

Susceptibility of oral pathogenic microorganisms to aqueous and ethanolic extracts of *Stryphnodendron adstringens* (barbatimão)

*Suscetibilidade de microorganismos patogênicos orais a extratos aquosos e etanólicos de *Stryphnodendron adstringens* (barbatimão)*

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ABSTRACT

Stryphnodendron adstringens, also known as "barbatimão", is a native plant from the "cerrado" area in Brazil. It has been reported that barbatimão extracts possess several biological activities, including antimicrobial and healing effects, among others. The aim of this study was to evaluate the in vitro susceptibility of oral pathogenic bacteria and yeast to barbatimão aqueous and ethanolic extracts. Susceptibility tests for *S. mutans* (ATCC 70069), *Staphylococcus aureus* (ATCC 12692), *Actinobacillus actinomycetemcomitans* (ATCC 29522) and *Candida albicans* (ATCC 18804) to barbatimão aqueous extract (BEA) and ethanolic extract (BEE) solutions were performed. MIC was also determined for each microorganism. The results showed that BEA possess activity against bacteria, and very small efficacy against yeast. BEE showed both antibacterial and antifungal activities. It can be concluded that barbatimão extracts have antimicrobial activity against oral pathogenic microorganisms. Barbatimão extracts could be considered a potential alternative for the treatment of infectious conditions of the oral cavity.

Key Words: oral microorganisms, susceptibility, MIC, barbatimão, *Stryphnodendron adstringens*

RESUMO

Stryphnodendron adstringens, também conhecido como "barbatimão", é uma planta nativa da região do cerrado do Brasil. A literatura documenta que extratos de barbatimão apresentam diversas atividades biológicas, incluindo efeitos antimicrobianos e cicatrizantes. O objetivo deste trabalho foi avaliar a suscetibilidade in vitro de bactérias e fungo patogênicos da cavidade oral a extratos aquosos e etanólicos de barbatimão. Testes de suscetibilidade para *S. mutans* (ATCC 70069), *Staphylococcus aureus* (ATCC 12692), *Actinobacillus actinomycetemcomitans* (ATCC 29522) e *Candida albicans* (ATCC 18804) a extratos aquosos (BEA) e etanólico (BEE) de barbatimão foram realizados. A determinação do MIC para cada microorganismo também foi avaliada. Os resultados mostraram que BEA apresentou somente atividade antibacteriana considerável, enquanto que BEE mostrou tanto atividade antibacteriana quanto antifúngica. Pode-se concluir que os extratos de barbatimão apresentam atividade antimicrobiana contra microorganismos patogênicos da cavidade oral. Tais extratos poderiam ser considerados uma possível alternativa terapêutica para condições infecciosas da cavidade oral.

Palavras-chave: microorganismos orais, suscetibilidade, MIC, barbatimão, *Stryphnodendron adstringens*.

INTRODUCTION

Since ancient times, plants have commonly been used in folk medicine for the treatment of several diseases. Detailed studies are necessary to prove their biological activity and provide necessary information about their therapeutic use.

Stryphnodendron adstringens (Martius) Coville (Leguminosae), popularly known as "barbatimão", grows abundantly in the central savana area of Brazil.

Decoction and infusion of the stem bark have been traditionally used by the native population of Brazil for the treatment of leukorrhea, diarrhoea, and gynaecological infections¹. Neves et al.² demonstrated that the aqueous extract of this plant has significant wound healing effect. It has also been demonstrated that the aqueous extract of *S. adstringens* possesses antiinflammatory, analgesic and protective effects on the gastric mucosa^{3, 4, 5}. Previous studies showed significant cicatrizing properties from this plant^{6, 7}.

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Despite the widespread use of *S. adstringens* in Brazilian folk medicine, few is known about the antimicrobial activity of barbatimão extract solutions against oral microorganisms associated with infectious conditions. The objective of the present study was to evaluate the *in vitro* susceptibility of oral pathogenic bacteria and yeast to barbatimão ethanolic and aqueous extracts.

MATERIAL AND METHODS

Plant material and extract preparation

The stem bark of *S. adstringens* was collected in Sabará (23° 43' 7.8" S, 50° 45' 23" W, 926m alt), state of Minas Gerais, Brazil, in July 1998, identified and prepared by the Laboratory of Pharmacognosy from the Federal University of Minas Gerais (UFMG). A voucher specimen was deposited at the Herbarium of the same laboratory. Air-dried stem bark was extracted with hexane, acetate, n-butanol and ethanol (for TB1, TB2, TB3 and TB4, respectively). The extract was filtered, evaporated under reduced pressure, and then lyophilized.

The lyophilized extract was stored at -20°C until further use. Aqueous and ethanolic solutions were prepared from this lyophilized product and tested *in vitro* for their inhibitory ability on microorganisms growth. Three samples of aqueous barbatimão extract (TB1, TB2, TB3) and one of ethanolic barbatimão extract (TBIV) were obtained. 100mg/mL barbatimão aqueous extracts were prepared with sterile Phosphate Buffered Saline (PBS). The 10% ethanolic extract was prepared using p/v 1:10 (absolute alcohol 99.2° GL-EMFAL-Brazil).

Microbial susceptibility test and Minimal inhibitory concentration (MIC)

The susceptibility tests of *S. mutans* (ATCC 70069), *Staphylococcus aureus* (ATCC 12692), *Actinobacillus actinomycetemcomitans* (ATCC 29522) and *Candida albicans* (ATCC 18804) to barbatimão aqueous (BEA) and ethanolic (BEE) extract solutions were performed using the agar diffusion test⁸. A 0.1 ml aliquot of over-night cultures of *S. mutans*

strains incubated at 37°C in 5% saccharose Brain Heart Infusion-BHI (Difco-USA), corresponding to 0.5 turbidity on the McFarland scale, was plated onto 30 ml Mitis salivarius bacitracin agar (Difco-USA) previously melted and distributed on Petri dishes measuring 150.0 mm in diameter. *S. aureus* and *A. actinomycetemcomitans* species were over-night incubated at 37°C in BHI (Difco-USA) and 0.1 ml aliquot was plated onto 30.0 ml Blood Agar (Difco-USA) supplemented with Hemin/Menadione (Sigma-USA). Petri dishes were incubated 18 hours at 37°C in anaerobic system (Difco-USA) vase. A 0.1 mL aliquot of 24 hour cultures of *C. albicans* was incubated at 37°C in Sabouraud dextrose broth (BIOBRÁS-Brazil), corresponding to 5.0 turbidity on the McFarland standard, and seeded in 30 mL Sabouraud dextrose agar (BIOBRÁS-Brazil).

All sensibility tests were made in Mueller Hinton agar plates for bacteria and Sabouraud agar for *C. albicans*. Sterile blank disks (CECON-Brazil) were soaked in 20 µl of the BAE and BEE solutions and applied to the agar surface previously seeded with the microorganism. Positive and negative controls of the discs containing 30µg of penicillin (for bacteria) and 30µg nystatin (for yeast), 20µl sterile phosphate buffered saline (PBS), and 20µl of Ethanol 93.2°C were used. The effect of 93.2° alcohol was also determined, because it is a BEE compound. After 48 hours of incubation at 37°C, the diameters of the inhibition zones were measured and compared. The presence or absence of inhibition zones was used as a criterion for the definition of active or inactive extracts. Tests were performed in triplicate.

MIC was determined as the lowest concentration of the extract which inhibited the growth of the tested microorganisms using the agar dilution method⁹.

Statistical analysis

The results of the diameters of the inhibition zones were reported as Means ± Standard Deviation (M±SD) and were analyzed statistically by the non-parametric Kruskal-Wallis test. Differences at the level of variation (p< 0.05) were considered to be significant.

RESULTS

Minimal inhibitory concentration (MIC) of *Stryphnodendron adstringens* against oral pathogenic microorganisms are shown in Table 01. TB3 showed greater MICs than the TB1 and TB2. No significant differences were observed in TB1 and TB2 MICs results. However, TBIV solution presented the strongest potential on inhibiting the growth of microorganisms when compared to the other solutions.

Microorganism	Stryphnodendron adstringens extract (µg/mL)			
	TB1	TB2	TB3	TBIV
<i>Staphylococcus aureus</i>	25,80	19,80	58,10	4,64
<i>Streptococcus mutans</i>	20,33	28,33	38,33	3,25
<i>Actinobacillus actinomycetemcomitans</i>	22,83	18,83	28,41	5,86
<i>Candida albicans</i>	441,66	441,66	883,33	4,25

Table 01: Minimal inhibitory concentration (MIC) of *Stryphnodendron adstringens* against oral pathogenic microorganisms.

Results on the susceptibility tests are shown in table 02. *S. mutans*, *S. aureus* and *A. actinomycetemcomitans* growth was inhibited by all the extracts tested. BAE showed antimicrobial activity for bacteria, but did not show significant efficacy against *C. albicans*. Only BEE showed considerable antifungal activity for *C. albicans*. The inhibition zones observed for BEE were significantly higher comparing with ethanol alone.

Extracts	<i>C. albicans</i>	<i>S. mutans</i>	<i>S. aureus</i>	<i>A. actinomycetemcomitans</i>
TB1	0.00±0.00	14.08±1.65	16.33±1.00	13.09±1.22
TB2	7.0±0.00	19.00±1.32	21.11±1.17	11.11±1.36
TB3	6.00±0.00	12.41±0.49	15.33±0.81	11.42±0.98
TBIV	12.94±1.37	18.50±1.81	18.55±2.24	15.44±3.46
Nystatin	22.87±0.64	-	-	-
Penicillin	-	21.50±1.93	36.00±1.51	16.40±1.94
Ethanol	7.42±1.72	7.50±6.67	0.00±0.00	7.40±4.57
Distilled Water	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

* All experiments were made in triplicate.

Table 02: Mean (mm)* and Standard Deviation (M±SD) of inhibition zones to susceptibility test of oral microorganisms to Barbatimão Aqueous Extract (BAE) and Ethanol (BEE).

DISCUSSION

Our experiments demonstrate that barbatimão extracts, traditionally used in Brazil to treat infectious diseases, exhibited *in vitro* antimicrobial activity against oral pathogenic microorganisms. The use of *S. adstringens* is very popular in folk medicine, despite the few studies documenting the efficacy of barbatimão extracts against pathogenic microorganisms. Soares et al.¹⁰ investigated the antibacterial activity of the crude hydroalcoholic extract of *S. adstringens* on dental caries microorganisms. The extract showed antibacterial activity against *Streptococcus mitis* and *Lactobacillus casei*.

Four different solutions were prepared and tested *in vitro* in the present study. BEA showed activity against *S. aureus*, *A. actinomycetemcomitans* and *S. mutans*. These microorganisms are relevant in Dentistry because they are related to dental abscesses, periodontal diseases, and dental caries, respectively. BEE also showed efficacy against these bacteria and also against *C. albicans*, which is associated with oral candidosis and denture stomatitis.

The chemical composition of *S. adstringens* was investigated by Mello et al.^{11, 12, 13} and the main isolated classes of constituents are flavan-3-ols, prodelphinidins and prorobinetidins. Leaves and bark show delphinidin, gallic acid, flavonols and tannins content¹⁴.

The precise profile of active constituents of *S. adstringens* is still not well known, however, tannins could be considered the most relevant substances due to its high content in the plant. *S. adstringens* contains a large quantity of condensed tannins, varying between 10 and 37%, depending on the place and season of the year in which the sample has been collected^{11, 15}. The screening of active molecules in the barbatimão extract is an important research activity that should be considered in future studies. It is very likely that tannin is the main antimicrobial constituent in the plant extract, but other studies need to clarify if there is a synergistic effect of molecules responsible for the antimicrobial activity.

It has been demonstrated that tannins and related compounds exert several biological and pharmacological activities, such as bactericidal, antiviral, molluscicidal, antihelminthic and antiprotozoan actions, antihepatotoxic action, inhibition of xanthine oxidase and mono-amino oxidase and inhibition of glucosyltransferases. It is believed that these properties derive from their antioxidant and radical scavenging activities, and from their ability to form complexes with other macromolecules, such as proteins and polysaccharides, and metal ions.¹⁶

Three hypothesis might explain the antimicrobial mechanism of tannins on bacteria, filamentous fungus, and yeast^{17, 18}: a) inhibition of enzyme activity by complexating with substrates of bacteria and fungi; b) direct action of tannins on the microorganism metabolism, through the inhibition of oxidative phosphorylation; c) a mechanism involving the complexation of tannins with metabolic ions, decreasing the availability of essential ions to the metabolism of the microorganisms.

It is very likely that ethanol may play an important role for the extraction of barbatimão active compounds, considering the differences found in the susceptibility test for aqueous and ethanolic solutions. Moreover, the ethanolic solution of barbatimão showed both antifungal and antibacterial activity, showing that this solvent may play an important role in extracting the active antimicrobial molecules from *S. adstringens*.

The use of barbatimão plant for decreasing the quantity of oral microorganisms is technically feasible. Our study suggests a potential use of barbatimão extracts as an alternative treatment of infectious conditions of the oral cavity.

CONCLUSION

It can be concluded that barbatimão aqueous extracts possess efficacy against the *in vitro* growth of bacteria tested in this study and presented small efficacy against *C. albicans*. The ethanolic extract of barbatimão presented efficient growth inhibition property for all the microorganisms tested. Further studies need to be carried out in order to evaluate if

those extracts are useful for the clinical treatment of infectious conditions of the oral cavity, such as denture stomatitis, dental caries, and periodontal diseases.

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