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Phenolic compounds, antioxidant and antibacterial activity of extract from leaves and bark of *Stryphnodendron adstringens* (Mart.) Coville[1]

Compostos fenólicos, atividade antioxidante e antibacteriana do extrato das folhas e casca de *Stryphnodendron adstringens* (Mart.) Coville

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ABSTRACT - There is currently growing interest in the investigation of bioactive compounds from natural sources. In this context, this study investigated the phenolic content, antioxidant capacity, and antimicrobial activity of the hydroalcoholic extract (EHA) of the stem barks and leaves of *Stryphnodendron adstringens*. Plant parts were extracted with ethanol:water (1:1) and filtered in a vacuum to obtain the crude extract. Phenolic content was determined by the Folin–Ciocalteu method. Antioxidant activity was measured using the DPPH test. The antimicrobial activity of extracts was evaluated against Gram-positive cocci: *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* (strain isolated from a patient); and Gram-negative bacilli: *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). The zone of inhibition and the minimum inhibitory concentration (MIC) of the plant extracts were evaluated by the agar diffusion disc method and broth microdilution, respectively. Total phenolic content of the EHAs was higher in stem barks (970.4 mg GAE/g) than in leaves (693.8 mg GAE/g). In the DPPH assay, all extracts showed high antioxidant activity, above 75% at the concentration of 100 μg/mL. About antimicrobial activity, all the EHAs showed effects against Gram-positive bacteria *S. aureus* and *S. epidermidis*, whose MIC ranged from 7.81 μg/mL to 62.5 μg/mL. This finding is important since these pathogens are generally resistant to a variety of widely known antibiotics. Therefore, these results are promising and indicate the use of *S. adstringens* (Mart.) Coville as a natural antioxidant in foods, medicines and also point to its use in antimicrobial therapy.

Key words: Cerrado. Natural products. Minimum inhibitory concentration.

RESUMO - Atualmente é crescente o interesse pela investigação de compostos bioativos de fontes naturais. Nesse contexto, o presente estudo investigou o conteúdo fenólico, capacidade antioxidante e atividade antimicrobiana do extrato hidroalcoólico (EHA) das cascas do caule e folhas de *Stryphnodendron adstringens* (Mart.) Coville. Partes da planta foram extraídas com etanol: água (1:1) e filtradas a vácuo para obtenção do extrato bruto. O conteúdo fenólico foi determinado pelo método de Folin-Ciocalteu. A atividade antioxidante foi medida pelo teste DPPH. A atividade antimicrobiana dos extratos foi avaliada contra cocos Gram-positivos: *Staphylococcus aureus* (ATCC 25923) e *Staphylococcus epidermidis* (cepa isolada de paciente); bacilos Gram-negativos: *Escherichia coli* (ATCC 25922) e *Pseudomonas aeruginosa* (ATCC 27853). A zona de inibição e concentração inibitória mínima (CIM) dos extratos vegetais foram avaliadas pelo método de disco difusão em ágar e microdiluição em caldo, respectivamente. O conteúdo fenólico total dos EHAs foi maior nas cascas do caule (970,4 mg GAE/g) do que nas folhas (693,8 mg GAE/g). No ensaio DPPH, todos os extratos apresentaram alta atividade antioxidante, acima de 75% na concentração de 100 μg/mL. Sobre a atividade antimicrobiana, todos os EHAs apresentaram efeitos contra bactérias Gram-positivas *S. aureus* e *S. epidermidis*, cuja CIM variou de 7,81 μg/mL a 62,5 μg/mL. Essa descoberta é importante, uma vez que esses patógenos geralmente são resistentes a uma variedade de antibióticos amplamente conhecidos. Portanto, esses resultados são promissores e indicam o uso de *S. adstringens* (Mart.) Coville (Barbatimão) como antioxidante natural em alimentos, medicamentos e também apontam para seu uso na terapia antimicrobiana.

Palavras-chave: Cerrado. Produtos naturais. Concentração inibitória mínima.

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INTRODUCTION

Foodborne diseases constitute a global health problem. During the infection, pathogenic bacteria and/or microbial toxins produced enter to the human body through the contaminated food or water. Pathogenesis varies according to the host's health conditions, the type of microorganisms and the amount of the agent to which the host is initially exposed. Common examples of food and waterborne outbreaks are the *S. aureus* (known for its numerous enteric toxins) and the *E. coli* (which may include enterotoxigenic and even enterohemorrhagic strains) infection (GALIÉ *et al.*, 2018; TAKÓ *et al.*, 2020).

Antibacterial drugs currently available are progressively becoming more inefficient, and therapeutic options are increasingly limited due to the emergence of resistance mechanisms against these therapeutic agents. Thus, the identification of new drugs is an urgent need, and the clinical challenge of treating bacterial infections becomes even greater due to the development of resistance, drug-related toxicity, significant drug interactions, and the high financial cost of treatment (HICKL *et al.*, 2018).

Therefore, the study of medicinal plants is important because it allows the discovery of natural products to develop new drugs with antibacterial action. In addition to their efficacy, natural products are mostly non-toxic, and therefore, they can be used as safe therapeutic strategies (RAHMAN *et al.*, 2018; SALAM; QUAVE, 2018). During the past two decades, much attention has been paid to plants as novel alternative therapeutic agents for the treatment of infectious diseases due to their bioactive natural compounds such as phenol, flavonoids, tannins, terpenoids, lectins, polypeptides, polyacetylenes, and alkaloids (ERDEM *et al.*, 2015; TAKÓ *et al.*, 2020).

Medicinal plants are potential sources for developing new drugs since natural plant compounds have different bioactivities, namely antitumor, antihypertensive, antioxidant, and antimicrobial. In this context, the *Stryphnodendron adstringens* (Mart.) Coville (Barbatimão), a member of the Fabaceae family, commonly found in Cerrado, the second-largest Brazilian biome, is commonly used in the treatment and asepsis of cutaneous and genitals wounds (RICARDO *et al.*, 2018). In previous studies, it was proven that stem bark of the Barbatimão had high levels of secondary metabolite tannin, a phenolic compound with healing properties and antifungal activity against the pathogenic fungi *Candida albicans* (SOUZA-MOREIRA; QUEIROZ-FERNANDES; PIETRO, 2018).

This work aimed to investigate the phenolic content, the antioxidant capacity, and antimicrobial activity of the hydroalcoholic extract (EHA) of the stem barks and leaves of *S. adstringens* (Mart.) Coville. To the best of our knowledge, there are no studies that establish total phenolics and evaluate antimicrobial and antioxidant activities of the hydroalcoholic extract from stem barks and leaves of *S. adstringens* (Mart.) Coville using the two different solvent withdrawal methodologies: rotoevaporated or heated on isomantle. Additionally, the present research may contribute to demonstrating that Barbatimão is a source of compounds for the development of new therapeutic drugs.

MATERIAL AND METHODS

Plant materials

S. adstringens (Mart.) Coville stem barks and leaves were collected in December 2018 from the rural area of Patos de Minas, Brazil (Lat.: -18.6851654; Long.: -46.4717834). The materials were identified by agronomist Dr. Terezinha Aparecida Teixeira. Voucher specimens (No. 78516) were deposited in the Uberlandense Herbarium of Biology Institute, Federal University of Uberlândia, Brazil (HUFU).

Preparation of *S. adstringens* extracts

Crude extracts were obtained using the method described by Pinho *et al.* (2012). An aliquot of 20 g of powdered *S. adstringens* (Mart.) Coville stem barks and leaves were separately extracted with a mixture of ethanol:water (1:1) in a water bath (60 °C) for 1 h. Then, the mixture was filtered on filter paper in a vacuum pump (200 Pa). To compare the solvent withdrawal methodologies, the extracts were divided: EHA1 (hydroalcoholic extract from stem bark rotoevaporated); EHA2 (hydroalcoholic extract from stem bark heated on an isomantle); EHA3 (hydroalcoholic extract from leaves rotoevaporated); EHA4 (hydroalcoholic extract from leaves heated on an isomantle). The samples that were rotoevaporated passed in a rotary evaporator (Fisatom, model: 802), heated at 45 °C under 500 Pa. The samples submitted to an isomantle were placed in the equipment at 40 ºC for 30 min. After removing the solvent, the remaining aqueous part was frozen in an ultra-freezer at -80 °C for 24 h and then freeze-dried for 48 h. Finally, a farinaceous powder was obtained, which was used for the dosage of total phenols and evaluation of antioxidant and antimicrobial activity.

Total phenolic content

The total phenolic content of extracts was determined by the method described by Singleton and Rossi (1965). An aliquot of 0.5 mL of the sample (100 μg/mL) was mixed with 2.5 mL of Folin–Ciocalteu reagent (0.2 mol/L). Subsequently, 2 mL of sodium carbonate solution (7.5%) was added to the mixture. Finally, the samples' absorbance was read at 765 nm after standing at ambient

temperature for 1 h. Quantification of total phenolic content was based on a standard curve of gallic acid (GA; 16.1–500 μg/mL). The phenolic content was expressed as mg gallic acid equivalents (GAE) per gram of the extract. The assays were performed in triplicate, as as required by the methodology.

Antioxidant activity evaluation by DPPH assay

Radical-scavenging activity of the extracts was determined using the DPPH radical scavenging activity assay method, according to Lopes-Lutz *et al*. (2008) with a slight modification. For the evaluation, 2.7 mL of DPPH solution (40 $\mu g/mL^{-1}$) was mixed with 0.3 mL of each extract concentration (100, 50, 25, and 12.5 μg/mL). Experiments were conducted in triplicate for each concentration. The percentage of antioxidant activity (AA%) was calculated in the following way:

$$AA\% = [(A_{blank} - A_{sample})/A_{blank}] \times 100, \quad (1)$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound.

To calculate the EC50 values (the concentration of the extract necessary to reduce 50% of the DPPH radical) of the different extracts, the antioxidant activity in different concentrations was calculated (conducted in triplicate for each concentration) to draw a curve between the antioxidant capacity of the respective extract and its concentration. These data were subjected to a regression analysis, and an equation of the line was obtained for the calculation of the EC50.

Antimicrobial activity

Bacterial strains

The antimicrobial activity of extracts was evaluated against four bacterial strains, including Gram-positive cocci: *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* (strain isolated from a patient with a bloodstream infection identified by the Vitek II automated method in HC-UFU); and Gram-negative bacilli: *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). All bacterial strains were obtained from the Laboratory of Molecular Microbiology, Institute of Biomedical Sciences (ICBIM), Federal University of Uberlândia. Microorganisms were stocked at -20 °C in tryptone soy broth (TSB) supplemented with 20% glycerol. Separately, each bacterium was reactivated in tubes with 5 mL Luria Bertani broth (LB). After 24 h of incubation at 37 °C, the microorganisms were seeded in Petri dishes containing tryptone soy agar (TSA) medium and then incubated for 24 h at 37 °C.

Disc diffusion method

This antimicrobial test was performed as described by the Clinical and Laboratory Standards Institute (2018). In this assay, a suspension of each bacterium adjusted to 0.5 McFarland turbidity (10^8 CFU/mL) was prepared and seeded on the surface of plates containing Mueller–Hinton agar (MHA). Then, 6 mm diameter filter paper disks loaded with 500 μg/mL EHAs were added in triplicate. As a negative control, disks containing with sterile distilled water were used. As a positive control, we used amoxicillin + clavulanic acid (AMC 20/10 μg) for *S. aureus*, azithromycin (AZT 15 μg) for *S. epidermidis*, aztreonam (ATM 30 μg) for *P. aeruginosa*, and ampicillin (AMP 10 μg) for *E. coli*. After the discs were added, the plates were incubated at 37 °C for 18 to 24 h, and then the inhibition zone was measured in mm. Inhibition zone ≥ 7 mm = positive result.

Minimum inhibitory concentrations (MIC) assay

The MIC of the EHAs was determined according to the methodology described by Clinical and Laboratory Standards Institute (2018). Only the microorganisms that were inhibited in the agar diffusion assay were tested. The MIC was based on the microdilution method using 96-well microtiter plates. In the microplate containing broth LB, serial dilutions were made for each EHAs of 500 to 3.91 μg/mL. Posteriorly, 10 μL of bacterial suspension (10^8 CFU/mL - 0.5 McFarland) was added to the wells. The microplate was incubated at 37 °C for 24 h. For evaluation of cell viability of bacteria, 10 μL of methylthiazolyldiphenyl-tetrazolium bromide (MTT) at 0.2 mg/mL was added to each well and incubated for 4 h. In the experiment, bacterial inoculum with chloramphenicol (10 μg/mL) was used as positive control and bacterial inoculum without EHAs and antibiotics as negative control.

Statistical analysis

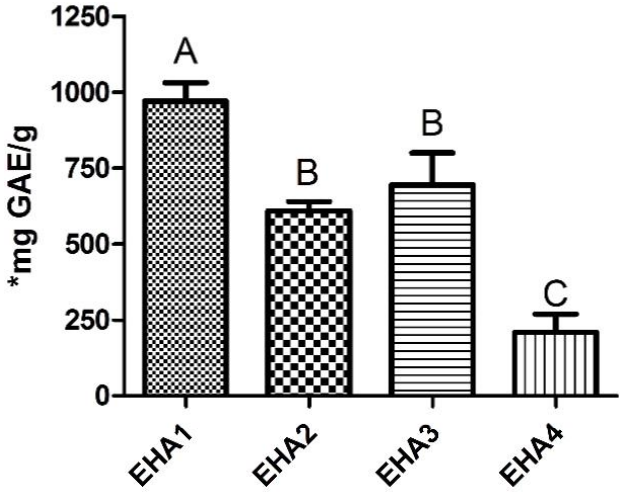
Statistical program Sisvar® (version 5.6) was used, and the data were submitted to analysis of variance, and the means were compared by the Scott-Knott test at the 5% probability level. Differences at $p < 0.05$ were considered significant. GraphPad Prism® (version 5.01) was used for the results that were plotted in bar graphs. The correlation (r) between the values of total phenol content and antioxidant activity of EHAs was calculated using Microsoft Excel® program.

RESULTS AND DISCUSSION

Content of phenolic compounds and antioxidant potential

The total phenolic in the extracts, using the Folin–Ciocalteu reagent, showed that extracts EHA1, EHA2, EHA3, and EH4 present 970.4, 608.7, 693.8, 208.7 mg GAE/g of EHA, respectively (Figure 1).

Figure 1 - Total phenolic contents of the extracts of *S. adstringens* (Mart.) Coville



Notes: The assay allowed calculating of mean values of total phenolic compounds contents in the EHAs of *S. adstringens* (Mart.) Coville. Means followed by the same letter do not differ significantly at 5% probability by the Scott-Knott test. EHA1 (hydroalcoholic extract from stem bark rotoevaporated); EHA2 (hydroalcoholic extract from stem bark heated on an isomantle); EHA3 (hydroalcoholic extract from leaves rotoevaporated); EHA4 (hydroalcoholic extract from leaves heated on an isomantle)

Extracts of stem bark (EHA1 and EHA2) showed higher dosages of total phenolic than the leaf extract (EHA3 and EHA4) when subjected to the same preparation process, indicating that in the stem bark, most of the compounds are found. This result is indicative that the stem bark of this species can be considered an important source of compounds with antioxidant action, which makes it potentially useful in the area of nutrition and for pharmaceutical purposes and the synthesis of a new medicine or cosmetic with antioxidant properties.

Also, it is notable that the samples submitted to an isomantle had lower total phenol content than rotoevaporated samples. Probably, this fact is due to the contact that these samples had with the heat source in the isomantle, since the phenolic compounds are thermosensitive and the extraction methods under high temperatures can degrade these metabolites (CVETANOVIĆ *et al.*, 2017). Also, regarding temperature control, the blanket is a more unstable and rustic piece of equipment than the rotary evaporator, and this may have influenced the deterioration of the metabolites present in the extracts. To the best of our knowledge, there are no recorded works that measure total phenols in the hydroalcoholic extract of the barks and leaves of *S. adstringens* (Mart.) Coville. In other studies, the content of total phenolic content in plants of the Fabaceae family is reported (DZOYEM; MCGAW; ELOFF, 2014). Differently, this present study reported lower values of total phenolics for the acetone extract from *Dalbergia nitidula* Padeiro and *Indigofera cylindrica* leaves, 14.39 ± 0.62 mg GAE/g and 8.94 ± 1.52 mg GAE/g, respectively. Generally, variations in compounds present in plant extracts may be due to temperature, climate variation, and collection time, and even in factors such as plant development and age (GOBBO-NETO; LOPES, 2007). However, there are studies using different solvents that determine total phenols in this plant's bark for different purposes of this study. Baldivia *et al.* (2018), with the objective of evaluating the anticancer activity of Barbatimão stem bark in a melanoma cell line, showed that the aqueous extract (SAAE) of this part at 200 µg/mL presented a significant dosage of total phenols, 195.16 mg GAE/g of SAAE. Therefore, these results also show that the Barbatimão bark is a remarkable supplier of phenolic compounds.

Natural antioxidants can decrease the deleterious effects of various oxidative stress-induced pathological conditions because these molecules can contain cellular components degraded by free radicals (RAHMAN *et al.*, 2018). The antioxidant activity assay of EHAs evaluated by the DPPH free radical scavenging method presented significant results, as observed in Figure 2. Currently, this method has been widely applied in several samples, such as vegetables, herbs, and medicinal plants, as it is a quick and easy method to apply and has high sensitivity (ALAM; BRISTI; RAFIQUZZAMAN, 2013).

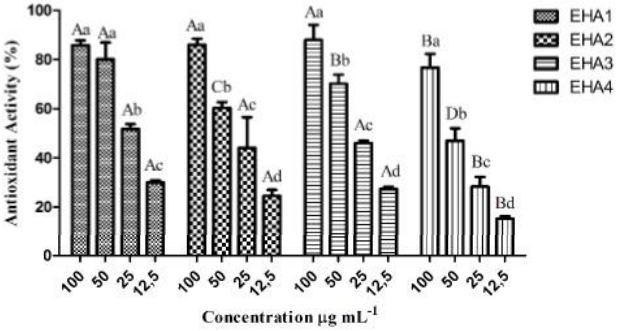
At the concentration of 100 µg/mL, all extracts exhibited antioxidant activity above 75%. Conversely, the concentration of 12.5 µg/mL had the lowest measurement, %AA below 30%. Therefore, there is an increasing relationship between EHA concentration and antioxidant activity; that is, the higher the concentration of the extract, the higher the measurements for %AA. At the concentration of 50 µg/mL, %AA was found to be higher in the rotoevaporated EHAs than the EHAs heated in an isomantle. This difference may be due to the solvent removal process in the isomantle being more deteriorating, which results in the loss of metabolites that would have antioxidant capacity. In addition, of all the extracts, EHA1 and EHA4 presented the highest and lowest %AA, respectively. This result indicates there is more preservation of antioxidant compounds if the rotary evaporator is used and the stem bark of the *S. adstringens* (Mart.) Coville has more antioxidant compounds than leaves. Similar to what was found in this study, other studies have shown that plants of the Fabaceae family have interesting antioxidant capacity. Sadiq *et al.* (2017) showed that the ethanol extract from the leaves and bark of *Acacia nilotica* present promising antioxidant effects.

The antioxidant activity with the EC50 values, using the DPPH method, from Barbatimão extracts is shown in (Table 1). The results were expressed in EC50

(extract concentration in μg/mL capable of reacting with 50% of the radical present in the solution DPPH). Therefore, the lower the EC50 value, the greater the antioxidant activity of the analyzed extract.

As can be seen in Table 1, EHA1 showed the highest antioxidant activity with an EC50 value of 24.1 ± 0.02 μg/mL, followed by EHA3 extract with an EC50 of 29.2 ± 0.03 μg/mL. The EHA4 extract showed the lowest antioxidant activity with an EC50 of 58.8 ± 0.01 μg/mL. Other studies have shown that plants in the Fabaceae family have strong activity in sequestering the DPPH radical. Dzoyem, Mcgaw and Eloff (2014) obtained results that corroborate our findings, with strong antioxidant activity for the acetone extract from *Indigofera cylindrica* (Fabaceae) leaves, with an EC50 value of 22.31 ± 9.92 μg/mL. However, these authors also observed that the acetone extract from the leaves of *Baphia racemosa* Hochst Baker (Fabaceae) and *Crotalaria capensis* Jacq (Fabaceae) have a weak capacity to degrade the DPPH radical, with EC50 values of 210.69 ± 65.48 μg/mL and 195.26 ± 30.64 μg/mL, respectively. Thus, demonstrating that using plants from the same family (Fabaceae), there can be a variation of secondary metabolites responsible for the sequestering activity of the DPPH radical. There are several factors that can influence the accumulation of secondary metabolites in plants, such as ultraviolet radiation, seasonality, as well as pathogen attack, herbivory, and water availability (GOBBO-NETO; LOPES, 2007).

Figure 2 - Antioxidant activity of the extracts of *S. adstringens* (Mart.) Coville by DPPH radical scavenging



Notes: The assay allowed calculating the percentage (%) of the antioxidant activity in relation to the analyzed concentrations. The means followed by different letters (A, B, C, and D) differ significantly at 5% probability by the Scott-Knott test, the uppercase letters to compare the concentration between the extracts and the lowercase letters to compare the concentration within each extract. EHA1 (hydroalcoholic extract from stem bark rotoevaporated); EHA2 (hydroalcoholic extract from stem bark heated on an isomantle); EHA3 (hydroalcoholic extract from leaves rotoevaporated); EHA4 (hydroalcoholic extract from leaves heated on an isomantle)

In addition, there are previous studies with results different from ours, as in the research by Baldivia *et al.* (2018), who found that the aqueous extract of the stem bark of *S. adstringens* had an EC50 of 3.81 ± 0.02 μg/mL. This divergence in the results can be explained by the difference in the extraction method, solvent used, or origin of the plant species.

In the results obtained, there was a positive correlation (r = 0.82) between total phenol contents and antioxidant capacity. In this way, the antioxidant activity was higher in extracts where the amount of total phenols dosed was higher. This correlation was also observed in the study by Sousa *et al.* (2007), in which the antioxidant capacity and total phenols of the ethanolic extract of the medicinal plants of the Cerrado *Terminalia brasiliensis* (Combretaceae), *Copernicia cerifera* (Arecaceae), and *Cenostigma macrophyllum* (Fabaceae) were evaluated. Thus, medicinal plants in the Cerrado can be considered a potential resource for obtaining new therapeutic compounds that may have potential antioxidant action and prevent pathological processes.

Antimicrobial potential

Agar diffusion

Certain food pathogens can survive under adverse environmental factors such as cold, heat, acidic and high salt conditions and have the capacity to form biofilms on biotic or abiotic surfaces. These properties can facilitate their growth and spread on food contact surfaces as well. On the other hand, the consumption of raw products, such as fruits and vegetables, packaged salads and ready-to-eat products has increased. This can cause diseases by exposing consumers to a greater variety of products potentially contaminated with food

Table 1 - Antioxidant capacity (EC50 in μg/mL) of hydroalcoholic extracts from the stem bark and leaves of *S. adstringens* (Mart.) Coville, using the free radical DPPH

	Barbatimão Extracts			
	EHA1	EHA2	EHA3	EHA4
EC50 in μg/mL	24.1 ± 0.02	41.2 ± 0.01	29.2 ± 0.03	58.8 ± 0.01

Notes: results are expressed as mean ± SEM (n = 3); EHA1 (hydroalcoholic extract from stem bark rotoevaporated); EHA2 (hydroalcoholic extract from stem bark heated on an isomantle); EHA3 (hydroalcoholic extract from leaves rotoevaporated); EHA4 (hydroalcoholic extract from leaves heated on an isomantle)

pathogens (TAKÓ *et al.*, 2020). Moreover, the misuse and overuse of anti-infective drugs against pathogenic microorganisms has generated greater resistance to clinical antibiotic therapy, acquiring the ability to survive at high drug concentration that cause serious diseases and/or chronic infections (HASHEMPOUR-BALTORK *et al.*, 2019). In this context, the search for a new, better, and more accessible antibiotic derived from a medicinal plant as an alternative or complement to the treatment of bacterial drug resistance is of paramount importance. Thus, species commonly used as herbal medicine show biologically active components as a good alternative due to the variety of plants' secondary metabolites and their potential to exert antimicrobial activities (CHOUNA *et al.*, 2009; KUETE, 2010).

In the present study, the antibacterial activity of the EHAs was tested against Gram-positive bacteria *S. aureus* and *S. epidermidis* and Gram-negative *E. coli* and *P. aeruginosa* by the qualitative method of diffusion in agar (Table 2).

All extracts at the concentration of 500 µg/mL inhibited the growth of Gram-positive bacteria *S. aureus* (ATCC 25923) and *S. epidermidis* (Clinical Strain), a significant finding as these bacteria are often resistant to a variety of well-known antibiotics (FERREIRA *et al.*, 2019; VERGARA *et al.*, 2017). *S. aureus* is able to multiply on the mucous membranes and skin of food handlers, a major issue for food factories (GIAOURIS *et al.*, 2014), because staphylococcal enterotoxins are heat-stable and are secreted during growth of this bacterium in a food matrix, eventually contaminated by the food handler or an animal. These enterotoxins bind to class II MHC in T cells, giving rise to their activation and to an acute toxic shock with diarrhea and vomiting (SCHELIN; SUSILO; JOHLER, 2017). Moreover, the emergence of methicillin-resistant *S. aureus* (MRSA) in farm animals has cause great concern because animal-derived foods are a primary contamination origin for this resistant pathogen and this bacterium is able to form biofilms on many different kinds of animal surfaces (VERGARA *et al.*, 2017). The positive result with Gram-positive bacteria is more common to verify since they are more sensitive to plant metabolites. Chakraborty *et al.* (2018) demonstrated that the leaf ethanolic extracts of three species of plants (*Cannabis sativa, Platycladus orientalis,* and *Psidium guajava* L.) presented inhibitory activity against strains of MRSA. Moreover, the authors attributed this effect to the phenolic compounds present in these extracts. The same class of secondary compounds present in abundance in the extracts was evaluated in this study. This emphasizes the importance of using plant phenolic as natural alternatives to synthetic compounds to eliminate pathogens and spoilage bacteria from food environments.

On the other hand, for the Gram-negative species *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853), the extracts showed no antibiotic action, which may be related to the lower susceptibility of Gram-negative bacteria to plant extracts, probably due to the more complex structure of their cell wall,

Table 2 - Antimicrobial activity of *S. adstringens* (Mart.) Coville extracts by agar well diffusion assay

		---------- Microrganisms ----------			
		---------- Inhibition zone diameters (mm) ----------			
Samples	Concentration	S. aureus (ATCC 25923)	S. epidermidis*	P. aeruginosa (ATCC 27853)	E. coli (ATCC 25922)
Extracts					
EHA 1	500 µg/mL	22.7 (± 1.5)	26.7 (±.1.5)	N/A	N/A
EHA 2	500 µg/mL	23.7 (± 2.1)	26.3 (± 1.5)	N/A	N/A
EHA 3	500 µg/mL	21.0 (± 2)	25.0 (± 1)	N/A	N/A
EHA 4	500 µg/mL	19.0 (± 1.5)	22.6 (± 2)	N/A	N/A
Controls					
AMC	20/10 µg	21.6 (± 2)	N/T	N/T	N/T
AZT	15 µg	N/T	49.6 (± 1)	N/T	N/T
ATM	30 µg	N/T	N/T	22.0 (± 1.5)	N/T
AMP	10 µg	N/T	N/T	N/T	19.1 (± 1.5)
Sterile water	10 µL	N/A	N/A	N/A	N/A

Notes: N/A: No activity; N/T: Not tested; EHA1 (hydroalcoholic extract from stem bark rotoevaporated); EHA2 (hydroalcoholic extract from stem bark heated on an isomantle); EHA3 (hydroalcoholic extract from leaves rotoevaporated); EHA4 (hydroalcoholic extract from leaves heated on an isomantle); AMC (amoxicillin + clavulanic acid); AZT (azithromycin); ATM (aztreonam); AMP (ampicillin); * (strain isolated from a patient with bloodstream infection identified by the Vitek II automated method in HC-UFU)

which is largely responsible for the impermeability and natural resistance to the penetration of antibiotic molecules (CHOI; LEE, 2019). This may have hampered the action of EHAs in these microorganisms. Also, the crude extract contains a mixture of several compounds, and it is likely that some antimicrobial compounds in Gram-negative bacteria may have been inhibited. Moreover, some metabolites that would act against these bacteria may have been lost during the solvent withdrawal process. Previously, Pinho *et al.* (2012) also verified, by the agar diffusion method, that the hydroalcoholic extract of the leaves of *S. adstringens* at 300 μg/mL inhibited the growth of *S. aureus*. In this case, the inhibition halos were 10 mm. This same extract also had no effect on *E. coli.* So, this study reinforces that the leaf of *S. adstringens* has antimicrobial activity in Gram-positive bacteria.

Minimum inhibitory concentration (MIC)

The extracts that showed antibacterial activity with inhibition halos equal to or greater than 7 mm against the Gram-positive species *S. aureus* (ATCC 25923) and *S. epidermidis* (Clinical Strain) were subjected to the determination quantitative test of Minimum Inhibitory Concentration (MIC) by microdilution (Table 3).

The results presented in this technique showed that bark extracts are better inhibitors than leaf extracts. In addition, *S. epidermidis* was more sensitive to EHAs than *S. aureus*. This result is noteworthy since the antibacterial effect demonstrated *in vitro* for EHA1 and EHA2 against *S. epidemidis* was more potent than the standard antibiotic. That is, the extracts of the bark demonstrated a lower MIC than the leaf extracts. According to Wamba *et al.* (2018) a plant extract is very active if MIC < 100 μg/mL, significantly active if 100 ≤ MIC < 512 μg/mL, moderately active when 512 < MIC ≤ 2048 μg/mL, and weakly active if MIC > 2048 μg/mL. Therefore, based on this scale, all EHAs in this study were considered to be very active because the MIC was < 100 μg/mL. Thus, these EHAs can be applied to a pharmaceutical composition as a modulator, adjuvant, or precursor for the synthesis of a new antibiotic in the future. In addition, the solvent withdrawal methods did not interfere in the results of this technique since the MICs were the same between EHAs (EHA1, EH2) and bark (EHA3, EHA4).

Other studies also report the antimicrobial potential in plants of the family Fabaceae. Corroborating our findings, Dzoyem, Mcgaw and Eloff (2014) reported significantly active antibacterial activity against *S. aureus* for the acetone extract of the leaves of *Dalbergia nitidula* (Fabaceae), with a MIC of 160.0 μg/mL. However, these authors also observed that the acetone extract from the leaves of *Baphia racemosa* (Fabaceae) does not have antibacterial activity against *P. aeruginosa*. Awouafack *et al.* (2013) showed that crude extract and isolated compounds of *Eriosema robustum* had significant activity against *S. aureus*, *E. faecalis*, and *E. coli.* The present study corroborated that the plants of the Cerrado belonging to the family Fabaceae are remarkable resources for the bioprospection of new antimicrobial drugs.

Extracts of the stem barks (EHA1 and EHA2) showed lower values for MIC, higher total phenol dosage, and better %AA. Thus, it suggests that there is a greater presence and availability of antioxidant and antimicrobial metabolites in the bark than in the leaf of *S. adstringens* (Mart.) Coville.

Although these extracts are considered promising, further investigations into the clinical evidence of their efficacy are necessary. It is also important to emphasize that for the medicinal use of these plants as antimicrobials, future studies, such as those related to *in vivo* pharmacokinetics, cytotoxicity, and clinical trials, should be performed.

Table 3 - Minimal inhibitory concentration (MIC) of hydroalcoholic extracts of (EHAs) of *S. adstringens* (Mart.) Coville

Samples	MIC (μg/mL)	
	Microorganisms	
	S. aureus (ATCC 25923)	S. epidermidis*
EHA1	31.25 μg/mL	7.81 μg/mL
EHA2	31.25 μg/mL	7.81 μg/mL
EHA3	62.5 μg/mL	15.6 μg/mL
EHA4	62.5 μg/mL	15.6 μg/mL
Chloramphenicol	10 μg/mL	10 μg/mL

Notes: EHA1 (hydroalcoholic extract from stem bark rotoevaporated); EHA2 (hydroalcoholic extract from stem bark heated on an isomantle); EHA3 (hydroalcoholic extract from leaves rotoevaporated); EHA4 (hydroalcoholic extract from leaves heated on an isomantle); * (strain isolated from a patient with bloodstream infection identified by the Vitek II automated method in HC-UFU)

CONCLUSIONS

1. Based on the investigation into its phytochemical profile, the extract of *S. adstringens* (Mart.) Coville bark stands out in all the tests that were performed, as it presented the highest content of total phenols. Considering this finding, it is reasonable to state that the antioxidant activity obtained by Barbatimão is expressive and is related to this group of metabolites;
2. In addition, EHAs showed strong *in vitro* antimicrobial activity against Gram-positive bacteria *S. aureus* (ATCC 25923) and *S. epidermidis* (strain isolated from a patient), as concentrations were less than 100 μg/mL. This finding is relevant, considering that these pathogens are often resistant to a variety of widely used antibiotics;
3. From these results, the future of this work would be to test the extracts against bacteria with a multi-resistant profile and with biofilm-forming species in the food industry. The results indicate that Barbatimão's EHAs appear as a viable alternative for bioactive compounds that inhibit the growth of pathogenic and food-damaging bacteria, such as *S. epidermidis* and *S. aureus*.

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