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

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
INTRODUCTION

In the last years there was scientific advance involving studies on the medicinal plants in order to obtain new composites with therapeutic properties. 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.

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.

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. 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. The safest way to remove this microorganism is the mechanical one; however, the chemical agents are useful. 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. Bacterial cells adhered to the surface of materials are a signal or a target for specific bacterial receptors to be liked.

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.

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.

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\textsuperscript{11,12}.

Owing to the use of plants, many times in an empirical way, this work evaluated the action \textit{in vitro} of anti-adherence action of two vegetable extracts, the basil extract (\textit{Ocimum basilicum} L.) and the coriander extract (\textit{Coriandrum sativum} L.) which presented great potential of application as antimicrobial agent, as medicinal agent, as flavor in food and also fragrance in pharmaceutical products\textsuperscript{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 \textit{Ocimum basilicum} L. (basil) and \textit{Coriandrum sativum} L. (coriander) extracts were used, and they were obtained from the enterprise Biotae/SP.

In this study, the \textit{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 % gluoses 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 $1.5 \times 10^8$ (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 $1.5 \times 10^8$ 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).

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 *Ocimum 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).


<table>
<thead>
<tr>
<th>Extract concentration/incubation time</th>
<th>Pure</th>
<th>1:2</th>
<th>1:4</th>
<th>1:8</th>
<th>1:16</th>
<th>1:32</th>
<th>1:64</th>
<th>1:128</th>
</tr>
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Frame 2. Evaluation of inhibition of the formation of biofilm of Coriandrum sativum L. extracts (coriander).

<table>
<thead>
<tr>
<th>Extract concentration/incubation time</th>
<th>Pure</th>
<th>1:2</th>
<th>1:4</th>
<th>1:8</th>
<th>1:16</th>
<th>1:32</th>
<th>1:64</th>
<th>1:128</th>
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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\(^\text{14,15}\). The inefficacy of traditional methods and the low cost\(^\text{16}\) 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\(^\text{17}\). 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. Rasekh et al. 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. Regarding to the world market, 80% of people use plants to heal illnesses. Authors like Cragg & Newma (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. The ideal characteristics of an antimicrobial: low toxicity, and knowing the interaction 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.

The action of phytotherapy against *S. mutans* is reported successfully in the literature.

Bezerra et al. (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. Therefore, the adhesion depends basically on the factors which involve characteristics of the microorganism, of the surface and of the medium conditions.

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.

Because they are very resistant to antimicrobial agents, the biofilm cells (sessile) are very difficult to remove.

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.

Meng et al. (2000), Ho et al. (2001) and Barbosa-Filho (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) and tannins.

Chipault (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 anticaries 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 analysis 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**


