SCIENTIA PLENA
www.scientiaplena.org.br
VOL. 21, NUM. 02 2025
doi: 10.14808/sci.plena.2025.029901

Crude extract of *Stryphnodendron adstringens* (Mart.) Coville: antifungal, antibacterial, antioxidant and hemolytic activities

Extrato bruto de *Stryphnodendron adstringens* (Mart.) Coville: atividades antifúngica, antibacteriana, antioxidante e hemolítica

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(Recebido em 07 de outubro de 2024; aceito em 16 de fevereiro de 2025)

The search for bioactive plant compounds has gained significant attention worldwide in recent decades. *Stryphnodendron adstringens* (Mart.) Coville, commonly known as barbatimao, is endemic to the Brazilian cerrado and is characterized by a high phenolic content in its bark, which possesses potential biological activity. This study aimed to evaluate the antibacterial, antifungal, and hemolytic activities of the hydroethanolic extract (EHE) of *S. adstringens* using the disk diffusion method. Additionally, it sought to quantify polyphenols using the Folin-Ciocalteu method, flavonoids through the complexation reaction with aluminum chloride, and assess antioxidant activity through DPPH radical scavenging. The extract exhibited a relative phenolic concentration (373.23 mg EAG/g) with a high flavonoid content (961.96 mg EQ/g). The extract showed antioxidant activity above 70%. At a concentration of 100 µg/mL, satisfactory antimicrobial activity was observed against *Escherichia coli*, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, and *Cryptococcus neoformans* var. grubii. The extract showed no hemolytic activity. Therefore, it can be inferred that *S. adstringens* exhibits potential biological activity, possibly attributed to its rich phenolic content.
Keywords: barbatimao, biological activity, medicinal plant.

A busca por compostos bioativos vegetais tem ganhado o cenário mundial nas últimas décadas. A planta *Stryphnodendron adstringens* (Mart.) Coville, popularmente denominada como barbatimao, é endêmica do cerrado brasileiro e caracteriza-se por apresentar em sua casca um alto teor de polifenois que possuem potencial capacidade biológica. Este estudo teve como objetivo avaliar atividade antibacteriana, antifúngica e hemolítica do extrato hidroetanólico (EHE) de *S. adstringens* pelo método de disco-difusão. Assim como quantificar polifenóis pelo método de Folin Ciocaulteau, flavonoides através da reação de complexação com cloreto de alumínio, e avaliar a atividade antioxidante pelo sequestro de radical oxidante de DPPH. O extrato apresentou relativa concentração fenólica (373,23 mg EAG/g) com elevado conteúdo de flavonoides (961,96 mg EQ/g). O extrato apresentou atividade antioxidante acima de 70%. Observou-se na concentração de 100 µg/mL atividade antimicrobiana satisfatória frente cepas de *Escherichia coli*, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis* e *Cryptococcus neoformans* var. grubii. O extrato não apresentou atividade hemolítica. Logo, infere-se que a planta *S. adstringens* apresenta potencial atividade biológica oriunda, possivelmente, de seu rico conteúdo fenólico.
Palavras-chave: barbatimão, atividade biológica, planta medicinal.

1. INTRODUCTION

Plants are a good source of bioactive compounds due to their high structural, metabolic, and bioavailability variability. The extraction of biologically active compounds from natural sources has grown and solidified as one of the major applications of biodiversity. Trends related to the

use of certain plant-derived compounds for product formulation have been successfully established [1, 2]. Some plant species have been used for therapeutic purposes for centuries by indigenous and quilombola communities. This is the case with *Stryphnodendron adstringens* (Mart.) Coville, a plant from the Fabaceae family, native to the cerrado biome, endemic to Brazil, and commonly known as barbatimao. Its common name is derived from the Tupi-Guarani language and means "tree that tightens." An adult of this species typically reaches a height of 4 to 5 meters with a trunk diameter of 20 to 30 cm. Its leaves are bipinnate with 6 to 8 pairs of leaflets per pinna [3].

Barbatimão is commonly used in the form of infusions or aqueous extracts by traditional populations for the treatment of various ailments, such as diarrhea, gonorrhea, leucorrhea, ulcers, hemorrhages, vaginal infections, and ringworm. Some studies highlight the biological capabilities of this plant, such as antioxidant activity [4], wound healing [5], cytotoxicity [6], potential antifungal [7], antibacterial [8], anti-inflammatory effects, as well as angiogenic and gastroprotective activities. However, few studies address its antiviral and antiparasitic potential [9]. These biological properties are related to the high content of polyphenolic compounds produced by this plant, especially flavonoids and tannins. These compounds have a high capacity to form complexes with metal ions, proteins, and polysaccharides, in addition to their antioxidant characteristics and free radical scavenging abilities [10]. Phenolic compounds are present in most plants, inhibiting the formation of free radicals and encompassing a large class of natural antioxidants, including flavonoids, tannins, coumarins, and lignins, among others. In general, antioxidants deactivate or stabilize free radicals and prevent cellular degeneration by inhibiting highly reactive compounds that can oxidize proteins, carbohydrates, lipids, and nucleic acids, thus maintaining the integrity of cells and tissues [5, 11.

Many natural compounds have been explored for antibacterial and antifungal activity [12-14]. This potential is related to their ability to complex polymers (peptides, polysaccharides, glycoproteins, etc.) of pathogen cell walls and membranes [15]. In recent years, there has been an increased search for bioactive compounds derived from plants, especially woody ones, capable of biological control [16]. The excessive use of synthetic antimicrobials has led to a significant increase in the resistance of certain strains, prompting more efforts to develop new, less cytotoxic, and effective drugs against bacterial and fungal infections [17, 18]. Due to its vast territorial extent and wide range of ecosystems, Brazil is established globally as a powerhouse of biodiversity, with the largest tropical rainforest on the planet and two major biodiversity hotspots (cerrado and Atlantic Forest). Many plant species can be explored, and even well-known species may have gaps in specific literature data. Thus, contributing to the science of natural products related to Brazilian plant species is an emerging task [4, 12, 19].

Considering the above, this study aimed to evaluate the presence of polyphenols as well as the antioxidant, antibacterial, antifungal, and hemolytic activities of the hydroethanolic extract derived from the bark of *Stryphnodendron adstringens* (Mart.) Coville.

2. MATERIAL AND METHODS

2.1 Collection of Plant Material

The collection was conducted in the rural area of Santa Rita do Sapucaí - MG (22°17'8.082"S, 45°48'18.174"W) in a natural ecotone area between the Cerrado and Atlantic Forest biomes, during spring in November 2021. An assessment of the available specimens in the designated study area was carried out, and a representative individual was selected. Consequently, bark, branches, leaves, and fruits of the plant specimen were collected. The samples were transported to the Botany and Phytotherapy Laboratory at the University of Vale do Sapucaí, where botanical analyses were performed. The species was identified based on anatomical criteria, and a herbarium specimen was deposited at the UNIVAS Herbarium with the registration number UNIVAS-007.

2.2 Preparation of Plant Extract

The samples of *S. adstringens* bark were dried at 50 °C for five days in a Pasteur oven. The dried samples were then processed in a knife mill to obtain a fine, homogeneous powder. The extract was prepared using the dynamic maceration method with a hydroethanolic solution of ethanol and distilled water (7:3) as the solvent. The extract was prepared at a 5% (w/v) concentration, using 25 g of the bark powder and 500 mL of the solvent in an Erlenmeyer flask, with constant agitation at 40 rpm for 48 hours. After this period, the extract was filtered and stored in an amber bottle, protected from light and refrigerated [20].

2.3 Determination of Total Soluble Solids Content

The total soluble solids content was evaluated by drying in an oven [12]. Aliquots of the extract were added to pre-weighed beakers, which were then placed in an oven at 55 °C for 5 days to dry. After this period, the mass of the dried extract was measured, and the total soluble solids content was determined using the following formula: Sst = mf – mi. Where Sst = total soluble solids content (mg/mL); mi = initial mass of the beaker (g); mf = final mass of the beaker (g).

2.4 Determination of Total Phenolic Compounds

The total phenolic content was determined using the Folin-Ciocaulteau colorimetric method [21], with modifications. A standard curve was constructed using gallic acid at concentrations of 1 µg/mL, 2 µg/mL, 3 µg/mL, 4 µg/mL, 5 µg/mL, 6 µg/mL, and 7 µg/mL in volumetric flasks. Each flask received 8 mL of Folin-Ciocaulteau reagent and 12 mL of 20% sodium carbonate solution and was allowed to rest for 2 hours in the dark. After this period, the volume was adjusted to 100 mL with distilled water. The extract was assessed using a colorimetric solution prepared in the same way as the standard curve, replacing gallic acid with 100 µL of the hydroethanolic plant extract. Samples were prepared in triplicate, and readings were taken with a spectrophotometer (BEL® UV-M51) at 760 nm. The standard curve equation was calculated using the least squares method. Results were expressed in milligrams of gallic acid equivalents per gram of plant extract (mg EAG/g).

2.5 Determination of Total Flavonoids

For this experiment, a standard quercetin curve was constructed using methanolic quercetin at concentrations of 12 µg/mL, 6 µg/mL, 3 µg/mL, and 1.5 µg/mL. Aliquots of 4 mL of each concentration were placed in test tubes, and 4 mL of 2% methanolic aluminum chloride solution was added to each tube. For the test group, 4 mL of extract was added to the tubes, followed by 4 mL of aluminum chloride. The tubes were allowed to rest for 30 minutes. Samples were analyzed using spectrophotometry at a wavelength of 425 nm, and all readings were made in triplicate. The standard curve equation was calculated using the least squares method. Values were expressed in milligrams of quercetin equivalents per gram of extract (mg EQ/g) [22].

2.6 Determination of Antioxidant Activity

Antioxidant activity was determined using the DPPH (2,2-Diphenyl-1-Picrylhydrazyl) radical scavenging method at 0.06 mM [22, 23]. An aliquot of 100 µL of the extract at a concentration of 100 µg/mL was added to a tube containing 3.9 mL of 0.06 mM methanolic DPPH solution. A control was performed using 100 µL of hydroethanolic solution in place of the plant extract. As a comparative standard, the flavonoid rutin at a concentration of 100 µg/mL was used. After the reaction, samples were analyzed using spectrophotometry. Analyses were conducted in triplicate, and the tubes were incubated for 30 minutes in the dark. The absorbance values obtained were converted into percentage inhibition of antioxidant activity using the following formula:

%I = ((ABS Control – ABS Sample) / ABS Control) x 100. Where %I = DPPH radical scavenging percentage; ABS Control = absorbance reading of the control solution; ABS Sample = absorbance reading for the test sample.

2.7 Determination of Antibacterial and Antifungal Activity

The antimicrobial activity of the hydroethanolic extract of *S. adstringens* bark was assessed using the disk diffusion method against strains from the American Type Culture Collection (ATCC), according to the Kirby-Bauer methodology [24, 25]. Reference strains used included: *Escherichia coli* ATCC 8739, *Mycobacterium tuberculosis* ATCC 25177, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimuriu* ATCC 14028, *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 25923, *Streptococcus pyogenes* ATCC 19615; *Candida albicans* ATCC 10231, *Candida glabrata* ATCC MYA 2950, *Candida krusei* ATCC 6258, *Candida parapsilosis* ATCC 22019, and *Cryptococcus neoformans* var. grubii ATCC 90113. A small fragment of colonies grown for 24 hours was placed in a 0.9% saline solution. The resulting suspension was homogenized in a vortex mixer and the turbidity was adjusted to a 0.5 McFarland standard using a spectrophotometer with absorbance at 625 nm. After standardizing the inocula, 200 µL of the microbial suspensions were spread over the surface of Mueller-Hinton agar (MHA) for bacteria and Mueller-Hinton agar supplemented with 2% glucose (MHAG) for yeasts. Then, 6 mm sterile Whatman filter paper disks impregnated with 10 µL of the extract at a concentration of 100 µg/mL (after complete evaporation of the solvent) were placed on the agar surfaces. As positive controls, disks impregnated with the antibiotics Erythromycin (ERIT 15 µg) and Amoxicillin with Clavulanic Acid (AMOX 30 µg), as well as the antifungal Fluconazole (FLUC 25 µg), were used. The tests were performed in sextuplicate. The plates were incubated at 35 °C for 24 hours for bacterial strains and 48 hours for yeasts. After this period, the zones of inhibition, if present, were measured, and the data obtained were subjected to analysis of variance (ANOVA), with means compared using the Tukey test, both at 5% significance, using Software R 2.5.1.

2.8 Determination of Hemolytic Activity

Hemolytic activity was assessed using the disk diffusion method on solid Blood Agar (Newprov®) [26] with modifications. Soluble solids from the *S. adstringens* extract were dissolved in phosphate buffer solution (PBS) at a concentration of 100 µg/mL. The surfactant Triton X-100 was used as a positive control, and the PBS was used as a negative control. The surfactant, PBS, and the soluble solids suspension were pipetted (10 µL) onto 6 mm Whatman filter paper disks and quickly placed on the Blood Agar medium in Petri dishes. The test was performed in sextuplicate. The plates were incubated for 24 hours at 37 °C. After this period, the halos were measured (mm), and the average and standard deviation were determined using Microsoft Excel Windows 10.

3. RESULTS AND DISCUSSION

3.1 Total Soluble Solids, Polyphenols, and Flavonoids

In the 1990s, two flavan-3-ols were isolated from *S. adstringens*: epigallocatechin 3-O-(3,5-dimethyl)-gallate and epigallocatechin 3-O-(3-methoxy-4-hydroxybenzoate), along with four new proanthocyanidins [27, 28]. The natural products literature also documents other phenolic molecules in *S. adstringens*, including gallic acid, caffeic acid, catechin, kaempferol, and rutin, with particular emphasis on gallic acid, its esters, catechin, and its derivatives (such as gallocatechin, epigallocatechin and others), which are found in higher concentrations [29].

In this research, the total soluble solids content of the extract was 20.90 ± 0.18 mg/mL, the total phenolic compounds concentration was 373.23 ± 3.90 mg EAG/g, and the total flavonoids content was 961.96 ± 5.00 mg EQ/g. As previously reported [6], the extract of barbatimão leaves

showed a rich composition of the flavonoid proanthocyanidin (242.17 ± 14.27 mg QE/g), and its derived fractions had high phenolic concentrations of gallic acid, proanthocyanidin dimers [epicatechin-(4β→8)catechin], and epigallocatechin [(-)-epicatechin-3-O-gallate (ECG)]. Proanthocyanidins constitute a class of structurally complex and highly antioxidant bioactive molecules [30].

The presence of major groups of flavonoids, tannins, and coumarins in the hydroethanolic extract of S. adstringens has been documented, highlighting the rich presence of flavonoids and tannins in this plant [31]. The extract of S. adstringens bark, produced by dynamic maceration using acetone and water as solvents at a concentration of 500 µg/mL, showed a satisfactory concentration of phenols (158.7 ± 4.0 mg GAE/g) and a high concentration of condensed tannins (914.6 ± 51.0 mg GAE/g). Compared to this research, the phenolic concentration obtained was more than double (373.23 ± 3.90 mg GAE/g), which may be related to the solvent used, temperature, sample collection season, among other variables [29]. Additionally, the phenolic composition of aqueous and hydroethanolic extracts derived from both the bark and leaves has also been reported, with the highest concentration observed in the bark (970.4 mg GAE/g), a value that exceeds that found in our research [32].

3.2 Antimicrobial, Antioxidant and Hemolytic Activities

In this study, the raw extract of *S. adstringens* showed an antioxidant activity of 73% (± 0.09), while the quercetin standard exhibited an antioxidant capacity of 90.62% (± 0.1). In a previous study, extracts of *S. adstringens* were obtained by percolation using 50% ethanol (EtOH 50), 70% ethanol (EtOH 70), acetone:water (7:3; v/v - Ac:H2O), and chloroform (CHCl). In that study, gallic acid, rutin, and vitamin C were used as positive controls, with antioxidant activities of 95.63%, 95.46%, 95.18%, 73.86%, 97.24%, 96.83%, and 98.14%, respectively. All controls and extracts were evaluated at a concentration of 250 µg/mL. As expected, the controls used by the authors showed high antioxidant percentages. The extracts obtained with hydroethanolic solutions (50% and 70%) demonstrated the highest DPPH radical scavenging capacity compared to other extracts, while chloroform was less effective in extracting antioxidant substances compared to the hydroethanolic solvent. In this research, the hydroethanolic extract of *S. adstringens* showed an antioxidant percentage of 73% at a concentration of 100 µg/mL, which is excellent when compared to the hydroethanolic extracts at 250 µg/mL mentioned above. Even at a lower concentration (100 µg/mL), the hydroethanolic extract showed an antioxidant percentage similar to that obtained by the authors using chloroform and more than double the concentration [33]. In another study, the aqueous extract obtained from barbatimao bark was evaluated at concentrations ranging from 0.1 to 500 µg/mL and showed a maximum antioxidant capacity of 89.92%. In this research, ascorbic acid (vitamin C) had a DPPH radical scavenging capacity of 87.44%. The same study used the traditional ABTS [2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)] radical capture method, yielding antioxidant percentages of 99.68% for the aqueous extract and 99.67% for the control [34]. Interestingly, hydroethanolic extracts of *S. adstringens* bark prepared by rotary evaporation and evaluated at 100 µg/mL showed antioxidant activity above 70%, similar to the values found for the extract evaluated in this research [32].

The results from this research and the studies reviewed reinforce the biological potential of this plant as a source of compounds with antioxidant activity, highlighting the high efficacy of hydroethanolic solvent in extracting antioxidant substances. This suggests that the choice of solvent for the extraction process, among other factors such as temperature, pressure, sample granulometry, can be an intrinsic factor for the effective extraction of antioxidant molecules. Interestingly, Cruz et al. (2001) [35] emphasize that biological mechanisms are associated or function similarly. For instance, the antioxidant and antimicrobial capacities of certain natural products are related to the interaction between chemical groups present in these molecules and their targets. Flavonoids such as rutin or quercetin act by neutralizing or stabilizing hydroxyl (OH-), nitric oxide (NO-), and hydrogen peroxide (H_2O_2) radicals, which can cause degenerative processes in cells and tissues. The same mechanisms associated with these interactions can be observed when evaluating the antimicrobial capacity of these molecules [36]. In other words, just

as certain compounds can complex, stabilize, and neutralize oxidizing radicals, they also act as complexing agents for bacterial or fungal cell walls. By complexing with the cell wall, the antimicrobial agent disrupts movement, growth, and damages cellular permeability, leading to microbial cell death [37].

In the antibacterial assay, except for *Streptococcus pyogenes*, the hydroethanolic extract of *S. adstringens* at a concentration of 100 µg/mL showed inhibitory activity against all tested strains, as demonstrated in Table 1, with inhibition zones ranging from 9.25 to 12.6 mm and a p-value < 0.05.

Table 1. Antibacterial activity of the hydroethanolic extract of Stryphnodendron adstringens.

Microorganism	Mean of the halos (mm)
S. aureus ATCC 25923	12,66[a]
P. aeruginosa ATCC 27853	11,25[ab]
S. aureus ATCC 6538	11,16[ab]
E. coli ATCC 8739	11[ab]
M. tuberculosis ATCC 25177	9,45[b]
S. typhimurium ATCC 14028	9,25[b]
S. pyogenes ATCC 19615	0[c]

Means followed by the same letters in the columns do not differ by the Tukey test, at a 5% probability level. Mm = millimeters. $p = 2.2x10^{-16}$.

Previously, the ethanolic extract obtained from the bark of *S. adstringens* was evaluated using the disk diffusion technique against *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) at concentrations of 200, 400, and 600 µg/mL [38].

The antimicrobial activity of hydroethanolic (50% and 70%), hydroacetic (50%), and chloroformic extracts of barbatimao was evaluated against *S. aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), and *E. coli* (ATCC 10536) at 50 mg/mL using the diffusion method on solid media. The chloroformic extract showed no antimicrobial activity, while the other extracts were effective against all tested strains, with the 50% hydroethanolic extract being more effective against *S. epidermidis* (inhibitory halos of 14.5 mm). The 70% hydroethanolic extract and the hydroacetic extract were more effective against *E. coli*, showing inhibitory halos of 14.3 mm and 14.0 mm, respectively. The results for *E. coli* and *Staphylococcus* spp. effectively corroborate the data obtained in this study, which, when evaluating the 70% hydroethanolic extract (barbatimao bark), determined average halos of 12.66 mm for *S. aureus* ATCC 25923, 11.16 mm for *S. aureus* ATCC 6538, and 11.0 mm for E. coli ATCC 8739 [33].

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the ethanolic extract of *S. adstringens*—obtained by maceration—were previously determined against *S. aureus* (ATCC 6538), *P. aeruginosa* (ATCC 25853), and *E. coli* (ATCC 8739). The extract was most effective against *S. aureus*, with a minimum inhibitory value of 100 µg/mL and a bactericidal concentration of 200 µg/mL [39]. The strains of *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), and *P. aeruginosa* (ATCC 15442) were also evaluated against the crude extract of *S. adstringens* bark at concentrations of 50 µg/mL, 250 µg/mL, and 500 µg/mL. *E. coli* was not susceptible to the extract, while the *S. aureus* strains (500 µg: 22 mm; 250 µg: 20 mm; 50 µg: 11 mm) and *P. aeruginosa* (500 µg: 15 mm; 250 µg: 13 mm; 50 µg: 08 mm) were susceptible—showing inhibitory halos—in all concentrations evaluated [40]. Interestingly, in another study, the strains of *S. aureus* (ATCC 25923) and *P. aeruginosa* (ATCC 27853) were also susceptible to the crude extract of barbatimao leaves, while E. coli showed no susceptibility [41].

Regarding antifungal activity, in this study, the hydroethanolic extract of *S. adstringens* exhibited inhibitory activity against all tested strains as shown in Table 2. The halos ranged from 7.75 mm (*C. parapsilosis*) to 12.3 mm (*C. albicans*), with a p-value of < 0.05.

Table 2. Antifungal activity of the hydroethanolic extract of Stryphnodendron adstringens.

Microorganism	Mean of the halos (mm)
C. albicans ATCC 10231	12,3[a]
C. krusei ATCC 6258	10,91[b]
C. neoformans var. grubii ATCC 90113	9,83[c]
C. glabrata ATCC MYA2950	8,75[d]
C. parapsilosis ATCC 22019	7,75[d]

Means followed by the same letters in the columns do not differ by the Tukey test, at a 5% probability level. Mm = millimeters. $p = 2.2x10^{-16}$.

Previously, the antimicrobial activity (minimum inhibitory concentration - MIC) of the 50% hydroethanolic extract from the leaves of *S. adstringens* was evaluated against *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 4352), *C. albicans* (ATCC 18804), *Candida tropicalis* (ATCC 13803), and *C. parapsilosis* (ATCC 22009). The *S. aureus* and *P. aeruginosa* strains were the most susceptible, both with a minimum inhibitory value of 0.19 µg/mL, while *K. pneumoniae* showed a minimum inhibitory value of 6.25 µg/mL, and the other less susceptible species were inhibited at a minimum concentration of 12.5 µg/mL [42]. Interestingly, the subfraction F2.4 of the *S. adstringens* bark extract showed a minimum inhibitory concentration of 5 µg/mL and a minimum fungicidal concentration of over 160 µg/mL for *C. neoformans* ATCC 28957. Regarding antifungal activity, studies have associated the use of compounds derived from barbatimao as potential agents against biofilm formation for different *Candida* species [43]. These potential molecules correspond to the group of condensed tannins [7] and polymeric proanthocyanidins [44].
The susceptibility of the evaluated strains to the adopted positive controls is an important indicator of the viability of the proposed assay. In this research, the strains exhibited variable susceptibility to standard antibiotics and antifungal (Table 3).

Table 3. Antibacterial and antifungal activity of the positive controls.

Microorganism	Treatments		
	Amox. Ác. cl. (30µg)	Eri. (15 µg)	Fluc. (25 µg)
S. aureus ATCC 25923	25,33 ± 9,4	22 ± 2,0	Nt
S. aureus ATCC 6538	20,83 ± 5,9	17,66 ± 1,9	Nt
S. pyogenes ATCC 19615	24,16 ± 7,5	8,33 ± 1,1	Nt
M. tuberculosis ATCC 25177	25,83 ± 1,4	14 ± 0,7	Nt
E. coli ATCC 8739	23,41 ± 1,3	0	Nt
S. typhimurium ATCC 14028	26,41 ± 0,8	0	Nt
P. aeruginosa ATCC 27853	34,33 ± 1,6	0	Nt
C. albicans ATCC 10231	Nt	Nt	27,23 ± 1,3
C. glabrata ATCC MYA2950	Nt	Nt	17,91 ± 1,4
C. krusei ATC 6258	Nt	Nt	28,25 ± 1,1
C. parapsilosis ATCC 22019	Nt	Nt	25,5 ± 0,9
C. neoformans var. *grubii* ATCC 90113	Nt	Nt	26,16 ± 1,2

Nt = Not tested; Amox. Cl. = Amoxicillin combined with clavulanic acid; Eri. = Erythromycin; Fluc. = Fluconazole.

In the hemolysis assay, the hydroethanolic extract of *S. adstringens* showed no hemolytic activity at the evaluated concentration of 100 µg/mL. The negative control, PBS, also showed no hemolytic activity. However, as expected, the positive control, represented by the surfactant Triton X-100, was able to produce hemolysis halos (20 mm ± 1.21) in blood agar, validating the adopted method.

To date, the hemolytic activity of *S. adstringens* using the disk diffusion method in blood agar has not been reported in the literature. The absence of hemolytic activity is a significant factor, especially for the development of derivative products for medical and veterinary applications. Some studies even highlight the potential of *S. adstringens* as a producer of molecules that act protectively in conjunction with blood system mechanisms. Interestingly, it has been demonstrated that the aqueous extract of *S. adstringens* neutralized proteases from the venom of the snake *Bothrops pauloensis*, inhibiting coagulative activity and prolonging plasma clotting time by 50%, in addition to showing 100% inhibition of hemorrhagic activity [45]. Moreover, regarding the anti-hemolytic potential of *S. adstringens*, it was found that the aqueous extract of this plant showed no hemolytic activity when incubated with human erythrocytes at concentrations of 125, 100, 75, and 50 µg/mL. Additionally, the extract exhibited protective activity when incubated with erythrocytes along with the oxidizing agent 2,2'-azobis-(2-amidinopropane) dihydrochloride (AAPH) at 50 mM [34]. The results described in the cited studies and reported in this article reinforce the non-hemolytic and protective potential of *S. adstringens.*

Notably, the biological potentials of *S. adstringens* are intrinsically linked to the effective capacity of phenolic substances to interact with other molecules, such as oxidizing radicals (e.g., superoxide and hydrogen peroxide) and polymeric complexes (such as peptidoglycan found in large quantities in the cell walls of Gram-positive bacteria) [15]. In summary, we should highlight the biological capacities of *S. adstringens*, especially regarding its antioxidant, antibacterial, and antifungal activities, which are intrinsically associated with the chemical composition of this plant, rich in phenolic acids (gallic acid, caffeic acid, and protocatechuic acid), tannins, and flavonoids (catechins—epicatechins, gallocatechins, epigallocatechins, quercetin, kaempferol, among other molecules) [10].

4. CONCLUSION

As observed, the hydroethanolic extract obtained from the bark of *S. adstringens* exhibits antibacterial activity against both Gram-negative and Gram-positive bacterial strains, as well as antifungal activity against yeasts of the genera *Candida* spp. and *Cryptococcus* spp. Additionally, the extract demonstrates a high capacity to scavenge DPPH free radicals and shows no hemolytic activity, which reinforces its protective effect. These results, combined with the high content of polyphenols and flavonoids, highlight that the bark of *Stryphnodendron adstringens* (Mart.) Coville represents a promising source of compounds with potential biological activity.

5. ACKNOWLEDGMENTS

To the Botany and Phytotherapy Laboratory of the Faculty of Philosophy, Sciences, and Letters Eugênio Pacelli at the University of Vale do Sapucaí (UNIVAS) and to the Research and Development Laboratory in Natural Products at BEEOTEC Bio Eco Evolution.

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