was added in all the wells of culture (Figure 3).

Figure 2. Microtiter plate with 96 wells. Lines A, B and C, column 1 correspond to the basil extract; lines D, E and F, column 1 correspond to the coriander extracts; both extracts in their concentrated form and other dilutions until the column 8.

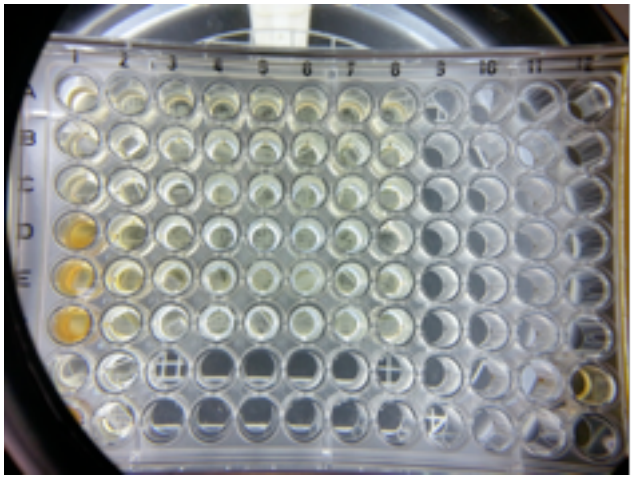
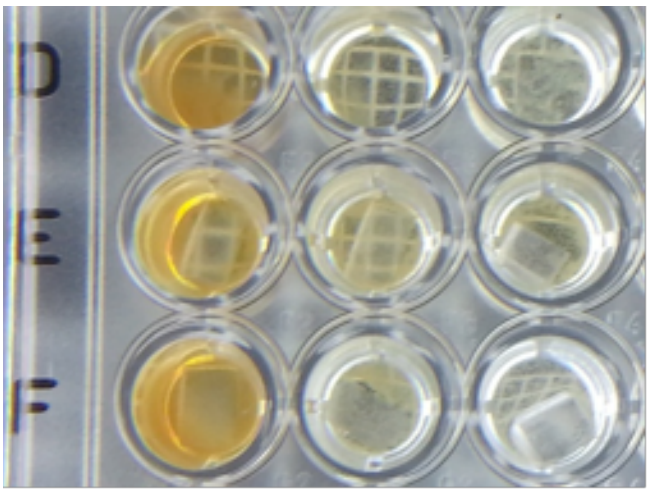


Figure 3. Plates containing specimens inoculated in TSB broth + extract.



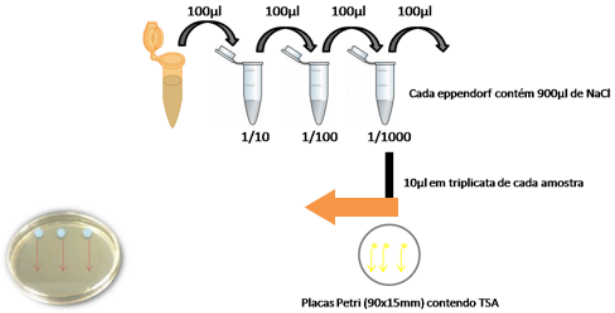
The plates were incubated at 35-37°C by 24, 48 and 72 hours with no agitation.

The positive control was that which was no exposed to any pharmacological agent; in other words, only the culture medium plus *S. mutans* suspension, while the negative control was the specimen with the culture medium. All the experiments were carried out in triplicate and for each time of contact (24, 48 and 72 hours).

After the incubation period, with a sterilized tweezers, the specimens were removed from their wells and washed in saline solution (0.09%) to remove non adhered bacteria. After the process of washing, the specimens referent to each incubation time and each dilution were placed in conical plastic tubes 15 ml (Falcon), containing 2.0 ml of saline solution (0.09%) and agitated by 60 seconds in a tube shaker (vortex), and submitted to sonication in the frequency 40±2 kHz by 5 minutes (Figure 4).

Figure 4. Ultrasound tank. Tubes with specimens are sonicated in order to provoke disintegration of bacterial biofilm.

Figure 5. Serial dilution of the sample in NaCl and plating.



Cell viability was determined by serial dilution of the sample in saline solution, and 10 µl of each dilution were spread by the Drop method in plates Petri (90x15 mm) containing tryptic soy agar (TSA). The plates were incubated at 35-37ºC in bacteriological incubator by until 48 hours (Figure 5).

RESULTS

Antimicrobial activity of *Ocimum basilicum L.* (basil) and *Coriandrum sativum L.* (coriander) extracts in biofilm of *S. mutans* was conducted evaluating the determination of the Inhibitory Minimum Concentration (IMC) of the pure extract and diluted on the formation of the biofilm in acrylic specimens as used in appliances in orthodontics.

The determination od the Inhibitory Minimum Concentration (IMC) by the microdilution broth method showed that the fluid extract of *Ocimun basilicum L.* and of the *Coriandrum sativum L.* presented inhibitory activity on the adherence face the *S. mutans* strain. The microorganism did not adhere to

the surface of specimen and consequently did not form the biofilm on its surface (Frames 1, 2 and Figure 6).

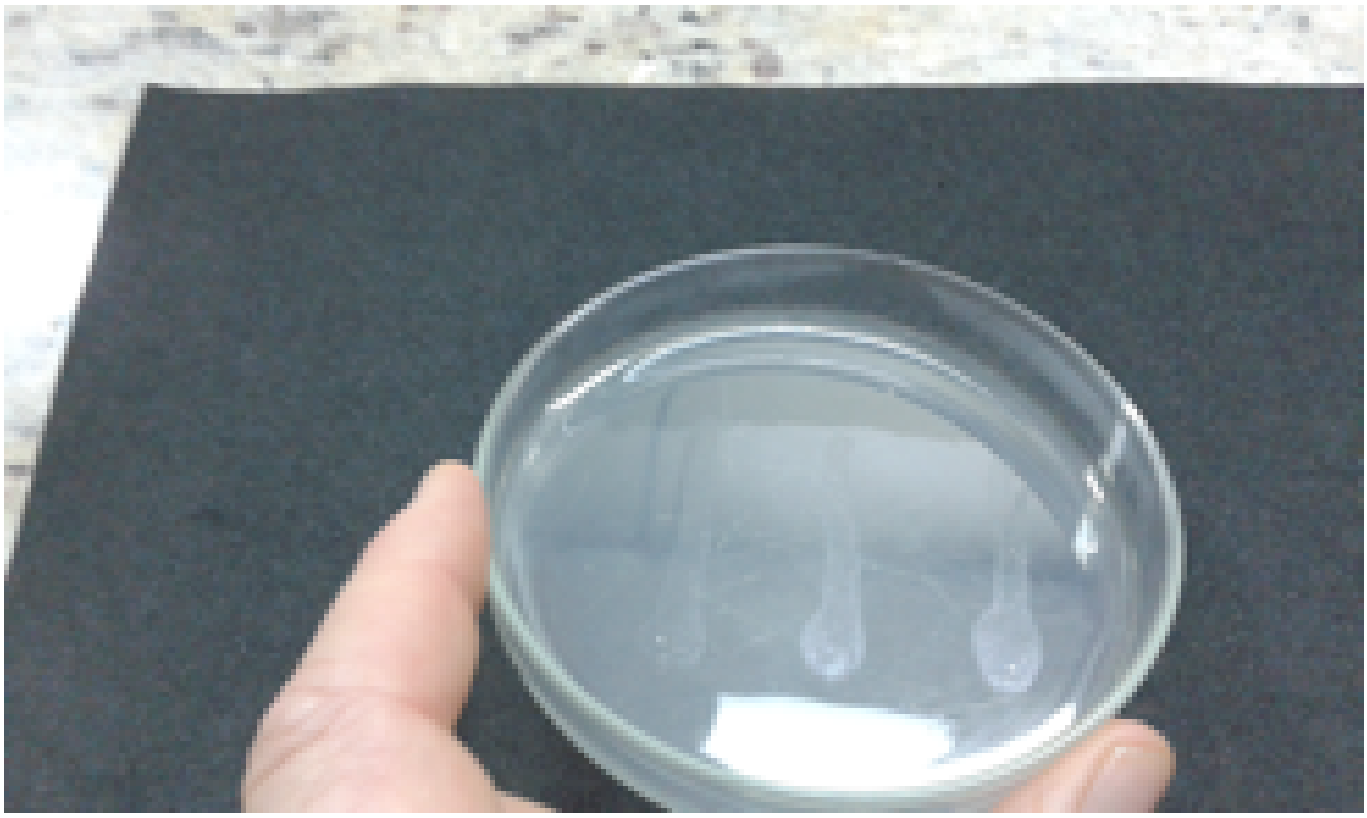
Frame 1. Evaluation of inhibition of the formation of biofilm of *Ocimum basilicum L.* extracts (basil).

Extract concentration/incubation time	Pure	1:2	1:4	1:8	1:16	1:32	1:64	1:128
24 hours	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
48 hours	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
72 hours	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.

Frame 2. Evaluation of inhibition of the formation of biofilm of *Coriandrum sativum L.* extracts (coriander).

Extract concentration/incubation time	Pure	1:2	1:4	1:8	1:16	1:32	1:64	1:128
24 hours	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
48 hours	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
72 hours	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.

Figure 6. No development of colonies in TSA representative of each dilution and extract. Only observing the inoculum mark in each triplicate.



DISCUSSION

In the ast years it has become common place the use of phytotherapy as alternative method to treat buccal diseases[14,15]. The inefficacy of traditional methods and the low cost[16] opened up ways to use base plants products as a supporting to the mechanical therapy. Phytoterapics are free of side effects and they can be used for a long period[17]. However, the use of medicinal plants might be aggressive, and there would be caution

regarding to the toxicological, microbiological and legal features relative to the natural composites and their combination.[11] Rasekh et al.[10] in 2011, evaluated the acute and sub chronic toxicity of the hydro alcoholic extract of *O. basilicum L*. in mice and they suggested that the hematologic system could be as a target to the oral toxicity of this plant.

In Brazil, there are more than 260 medicinal plants cataloged and distributed into 19 different indications for use in dentistry[18,19]. Regarding to the world market, 80% of people use plants to heal illnesses. Authors like Cragg & Newma[12] (1999) believe that the use of substances derivated from herbs represent 25% of medical prescription in industrialized countries.

In the literature, most of publications with phytoterapics is related to the antimicrobial and antifungal activities by the significant advance of development of natural derivatives with bacterial or fungal activity[20-23]. The ideal characteristics of an antimicrobial: low toxicity, and knowing the inter action of the product with oral epithelium; low permeability, no provoking unbalances which could lead to other diseases recurrent; and good retentiveness (substantivity) to could be slowly released.[24]

The action of phytotherapy against *S. mutans* is reported successfully in the literature[20-24].

Bezerra et al.[16] (2013) described that microorganisms of tooth biofilm are associated to caries. However, some bacteria are more active than others, and the *Streptococcus mutans* is one of the oral microorganisms more related to the initial caries because it is able to colonize teeth, produce intra and extra cellular polysaccharides, is highly acidogenic further metabolize several salivary glycoproteins .

The formation of conditioning film is a precursor step of all the process of development of the biofilm in surfaces in contact with water; this pellicle is formed by adsorption of organic molecules dissolved in the aqueous medium on the surface. Next, a displacement of microorganisms and particles occurs to the surface[25]. Therefore, the adhesion depends basically on the factors which involve characteristics of the microorganism, of the surface and of the medium conditions[12].

In any study on the biofilm production, it always should be considered the experimental conditions and the influence of factors like temperature and surface composition of the material in the process of bacterial adhesion. For some researchers, bacterial adhesion and biofilm production result from a multi factorial process[12].

Because they are very resistant to anti-microbial agents, the biofilm cells (sessile) are very difficult to remove[26].

High concentrations of antimicrobial are not also too much effective, because these

agents cannot kill the cells due to the limitation of the drug diffusion; not all the good antibiotics are effective against the biofilm.

In the case of the oral biofilm, several agents are available in the Market with different ways of action, and they were tested to intervene on the biofilm formation and metabolism. However, due to the various undesirable side effects associated to these agents, the research for alternative agents is a need[27].

Meng et al.[24] (2000), Ho et al.[25] (2001) and Barbosa-Filho[26] (2006) highlighted the importance to find effective and low toxic substances in the run against the resistance and the appearance of pathogenic microorganisms. The evaluation of the therapeutic potential of medicinal plants and some of their constituents such as flavonoids, alkaloids, triterpenes, sesquiterpenes, tannins, lignans, among others, have been studied as medicinal agents.

The phytochemical characteristic of commercial extracts provided by the enterprise Biotae revealed the presence of flavonoids (polyphenols) e tannins.

Chipault[27] (1962) describes that antioxidants are generally composites which can inhibit the oxidation of lipids or other molecules by removal of free radicals and that several composites found in all the types of plants, vegetables and fruit were observes because they have the same effect.

Phenolic composites (polyphenolics) in the concentrated extracts seem to have anti-caries properties that can be effective, even in the presence of saccharose and in very low doses.

The mechanism responsible by antimicrobial activity of phenolic composites present in extract of plants is not completely understood. However, several studies have suggested that these composites might inactivate important enzymes and damage the cytoplasmic membrane.

Thereby, antimicrobial properties of basil and coriander extracts showed potential for use in oral health as prophylaxis in the care of prosthesis confectioned in acrylic.

As these polyphenols are known as antioxidant, it was suggested that the antimicrobial effect might be caused by the antioxidant effect. The results indicated there are relations between the antimicrobial effect and the antioxidant capacity of polyphenols.

The data obtained in this research showed absence of viable sessile cells after the contact with basil and coriander extracts, what suggests a more metabolic action instead of an antimicrobial mechanism. Meanwhile, more investigations are necessary because to distinguish the effects of polyphenols from the antimicrobial effects, a separated experimental environment is required, and in this study, the activity of inhibition to adherence was tested thinking about the antimicrobial action; we

noticed out along the study and the analisis of results a biochemical reaction occurring.

To verify the hypothesis that the enzymes produced by *S. mutans* can be inhibited by polyphenols will be necessary other studies, and thus confirm our findings.

CONCLUSION

- Polyphenolic compounds present in the basil and coriander extracts inhibited the formation of biofilm of *S. mutans in vitro* in the concentrations pure until 1:128;
- Polyphenolics interfered on the glucan synthesis engine by inhibition of the enzymes (glucosyl- and fructosyl transferase) which synthesize the extracellular polymers, also acting as antioxidant, and therewith they presented antimicrobial activity.

REFERENCES

1. Vieira DRP, Amaral FMM, Maciel MCG, Nascimento, Flávia FRF, Libério AS. Plantas e constituintes químicos empregados em Odontologia: revisão de estudos etnofarmacológicos e de avaliação da atividade antimicrobiana in vitro em patógenos orais. Rev Bras Pl Med 2014;16:135-67.

2. Gibbons RJ. Bacterial Adhesion to Oral Tissues: A Model for Infectious Diseases. J Dent Res 1989;68(5): 750-60.

3. Jeon JG, Klein MI, Xiao J, Gregoire S, Rosalen PL, Koo H. Influences of naturally occurring agents in combination with fluoride on gene expression and structural organization of Streptococcus mutans in biofilms. BMC Microbiology 2009;9:228-38.

4. Almeida LS, Murata RM, Yatsuda R, Dos Santos MH, Nagem TJ, Alencar SM, et al. Antimicrobial activity of Rheedia brasiliensis and 7-epiclusianome against Streptococcus mutans. Phytomed 2008;15:866-91.

5. Lawrence JR, Zhu B, Swerhone GD, Topp E, Roy J, Wassenaar LI, et al. Community-Level Assessment of the Effects of the Broad-Spectrum Antimicrobial Chlorhexidine on the Outcome of River Microbial Biofilm Development. Applied and Environmental Microbiology 2008;74(11):3541–50.

6. Rodrigues ACC, Guedes MLS. Utilização de plantas medicinais no Povoado Sapucaia, Cruz das Almas – Bahia. Rev Bras Pl Med 2006;8(2):1-7.

7. Ostrosky-Zeichner L, Rex JH, Pfaller MA, Diekema DJ, Alexander BD, Andes D, Brown SD, et al. Rationale for reading fluconazole MICs at 24 hours rather than 48 hours when testing Candida spp. by the CLSI M27-A2 standard method. Antimicrob Agents Chemother 2008;52(11):4175-7.

8. Malhotra R, Grover V, Kapoor A, Saxena D. Comparison of the effectiveness of a commercially available herbal mouthrinse with chlorhexidine gluconate at the clinical and patient level. J Indian Soc Periodontol 2011;15:349-52.

9. Gupta D, Bhaskar DJ, Gupta RK, Karim B, Jain A, Singh R, et al. A randomized controlled clinical trial of Ocimum sanctum and chlorhexidine mouthwash on dental plaque and gingival inflammation. J Ayurveda Integr Med 2014;5:109-16.

10. Rasekh HR, Hosseinzadeh L, Mehri S, Nejad MK, Aslani M, Tanbakoosazan F. Safety Assessment of ocimum basilicum hydroalcoholic extract in wistar rats: acute and subchronic toxicity studies. Iran J Basic Med Scie 2011;15(1):645-65.

11. Albuquerque UP, Hanazaki N. As pesquisas etno dirigidas na descoberta de novos fármacos de interesse médico e farmacêutico: fragilidades e pespectivas. Bras Farmacogn 2006;16:678-89.

12. Cragg CM, Newman DJ. Discovery and development of antineoplasic agentes from natural sourcer. Cancer Invest 1999;17:153-63.

13. Opalchenova, G., Obreshkova, D. Comparative studies on the activity of basil - an essential oil from Ocimum basilicum L.-against multidrug resistant clinical isolates of the genera Enterococcus and Pseudomonas by using different test methods. J Microbiol Methods 2003;54(1):105-10.

14. Alves, PM, Queiroz LMG, Pereira JV, Pereira MSV. In vitro antimicrobial, antiadherent and antifungal activity of Brazilian medicinal plants on oral biofilm microorganisms and strains of the genus Candida. Rev Soc Bras Med Trop 2009;42(2):222-4.

15. Cavalcanti et al. Atividade antifúngica de extratos vegetais brasileiros sobre Cepas de Candida. R Bras Cien Saúde 2012;16(1):43-8.

16. Bezerra LMD, Ferreira GLS, Silva ICG, Castro RDC. Atividade antibacteriana in vitro de fitoconstituintes sobre microrganismos do biofilme dentário. Rev Bras Cie Saúde 2013;17:79-84.

17. Bott TR. Aspects of biofilm formation end destruction. Corrosion Reviews 1993;11:1-24.

18. Jefferson KK. What drives bacteria to produce biofilm? FEMS Microbiol Lett 2004;136:227-31.

19. Wimpenny J., Manz W., Szewzyk U. Heterogeneity in biofilms. FEMS Microbiol Reviews 2000;24:661-71.

20. Zhang TC, Bishop PL. Evaluation of substrate and pH effects in a nitrifying biofilm. Water Environ Res 1996;68(7):1107-15.

21. Puverdorj-Gage B, Costertton JW, Stoodley P. Phenotypic differentiation and seeding dispersal in non-mucoid and mucoid Pseudomonas aeruginosa biofilms. Microbiology 2005;145:1569-76.

22. Ferrazzano GF, Amato I, Ingenito A, De Natale A, Pollio A. Anti-cariogenic effects of polyphenols from plant stimulant beverages (cocoa, coffee, tea). Fitoterapia 2009;80:255-62.

23. Araujo CRF, Pereira JV, Pereira MSV, Alves PM, Higino JS, Martins AB. Bactericidal minimum concentration of extract of popularily known as “cajueiro” upon bacterias of dental biofilm. Pesq Bras Odontoped Clin Integr 2009;9(2):187-91.

24. Meng JC, Zhu QX, Than RX. New antimicrobial mono and sesquiterpenes fron Soroseris hookeriana subsp Erysimoides. Planta Med 2000;66:541-4.

25. Ho KY, Tsai CC, Huang HS, Chen CP, Lin TC, Lin CC. Antimicrobial activity of tannin components from Vaccinium vitis – idaea L. J Pharm Pharmacol 2001;53:187-91.

26. Barbosa Filho JM, Medeiros KCP, Diniz MFFM, Batista LM, Athayde Filho PF, Silva MS, et al. Natural products inhibitors of the enzyme acetycholinesterase. Ver Bras Farmacogn 2006;16:258-285.

27. Chipault JR. Autoxidation and Antioxidants. New York: Wiley; 1962.