International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
www.florajournal.com
IJHM 2022; 10(1): 32-39
Received: 15-11-2021
Accepted: 20-12-2021

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A analysis of the phytochemical composition and antioxidant, antimicrobial and cytotoxic activities of extracts from *Aspidosperma pyrifolium* Mart. (Apocynaceae)

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Abstract
Brazil's biodiversity combined with its sociodiversity contributes to the development of phytotherapy in the country, and the Caatinga biome is the main and largest ecosystem in northeastern Brazil. This biome is composed of several vegetal species, among them, *Aspidosperma pyrifolium*, popularly known as pereiro, and widely used by folk medicine. This article aimed to determine the phytochemical characterization and antioxidant, hemolytic and antimicrobial activity of aqueous, ethanolic and methanolic extracts from leaf and stem bark of *A. pyrifolium*. It was possible to identify the presence of cinnamic derivatives and flavonoids in all extracts of *A. pyrifolium*. The activity of removal of free radicals from *A. pyrifolium* extracts ranged between 5196 to 748.8 µg/mL, whereas ABTS values ranged from 1891 to 223 µg/mL, while TAC from 3827 to 726.4 µg/mL. The total phenol content of *A. pyrifolium* ranged from 185 to 54 mg GAE/g and the flavonoids remained in the range of 6.13 to 2.33 mg QE/g. The hemolytic percentage of aqueous, methanolic and ethanolic extracts from leaf were 4.07%, 11.46% and 29.27%, respectively, in the highest concentration tested (2000 µg/mL). Aqueous stem bark extract was responsible for 3.26% of hemolytic activity, followed by methanolic (9.07%) and ethanolic (37.46%). It was not possible to observe antimicrobial activity against bacterial strains tested at concentrations from 2000 to 125 µg/mL. Therefore, other studies in higher concentrations of aqueous, ethanolic and methanolic extracts from the leaf and stem bark of *A. pyrifolium* are necessary, considering the obtaining of bioactive substances for therapeutic purposes.

Keywords: *Aspidosperma pyrifolium*, pereiro, apocynaceae, caatinga, phytotherapy

Introduction
Medicinal plants have been used for a long time in the prevention and treatment of diseases, becoming the main source of drugs in folk medicine, and they are essential in the development of herbal medicines today [1-2]. Brazil benefits from having the largest biodiversity on the planet, being the country with the largest number of vascular plants and endemic species in the world [3-4]. Brazil is the Earth's biodiversity champion with the largest number of vascular plants and endemic species [3], and the Caatinga is the largest biome in the northeastern Brazil (Alagoas, Bahia, Ceará, Paraíba, Pernambuco, Piauí, Rio Grande do Norte, Sergipe, and northern Minas Gerais), containing 4,322 species of seed plants, and of these, 744 species are endemic plants, considered an important natural laboratory [3, 5]. Among the plant species found in the Caatinga, *Aspidosperma pyrifolium* Mart., which belongs to Apocynaceae family, is popularly known as: pereiro, pereiro-branco, pereiro-preto, pereiro-do-sertão and peroba-rosa [6-8]. *A. pyrifolium* exhibits great importance for its wide use in folk medicine as an anti-inflammatory of urinary tract [9-11], in addition to several biological activities (antioxidant, antimicrobial and antiplasmodial) due to the presence of secondary metabolites [12-15], making it very attractive for bioprospecting [5, 16-17]. Several discussions have been realized about healthcare-associated infection (HAI), which is one of the main public health problems in worldwide [18-20], given the decrease in options for the treatment of infections by drug-resistant bacteria [16, 21]. Therefore, the use of plant extracts can support a new alternative in the treatment of infectious diseases, or complement conventional medicine, through of medicines of plant origin and especially accessibility to low-income people [22-23]. A broad systematic review on the antimicrobial potential of plant extracts of interest to the Brazilian Health

System between 2010 and 2013 found 21,357 articles that discuss new therapeutic alternatives for bacterial infections [24]. Another study confirmed the wide biological action of medicinal plants extracts from Brazil and their applicability in the treatment of diseases, including antimicrobial and antioxidant action, analgesic and anti-inflammatory properties, etc. [25]. Anand *et al*. (2020) suggest the biopharmaceutical potential of species of the Apocynaceae family in the treatment of several diseases, including urinary, respiratory, skin and gastrointestinal infections, in addition, it was possible to observe their antimicrobial action in *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Escherichia coli* [26].

In view of the biological activity of plant species obtained from Caatinga, it is necessary to evaluate the antioxidant, antimicrobial and cytotoxic activities of leaf and stem bark extracts of *A. pyrifolium*, and to evaluated their phytochemical profile, which may support other studies and possible new therapeutic options for HAI.

Materials and methods

Plant Material

Leaf and stem bark of *A. pyrifolium* were collected in National Park of Catimbau (PARNA do Catimbau), located in the state of Pernambuco, Brazil (8° 30' 57 " S and 37° 20' 59" W). Samples were identified by Dr. Alexandre Gomes da Silva (*in memoriam*), and the specimen was deposited in Herbarium Dárdano de Andrade Lima of the Instituto Agronômico de Pernambuco under identification number 85,734. The plant material was registered in the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen) at number A08E18B.

Preparation of extracts

Biological materials were stored in cold chamber and incubated at 40 ºC until complete drying. Stem bark and leaf were dried, and processed in industrial mill. After, the material was processed in industrial mill and 10 g were weighed, followed by dilution in 100 mL of distilled water, ethanol or methanol to obtain the aqueous, ethanolic and methanolic extract, respectively. These were shaken and incubated in water bath under certain boiling points of each solvent for 30 min, followed by filtration and storage at -80 ºC. Finally, all extracts were lyophilized for 48 h.

Phytochemical Analysis

Total Phenols and Flavonoids Content

The total phenolic content was estimated using the Folin-Ciocalteau method [27] with some modifications. Aliquots (20 µL) of each extract (1 mg/mL) were diluted in 100 µL of the Folin-Ciocalteau reagent and then 80 µL of the sodium bicarbonate solution (0.7 M) was added, and incubated at 25 ºC. Absorbance was measured at 735 nm, methanol or distilled water was used as a negative control and gallic acid as a standard. The results were calculated using the gauge acid calibration curve (10-100 µg/mL) and expressed in mg equivalent of gallic acid per gram of extract (GAE/g extract).

Flavonoid content was determined by the aluminum chloride colorimetric method [28]. Extracts were tested at a concentration of 1 mg/mL and quercetin was used to obtain the standard calibration curve (10-100 µg/mL). Each extract (100 µL) was mixed with 100 µL of the reagent (2% $AlCl_3$ in methanol) and incubated for 1 h at 25 ºC for later measurement of absorbance at 420 nm. The results were expressed in mg equivalent to quercetin per gram of extract (QE/g extract). Each assay was performed in triplicate in independent experiments.

High Performance Liquid Chromatography (HPLC)

The extracts were analyzed by HPLC with 5 mg solubilized in 10 mL of 50% ethanol (v/v), followed by filtration through PVDF filters (0.45 µm). The analysis was performed in Ultimate 3000 HPLC system (Thermo Fisher Scientific®, USA), coupled to a photodiode array detector (DAD; Thermo Fisher Scientific®) and equipped with binary pump (HPG-3x00RS, Thermo Fisher Scientific), degasser and automatic sampler with 20 µL loop (ACC-3000, Thermo Fisher Scientific®). The wavelength was fixed at 270 nm and the chromatographic separations were obtained with C_{18} column (250 mm x 4.6 mm d.i., 5 µm) Dionex® with security guard (C_{18}; 4 mm x 3.9 µm; Phenomenex®). Separations were realized at 27 ± 1 ºC and mobile phase consisted of ultrapure water (Purelab Classic UV, Elga®) and methanol (HPLC grade, Tedia®), both acidified with 0.05% trifluoroacetic acid (Vetec®) and flow adjusted to 0.7 mL/min. A gradient program was applied: 0-10 min, 15-25% B; 10-15 min, 25-40% B; 15-20 min, 40-80% B; 20-25 min, 80% B; 25-28 min, 80-40% B; 28-30 min, 40-15% B. The data were analyzed after injection in triplicate and processed using the Chromeleon 6.8 program (Dionex/Thermo Fisher Scientific®, USA). The contents were calculated according to the equation of the pattern for rutin (>94%, Sigma-Aldrich®), caffeic acid (>95%, Sigma-Aldrich®) and chlorogenic acid (>97%, Sigma-Aldrich®).

Antioxidant activity

DPPH assay

The antioxidant activity of the extracts was measured by the DPPH method (2,2-diphenyl-1-picryl-hydrazil) in triplicate with ascorbic acid (positive control) and methanol (negative control). The absorbance was measured at 517 nm (BioTek UQuant MQX200) and the percentage of inhibition (I%) was calculated as: I% = [(Ac-As)/(Ac)] x 100 (Ac is the absorbance and As is the absorbance of the extract) [29].

ABTS assay

The ABTS radical solution was diluted in ethanol to adjusted the absorbance to 0.7 nm (± 0.02) at 734 nm. Samples (10 µL) of the extracts (1000 to 31.25 µg/mL) were diluted to 1 mL of the ABTS radical in triplicate and measured at 734 nm. Ascorbic acid was used as a standard antioxidant and inhibition activities were calculated: % = [(Ac-As) / (Ac)] x 100 (Ac is the absorbance of the control and As is the absorbance of the extracts) [30].

Total Antioxidant Capacity (TAC)

Samples (100 µL) of the extracts (1000 to 31.25 µg/mL) were associated with 1 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and incubated at 95° C for 90 min, and absorbance was measured at 695 nm. The control reaction contains 100 µL of methanol and 1 mL of reagent solution and the standard used was ascorbic acid (1 mg/mL). Antioxidant capacity of the samples was calculated in triplicate: TAC% = [(Aa-Ac) / (Aaa-Ac)] x 100 (Aa is the absorbance of the sample, Ac is the absorbance of the control and Aaa is the absorbance of the acid ascorbic) [31].

Hemolytic action

The hemolytic analysis of *A. pyrifolium* extracts was determined in erythrocytes [32] in quadruplicate. Extracts were diluted DMSO (ethanolic and methanolic) and sterile distilled water (aqueous), followed by microdilution (2000 at 125 µg/mL) in 96-well microplates. Then, 2 mL of blood were dissolved in sterile saline, centrifuged at 2,500 rpm for 2 min. Triton X-100 was used as positive control and sterile saline as negative control. The microplates were incubated for 3 h at 37 ºC under constant agitation, and later they were centrifuged at 3,500 rpm for 4 min at 24ºC with supernatant removed to another 96-well microplate for reading in spectrophotometer (Benchmark plus, BioRad®, California, USA) at 540 nm.

Reference strains

Bacterial strains were used for antimicrobial resistance testing according to Clinical & Laboratory Standards Institute (CLSI, 2019) [33]: *Staphylococcus aureus* (ATCC 29213), *Klebsiella pneumoniae* (ATCC 700603), *Acinetobacter baumannii* (ATCC 19606), *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922). Strains were incubated in Brain Heart Infusion (BHI) broth at 37° C for 24h. Then, were seeded and cultivated on BHI agar under the same conditions for bacterial isolation.

Minimum inhibitory and bactericidal concentration

Antimicrobial action of the extracts was determined by microdilution in broth in 48-well microplates in triplicate, adapted from CLSI (2019) [33]. Extracts were tested at concentrations of 2000 at 31.25 µg/mL [12] and diluted in DMSO (methanolic and ethanolic) and sterile distilled water (aqueous). Then, each solution was dissolved in Mueller Hinton broth to obtain a final non-toxic solution to bacterial cells (1% DMSO). After 24h of incubation at 37ºC, the minimum inhibitory concentration (MIC) was determined, and the minimum bactericidal concentration (CBM) was performed by count of colony forming units (CFU) in the concentrations that did not exhibit visible bacterial growth. The results obtained from MIC were validated by colorimetry test with resazurin [32].

Statistical analysis

Hemolytic percentage and antioxidant activity were analyzed by linear regression using SPSS 18.0 program (IBM Co., New York, USA) for Windows.

Results

Phytochemical analyzes of the extracts were realized from the dosage of phenolic and flavonoid, and high-performance liquid chromatography to identify and quantify the different classes of secondary metabolites, alkaloids, glycosides, polyphenols, saponins, terpenes and anthraquinones. It was observed that total phenolic content of the extracts ranged from 54.55 to 185.86 mg GAE/g with the highest value obtained of the aqueous leaf extract (185.86mg GAE/g), followed by the methanol stem bark extract (138.32 mg GAE/g) and lower for methanol leaf extract (54.55 mg GAE/g) and aqueous stem bark extract (59.96 mg GAE/g), as described in table 1.

Flavonoid compounds remained in a range of 2.33 to 6.13 mg QE/g, in which the ethanolic leaf extract presented a higher concentration among the six extracts (6.13 mg QE/g) (Table 1).

Table 1: Total content of phenols and flavonoids in aqueous, ethanolic and methanolic extracts of the leaf and stem bark of *Aspidosperma pyrifolium* Mart.

Extracts	Phenols (mg GAE/g)	Flavonoids (mg QE/g)
Stem bark		
Aqueous	59.96	3.27
Ethanolic	130.45	4.24
Methanolic	138.32	4.33
Leaf		
Aqueous	185.86	2.33
Ethanolic	78.98	6.13
Methanolic	54.55	6.00

mg GAE/g: mg equivalent of gallic acid per gram of extract;
mg QE/g: mg equivalent of quercetin per gram of extract.

In aqueous stem bark extract, cinnamic derivatives were observed: caffeic and chlorogenic acid, and only a peak of cinnamic derivatives was observed in the ethanolic and methanolic extracts (with maximum absorption at 217, 240 and 326 nm). Aqueous, ethanolic and methanolic leaf extracts exhibit peaks of cinnamic derivatives, and the presence of caffeic acid was confirmed (Figure 1). The major compounds present in all extracts of *A. pyrifolium* are caffeic acid, and rutin was also observed in the leaf extracts (maximum absorption at 201, 265 and 357 nm) (Table 2).

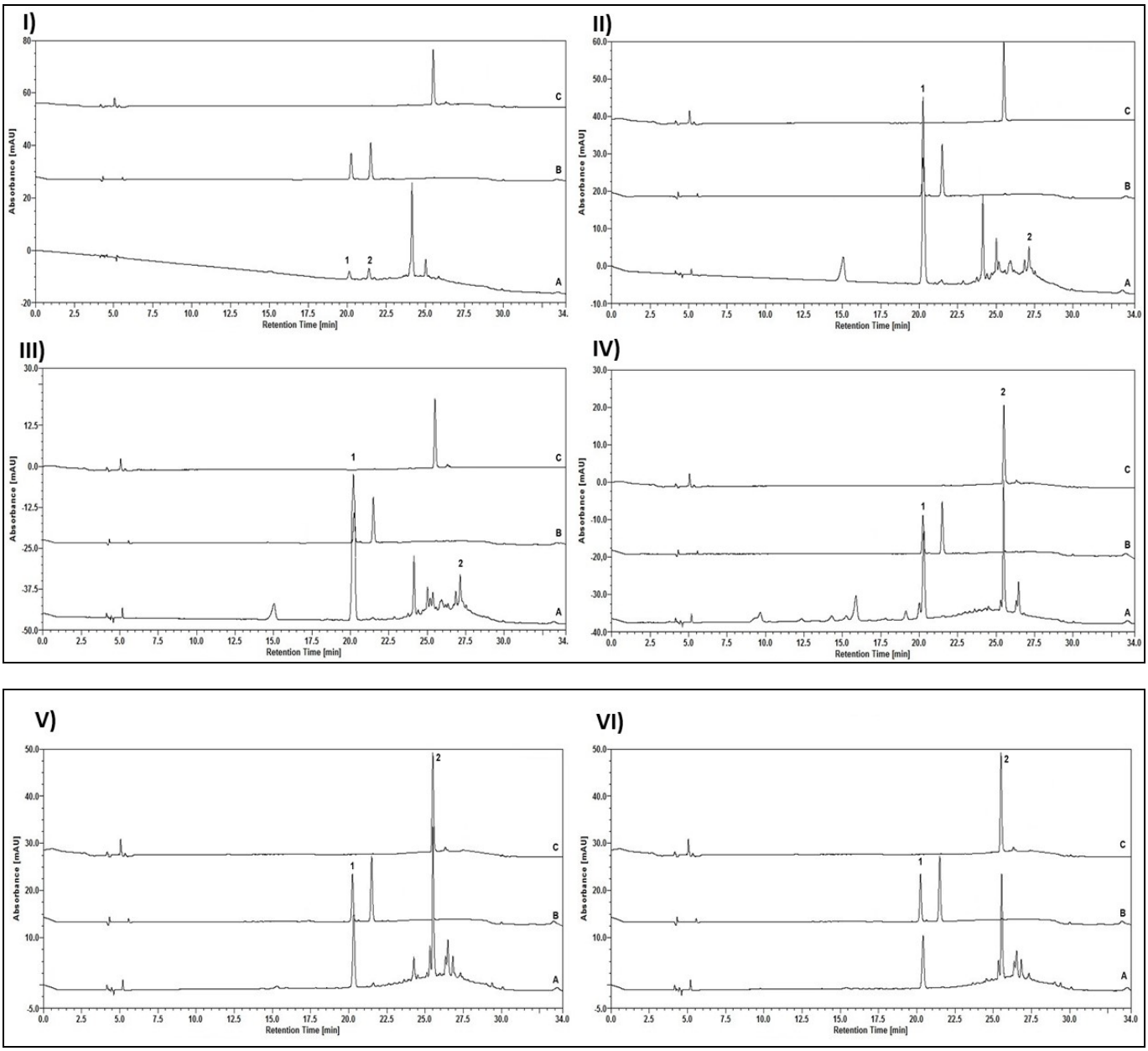

Fig 1: HPLC chromatographic profile of aqueous, ethanolic and methanolic extracts of the leaf and stem bark of *Aspidosperma pyrifolium* Mart

HPLC of the stem bark aqueous extract; A: Chromatogram of the bark aqueous extract; B: Chromatogram of cinnamic derivatives; C: Chromatogram of the flavonoid; 1: Caffeic acid; 2: Chlorogenic acid. II) HPLC of the stem bark ethanolic extract; A: Chromatogram of the stem bark ethanol extract; B: Chromatogram of cinnamic derivatives; C: Chromatogram of the flavonoid; 1: Caffeic acid; 2: Flavonoid derivative. III) HPLC of the stem bark methanol extract; A: Chromatogram of the stem bark methanol extract; B: Chromatogram of cinnamic derivatives; C: Chromatogram of the flavonoid; 1: Caffeic acid; 2: Flavonoid derivative. IV) HPLC of leaf aqueous extract; A: Chromatogram of the leaf aqueous extract; B: Chromatogram of cinnamic derivatives; C: Chromatogram of the flavonoid; 1: Caffeic acid; 2: Rutin. V) HPLC of the leaf ethanolic extract; A: Chromatogram of the leaf ethanolic extract; B: Chromatogram of cinnamic derivatives; C: Chromatogram of the flavonoid; 1: Caffeic acid; 2: Rutin. VI) HPLC of the leaf methanol extract; A: Chromatogram of the leaf methanol extract; B: Chromatogram of cinnamic derivatives; C: Chromatogram of the flavonoid; 1: Caffeic acid; 2: Rutin.

Table 2: Chemical composition of aqueous, ethanolic and methanolic extracts of the leaf and stem bark of *Aspidosperma pyrifolium* Mart.

Extract	Cinnamic Derivatives		Flavonoid
	Caffeic acid	Chlorogenic acid	Rutin
Stem bark			
Aqueous	0.02 g% (0.79%)	0.02 g% (0.41%)	-
Ethanolic	0.05 g% (0.03%)	0.21 g% (0.03%)	0.35 g% (0.01%)
Methanolic	0.04 g% (0.34%)	0.17 g% (0.06%)	0.45 g% (0.16%)
Leaf			
Aqueous	0.04 g% (0.84%)	1.25 g% (0.20%)	1.11 g% (0.04%)
Ethanolic	0.07 g% (0.18%)	0.99 g% (0.90%)	0.41 g% (0.29%)
Methanolic	0.05 g% (0.41%)	0.48 g% (0.12%)	0.73 g% (0.18%)

-, not found. Mean (standard deviation)

The values of antioxidant activity of the extracts are detailed in table 3. The methanolic and ethanolic extracts of the stem bark exhibited the lowest IC_{50} values through ABTS assay, respectively, 223 µg/mL and 426 µg/mL, followed by the ethanolic extract of the leaf (702 µg/mL). These extracts also showed a high elimination of the free radical. Through TAC assay was possible to observe that the methanolic extract of

the stem bark remained with greater antioxidant action (726.4 µg/mL) and the lowest action was observed in the aqueous extract of (3827 µg/mL).

Table 3: Antioxidant activity of aqueous, ethanolic and methanolic extracts from the leaf and stem bark of *Aspidosperma pyrifolium*.

Extracts		IC50(µg/mL)	
Stem bark	**DPPH**	**ABTS**	**TAC**
Aqueous	5196	1024	1567
Ethanolic	854,6	426	1609
Methanolic	748.8	223	726.4
Leaf			
Aqueous	2456	1891	3827
Ethanolic	1072	702	1418
Methanolic	2112	1780	1975
AA	72.54	12.74	185.5

AA: ascorbic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2'-azino-bis (3-ethylbenzothiazoline) 6-sulfonic acid; TAC, Total Antioxidant Capacity; $IC_{50,}$ Extract concentration required to inhibit 50% of free radicals.

Through analysis of hemolytic activity, it was observed that the stem bark and leaf aqueous extracts exhibited percentages of 3.26% and 4.07%, respectively. Already the ethanol extracts showed a higher percentage compared to controls (Table 4).

Table 4: Hemolytic percentage of the aqueous, ethanolic and methanolic extracts of the leaf and stem bark of *Aspidosperma pyrifolium*.

Extract	Hemolysis (%)
Stem bark	
Aqueous	3.26 (±0.74)
Ethanolic	37.46 (±10.75)
Methanolic	9.07 (±3.22)
Leaf	
Aqueous	4.07 (±0.7)
Ethanolic	29.27 (±6.46)
Methanolic	11.46 (±1.97)

% (standard deviation)

In the concentrations evaluated of the *A. pyrifolium* extracts it was not possible to observe antimicrobial activity against the bacterial strains used (MIC > 2000 µg/mL), as seen in table 5.

Table 5: Minimum inhibitory and bactericidal concentration of aqueous, ethanolic and methanolic extracts from the leaf and stem bark of *Aspidosperma pyrifolium* Mart. against reference bacterial strains.

ATCC strains	***A. pyrifolium***					
	Stem bark			**Leaf**		
	AQU	**ETH**	**MET**	**AQU**	**ETH**	**MET**
	MIC/MBC			MIC/MBC		
S. aureus (29213)	>2000			>2000		
K. pneumoniae (700603)	>2000			>2000		
A. baumannii (19606)	>2000			>2000		
P. aeruginosa (27853)	>2000			>2000		
E. coli (25922)	>2000			>2000		

AQU: Aqueous; ETH: Ethanolic; MET: Methanolic; MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration; The concentrations are being expressed in µg/mL.

Discussion

The Apocynaceae family is one of the ten largest plant families in worldwide, in which several species have been reported for its medicinal use and chemical constituents, such as *A. pyrifolium*. These constituents are results of plant secondary metabolism in response, mainly to the environmental conditions and in defense to predators. Thus, these metabolites represent an excellent source of new bioactive substances [34-35].

Among the secondary metabolites produced, phenols and flavonoids (polyphenols) stand out due to protect against herbivory and damage caused by ultraviolet radiation [36-37]. In the present study results showed that the extracts (ethanolic and methanolic) obtained from the stem bark have a high content of phenolic compounds, whereas in the extracts obtained from the leaf, the aqueous extract showed a greater quantity. These data are superior to those obtained by Nunes *et al*. (2018) [37], who evaluated the photoprotective and antioxidant capacity of *A. pyrifolium*; on the other hand, the flavonoid content was higher than that obtained in this study. This difference in the quantification of these compounds may be associated with seasonality and anthropized areas, as well as other abiotic factors (light, ultraviolet radiation, temperature, water stress and salinity), acting on the activation, regulation and gene expression of biosynthesis of secondary metabolites on plants [38-39].

In this study, phenolic compounds, chlorogenic and caffeic acids, and flavonoids (rutin) were identified, corroborating the results described by Souza-Lima *et al*. (2017) in the leaf aqueous extract [11]. Moreover, De Araújo *et al*. (2007) revealed the presence of monoterpenic indole alkaloids, such as aspidofractinin, 15-demethoxypyrifoline and N-formilaspidofractinin in indole fractions of stem bark [7]. Nogueira *et al*. (2014) found other types of alkaloids (plumerans), glycosides in the ethanolic extract of the seed [40]. In the study by Ceravolo *et al*. (2018), only two components were identified in fractions rich in stem bark alkaloids, alkaloid bisindol leucoridin B and another compound not identified in databases [14]. It can be seen that different parts of plant, as well as different forms of extraction can generate different chemical constituents. This is the first report of phytochemical characterization of ethanolic and methanolic extracts from leaf, and aqueous, ethanolic and methanolic extracts from stem bark of *A. pyrifolium*.

The antioxidant effect of medicinal plants is highly applicable in the establishment of immunity, protection of biomembranes against lipid peroxidation induced by free radicals, in addition to attenuating the pathogenicity of several diseases, such as Parkinson's [15, 41-43]. It was perceived the need for higher concentrations of extract to inhibit 50% of free radicals (IC_{50}) when compared to the standard (ascorbic acid). This data corroborates the study by Nunes *et al*. (2018) who obtained IC_{50} high values of ethanolic extract of stem bark (132.24 µg/mL); however, using another standard component,

quercetin (5.01 µg/mL), it remains elusive about the levels of antioxidant activity [37].

In addition, another study observed that the antioxidant properties of the ethanol-aqueous fraction of *A. pyrifolium* of the seed, having been derived from glycosylated benzoic acid as the main component, were described as neuroprotective associated with antioxidant properties, decreasing lipid peroxidation by more than 60% in rats with degenerated neurons [15].

Although medicinal plants are widely used traditionally for medicinal purposes, mainly because of their easy accessibility and low cost, these plants, when administered improperly and indiscriminately, can cause toxicity, revealing the mistaken idea of the absence of adverse effects by natural products [44-46]. Some studies have reported cases of abortions, neonatal loss, intoxication and poisoning by eating *A. pyrifolium* by other mammals [47-49]; however, this is the first study to show low to medium hemolytic percentages in human erythrocyte cells. Such effects caused to the cell membrane occurred due to the chemical composition of natural products that act on specific pathways of ion transport, induction of oxidative damage of the cell membrane, disturbance of the structure of the lipid layer and change of the regulatory mechanisms of cell volume [50-54].

In the present study antimicrobial activity was observed in aqueous, ethanolic and methanolic extracts of the stem and leaf bark of *A. pyrifolium* at concentrations above 2000 µg/mL against tested bacterial strains. One study suggests that there is antimicrobial activity in concentrations above 1000 µg/mL of ethanolic extracts from stem bark of *A. pyrifolium* in *S. aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6623), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) [55]. Furthermore, Pessini *et al*. (2012) tested ethanol extracts from stem bark, fruit, flower, root, root bark and alkaloid fractions at concentration of 1000 µg/mL in *S. aureus* (ATCC 25923), *B. subtilis* (ATCC 6623), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 15442), *Candida albicans* (ATCC 10231), *C. parapsilosis* (ATCC 22019) and *C. tropicalis*; however, the authors describe antimicrobial activity only in alkaloid fractions in *S. aureus* (MIC = 125 µg/mL), *B. subtilis* (MIC = 250 µg/mL), *C. parapsilosis* (MIC = 500 µg/mL) and *C. tropicalis* (MIC = 500 µg/mL) [12]. Therefore, it can be inferred that the alkaloid fractions concentrated can potentiate the biological activity, in addition to being chemotaxonomic substances of the plant genus [6, 7].

Still, it was possible to observe antimicrobial activity of aqueous extracts of the bark of the stem of medicinal plants from Caatinga/Pernambuco, including aqueous extract of *A. pyrifolium*, however, in phytopathogenic bacteria, presenting MIC ranging between 12.5 and 25 mg/mL[13], in concentrations higher than those tested in this study. In summary, it is important to highlight that the alkaloid fractions of *A. pyrifolium* extracts can play a relevant role in antimicrobial activity, at concentrations of 1000 µg/mL or ≥ 1000 µg/mL. Furthermore, it is valid to consider analyzes with higher concentrations *of A. pyrifolium* extract in medically important bacteria. Furthermore, it is essential that other solvents be explored, since different solvents extract different chemical substances. Based on this, future studies can be carried out taking these considerations into account.

Conclusion

It is important to emphasize the need for studies with medicinal plants of the Caatinga, due to the high phototherapeutic potential that is still little explored, in addition to the existence of several species that may be possible sources of new therapeutic molecules. The analysis of the extracts, particularly *A. pyrifolium*, is necessary to add scientific data to the empirical ethnobotanical use of medicinal plants, as well as to describe the biological activities of natural extracts aiming at new therapeutic alternatives for treatment of HAI, in view of the increased of microbial resistance to antimicrobials.

This is the first report of the phytochemical characterization of the ethanolic and methanolic extracts of the leaf, and aqueous, ethanolic and methanolic extracts of the stem bark of *A. pyrifolium*, as its antioxidant and hemolytic activity in humans' erythrocytes. In phytochemical analyzes, it was observed the representative content of phenolic and flavonoid compounds in the extracts. In antioxidant activity, the methanolic extract of the stem bark showed greater antioxidant action. At the tested concentrations of the extracts of *A. pyrifolium*, it was not possible to obtain antimicrobial activity against bacterial strains.

Funding

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), FACEPE - APQ-0493-4.03/14, CNPq – 405297/2018-1, CNPq - 307110/2018-4 and Aggeu Magalhães Institute (FIOCRUZ) Pernambuco/Brazil.

Declaration of competing interest

All authors declare no any conflict of interest.

Acknowledgment

Thanks to Dra. Márcia Vanusa da Silva and Alexandre Gomes da Silva (*in memoriam*) who kindly provided the products evaluated in this study.

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