

## Antimicrobial Activity of Barbatimão Glycol Extract on *Streptococcus mutans*

### Atividade Antimicrobiana do Extrato Glicólico Barbatimão Sobre o *Streptococcus mutans*

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#### Abstract

The study of medicinal plants with antimicrobial action is a current issue, mainly due to the increase of bacterial resistance and the need for new antimicrobials with equal or greater efficacy, low cost and low side effects. The constant search for new products with bactericidal potential on oral microorganisms, mainly of natural origin, has grown considerably in recent years. The objective of this research was to evaluate the efficiency of concentrations of Barbatimão glycolic extract solution (*Stryphnodendron adstringens*) in inhibiting the bacteria growth associated with in vitro dental caries. Sixteen different concentrations of Barbatimão glycolic extract were prepared (0%, 1.56%, 3.12%, 4.68%, 6.25%, 7.81%, 9.37%, 10.93, 12, 5%, 25%, 37.5%, 50%, 62.5%, 75%, 87.5%, 100%) and the 2% chlorhexidine solution were used as the control group. The prepared solutions were tested on the bacterial strain of *Streptococcus mutans*. Bacterial growth was observed on plates containing BHI culture medium. Barbatimão concentrates were placed in wells made in the culture medium and each plate was incubated in a CO<sub>2</sub> oven (37 ° C / 24 hours). The test was performed in triplicate, resulting in the absence of bacterial inhibition at the tested barbatimão concentrations, with bacterial inhibition of 2% chlorhexidine being positive. It is concluded that the barbatimão glycolic extract in the analyzed concentrations does not present bactericidal action on *Streptococcus mutans*.

**Keywords:** Dental Caries. Microbiota. *Stryphnodendron barbatimam*

#### Resumo

*O estudo de plantas medicinais com ação antimicrobiana é um assunto atual, principalmente devido ao aumento da resistência bacteriana e a necessidade de novos antimicrobianos com igual ou maior eficácia, baixo custo e baixos efeitos colaterais. A busca constante de novos produtos com potencial bactericida sobre os micro-organismos orais, principalmente de origem natural tem crescido bastante nos últimos anos. O objetivo desta pesquisa foi avaliar a eficiência de diferentes concentrações de solução de extrato glicólico de Barbatimão (*Stryphnodendron adstringens*) na inibição do crescimento de bactérias associadas a cárie dental in vitro. Foram preparadas dezesseis concentrações do extrato glicólico do Barbatimão (0%, 1,56%, 3,12%, 4,68%, 6,25%, 7,81%, 9,37%, 10,93, 12,5%, 25%, 37,5%, 50%, 62,5%, 75%, 87,5%, 100%) e como grupo controle foi utilizada a solução de clorexidina 2%. As soluções preparadas foram testadas sobre a cepa bacteriana de *Streptococcus mutans*. O crescimento bacteriano foi observado em placas contendo meio de cultura BHI. As concentrações do barbatimão foram colocadas em poços realizados no meio de cultura e cada placa foi incubada em estufa de CO<sub>2</sub> (37°C / 24 horas). O teste foi realizado em triplicata, tendo como resultado a ausência de inibição bacteriana para as concentrações do barbatimão testadas, sendo positiva a inibição bacteriana para clorexidina a 2%. Conclui-se que o extrato glicólico de barbatimão nas concentrações analisadas, não apresenta ação bactericida sobre o *Streptococcus mutans*.*

**Palavras-chave:** Cárie Dentária. Microbiota. Barbatimão

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#### 1 Introduction

Dental caries, among the pathologies affecting the oral cavity, are the one that presents the highest world prevalence, being considered a multifactorial disease, since its origin depends on the interaction of essential factors, such as: host (tooth), micro-organism, diet and time<sup>1</sup>.

Concerning the micro-organisms present in the oral cavity, there are species that participate in the formation and progression process of dental caries in an important way, *Streptococcus mutans* being considered the micro-organism that presents the greatest cariogenic potential and is directly associated with its onset<sup>2</sup>.

Bacterial plaque control plays an important role in the dental caries prevention, not only in the decrease of oral bacteria, but also in the cavities cleaning process after cavity preparations, with the objective of avoiding a new incidence of dental caries. The use of antimicrobial agents in this control is widely used and presents satisfactory results, and chlorhexidine is the most widely used and the most effective, mainly because it is a potent antimicrobial agent and plays an important role in the inhibition of metalloproteinases present in the dental matrix<sup>3</sup>.

The constant search for new products with bactericidal potential on oral microorganisms, mainly of natural origin,

has brought about several studies with that objective. This is due to the inefficiency of some synthetic products, their side effects and also to the search for products with lower production costs and lower adverse effects<sup>4</sup>.

Phyto therapy medicinal products are pharmaceutical preparations (extracts, dyes, ointments and capsules) of medicinal herbs obtained from one or more plants. They exhibit several advantages for therapeutic use, such as low cost and reduced adverse effects, in relation to synthetic drugs. Among these phytoterapeutic products, barbatimão (*Stryphnodendron adstringens*), a medicinal plant, present mainly in the Brazilian cerrado, exhibits antibacterial, anti-inflammatory, antiseptic and hemostatic efficacy<sup>5</sup>.

Barbatimão seems to present antimicrobial activity on *Streptococcus mutans* and other bacteria involved in the caries process. Therefore, this research aimed to evaluate the efficacy of different concentrations of Barbatimão glycolic extract solution (*Stryphnodendron adstringens*) in the inhibition of *Streptococcus mutans* growth *in vitro*.

## 2 Material and Methods

The tests were conducted at the Laboratory of Oral Microbiology, at the State University of Londrina (UEL), Londrina-PR.

### 2.1 Obtaining Barbatimão extract

The barbatimão glycolic extract (*Stryphnodendron adstringens*) was purchased in its pure, certified composition from the company Empresa Império do Banho – ME. The glycolic extraction does not present cytotoxicity. Sixteen concentrations of the extract were obtained from the same company, using propylene glycol solvent in water, namely: 0%, 1.56%, 3.12%, 4.68%, 6.25%, 7.81%, 9.37%, 10.93, 12.5%, 25%, 37.5%, 50%, 62.5%, 75%, 87.5%, 100%. As a control group the 2% chlorhexidine solution was used which was purchased from Maquira Indústria de Produtos Odontológicos Ltda.

### 2.2 Cultivation of Microorganisms

The bacteria used for the tests were *Streptococcus mutans* ATCC 700610 strain. The plates containing these microorganisms were maintained at a temperature from 4 °C to 8 °C in BHI-agar medium (Brainheartinfusion agar). To perform the tests, five colonies of each *Streptococcus mutans* plate with disposable handle were collected in a sterile environment that were immersed and dispersed in Falcon tubes containing 10 mL of BHI broth (Brainheartinfusionbroth). They were then incubated for 16 hours in a CO<sub>2</sub> oven (5%) at 37 °C without agitation.

### 2.3 Antimicrobial test: in agar diffusion

After 16 hours of incubation, the inocula were washed three times with phosphate-saline buffer (PBS) and a bacterial

suspension diluted in PBS with turbidity similar to the McFarland 0.5 scale in a transparent glass tube, in sterile environment, totaling 1.5 x 10<sup>8</sup> CFU/mL.

A thin layer of agar/water was then deposited at the bottom of the plate (10ml), followed by a layer of 20 ml BHI-agar (1,5%) containing 200 µl of the microorganism suspension, deposited on the agar/water. This same layer of BHI-Agar was perforated for the production of wells with a blue 1 ml tip, after perforation, the lids were removed from each well, resulting thus in an agar/-water layer bottom. In each well, about 0.2 ml of the barbatimão formulations containing the concentrations respectively: 0%, 1.56%, 3.12%, 4.68%, 6.25%, 7.81%, 9.37%, 10.93, 12.5%, 25%, 37.5%, 50%, 62.5%, 75%, 87.5%, 100% were added. The control group was made with 2% chlorhexidine. After 24 hours, inhibition halos were measured with the aid of an electronic caliper. Then, the inhibition halo was subtracted by the well diameter. The results were expressed in millimeters.

## 3 Results and Discussion

It was observed that none of the tested concentrations of the barbatimão glycolic extract (*Stryphnodendron adstringens*) showed potential for inhibition of bacterial growth of *Streptococcus mutans*.

The current society demonstrates a concern about nature conservation, as well as the search for knowledge of the use of plant species. The transformation of a plant into a technically elaborated product requires studies to validate plant species in order to prove safety and efficacy. More than a hundred compounds derived from natural products are in the laboratory test phase, for the most diverse uses in treatment of diseases. The evaluation of antimicrobial activity of glycolic extract of barbatimão barks (*Stryphnodendron adstringens*) corroborates this current trend of developing and testing drugs produced from natural plants, mainly for micro-organism control<sup>6</sup>.

Medicinal plants produce a wide aspect of pharmacological actions, with the emphasis on anti-inflammatory and antimicrobial activities<sup>7</sup>, and the barbatimão plant extract is a natural product widely used as a healing, anti-inflammatory, hemostatic, anti-edematogenic, antiseptic agent<sup>8</sup>.

Orlando<sup>9</sup> observed *in vitro* antimicrobial activity of crude hydroalcoholic extract of barbatimão bark (*Stryphnodendron adstringens*) for *Enterococcus faecalis*, *Kocuriarhizophila*, *Escherichia coli*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Candida albicans* and *Candida krusei*.

Soares *et al.*<sup>10</sup> evaluated the efficacy of barbatimão crude extract (*Stryphnodendron adstringens*) obtained by means of hydroalcoholic extraction, before micro-organisms responsible for dental caries. Strains of the following micro-organisms were used: *Enterococcus faecalis*, *Streptococcus salivarius*, *Streptococcus sanguinis*, *Streptococcus mitis*, *Streptococcus*

*mutans*, *Streptococcus sobrinus* and *Lactobacillus casei*. Antibacterial activity was observed on all of them, and the best results were on *S. mitis* and *L. casei* strains, the minimum inhibitory concentration (MIC) varied from 350 to > 400µg/mL.

Pereira *et al.*<sup>11</sup> also confirmed the efficiency of the barbatimão hydroalcoholic extract (*Stryphnodendronadstringens*) in relation to the following pathogenic microorganisms in the oral cavity: *Candida albicans*, *Streptococcus mutans*, *Staphylococcus aureus* and *Aggregatibacter actinomycetemcomitans*. The barbatimão extracts showed antibacterial activity against all the tested micro-organisms, except for *A. actinomycetemcomitans*.

Miranda<sup>12</sup> used barbatimão hydroalcoholic extract (*Stryphnodendronadstringens*) as endodontic medication. The tests were performed in microplates with diffusion in wells of the extract, incubation at 35° from 24 to 48 hours, where the bactericidal action for the micro-organisms was verified: *Prevotellana nigrescens*, *Actinomyces naeslundii*, *Porphyromonas gingivalis*, *Enterococcus faecalis* and *Haemophilus actinomycetemcomitans*.

Although the above studies demonstrate the barbatimão action (*Stryphnodendronadstringens*) as antimicrobial, the use of hydroalcoholic extraction, the control of the solvent (alcohol) does not appear in the studies, which does not clarify whether there was interference of the solvent used in the barbatimão bactericidal action<sup>9-12</sup>.

The results of the present study contradict data from previous studies<sup>9-12</sup> where inhibition of bacterial growth was observed, with barbatimão bactericidal action (*Stryphnodendronadstringens*) in its presentation as hydroalcoholic extract, which was not confirmed in its presentation as glycolic extract used in this study, suggesting an interference in the form of extraction of the barbatimão active principles (tannins) in relation to the solvent used.

Cowan<sup>13</sup> emphasizes that there is a better extraction of the active principles (tannins phenolic compounds, flavonoids and terpenoids) by means of hydroalcoholic extraction. Whereas Ardisson *et al.*<sup>14</sup> found a better characterization of the barbatimão (tannins) properties in the use of propylene glycol in water. The concentration of 80% propylene glycol was more selective.

Chlorhexidine as a positive control obtained a satisfactory result for the inhibition of the growth of *Streptococcus mutans*, confirming its great efficacy as a bactericidal agent<sup>15,16</sup>. Growth inhibition halo was observed above 20 mm, which corroborates the findings of Festuccia *et al.*<sup>17</sup>.

Moreira *et al.*<sup>18</sup> in the *in vitro* evaluation of antimicrobial activity of buccal antiseptics, verified inhibition halo with chlorhexidine above 20 mm. Costa *et al.*<sup>19</sup> observed *in vitro* the antimicrobial action of plant extracts on *Enterococcus faecalis*, using chlorhexidine as a positive control, obtaining an inhibition halo of 24.52 mm.

## 4 Conclusion

Based on the results obtained in the present study, it can be concluded that, by the methodology applied, the different concentrations of barbatimão glycolic extract (*Stryphnodendronadstringens*) were not able to foster bactericidal action in the colonies of *Streptococcus mutans*.

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