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Antibacterial effect of *Stryphnodendron adstringens* (Mart) Coville (Fabaceae) extract on dental caries

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Abstract
It is common knowledge the current trend of developing and testing products accrued from natural plants. On that premise, *Stryphnodendron adstringens* (Mart) Coville is highlighted and was the aim for evaluating its antibacterial effect on dental caries. A crude dried methanolic extract was prepared and applied from 50 to 500 mg/mL to determine the Minimum Inhibitory Concentration (MIC) on seven distinguished bacterial cultures. Results revealed by 0.01% resazurin demonstrated *Stryphnodendron adstringens* (Mart) Coville extract exerted antibacterial effect on all tested bacterial strains, with MIC > 250 mg/mL for *Lactobacillus casei*, *Streptococcus mitis* and *Streptococcus salivarius* and MIC > 400 mg/mL for Enterococcus faecalis, *Streptococcus mutans*, *Streptococcus sanguinis* and *Streptococcus sobrinus*. In view of outcomes, the tested crude vegetal extract presents antibacterial potential for application in dental caries.

Keywords: Antibacterial potential, *Stryphnodendron adstringens* (Mart) Coville, oral microbiome, caries

Introduction
Currently, there is an interest increase in medicinal plants, due to the great demand for alternative therapies [1]. That is mainly owing to the ineffectiveness of some synthetic products, the cost of allopathic medicines and the search for less aggressive treatments [2]. Herbal medicines are pharmaceutical preparations (extracts, tinctures, ointments and capsules) obtained from one or more plants and used for the treatment of various diseases. There are countless advantages for their therapeutic use, such as low cost and wide availability for the low-income population [3].
Stryphnodendron adstringens (Mart) Coville (*S. adstringens*) is a legume considered as a medicinal plant widely distributed throughout the Brazilian Cerrado [4]. Mainly known as barbatimão, also receives several popular names, which vary according to the region: alaramotemo, barba-detimam, charãozinho-roxo, ilatimó, ulatimó, casca do Brasil, casca-da-virgindade e casca-da-mocidade [5]. It presents as chemical constituents alkaloids, flavonoids, terpenes, stilbenes, steroids, protease inhibitors (such as trypsin) and particularly tannins [6, 7, 8]. Tannins are the major components of *S. adstringens*, and these compounds have been associated to its antimicrobial effects [6, 7]. There are three general properties of tannins that are responsible for most of the pharmacological activities: the formation of complexes with metallic ions (iron, aluminum, calcium, copper, etc.), the antioxidant activity and scavenging of free radicals and the ability to form complexes with other molecules such as proteins and polysaccharides [9].
S. adstringens has been used in folk medicine as an antimicrobial, healing, anti-inflammatory, hemostatic, anti-edematogenic, antiseptic and anti-diarrheal agent [10, 11, 12]. However, a small number of studies have been carried out to evaluate the antimicrobial activity of *S. adstringens* on microorganisms related to oral diseases, such as dental caries [13, 14].
The versatile therapeutic potential of *S. adstringens* made it be included in the National List of Medicinal Plants of Interest to SUS [15] and recommended in the Brazilian Pharmacopoeia 5th [16]. Based on its potential, this work aimed to evaluate the antibacterial effect of *S. adstringens* extract on dental caries.

Material and Methods

Extract obtainment

S. adstringens crude dried methanolic extract was prepared as described by Santana *et al.* (2023) [17].

Evaluation of antibacterial activity

To determine the Minimum Inhibitory Concentration (MIC), the Kirby-Bauer method of plate diffusion was applied [18]. Seven bacterial cultures from the American Type Culture Collection (ATCC) were used: *Enterococcus faecalis* (ATCC 4082), *Lactobacillus casei* (ATCC 11578), *Streptococcus mitis* (ATCC 49456), *Streptococcus mutans* (ATCC 25175), *Streptococcus salivarius* (ATCC 25975), *Streptococcus sanguinis* (ATCC 10556) and *Streptococcus sobrinus* (ATCC 33478). The strains belong to the Laboratory of Clinical Microbiology from the University Center Brasília de Goiás. As a negative control, dimethyl sulfoxide (DMSO, Merck®) from 1 to 10% was applied; and as a positive control, a solution of chlorhexidine digluconate (Sigma®) at concentrations from 0.015 to 5.9 mg/mL was used. To define the sensitivity of bacterial strains to the concentrations of *S. adstringens* crude extract, serial dilutions were performed: 50 mg/mL, 100 mg/mL, 150 mg/mL, 200 mg/mL, 250 mg/mL, 300 mg/mL, 350 mg/mL, 400 mg/mL, 450 mg/mL and 500 mg/mL.

The sterility control of the broth, solvent and culture was performed. The inoculum was standardized by comparing it with the 0.5 tube of McFarland scale (0.1 mL of 1.0% barium chloride + 9.9 mL of 1.0% sulfuric acid) by checking the absorbances at 625 nm (Quimis Q898UV-DB) [19]. Microplates were sealed with parafilm and incubated at 35 ± 2 °C for 24 hours. After the incubation period, 30 µL of 0.01% resazurin (Sigma®) was added to each well. This revealing system allows immediate observation, whereby the blue color represents the absence of microbial growth and the red color, the positivity. Microplates were reincubated for 30 minutes and then descriptively analyzed.

Results

S. adstringens crude dried methanolic extract generated 20.14% yield (w/w) and a content determination for extractives content of 11.88% [17]. As presented in Table 1, *S. adstringens* demonstrated antibacterial effect on all tested bacterial strains, with MIC > 250 mg/mL for *Lactobacillus casei*, *Streptococcus mitis* and *Streptococcus salivarius*; and MIC > 400 mg/mL for *Enterococcus faecalis*, *Streptococcus mutans*, *Streptococcus sanguinis* and *Streptococcus sobrinus*.

Table 1: Minimum Inhibitory Concentration (MIC) of *S. adstringens* extract and the positive control, chlorhexidine digluconate.

Bacterial strains	*S. adstringens*	Chlorhexidine digluconate
	MIC (mg/mL)	MIC (mg/mL)
Enterococcus faecalis	> 400	0.290
Lactobacillus casei	> 250	0.190
Streptococcus mitis	> 250	0.310
Streptococcus mutans	> 400	0.030
Streptococcus salivarius	> 250	0.030
Streptococcus sanguinis	> 400	0.190
Streptococcus sobrinus	> 400	0.190

Discussion

The antibacterial properties evaluation of crude methanolic extract from *S. adstringens* leaves follows the current trend of developing and testing products accrued from natural plants [12]. *S. adstringens* is a promising plant, since it has proven healing, anti-inflammatory, hemostatic, antiseptic, anti-diarrheal and anti-edematogenic activities [11, 20, 21, 22]. Even so, few researchers have evaluated its antimicrobial activity on oral bacteria, a fact that motivated this study [7, 13]. In South America, the leaves and bark from *S. adstringens* are widely used by folk medicine [23]. Studies on the antimicrobial activity are also preferentially carried out with these parts of the plant, since they contain high tannins concentration (about 20%), which are the responsible for the plant medicinal properties [6, 7, 8].

According to the results obtained in this study, *S. adstringens* extract stated activity against strains of *Lactobacillus casei*, *Streptococcus mitis* and *Streptococcus salivarius* from MIC of 250 mg/mL on. These outcomes were considered the most satisfactory due to the activity at a lower concentration when compared to the other evaluated strains, whereby MICs were observed from 400 mg/mL on.

Soares *et al.* (2008) [7] and Almeida *et al.* (2017) [24] have developed similar antimicrobial activity with *S. adstringens* extract and observed effect at concentrations analogous to this study in strains of *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Kocuria rhizophila*, *Shigella flexneri* and *Staphylococcus aureus*. It is thought that the difference found in relation to the MICs of *S. adstringens* may be related to the conditions inherent to the microorganisms themselves.

Bacteria selected for the antimicrobial activity evaluation of *S. adstringens* crude extract are constituents of dental biofilm and their inclusion in the microbial consortium occurs at contrasting times [25]. *Streptococcus mitis* is one of the first bacteria to adhere to enamel, along with *Streptococcus sanguis* and *Streptococcus salivarius*. They favor the colonization of the tooth surface by other bacteria [26]. On the other hand, *Lactobacillus casei* is found in caries cavities already installed [27]. Accordingly, this paper results reveal a promising proposal for the development of products capable of acting on the development of dental biofilm, causing its inhibition and, consequently, preventing dental caries.

In this work, the positive control used was chlorhexidine digluconate. Results obtained with *S. adstringens* were not directly compared to chlorhexidine digluconate, since it is a pure substance and *S. adstringens* crude dried methanolic extract is a complex mixture. *S. adstringens* antimicrobial potential was previously demonstrated [6, 7, 8, 10, 11, 13, 14, 21, 24] and is suggestively due to the richness in tannins as the main component. Howbeit, in studies of antimicrobial activity with crude extracts from plant species, the potential effectiveness is not exclusively due to a single substance, but from a group of them [28]. The use of a plant species extract with satisfactory bactericidal effect could dispense the necessity of isolation processes for developing active substances, reducing chemical steps and, consequently, financial costs. That would enable possible use of a crude extract as an herbal medicine.

Conclusion

In view of the obtained results, it is believed that *S. adstringens* crude dried methanolic extract presents antibacterial potential for application in dental caries.

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Declaration of interest statement

Authors report no declarations of interest. Authors alone are responsible for paper content and writing.

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