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Original Article

Phytochemical characterization, and antioxidant and antibacterial activities of the hydroethanolic extract of *Anadenanthera peregrina* stem bark

Caracterização fitoquímica, atividade antioxidante e antibacteriana do extrato hidroetanólico de cascas do caule de *Anadenanthera peregrina*

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Abstract

The Brazilian Cerrado biome consists of a great variety of endemic species with several bioactive compounds, and *Anadenanthera peregrina* (L.) Speg is a promising species. In this study, we aimed to perform phytochemical characterization and evaluate the antioxidant and antibacterial activities against *Staphylococcus aureus* and *Escherichia coli* of the hydroethanolic extract of *A. peregrina* stem bark. The barks were collected in the Botanical Garden of Goiânia, Brazil. The hydroethanolic extract was obtained by percolation and subjected to physicochemical screening, total phenolic content estimation, high-performance liquid chromatography (HPLC) fingerprinting, and antioxidant (IC_{50} values were calculated for the 2,2-diphenyl-1-picrylhydrazyl assay - DPPH) and antibacterial activity determination. The pH of the extract was 5.21 and density was 0.956 g/cm^3. The phytochemical screening indicated the presence of cardiac glycosides, organic acids, reducing sugars, hemolytic saponins, phenols, coumarins, condensed tannins, flavonoids, catechins, depsides, and depsidones derived from benzoquinones. The extract showed intense hemolytic activity. The total phenolic content was 6.40 g GAE 100 g^{-1}. The HPLC fingerprinting analysis revealed the presence of gallic acid, catechin, and epicatechin. We confirmed the antioxidant activity of the extract. Furthermore, the extract did not inhibit the growth of *E. coli* colonies at any volume tested, but there were halos around *S. aureus* colonies at all three volumes tested. These results contribute to a better understanding of the chemical composition of *A. peregrina* stem bark and further support the medicinal applications of this species.

Keywords: products with antimicrobial activity, high pressure liquid chromatography, HPLC, medicinal plants, phenolic compounds.

Resumo

O bioma Cerrado brasileiro apresenta em uma grande variedade de espécies endêmicas com diversos compostos bioativos, e *Anadenanthera peregrina* (L.) Speg é uma espécie promissora. Neste estudo, objetivamos realizar a caracterização fitoquímica e avaliar as atividades antioxidantes e antibacterianas contra *Staphylococcus aureus* e *Escherichia coli* do extrato hidroetanólico de cascas do caule de *A. peregrina*. As cascas foram coletadas no Jardim Botânico de Goiânia, Brasil. O extrato hidroetanólico foi obtido por percolação e submetido a triagem físico-química, estimativa de conteúdo fenólico total, impressão digital por cromatografia líquida de alta eficiência (HPLC) e determinação da atividade antioxidante (valores de IC50 foram calculados para o ensaio 2,2-difenil-1-picril-hidrazil) e antibacteriana. O pH do extrato foi de 5,21 e a densidade foi de 0,956 g/cm^3. A triagem fitoquímica indicou a presença de glicosídeos cardíacos, ácidos orgânicos, açúcares redutores, saponinas hemolíticas, fenóis, cumarinas, taninos condensados, flavonóides, catequinas, depsídios e depsidonas derivados de benzoquinonas. O extrato mostrou intensa atividade hemolítica. O conteúdo fenólico total foi de 6,40 g de GAE 100 g^{-1}. A análise por impressão digital por HPLC revelou a presença de ácido gálico, catequina e epicatequina. Confirmamos a atividade antioxidante do extrato. Além disso, o extrato não inibiu o crescimento de colônias de *E. coli* em nenhum

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volume testado, mas houve halos em torno das colônias de *S. aureus* nos três volumes testados. Estes resultados contribuem para uma melhor compreensão da composição química da casca de *A. peregrina* e apoia ainda mais as aplicações medicinais desta espécie.

Palavras-chave: produtos com ação antimicrobiana, cromatografia líquida de alta velocidade, HPLC, plantas medicinais, compostos fenólicos.

1. Introduction

The World Health Organization (WHO) encourages countries to generate evidence-based policies and strategic plans for the use of medicinal plants (WHO, 2019). In this context, in Brazil, there are several medicinal plants that are used as herbal medicines by the rural and urban populations (Dutra et al., 2016; Pio et al., 2019). A single species can produce numerous chemical compounds with diverse pharmacological activities (Mendonça et al., 2019), including antibacterial (Pandini et al., 2018; Emre et al., 2020; Pacheco et al., 2020), anti-inflammatory (Ribeiro et al., 2018; Almohawes and Alruhaimi, 2020), antioxidant (Barth et al., 2018; Pontes et al., 2019; Vale et al., 2019), wound-healing properties (Ribeiro Neto et al., 2020), antihyperglycemic effect (Silva et al., 2020), and cardiovascular activity (Moreira et al., 2019).

The Cerrado, considered a hotspot of global biodiversity, is the second largest biome in South America, covering approximately 22% of the national territory. It is recognized as the richest savanna in the world, with 11.627 native plant species. In addition to environmental aspects, the Cerrado has social importance to the local population that uses its natural resources, including 220 medicinal species (Brasil, 2019). Some species in the Cerrado, such as *Stryphnodendron adstringens* (barbatimão) (Almeida et al., 2010; Ribeiro et al., 2014; Queiroz et al., 2021), *Macairea radula* (capuchina), and *Pterodon emarginatus* (sucupira) (Vila Verde et al., 2018), have been studied.

Phenolic compounds, mainly tannins, are responsible for the therapeutic activity of different plants in the Cerrado. They have been identified in the following plants in the Cerrado: *Hymenaea stignocarpa* (jatobá-do-cerrado) (Silva et al., 2019), *Caryocar* spp. (pequi) (Nascimento-Silva and Naves, 2019), *Inga laurina* (ingá) (Martins et al., 2019), *Annona crassiflora* (araticum) (Arruda et al., 2018), and *Passiflora alata* (maracujá-doce) (Pereira et al., 2018), making this biome a source of promising medicinal species for bioprospecting studies (Bailão et al., 2015).

The genus *Anadenanthera* has two species, *Anadenanthera colubrina*, which has a wide geographical coverage, and *Anadenanthera peregrina*, typical to the Brazilian Cerrado (Morim, 2015; Carvalho, 2003). *Anadenanthera peregrina* is distributed in the drainage areas, gallery forests, and rocky fields in the Cerrado. Popularly known as angico, *A. peregrina*, a rustic species of canopy-forming trees, resists drought and fire due to its thick bark that protects the plant (Souza et al., 2014). According to Mota et al. (2017), the bark of *A. peregrina* is a potential source of polar extracts, enabling the extraction of tannins that represent approximately 17% of the bark (173.3 mg CE g^{-1} bark) and 59% of the hydroalcoholic extract (in catechin equivalents). The bark and seeds of *Anadenanthera* are used to treat wounds (Pessoa et al., 2012, 2015) and respiratory diseases, owing to the antioxidant, anti-inflammatory, and antimicrobial properties (Weber et al., 2011; Gama et al., 2018).

A pharmacological study on the major species in the Cerrado reported that the stem and resin of *A. peregrina* prepared as decoctions or syrups, or macerated in cachaça and wine have potential to treat bronchitis and influenza (Souza et al., 2016). However, data on the phytochemical properties, and antioxidant (Mota et al., 2017) and antibacterial activities of *A. peregrina* stem bark, which is an important part of the plant used for therapeutic purposes, are limited. Therefore, in this study, we aimed to perform phytochemical analysis and evaluate the antioxidant and antibacterial activities of the hydroethanolic extract of *A. peregrina* stem bark.

2. Materials and Methods

2.1. Plant material and extraction

The stem barks of *A. peregrina* were collected from three plants in the Botanical Garden of Goiânia, Goiás State, Brazil (16°43'22"S, 49°22'54"W), with a diameter at breast height (DBH) of 110, 105, and 107 cm. The species was identified and authenticated by Dr. Lorena Lana Camelo Antunes, at the Laboratory of Plant Morphology and Taxonomy of the Federal University of Goiás, and a sample has been deposited in the herbarium of the same University (voucher code number: 61.014).

To obtain the extract, the barks were ground in a knife mill with Tamis 20 mesh (TE-625; Tecnal Ltd., Piracicaba, São Paulo, Brazil); then, 1000 g of the ground barks sample was percolated (Revitec Ltd., São Paulo, São Paulo, Brazil) with 5000 mL of hydroethanolic solution (50:50 v/v) for 24 h in a metal percolator with a Tamis 200 mesh lined with a layer of paper towel and cotton to filter the barks particles. Next, it was extracted exhaustively (0.2 mL. min^{-1}) at room temperature (percolation phase). Subsequently, the extract was evaporated at 40 °C in a rotary evaporator (TE211; Tecnal Ltd., Piracicaba, São Paulo, Brazil) under reduced pressure (vacuum pump - TE0581; Tecnal Ltd., Piracicaba, São Paulo, Brazil). The extract obtained (2500 mL) was stored in a closed refrigerated container (-2 °C to +8 °C) until further analysis. Posteriorly, after the rotavaporated hydroalcoholic extract was produced and using the Moisture Meter with an infrared heat source (ID 200; Scientific Mars), at 150 °C, the extract concentration was determined as 124 mg/mL, based on the content of solids in triplicate (Brasil, 2010).

2.2. pH and density

The pH and relative density of the hydroalcoholic extract were determined as described by Longhini et al. (2007).

2.3. Preliminary phytochemical screening

Phytochemical screening was performed according to the procedure described by Menezes Filho and Castro (2018), for each phytochemical test, 3 mL of the extract was used. The reaction intensity was visually determined using the cross test: (+++) highly positive, (++) moderately positive, (+) less positive, and (-) negative (Marín et al., 2018). Hemolytic activity was determined at 1 and 10 min of reaction of the extract with 5% red cell suspension. Optical micrographs were obtained to observe the hemolysis reaction.

2.4. Determination of phenolic compounds

The total phenolic compounds were determined as described by Menezes Filho et al. (2018), using the colorimetric method with Folin-Ciocalteu reagent. The results are expressed as gallic acid equivalent (GAE) 100 g^{-1} dry weight.

2.5. High-Performance Liquid Chromatography (HPLC) fingerprinting

The HPLC analysis was performed using Waters Alliance with the e2695 separation module and 2998 photodiode array detector; data were acquired using *Empower* software. Chromatographic separations were carried out using the Zorbax Eclipse XDB-C18 reversed-phase column (250 mm × 4.6 mm, 5 µm). The column temperature was maintained at 35 °C and the injection volume was 10 µL. The mobile phases were 0.05% formic acid in acetonitrile (pH = 3.45) (solvent A) and 0.05% formic acid in water (pH = 3.15) (solvent B) at a flow rate of 1 mL min^{-1}. The gradient applied was as follows: 0-5% A (0-5 min); 5-10% A (5-15 min); 10-15% A (15-25 min), 15-20% A (25-35 min), and then isocratic 15 min of 20% A (35-50 min). The mobile phases were filtered through a 0.45-µm polyvinylidene fluoride (PVDF) membrane and degassed using an ultrasonic bath.

For the analysis, 1 mL of the extract was diluted in 5 mL of methanol in a volumetric flask. To identify the peaks separated by HPLC, stock solutions (0.1 mg mL^{-1} in methanol) of the following standards, procured from Sigma Aldrich, were used: caffeic acid, caffeine, catechin, chlorogenic acid, ellagic acid, epicatechin, gallic acid, hesperidin, kaempferol, *p*-coumaric acid, quercetin, and rutin. The chromatograms were recorded at the wavelengths of 254, 327, and 366 nm, according to the different absorptions of each compound evaluated. The compounds were identified by comparing the HPLC chromatograms of the extract and those of the pure standards based on the retention time (R_t) and UV spectra in the wavelength range of 190-400 nm. Before injection, the solutions were filtered through a 0.45 µm PVDF membrane.

2.6. Antioxidant activity determination

The antioxidant activity was determined as described by Menezes Filho et al. (2018). The results are expressed as the extract concentration at which 50% of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals were inhibited (IC_{50}).

2.7. In vitro antibacterial activity of A. peregrina extract

Under a laminar flow hood, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 samples were thawed to room temperature, and then transferred to trypticase soy broth (TSB) liquid culture medium for sample dilution and incubated at 37 °C for 4 h. The activated strains were inoculated on Cled Agar and incubated at 37 °C for 24 h for the isolation of colonies. Using a sterile loop, the colonies were transferred to selective media, MacConkey for *E. coli* and mannitol salt agar for *S. aureus*; after 24 h, the isolated colonies were verified.

Using sterile loops, the isolated colonies were collected from each selective medium, and then a bacterial suspension in saline solution (0.85% NaCl) was prepared for each strain until the turbidity reached 0.5 on the McFarland scale. For this procedure, a McFarland 0.5 calibrated tube was used as the reference. A swab soaked in bacterial suspension solution was inoculated (for each sample) on Mueller-Hinton agar, covering the entire plate. Immediately, wells of diameter 10 mm were created in the agar plate using autoclave and ultraviolet light-sterilized glass tubes. Each well was identified with letters A, B, and C and filled with 50, 100, and 200 µL *A. peregrina* extract, respectively. Meropenem discs were used as the positive control and 200 µL of saline solution as the negative control. The plates were incubated in a bacteriological oven for 24 h. There were five replicates for each microorganism on different days (Silveira et al., 2009).

3. Results

3.1. Preliminary phytochemical screening

The extract was clear, homogeneous, and dark brown. Table 1 presents the results of the preliminary phytochemical screening. The hydroethanolic extract of *A. peregrina* stem bark was positive for glycosides, based on the moderate-intensity reaction with Kedde and Keller-Kiliani reagents, and a highly positive reaction with Raymond-Marthoud reagent. However, the result of Baljet reagent test was negative. The reaction with Baljet and Kedde reagents was positive due to the presence of compounds with cardenolide unsaturated pentagonal lactone ring. The reaction with Keller-Kiliani reagent was positive due to the presence of deoxygenating compounds (deoxysugar) with a free end. The reaction was positive with Raymond-Marthoud reagent due to the presence of an aglycone (genin), a non-glycidyl group that forms a part of glycosides.

The extract showed negative results in the tests for alkaloids, including the Libermann-Bouchardat, Wagner, and Mayer tests. The test for organic acids was positive with medium intensity according to the cross test. The test with Fehling's reagent for reducing sugars was also positive. The test for coumarins was positive. Foamed saponins were not observed in the extract.

A strong hemolysis was observed in a short time, that is, 1-10 min after incubation of the hydroethanolic extract with red blood cell suspension (Figure 1). Furthermore, in the micrographs, erythrocyte hemolysis was apparent.

The extract showed a positive reaction for condensed tannin compounds and intense reaction in the tests for

Table 1. Phytochemical prospecting of the main secondary metabolite groups of the hydroethanolic extract of *A. peregrina* stem bark.

Secondary metabolite	Hydroethanolic extract of *A. peregrina*
Cardiac glycosides	
Kedd reagent test	++
Keller–Kiliani reagent test	++
Baljet reagent test	-
Raymond–Marthoud reagent test	+++
Alkaloids	
Libermann–Bouchardat reagent test	-
Wagner reagent test	-
Mayer's reagent test	-
Organic acids	
Pascová reagent test	++
Reducing sugars	
Fehling reagent test	++
Non-reducing sugars	
Fehling + HCl test	-
Coumarins	
UV light 254 and 365 nm	+
Saponins	
Foamy	-
Haemolytic	+++
Polysaccharides	
Reactive lugol	-
Phenols	
$FeCl_3$	+++
Tannins	
$FeCl_3$	Gr
Flavonoids	
$Pb(C_2H_3O_2)_2$	++
Purines	-
Catechins	+++
Benzoquinone derivatives	+++
Depsids and depsidones	+++
Steroids and triterpenoids	-
Sesquiterpenolactones	-

Cross test: (+++) positive high, (++) moderate positive, (+) low positive and (-) negative; Gr = Green (catechins).

catechins and flavonoids. Benzoquinone and depside and depsidone derivatives were detected in the extract, based on the positive results with an intense reaction in the respective tests. The tests for purine compounds, steroids, triterpenoids, and sesquiterpene lactones were negative.

3.2. HPLC fingerprinting analysis

The data obtained from the HPLC fingerprinting analysis revealed that the following compounds were present in the extract: gallic acid (the R_t for extract and standard was 6.735 and 6.648 min, respectively), catechin (the R_t for extract and standard was 16.375 and 16.479 min, respectively), and epicatechin (the R_t for extract and standard was 21.335 and 21.387 min, respectively); the UV spectra of the extract and standard were identical. The chromatogram is shown at the wavelength of 254 nm, at which all compounds identified can be visualized (Figure 2).

3.3. Physicochemical properties and antioxidant activity

Table 2 presents the results of the physicochemical analysis and antioxidant activity assays, reduction of DPPH free radical and total phenol content expressed in g of gallic acid 100 g^{-1} extract. The pH of the hydroalcoholic extract of the stem bark of *A. peregrina* was 5.21. The relative density was 0.956 g/cm^3. The antioxidant activity expressed as IC_{50} was 44.13 mg mL^{-1} for extract and 0.25 mg mL^{-1} for butylated hydroxy toluene (BHT). Although the IC_{50} value of BHT was lower than that of the extract, the results showed that the plant material possess antioxidant activity. The total phenolic compound content was 6.40 g GAE 100 g^{-1} extract.

3.4. Antibacterial activity

The antibacterial activity of the extract is presented in Table 3. After 24 h of incubation, the extract did not inhibit *E. coli* colonies at any extract volume tested. There were halo regions around *S. aureus* colonies at all three volumes tested. Using a millimeter ruler, the diameter of inhibitory zones was measured, excluding the diameter of the wells.

4. Discussion

The hydroethanolic extract of *A. peregrina* stem bark showed a positive result in the Raymond-Marthoud, Kedde, and Keller-Kiliani tests for glycosidic compounds, although the result was negative in the Baljet test. Aglycones or genin compounds are characterized by the cyclopentanoperhydrophenanthrene structural core. There are two groups of cardiac glycosides: (1) cardenolides, 23-carbon chain compounds in which the unsaturated lactone ring is attached to the pentacyclic C-17 and (2) bufadienolides, compounds with 24 hexacyclic carbons (Kloss et al., 2016). These cardiotonic compounds directly

Table 2. Physicochemical properties, antioxidant activity, and total phenolic content of the hydroethanolic extract of *A. peregrina* stem bark.

Sample	pH	Density (g cm^3)	DPPH (IC_{50})	Total Phenolics (g GAE 100 g^{-1})
***A. peregrina* extract**	5.21 ± 0.01	0.956	44.13 mg mL^{-1}	6.40 ± 0.08

Means of three experiments followed by (±) standard deviation.

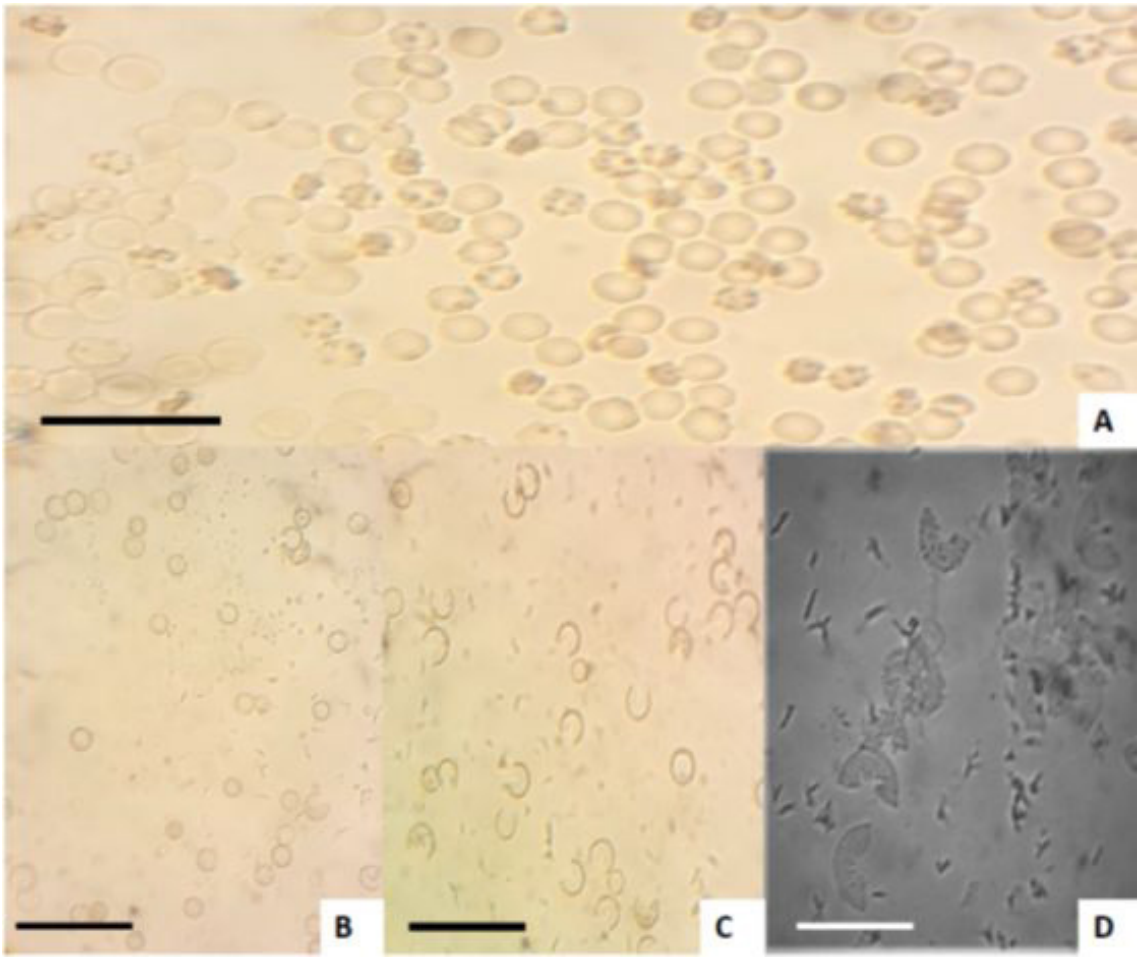

Figure 1. Erythrocyte hemolysis in a 5% red blood cell suspension by the hydroethanolic extract of *A. peregrina* stem bark. (A) 5% suspension of red blood cells; (B) hemolysis after 1 min of reaction; (C) advanced hemolysis after 5 min; and (D) completely hemolyzed red blood cells within 10 min of reaction. Bars: At (A) 1.000×; (B) 500×; (C) 650×; and (D) 1.800×.

Table 3. Diameter of the inhibitory zone of the hydroethanolic extract of *Anadenanthera peregrina* stem bark against *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922).

Strain	*A. peregrina* extract concentration			C +	C -
	50 µL	100 µL	200 µL		
E. coli	-	-	-	29 mm	-
S. aureus	10 mm	16 mm	20 mm	35 mm	-

Positive control (C+) = meropenem; negative control (C-) = saline solution.

act on the myocardium to alleviate heart failure and intoxication (Vickery and Vickery, 1981), a beneficial characteristic for medicinal plants.

In the present study, alkaloids, non-reducing sugars, foamed saponins, polysaccharides, purines, steroids, triterpenoids, and sesquiterpenelactones were not detected in *A. peregrina* stem bark extract. There are numerous factors that influence the production of certain class of compounds by plants, including the seasonality, circadian rhythm, and full development of the plant. Therefore, frequent sample collection in different seasons is necessary (Gobbo-Neto and Lopes, 2007). It is noteworthy that the alkaloid 5-Hydroxy-N,N-dimethyltryptamine (bufotenine) has been identified in the seeds of *A. peregrina* (Blackledge and Phelan, 2006). Foamed saponins were not present in the extract, and it is possible that during the sample collection period, this class of compounds was produced in minimal detectable quantities or was not produced (Ndamba et al., 1994).

Here, we observed erythrocyte hemolysis at different time points, suggesting that the extract is toxic to the hematopoietic system. The interaction of the extract

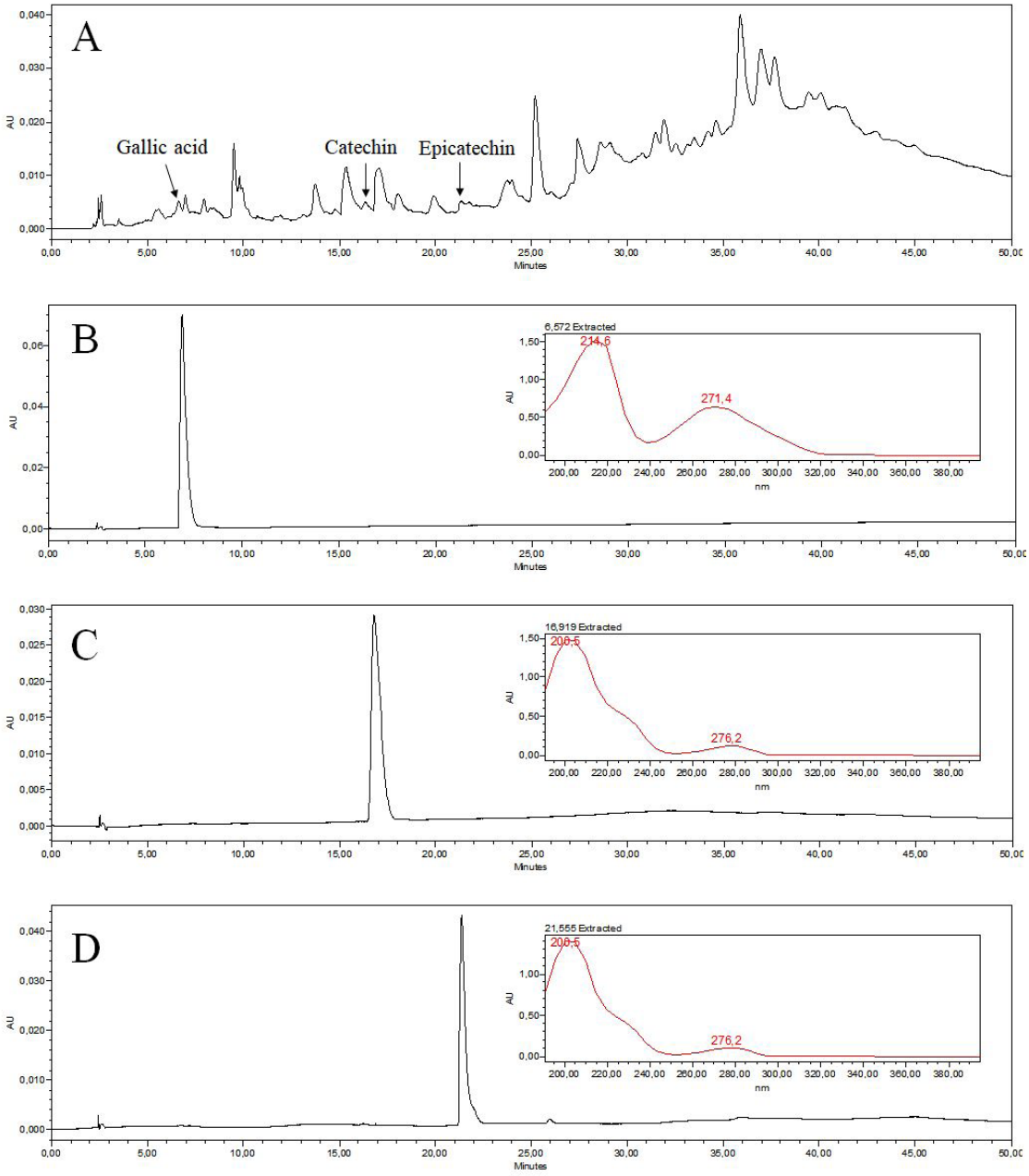

Figure 2. HPLC-PDA chromatographic profiles (λ = 254 nm) of: (A) sample extract; (B) gallic acid standard; (C) catechin standard; and (D) epicatechin standard, followed by UV spectra (190-400 nm).

with the erythrocyte membrane constituent sterols leads to the formation of pores in the membrane causing hemolysis, resulting in hemoglobin dispersion to the external environment (Sousa et al., 2018); furthermore, the platelets aggregated in small clusters. Further studies should be performed to evaluate the cytotoxic action of the stem bark extract of *A. peregrina* with respect to its action on platelets.

Starch and mucilage are the well-known polysaccharides with phytotherapeutic action against pneumological inflammation (Menezes Filho and Castro, 2018). In the present study, polysaccharides were not observed, although *A. peregrina* extract has been reported to exhibit this function (Souza et al., 2016).

The absence of purine compounds, steroids, triterpenoids, and sesquiterpene lactones in the stem bark extract of *A. peregrine* reinforces seasonal variations in the production of secondary metabolites (Gobbo-Neto and Lopes, 2007). Other important factors associated with the production of secondary metabolites are age, development, and different organs of plants, influencing the content and relative proportion of components in

the extract (Hendricks et al., 1997). Purine compounds with at least one structural nitrogen (N) atom have potential use in the production of new drugs, owing to their antiangiogenic and cytotoxic activities; thus, these compounds are important to the pharmaceutical and agricultural industries (Palkar et al., 2015). Furthermore, purines are associated with the wound-healing property of *A. peregrina* extract, favoring angiogenesis. The potential agronomic benefits of the extract are associated with steroidal and triterpene compounds, which are involved in pollen tube growth, internode elongation, and plant growth regulator production (Carvalho et al., 2015).

In the presented study, organic acids were detected in the extract; they, especially malate, citrate, and oxalate, play a role in the tolerance mechanisms of plants to aluminum silicates (Hartwig et al., 2007). Citrate is the most common organic acid in plants; it is a tricarboxylated anion that can form chelates with Al_3 with stable bonds (Jian Zheng et al., 1998; Piñeros et al., 2002).

In the present study, the extract was positive for reducing sugars and coumarins. Glucose and fructose are the reducing sugars that are present in diverse plant organs. Glucose acts synergistically on the central nervous system supplying energy and on the gastrointestinal system. Fructose acts as a source of energy to the musculoskeletal system (Barreiros et al., 2005; Araújo and Martel 2009). Among the coumarins, phytoalexins exhibit phytopathogenic activity as a fungicide. Phytoalexins act on cytoplasmic granulation systems, disorganize cellular content, cause plasma membrane disruption, and inhibit fungal enzymes, and thereby reduce and inhibit mycelial growth (Schwan-Estrada et al., 2000); these findings validate their antimicrobial use (Souza et al., 2016).

In the present study, we detected tannins in the extract. Tannins are categorized as flavanones, procyanidins, and condensed and hydrolysable tannins. Condensed tannins include true non-hydrolysable tannin compounds, and they are more resistant to fragmentation and are associated with flavonoid pigments, with the flavan-3-ol polymeric structure. Tannins are responsible for the reddish coloration of the stem bark (SBFGONOSIA, 2009). Tannins are known for their medicinal properties, including antioxidant and bactericidal activities (Ogawa and Yazaki, 2018; Cruz et al., 2020).

Hydrolyzable tannins such as gallotannins (meta-digalloyl groups > penta-*O*-galloyl-β-*D*-glucose (PGG) and ellagitannins (hexahydroxydiphenoyl (HHDP) > strictinin: R1=(β)-OG, R2=R3=H) are chemically composed of several molecules of phenolic acids, such gallic and ellagic acids, joined with a central glucose structure. The ester bonds are easily susceptible to hydrolysis by acids or enzymes, and in a solution, hydrolyzable tannins present a bluish color with ferric chloride, like gallic acid (Fernandes et al., 2018; Dai et al., 2020).

During the rainy season, grasses produce higher quantities of tannins, which are considered an antinutritional factor, reducing the consumption of grasses by ruminants, causing nutritional deficit (Nepomuceno et al., 2013). The ingestion of large amounts of tannin compounds can interfere with the digestibility, absorption, and bioavailability of nutrients (Lamy et al., 2011). However, in granular sorghum under short and medium cycle intercropping, condensed tannins improved grain yield, ranging from 1.285 to 8.710 kg ha^{-1}, dietary protein content, growth rate, fertility, and animal welfare (De Souza et al., 2019; Cuitiño and Vera, 2016).

Flavonoids are secondary metabolites known for their allelopathic effect in plants with various biological properties, especially, anti-inflammatory (Serafini et al., 2010) and antioxidant activities (Khater et al., 2019). In the present study, flavonoids were detected in the extract, validating its medicinal use. Some characteristic flavonoid phytoalexins identified in sorghum (3-deoxiantocyanidine flavonoids) include the following: luteolinidine, 5-methoxyluteolinidine, apigeninidin, and arabinosil-5-*O*-apigeninidin caffeic acid ester (Nicholson et al., 1987). In soybean, the phytoalexin glyceollin (pterocarpanoid) has been identified (Burden and Bailey, 1975).

In the present study, catechins were detected in the extract. Catechins include a diverse group of allelopathic compounds involved in plant-plant interaction, and they are widely used as an insecticide and a natural herbicide. These findings highlight the potential use of the extract as a natural defensive agent (Rabaioli and Silva, 2016).

Here, benzoquinone and depside and depsidone derivatives were also detected in the extract. The *p*-benzoquinone sorgoleone acts as a natural herbicide, via allelopathic effects on sorghum (Carvalho et al., 2015). This compound acts as a potent inhibitor of mitochondrial respiration and photosynthesis, via its action in the electron transport chain of photosystem II, competing for the same site of action of synthetic herbicides (atrazine and diuron) (Gonzalez et al., 1997). Depsides are polyketides and are produced via the biosynthetic reaction of orselinic acid synthase, where chain cyclization occurs in the formation of this acid. The derivatives of this compound have potential anticancer and anti-inflammatory activities (Kamiya et al., 2018).

In the present study, the test for phenols was positive and the total amount was evaluated in GAE. Phenolic compounds are important for the therapeutic properties of plants. The relationship between polyphenols and human health has been explored with an emphasis on cardiovascular diseases and metabolic syndrome, highlighting the relevance of these bioactive compounds (Durazzo et al., 2019). Phenolic compounds can alleviate the deleterious effects of free radicals acquired or internally produced in an organism. Several plant phenols effectively protect cells under oxidative stress. Studies have explored the potential of phenolic compounds and its derivatives in the treatment of inflammatory diseases, via autophagy mechanisms (Zenkov et al., 2016) and antimicrobial activity (Pinheiro et al., 2018).

In the present study, gallic acid, catechin, and epicatechin were detected in the extract by HPLC fingerprinting; they are common precursors of tannins. Thus, it is possible that the extract contained hydrolyzable tannins (gallotannins) and condensed tannins (catechin tannins and proanthocyanidins). These findings corroborated with those of the preliminary phytochemical screening, contributing to a better understanding of the chemical composition of this species. According to Monteiro et al. (2005), Azêvedo et al. (2017), and Dai et al. (2020), the

proportion of hydrolyzable and condensed tannins varies considerably, irrespective of whether both groups are present in the same plant; their content is influenced by natural factors (such as rain, soil, soil nutrition, and solar radiation), as well as by factors between species of the same group, genus, or family.

The qualitative analysis by HPLC of *A. colubrina* aerial parts indicated the presence of quercetin and low levels of gallic acid, catechin, and p-coumaric acid (Araújo et al., 2019). Gallic acid, catechin, and epicatechin have been shown to possess a wide variety of pharmacological activities, including antimicrobial, anti-inflammatory, and antioxidant activities (Abdulah et al., 2017; Kahkeshani et al., 2019; Pedro et al., 2019;), elucidating the therapeutic uses of *A. peregrina*.

Although other compounds evaluated were not found in the extract in the present study, it does not necessarily indicate that they are not produced by this species, as several factors may be involved in the biosynthesis of secondary metabolites, including environmental and genetic factors (Gobbo-Neto and Lopes, 2007; Pavarini et al., 2012). Further studies may highlight the potential of *A. peregrina* stem bark as an alternative source of these compounds, which may also be used as chemical markers of the species for quality control.

In the present study, the DPPH assay revealed the antioxidant activity of the hydroethanolic extract of *A. peregrina* stem bark. Although the IC_{50} value of BHT was lower than that of the extract, the results showed that the plant possesses antioxidant activity. The chemical compounds produced by plants can be altered by several abiotic factors, which interfere in the expression of allelopathic compounds (Pilatti et al., 2019). It is suggested that the antioxidant activity may be related to the presence of phenolic compounds, especially tannins that have antioxidant activity, and they were identified in the present study. Moreover, the dark color of the extract might be due to the presence of high levels of chlorophyll A and B pigments, and these pigments possibly masked the colorimetric reaction to reduce the purple coloration of the radical.

Mota et al. (2017) evaluated the hydroethanolic 50% (v/v) extract of *A. peregrina* bark. They reported a high content of total phenolic compounds (583 mg of GAE g^{-1} extract) and antioxidant activity of moderate intensity with an average IC_{50} value of 13 µg mL^{-1} compared with 2 mg mL^{-1} for Trolox. Furthermore, the Trolox equivalent antioxidant capacity was 237.6 mg Trolox g^{-1}. The phenol content in the bark extracts is highly variable between species, and the phenol content reported by Santos et al. (2012) in the bark extracts of *Eucalyptus grandis* (386 mg of GAE g^{-1}), *E. urograndis* (347 mg of GAE g^{-1}), and *E. maidenii* (204 mg of GAE g^{-1}) was lower than that reported on the bark of *A. peregrina* by Mota et al. (2017). Moreover, the methanolic extract obtained from eucalyptus barks is reported to exhibit antioxidant activity, validated by the presence of phenolic compounds and flavonoids (Mishra et al., 2010; Srivastava and Vankar, 2012). Just like as eucalyptus barks, *A. peregrina* barks are a source of polar extracts due to the presence of tannins and other phenolic compounds and can be used in the pharmaceutical sectors due to their antioxidant potential (Sartori et al., 2013).

Other studies on *Anadenanthera* species revealed their antimicrobial activity against *S. aureus* and *E. coli* (Araújo et al., 2015), potentiated action of neomycin and amikacin (Barreto et al., 2016), of cephalexin related to the amount of bark tannins (Araújo et al., 2018) and synergistic when combined with fluconazole (Nunes et al., 2015). In addition, *Anadenanthera* species have been reported to possess antifungal potential (Lima et al., 2014), inhibit biofilms (Trentin et al., 2013), and assist in pain management (Santos et al., 2013; Damascena et al., 2014). Angico hydroalcoholic extract (*Anadenanthera colubrina* var. *cebil*) has been reported to accelerate wound healing in rats. Furthermore, reducing sugars (++), flavonoids (quercetins) (+), condensed proanthocyanidins (+++), leucoanthocyanidins (++), saponins (saponosides) (+), and triterpenes and steroids (+) were found in the extract (Pessoa et al., 2012, 2015).

In conclusion, the phytochemical analysis of the hydroethanolic stem bark extract of *A. peregrina* showed the presence of a wide variety of chemical compounds with importance in the pharmaceutical, food, and agricultural fields. The HPLC fingerprinting analysis revealed the presence of gallic acid, catechin, and epicatechin in the extract. The extract showed hemolytic action, necessitating further toxicological assessment. Furthermore, the extract showed antibacterial and antioxidant activities. The results contribute to a better understanding of the chemical composition of *A. peregrina* stem bark extract and further strengthen its application in traditional medicine practices.

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