Acta Scientiarum
http://periodicos.uem.br/ojs
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v46i1.68903

Biological activities of *Stryphnodendron adstringens* bark decoction

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ABSTRACT. *Stryphnodendron adstringens,* called 'barbatimão', is used in Brazilian folk medicine given its healing, astringent, anti-inflammatory, and antimicrobial properties. However, data on its safe use remain inconclusive due to controversy about the risk posed by its ethnopharmacological intake to human health. Thus, the present study aims to quantify the main classes of compounds found in *S. adstringens* bark powder and investigate the biological activity of 'barbatimão' bark decoction against pathogenic bacterial strains. We also investigated its toxic activity using *the Artemia salina* test and its cytogenotoxicity using the *Allium cepa* test. Phenols (24.5 ± 1.5 mg mL^{-1}), tannins (15.08 ± 0.02 mg mL^{-1}), and flavonoids (1.33 ± 0.02 mg mL^{-1}) were quantified in the *S. adstringens* decoction. This decoction exhibited antibacterial activity against Gram-positive strains and toxicity against *A. salina*. It was not possible to observe the *S. adstringens* bark decoction genotoxicity in the *A. cepa* roots because the cell cycle stopped at the prophase, indicating the decoction's anti-proliferation effect. *S. adstringens* is a plant with great herbal potential; therefore, other study models must be used to investigate its potential antibacterial and anti-proliferation applications.

Keywords: *Allium cepa*; antibacterial; *Artemia salina*; cerrado; medicinal plants.

Received on June 12, 2023
Accepted on February 26, 2024

Introduction

According to the World Health Organization, approximately 88% of its member States acknowledge the relevance of using folk and complementary medicine for primary healthcare (World Health Organization [WHO], 2019). Plants are a source of compounds with several biological activities due to their secondary metabolism (Muñoz et al., 2021). Thus, the use of medicinal plants has been part of human history since its early stages. For centuries, people used plants to heal or to prevent many pathogenic microorganisms and diseases (Raskin et al., 2002).

Accordingly, given their historical use in humans, phytochemicals are safer for treating and preventing diseases (Raskin et al., 2002). Besides, a fraction of the population believes 'natural' is better, healthier, and safer than 'non-natural' or synthetic medicines (National Center for Complementary and Integrative Health [NCCIH], 2021). However, several scientific investigations based on bioassays suggest that some plants used in folk medicine have toxic, cytotoxic, and genotoxic effects (Basu & Tripura, 2021; Espadero, Gavilanes, Mosquera, & Alvarez, 2022).

Genus *Stryphnodendron* (Fabaceae) holds 42 species distributed in the neo-tropical region between Costa Rica and Brazil, most distributed in Brazil (Occhioni, 1990). Tree species belonging to this genus are commonly known as 'barbatimão' in Brazil. This common name came from the Tupy-Guarany indigenous language and meant a tree that squeezes, given its astringent properties (Vilar, D'Oliveira, Santos, & Chen, 2010). *Stryphnodendron adstringens* (Mart.) Coville (1910) is native to Brazil and presents a wide geographic distribution in the *Cerrado* and *Caatinga* biomes (Barbosa, Oliveira, Terribile, & Diniz-Filho, 2019). *S. adstringens* is often used in Brazilian folk medicine (Nascimento, Veríssimo, Bastos, & Bernardo, 2016). It is a small deciduous tree with a light color thick, rough, crooked stem. It has alternate, bipinnate compound leaves (Pellenz et al., 2019), and its stem bark has high tannin contents (Vilar et al., 2010).

Barbatimão has wound healing, anti-inflammatory, antioxidant, and diuretic properties and is applied to gastric ulcers and diabetes (Souza-Moreira et al., 2018) and as an inhibitor of the proteolytic, coagulant, hemorrhagic, edematogenic, and myotoxic activity of *Bothrops jararacussu* poison (Pereira Junior et al., 2020). The bark is the most used part of the plant for ethnopharmacological purposes, mainly after its decoction. The empirical and scientific effects attributed to *S. adstringens* bark may derive from the high concentrations of tannins, flavan-3-ol, gallic acid, proanthocyanidins, and chromones (Pellenz et al., 2019).

Despite all the beneficial 'barbatimão' activities, it is important to highlight that the generalized use of medicinal plants by the population justifies the research on the potential risks associated with their intake (Costa et al., 2010). Accordingly, investigations were conducted through several bioassays to check the risks posed by the medical intake of species belonging to the genus *Stryphnodendron*. Some investigations showed that 'barbatimão' is safe; on the other hand, other investigations evidenced that its intake can be risky (Souza-Moreira et al., 2018). Consequently, parameters set for its safe consumption remain inconclusive due to controversy about safety and risk issues related to its intake since it can pose a risk to the health of individuals who use this plant in folk medicine.

In this sense, the present study contributes with data about 'barbatimão' safe use in folk medicine and assesses some of its ethnopharmacological properties. Thus, *S. adstringens* was adopted because it is the species most often used in folk medicine. Stem bark was chosen because it is the most used plant part, mainly after its decoction (Souza-Moreira et al., 2018). The current study aimed to quantify the main classes of compounds found in *S. adstringens* bark powder and investigate their biological activity against some bacterial strains causing human pathologies. Additionally, its toxicity was investigated using *the Artemia salina* test*;* and its cytogenotoxicity was analyzed using the *Allium cepa* test.

Materials and methods

Plant collection

Stryphnodendron adstringens barks were collected at the Ecological Reserve of the *Universidade Estadual de Goiás* - REC/UEG (16° 22' 56.60' S, 48° 56' 39.16' W; approximate altitude of 1,017 m), Anápolis County, Goiás, Brazil, in January 2018 (Figure 1). Botanical identification was confirmed by Ph.D. Professor Mirley Luciene dos Santos. A voucher species (HUEG - 11679) was deposited in the Herbarium of the *Universidade Estadual de Goiás*. Barks were dried in an oven, at 39 °C, for four days. After drying, they were macerated with a knife mill (SPLabor®, Presidente Prudente, São Paulo State, Brazil). The resulting powder was kept in plastic containers and stored in a humidity-free location, in the dark, until its use.

Preparing decoction and aqueous fractions

Given its popular use (Souza-Moreira et al., 2018), decoction was adopted as a technique to extract compounds found in the assessed plant's bark. Aqueous extract solution concentration was based on the study by (Coelho et al., 2010) and (Martins, Figueiredo, Oliveira, & Casteluber, 2019), at the ratio of 20 g bark powder:100 mL distilled water (2:10 p:v). The mix was boiled for 20 min. and, subsequently, filtered in filter paper and left to cool at room temperature. After cooling, the aqueous solution (at a concentration of 0.2 g mL^{-1}) was freeze-dried (LS 3000, Terroni, São Carlos, São Paulo State, Brazil); it led to a 6.2% solid content. The freeze-drying procedure allowed identifying the amount of plant material extracted through the decoction process - the real concentration of the gross extract was 0.062 g mL^{-1}. Aqueous sub-fractions were prepared at concentrations 0.031, 0.016, and 0.008 g mL^{-1}.

Humidity determination

A scale with a halogen lamp (MOC63u UniBloc, Shimadzu) was used to determine moisture content due to *S. adstringens* bark powder decoction. The process was carried out according to the Brazilian Pharmacopoeia, 5th edition (Brasil, 2010). To do so, 1 g *S. adstringens* bark powder samples were heated at 105 °C for 7 min. The test was carried out in triplicate, and the mean was calculated.

Phytochemical prospecting

Decoction and each point of the standard curve were analyzed in triplicate in all colorimetric essays.

Total phenols' quantification

Total phenols' quantification was carried out based on the methodology by Mole and Waterman (1987), which takes into account the complexation of phenolic substances with ferric chloride ($FeCl_3$) - it was measured in a spectrophotometer (IL-592 - Kazuaki). A test tube filled with 1 mL of decoction (0.062 g mL^{-1}) was added with 2 mL sodium lauryl sulfate-LSS solution (1% w v^{-1})/triethanolamine (5% v v^{-1}) and 1 mL of $FeCl_3$. The color produced by the mix was read at 510 nm, after 15 min. at rest, at room temperature. In total, 2 mL of LSS/triethanolamine, 1 mL of $FeCl_3$, and 1 mL of distilled water were used as white color. The standard curve (R^2= 0.997) was plotted with tannic acid at the following dilutions: 0.15, 0.20, 0.25, 0.30, and 0.35 mg mL^{-1}. The decoction's total phenols concentration (mg mL^{-1}) was calculated based on the plotted standard curve.

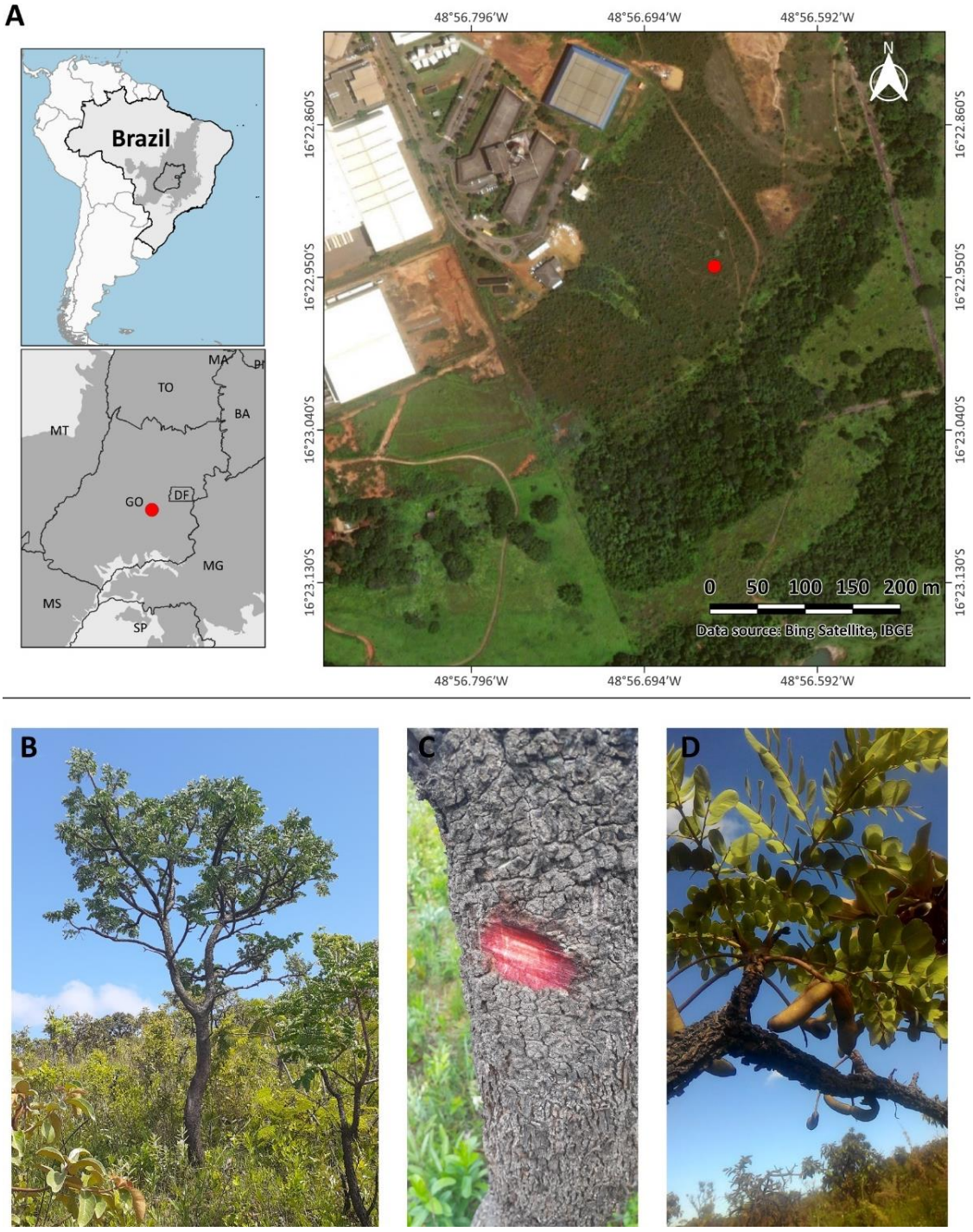

Figure 1. *Stryphnodendron adstringens* (Mar.) ('barbatimão'). (A) Collection location map: Ecological Reserve of the *Universidade Estadual de Goiás*, Anápolis County, Goiás, Brazil. The red dot on the map pinpoints the tree's location. (B) *S. adstringens* specimen. (C) 'barbatimão' bark used for decoction preparation. It has a light color, thick, rough, crooked stem. When the cortex part is removed, one finds a red sap wood. (D) *S. adstringens* leaves and fruits.

Tannins' quantification through protein precipitation

Tannins were quantified using the methodology by Mole and Waterman (1987), which is based on tannins' precipitation in an aqueous solution in the presence of protein. It was followed by tannins' complexation with $FeCl_3$ measured in a spectrophotometer (IL-592 - Kazuaki). Decoction (0.062 g mL^{-1}) was precipitated with Bovine serum albumin (1 mg mL^{-1}). The precipitate was dissolved in LSS/triethanolamine after centrifugation. Tannins were complexed with $FeCl_3$; the colored complex was read at 510 nm, after 15 min resting at room temperature. The white color was prepared with 4 mL LSS/triethanolamine and 1 mL $FeCl_3$. The standard curve (R^2 = 0.988) was plotted with tannic acid at the following dilutions: 0.10, 0.20, 0.30, 0.40, and 0.50 mg mL^{-1}. Tannins' concentration (mg mL^{-1}) in decoction was calculated based on the equation deriving from the standard curve.

Flavonoids' quantification

The method adapted from the assay described by (Rolim et al., 2005) was used; it considers flavonoids' ability to absorb radiation at the wavelength of ultra-violate light. Decoction (0.062 g mL^{-1}) was added to 0.02 M methanol:acetic acid (99:1 v v^{-1}). This mix was heated in boiled water under reflux at 90-100 °C for 40 min. The samples were analyzed in a spectrophotometer at 361 nm after cooling (IL-592 - Kazuaki). Methanol:0.02 M acetic acid (99:1) was used as blank. The standard curve was plotted with rutin at the following dilutions: 0.1, 0.2, 0.3, 0.4, and 0.5 mg $mL^{-1.}$ Flavonoid concentrations in decoction were calculated based on the equation deriving from the standard curve.

Antibacterial activity

The antibacterial activity was assessed against the following standard strains: (i) Gram-positive bacteria: *Enterococcus faecalis* (ATCC 19433), *Staphylococcus aureus* (ATCC 29213), and *Staphylococcus epidermidis* (ATCC 12228); (ii) Gram-negative bacteria: *Escherichia coli* (ATCC 25312) and *Pseudomonas aeruginosa* (ATCC 27953). The strains were collected at the microorganisms' bank of the *Laboratório de Microbiologia* at UEG Central Campus. The test was carried out using the disk diffusion method (Clinical and Laboratory Standards Institute [CLSI], 2015). Sterile blanc disks (5 mm) were soaked in 3 µL of decoction concentrations (0.062, 0.031, 0.016, and 0.008 g mL^{-1}) and applied over the inoculated in agar Mueller-Hinton (Kasvi). Chloramphenicol (30 µg) was used as the positive control. Inhibiting zones' diameters were measured and compared after 24 hours incubation, at 37 °C. The tests were performed in triplicate. Minimum inhibitory concentration assays were canceled because the decoction's dark color may have interfered with the spectrophotometric reading.

Toxicity in Artemia salina

The total aliquot of 36% (p v^{-1}) synthetic seawater added with yeast extract was used to incubate *A. salina* eggs (0.250 g) – oxygenation was constant at 25 °C for 36 hours. In total, 10 nauplii, 100 µL of saline solution, and 100 µL of the corresponding *S. adstringens* bark decoction dilution (0.062, 0.031, 0.016, or 0.008 g mL^{-1}) were taken to 96-well microtiter plates for the toxicity assay. Synthetic marine solution and potassium dichromate (12.5 mg mL^{-1}) were used as the negative and positive control, respectively. Survivals were counted after 24 hours, and LC_{50} was calculated using the Probit method (software STATISTICA®). The experiment was carried out in experimental and biological triplicates, totaling 90 nauplii per condition.

Allium cepa test

Bulbs of *A. cepa* (diameter 5-6 cm) were acquired in a local market. They were cleaned by removing the external bark - the dry basis of the stem was scraped to expose the root beginnings. Subsequently, the bulbs were washed in distilled water, dried with a paper towel, and placed in mineral water for 48 hours to root. Then, the bulbs were separated into six groups (n = 36) and treated with tested decoction concentrations (0.062, 0.031, 0.016, and 0.008 g mL^{-1}). Bulbs' roots in the negative and positive control groups were soaked in mineral water and ethyl methanesulfonate (166 µg mL^{-1}), respectively. All bulbs were exposed for 24 hours at ~ 21 °C. After the exposure time, four roots (from 2 to 2.5 cm) were removed from the six bulbs of each group and fixed in Carnoy solution (ethanol:glacial acetic acid, 3:1 v v^{-1}) for 24 hours. Subsequently, they were washed in distilled water and stored in 70% ethanol at 4 °C.

The tips of the collected roots were washed in distilled water and hydrolyzed in HCl 1 N for 10 min. Once more, the roots were washed in distilled water, and the meristems from four roots were macerated with a razor

blade on a microscope slide and inked with 2% acetic carmine. Then, meristems were covered with coverslips and carefully smashed. The slides were analyzed in a Primo Star Zeiss optical microscope (Zeiss, Oberkochen, Alemanha) at 100X magnification. In total, 1,000 cells from each bulb (totaling 6,000 cells per treatment) were assessed to evaluate chromosomal aberrations (CA) and nuclear anomalies (NA). The total number of cells in mitosis per 1,000 cells observed in each bulb was taken as Mitotic Index (MI). The analyses were carried out in sextuplicate.

Data analysis

Cytotoxicity, genotoxicity, and mutagenicity data were expressed as the mean + standard deviation (SD) of six bulbs per treatment (n = 6,000 cells). Data that do not present normal distribution in the Shapiro-Wilk test were transformed through the root-square method (software Past 3.0). Data were analyzed through ANOVA with post hoc Tukey test. $p \leqslant 0.05$ was considered statistically significant. Statistical tests were carried out in Past 3.0 software.

Results

Phytochemical prospecting

Stryphnodendron adstringens samples were analyzed based on some quality control parameters applied to plant medicines and to the main classes of metabolites identified for this species (Table 1).

Antibacterial activity

Stryphnodendron adstringen bark decoction inhibited bacterial growth (Table 2). Inhibition was detected against Gram-positive *Enterococcus faecalis*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* strains. Inhibition zones in all sensitive strains were dependent on doses lower than those in the positive control. *Enterococcus faecales* was the only strain sensitive to the lowest decoction concentration (0.008 g mL^{-1}).

Table 1. Quantitative analysis of *Stryphnodendron adstringens* bark powder.

Variable	Result	
	Weight (g)/ 20 g	%
Solid-contents	6.2 g	6.2
Extraction-yield	6.2 g	31.0
VCs[1]	2.268 g	11.3 (± 0.09)
	[mg mL^{-1}]	%
Total Phenols[2]	24.5 (± 1.5)	13.8
Tannins[2]	15.08 (± 0.02)	8.5
Flavonoids[2]	1.33 (± 0.02)	0.75

[1]VCs: volatile compounds. [2]By considering loss due to desiccation.

Table 2. Gram-positive inhibition halo measurements (mm) at different (g mL^{-1}) *Stryphnodendron adstringens* decoction concentrations.

	0.008	0.016	0.031	0.062	PC[1]
Enterococus faecales	0.2 ± 0.3^{b}	0.2 ± 0.4^{b}	0.8 ± 0.1^{b}	3.2 ± 5.9^{b}	20 ± 0^{a}
Staphylococus aureus	0^{c}	0.8 ± 0.1^{c}	0.8 ± 0.1^{c}	9.8 ± 1.9^{b}	20 ± 0^{a}
Staphylococus epidermides	0^{b}	0.6 ± 0.4^{b}	5.3 ± 6.1^{b}	7.3 ± 5.6^{b}	20 ± 0^{a}

[1]PC: positive control. Different lowercase letters in the same line point out significant statistical differences using ANOVA with post hoc Tukey test ($p < 0.05$).

Toxicity evaluation in *Artemia salina*

Stryphnodendron adstringens bark decoction toxicity in *A. salina* analysis showed LC_{50} = 0.0146 g mL^{-1} (Figure 2). There was no nauplii death at the concentration of 0.008 g mL^{-1}.

Cytogenotoxicity assessment using the *Allium cepa* test

Mitotic index (MI), chromosomal aberrations (CA), and nuclear anomalies (NA) frequencies were not statistically different ($p > 0.05$) from the negative control. However, they were significantly different from the positive control ($p < 0.05$) (Table 3). Cells at all cell-division stages were observed in the negative and positive controls. However, cells were only at the interphase, and in the initial prophase stages in groups exposed to the tested decoction concentrations (Table 3). Cell-cycle stopping may have been the cause of non-significant CA and NA frequencies. It is so because CA and NA formation depends on the stressor's action at the mitotic stages; phase blocking probably avoided their formation.

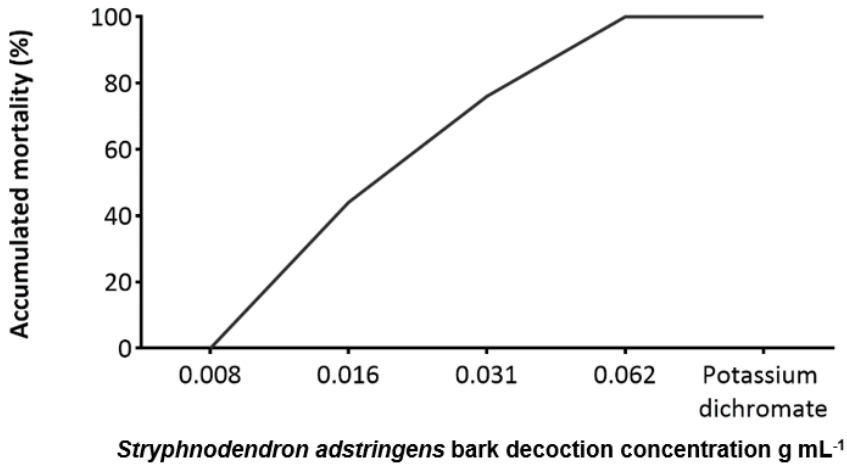

Figure 2. Frequency of (%) nauplii death after 24 hours exposure to the four *Stryphnodendron adstringens* bark decoction concentrations.

Table 3. Mitosis phases, mitotic index (MI), chromosomal aberrations (CA), and nuclear anomalies (NA) observed in the root meristem of *Allium cepa* treated with *Stryphnodendron adstringens* bark decoction.

Treatments	Number of cells per cycle phase					Activity		
	Inter_	Pro_	Meta_	Ana_	Telo_	[1]MI (%) ± SD*	[2]CA (%) ± SD*	NA (%) ± SD*
0.008 g mL^{-1}	5,055	945	0	0	1	15.76± 3.8[a]	0.33±0.51[a]	1.33±0.51[a]
0.016 g mL^{-1}	5,051	949	0	0	0	15.81± 2.8[a]	0.33±0.51[a]	1.33±0.81[a]
0.031 g mL^{-1}	4,978	1,022	0	0	0	17.03±4.7[a]	0.33±0.81[a]	1.00±0.89[a]
0.062 g mL^{-1}	5,270	730	0	0	0	12.16±3.8[a]	0.16±0.40[a]	0.83±0.75[a]
NC	4,878	972	54	45	51	16.2±3.2[a]	0.33±0.51[a]	0.83±0.98[a]
PC	5,274	698	11	5	12	11.4 ±2.08[b]*	4.8±3.1[b]	2.66±1.03[b]

*Data were expressed as mean ± SD (standard deviation). Different lowercase letters in the same column point out significant statistical differences ($p < 0.05$). MI: mitotic index. CA: chromosomal aberration. NA: nuclear anomalies. NC: negative control. PC: Positive control.

Discussion

Total phenols, tannins, and flavonoid contents of the samples obtained in the present study corresponded to the chemical digital print of *S. adstringens* bark (Brasil, 2021). Moreover, sample humidity content (11.3%) was within the limit set by the Brazilian Pharmacopoeia, 5th edition (Brasil, 2010), according to which plant drug humidity content must correspond at most to 14%. It is important to have humidity content lower than 14% since water excess in plant material enables microorganisms and insects' growth, hydrolysis reactions, and, consequently, material deterioration. Accordingly, ideal humidity limits range from 8 to 14% (Nascimento & Cardoso Filho, 2018); thus, humidity content ensures samples' adequate preservation.

Phenols' content in the extracts and fractions from *S. adstringens* bark can range from 1.5% to 80%. The highest values were observed for hydroalcoholic and hydroacetonic extracts (Brasil, 2021). Phenols are natural bioactive molecules formed by plants' secondary metabolism, which accounts for pigmentation and astringency. Phenols have shown interesting biological activities, such as antioxidant, antimicrobial, anti-inflammatory, and antiproliferative (Albuquerque, Heleno, Oliveira, Barros, & Ferreira, 2021).

Stryphnodendron adstringens is tannin-rich, which accounts for its astringent features and the species' name. Tannins are natural secondary phenolic metabolites widely distributed and omnipresent in the vegetal world. Nowadays, it is known that tannins are anticancer, virucidal, antimicrobial, antioxidant, anti-inflammatory, antidiarrheal, and antidiabetic substances. Furthermore, they help with cardiovascular protection, wound repair, and structural bone repair and can be used as a biopesticide (Pizzi, 2021). Any toxicity associated with tannins can derive from their complexation with metallic ions, mainly because biological systems require metallic ions as enzymatic cofactors (Scalbert, 1991). Moreover, tannins can react with proteins irreversibly (Pizzi, 2021). The minimum content of tannins in *S. adstringens* bark is 8% (Brasil, 2021).

Flavonoids, in turn, are an important class of natural polyphenolic compounds. They have beneficial effects on heart and metabolic diseases and autoimmune and neurological disorders. Furthermore, they have anti-oxidative, anti-inflammatory, anti-apoptotic, and immunomodulatory properties (Hamsalakshmi, Marappa, Joghee, & Chidambaram, 2022) and the ability to modulate enzymatic functions (Panche, Diwan, & Chandra,

2016). They also have antibacterial activity against a wide range of pathogenic microorganisms. Such activity has been calling attention to their potential to replace antibiotics (Xie, Yang, Tang, Chen, & Ren, 2015).

The activity of *S. adstringens* secondary metabolites against Gram-positive bacteria has already been reported (Soares & Pereira, 2016). Tannins are considered good bactericides because they react with proteins irreversibly, complexing within bacterial membranes and neutralizing their activity (Pizzi, 2021). Flavonoids can present the following antibacterial mechanisms: a) nucleic acid synthesis inhibition; b) cytoplasmic membrane function inhibition; c) energy metabolism inhibition; d) biofilm attachment and formation inhibition; e) porin inhibition in the cell membrane; f) membrane permeability change; and g) pathogenicity mitigation (Xie et al., 2015). On the other hand, the resistance of Gram-negative strains can be explained by the external membrane acting as a molecule barrier. Furthermore, periplasmic space enzymes can act in the extracellular degradation of secondary metabolites (Duffy & Power, 2001).

Artemia salina bioassay is a method accounting for sensitivity advantages, low cost, and easy handling (Costa et al., 2010). Consequently, it is used as a bioassay to evaluate plant extracts' toxicity (Indriaty, Ginting, Hasballah, & Djufri, 2022). Based on the *A. salina* test, *S. adstringens* bark decoction showed LC_{50} = 0.0146 g mL^{-1}; consequently, tested concentrations higher than LC_{50} (0.031 and 0.062 g mL^{-1}) were toxic because they led to death rates higher than 50% in the *A. salina* population. This outcome corroborated the results by Costa et al. (2010), who used *S. adstringens* bark extract obtained through turbo-extraction in acetone and water; they concluded that LC_{50} in this extract would be higher than 0.001 g mL^{-1}.

Allium cepa test is quite used to assess the cytogenotoxicity of different plant extracts; therefore, it emerges as adequate for such purpose given its reliable results (Basu & Tripura, 2021). Among the advantages of this test, one finds: a) its results can be extrapolated to other organisms; b) low cost; c) efficacy; and d) reliability (Carmo, Leal, & Ribeiro, 2020). So, the *A. cepa* bioassay is promising for the initial screening of cytogenotoxicity in aqueous medicinal plant extracts, not to mention its validation in regulating organs (Carmo et al., 2020).

In the current study, MI, CA, and NA frequencies were not significantly different at the tested *S. adstringens* bark decoction concentrations compared to the negative control. However, it is interesting to observe that either the negative or the positive controls presented cells at all cell-division stages. Nevertheless, all assessed *S. adstringens* bark decoction concentrations only allowed visualizing cells at the prophase, and this finding indicates cell-cycle stopping. This outcome points towards decoction antiproliferative and cytotoxic activity at all tested concentrations. A similar result was found by Reis et al. (2020) when they assessed the aqueous extract of 'barbatimão' bark in *A. cepa* seeds. These authors found that the two assessed concentrations (50 and 100 mg mL^{-1}) did not present cytotoxic and genotoxic effects. Silva et al. (2021) found similar results for cell-cycle stopping. They concluded that *S. adstringens* has a cytotoxic effect since there was cell-cycle inhibition in all four concentrations and exposure times, either in *A. cepa* or *Pisum sativum*.

Cell-cycle stopping may have been the cause of non-significant CA, and NA frequencies since their formation depends on mitotic stages to form themselves (Camilo-Cotrim, Bailão, Ondei, Carneiro, & Almeida 2022). Consequently, cell-cycle stopping may have interfered with CA and NA formation, a fact that masked decoction's likely genotoxic effects. Accordingly, it is not possible to state the genotoxic safety of *S. adstringens* bark decoction.

Cell-cycle stopping is reported in the literature as an effect in cell-cycle checking points that can block or delay mitosis progression due to cell and environmental stressors (Burgess, Rasouli, & Rogers, 2014). The CHFR checkpoint is an example; it is a mitotic stress checkpoint that causes prophase stopping due to substances acting in the microtubules, contributing to genomic stability maintenance (Privette & Petty, 2008). CHFR activation implies reducing the other mitotic phases and accumulating cells at prophases. This finding would justify that only the prophase stage was visualized in the current study.

Conclusion

The *S. adstringens* bark decoction showed antibacterial activity against Gram-positive strains. Concentrations of 0.031 and 0.062 g mL^{-1} caused over 50% mortality in A. salina, indicating toxicity. The decoction also had an antiproliferative effect, likely due to secondary metabolites. Our data suggest the genotoxicity of *S. adstringens* is not entirely conclusive, possibly marked by cell-cycle arrest. Further studies with varying concentrations, exposure times, and extraction methods are needed. Moderate consumption is advised until its safety is confirmed. The antibacterial and antiproliferative properties should be further studied for potential therapeutic applications, and sustainable exploitation of this non-cultivated species is essential.

Acknowledgments

KRXS was supported by FapeG student fellowship (201810267000075). CFCC was supported by Capes student fellowship (88887.483362/2020-00). LLS was supported by Capes student fellowship (88882.448042/2019-01).

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