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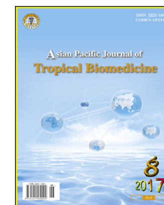

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Chemical profile and antimicrobial activity of *Secondatia floribunda* A. DC (Apocynaceae)

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ABSTRACT

Objective: To establish the chemical profile, and to evaluate the antibacterial and modulatory activity of the ethanolic extracts of the stalk's inner bark and heartwood of *Secondatia floribunda*.

Methods: Quantification of total phenols and flavonoids was determined by the Folin-Ciocalteu method and aluminum chloride, respectively. Phenolic compounds were identified and quantified by HPLC-DAD (High Performance Liquid Chromatography-Diodearray Detector) and the Infrared Spectroscopy was performed using the measure by Attenuated Total Reflectance with Fourier Transform (ATR-FTIR). Antibacterial assays for determination of the Minimum Inhibitory Concentration (MIC) and modification of aminoglycosides were performed by microdilution.

Results: Infrared spectra showed similar characteristics, having among its main absorption bands hydroxyl group (OH). The antibacterial activity showed clinically significant results for the strains of *Staphylococcus aureus* and *Escherichia coli*. In modulation assay, synergic and antagonistic effect for both extracts was observed. Heartwood extract in combination with antibiotics showed a significant MIC reduction at 19.8% ($P < 0.000\ 1$)-79.3% ($P < 0.01$).

Conclusions: This study is the first report of chemical and biological information of *Secondatia floribunda* suggesting that it is clinically relevant source of a new antibacterial therapy, especially due to the presence of significant levels of phenolic compounds.

1. Introduction

Over the years, various studies have been performed to demonstrate the efficacy of medicinal plants regarding their biological activities [1]. Previous to this, various plants were already used for this purpose as an alternative therapeutic option [2,3]. Through traditional medical knowledge a multitude of plant extracts became known to have active substances which are of interest for the development of new drugs. Among these substances, great attention has been given to studies of phenolic compounds, both by their chemical properties and pharmacological actions [4,5], thus, many compounds of this class have been isolated to better understand their potentials [6,7].

Among the activities that related to phenolic compounds, antimicrobial has shown its importance, because these

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substances possess favorable characteristics to affect various components and functions of the bacterial cell and even facilitate absorption into the cell [8]. Thus, it becomes relevant to carry out researches aiming to find mechanisms that act not only directly against microorganisms, but also the resistance modifying agents [9,10].

The *Secondatia* genus (Apocynaceae) contains 12 species distributed exclusively in South America. Three species can be found in Brazil, two being endemic [*Secondatia floribunda* (*S. floribunda*) and *Secondatia duckei*], occurring in the North, Northeast and Midwest, being present in savanna environments, caatinga, Atlantic Forest and the Amazon rainforest. The family of this genus is one of the largest and most representative in Brazil [11]. Its representatives stand out as important sources of useful chemical constituents of traditional and modern medicine, including species rich in phenolic compounds and iridoids [9,12].

In this context, we highlight the species *S. floribunda* A. DC (Apocynaceae) that is often found in areas of the cerrado in the Chapada do Araripe, south of the state of Ceará. Popularly known in the region as 'catuaba-de-rama' or 'catuaba-de-cipó', the species has excelled in ethnobotanical studies where local populations report medicinal indications for sexual impotence, aphrodisiac, nervous complications, depression, weakness, rheumatism and various inflammatory conditions by using the latex, roots and especially its peelings in the preparation of medicines [1,3,13,14]. However, there are no reports in the literature on chemical, biological and pharmacological studies about the species.

Thus, considering the medicinal importance of this species for the communities and the absence of scientific information related to chemical and biological data, this study is the first report on the chemical profile of the stalk's inner bark and heartwood of *S. floribunda*, as well as its antibacterial and modulatory activity.

2. Materials and methods

2.1. Collection of plant materials

Samples of stalk's inner bark and heartwood of *S. floribunda* were collected from Floresta Nacional do Araripe-Apodi (FLONA-Araripe) (07° 11′ S, 39° 13′ W), Crato, Ceará, Brazil. A voucher specimen (no. 9259) was deposited in the Herbarium Caririense Dardano de Andrade Lima (HCDAL) of the Regional University of Cariri (URCA). Authorization to collect botanical material was provided by the Authorization and Biodiversity Information System (SISBIO) of the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA), registered under No. 51674-1.

2.2. Preparation of extracts

The extracts were prepared using 334 g of dry stalk's inner bark and 294 g of dry heartwood. The samples were added separately in flasks with 2 L of ethanol and left in ambient temperature for 72 h. The ethanol was removed using a rotary vacuum evaporator (Model Q-214M2, Quimis, Brazil) and ultrathermal bath (Model Q-214M2, Quimis) under reduced pressure at a temperature of 50 °C. The yields to the extracts were 36.0% and 8.8% (w/w) for stalk's inner bark and heartwood, respectively.

2.3. Drugs, reagents and equipments

All chemical were of analytical grade. Methanol and trifluoroacetic acid were purchased from Merck (Darmstadt, Germany). Cyanidin was acquired from ChromaDex. Gallic acid, catechin, chlorogenic acid, caffeic acid, cinchonain, quercetin and apigenin were acquired from Sigma Chemical Co. (St. Louis, MO, USA). Amikacin, gentamicin and neomycin were obtained from Sigma Chemical Co., dissolved in sterile water before use. High performance liquid chromatography (HPLC-DAD) was performed with a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A diode array detector and LC solution 1.22 SP1 software. The spectra of the FT-IR transmittance were obtained in the spectrometer Agilent Technologies Cary 600 series. The absorbance measurements as a function of the concentrations were performed in a UV–visible spectrophotometer (Thermo Fisher mod. G10S UV–Vis).

2.4. Qualitative phytochemical tests

Phytochemical tests were used to detect the presence of alkaloids, heterosides, tannins, steroids, triterpenes, cumarins, quinones and organic acids were carried out as described by Matos [15] based on observation of color change and/or precipitate formation after addition of specific reagents.

2.5. Content of total phenols and flavonoids

The determination of the phenolic content followed the method of Singleton *et al.* [16], in which extract dilutions were prepared at concentrations ranging from 0.05 to 5.00 μg/mL. The solutions were remained for 15 min in an oven at 45 °C. After this period, the reading was performed in a spectrophotometer at a wavelength of 765 nm. The test was performed in triplicate and the content of phenolic compounds was calculated from the calibration curve using gallic acid in μgEq.GA/g.

The flavonoid content was performed according to the methodology of Kosalec *et al.* [17], with adaptations, utilizing aluminum chloride ($AlCl_3$). Samples from each extract were prepared at an initial concentration of 20 μg/mL and diluted to 10, 5, 2 and 1 μg/mL concentrations. A total of 760 μL of methanol, 40 μL of potassium acetate (10%), aluminum chloride (10%) and 1.120 μL of distilled water were added to 50 μL of each extract concentration. For the blank assay, the volume of aluminum chloride and 10% potassium acetate was replaced by distilled water. Incubation took place at room temperature for 30 min and the spectrophotometer reading was performed at 415 nm. The calibration curve was performed with quercetin, the test was performed in triplicate and the mean value expressed in μgEq.Q/g.

2.6. Quantification of phenolic and flavonoid compounds by HPLC-DAD

Reverse phase chromatographic analysis was carried out using a C18 column (250 × 4.6 mm *i.d.*) with particle diameter 5 μm. The mobile phase consisted of phase A = methanol and

phase B = water with 0.05% trifluoroacetic acid, previously degassed using an ultrasonic bath. A gradient program, as follows = 0 min, 15% phase A; 35 min, 30% phase A; 45 min, 40% phase A, and continued at 60 min, was followed by a 2 min post-time to re-equilibrate the system, at a flow rate of 0.5 mL/min [18]. The eluent was monitored by a PDA, and the detection wavelengths were 254 nm for gallic acid, 280 nm for catechin and cinchonain; 327 nm for chlorogenic and caffeic acids; 356 nm for quercetin and apigenin; and 520 nm for cyanidin. The sample injection volume was 50 μL. Ethanolic extract (stalk's inner bark and heartwood) of *S. floribunda* and mobile phase were filtered through 0.45 μm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use, the *S. floribunda* extracts were analyzed at a concentration of 20 mg/mL. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration range of (0.03–0.50) mg/mL. Chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200–700 nm). All chromatography operations were carried out at ambient temperature and in triplicate.

The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves. LOD and LOQ were calculated as 3.3 and 10.0 σ/S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve [19].

2.7. Fourier transform-infrared spectroscopy (FT-IR)

The FT-IR transmittance measurements of the *S. floribunda* extracts were obtained in the mid-infrared region (4 000 to 600 cm^{-1}) with spectrometer Agilent Technologies Cary 600 series. Approximately 1 mg of sample was crushed with about 100 mg of potassium bromide (KBr), then pressed into pellets of 1 mm under pressure at about 7 (seven) tons. Then the spectra were obtained with 32 scans and a resolution of 4 cm^{-1}.

2.8. Determination of antibacterial and modulatory activity of aminoglycosides

Antibacterial activity of the extracts was evaluated using the broth microdilution method [20]. Four standard bacteria strains were used provided by the Oswaldo Cruz Foundation-FIOCRUZ, *Staphylococcus aureus (S. aureus)* (ATCC 12692), *Enterococcus faecalis* (ATCC 4083), *Escherichia coli (E. coli)* (ATCC 25922), *Proteus vulgaris* (ATCC 13315) and the multiresistant clinical isolates: *E. coli* (27) obtained from sputum and *S. aureus* (358) from a surgical wound, donated by Federal University of Paraíba (UFPB). A bacterial inoculum of 100 μL suspended in 10% BHI broth in microtiter plates with 96 wells was used for the minimum inhibitory concentration (MIC). Then, 100 μL of each extract was added to each well with serial dilutions (1: 2) obtaining concentrations in the range of (512–518) μg/mL. For the controls, the last wells of each column were used and the microtiter plates were incubated at 37 °C for 24 h. Antibacterial activity was detected by the addition of 25 μL of the resazurin reagent [21]. The MIC was defined as the lowest concentrations for growth inhibition.

In order to evaluate the extracts as modulators of resistance to antibiotics of the aminoglycoside class (neomycin, gentamicin, and amikacin) a Gram positive strain (*S. aureus* ATCC 12692) and Gram negative strain (*E. coli* ATCC 25922) were used. The modulation pattern was determined in the presence or absence of the extracts in triplicate. Bacterial inoculum in 10% BHI was distributed on plates with the addition of 100 μL of the antibiotic solutions (1 024 μg/mL) and 100 μL of each extract in concentrations of MIC/8, with range at concentrations from 512.0 to 0.5 μg/mL and following the microdilution and colorimetric assay previously described. The same controls used in the evaluation of the MIC for the extracts were used for the modulation [22].

2.9. Statistical analysis

Differences between groups of HPLC were assessed by an analysis of variance model and Tukey's test. The microbiological results were expressed as geometric mean and analysis of results was applied to two-way ANOVA followed by Bonferroni posttests using GraphPad Prism 6.0 software. Results with $P < 0.05$ were considered as statistically significant.

3. Results

3.1. Phytochemical tests

The chemical prospecting of the *S. floribunda* extracts indicated the presence of tannins flobatenics and flavonoids (Flavones, Flavonols, Xanthones, Chalcones, Auron, Flavonons, Leucoantocyanidins, Catehins and Flavonones) (Table 1), revealing no phytochemical difference between the distinct parts of the plant.

3.2. Total content of phenols and flavonoids

The phenolic content obtained for the ethanolic extract of the stalk's inner bark and heartwood of *S. floribunda* was (37.71 ± 0.40) μgEq.AG/g and (33.65 ± 1.40) μgEq.AG/g respectively. Additionally, flavonoid content obtained was (27.84 ± 1.60) μgEq.Q/g for the stalk's inner bark and (45.62 ± 3.00) μgEq.Q/g for the heartwood. These data indicate slightly variable concentration of these compounds among the

Table 1

Identification of the main chemical classes of the ethanolic extracts of *S. floribunda*.

Extracts	Secondary metabolites classes														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Inner bark	–	–	+	–	–	+	+	+	+	+	+	+	+	+	–
Heartwood	–	–	+	–	–	+	+	+	+	+	+	+	+	+	–

1: Phenols; 2: Tannins Pyrogallics; 3: Tannins Flobatenics; 4: Anthocyanins; 5: Anthocyanidins; 6: Flavones; 7: Flavonols; 8: Xanthones; 9: Chalcones; 10: Auron; 11: Flavonons; 12: Leucoantocyanidins; 13: Catehins; 14: Flavonones; 15: Alkaloids; (+) presence; (–) absence.

vegetal parts, in the heartwood had a significantly higher concentration of flavonoids when compared to that obtained for the inner bark, which showed high amount of phenols.

3.3. HPLC-DAD analysis

HPLC analysis of the ethanolic extract of *S. floribunda* (inner bark and heartwood) revealed the presence of gallic acid (retention time-tR = 10.45 min; peak 1), cyanidin (tR = 15.09 min; peak 2), catechin (tR = 19.54 min; peak 3), chlorogenic acid (tR = 25.41 min; peak 4), caffeic acid (tR = 27.83 min; peak 5), cinchonain (tR = 33.01 min; peak 6), quercetin (tR = 43.76 min; peak 7) and apigenin (tR = 52.95 min; peak 8) (Figure 1 and Table 2).

According to the plant parts analyzed, the results of total and individual contents of the compounds show quantitative variability. The stalk's inner bark extract showed a higher concentration of phenolic compounds in relation to the heartwood extract that did not reveal the presence of the apigenin compound (Figure 2 and Table 2). This result was corroborated with total content of phenols and flavonoids show in this work.

Among the compounds, cinchonain presented substantially more quantity between the extracts evaluated, with values of (10.17 ± 0.01) mg/g for the stalk's inner bark and (5.03 ± 0.01) mg/g for the heartwood extract. Although it had the highest values for both plant parts, cinchonain was quantitatively higher for the stalk's inner bark. On the other hand, the catechin compound was shown in lower quantity for the stalk's inner bark extract (0.61 ± 0.03 mg/g), whereas for the heartwood extract it presented a considerably higher value (3.29 ± 0.02 mg/g). Additionally, the heartwood extract presents the flavonoid quercetin in a similar amount to the cinchonaine compound and

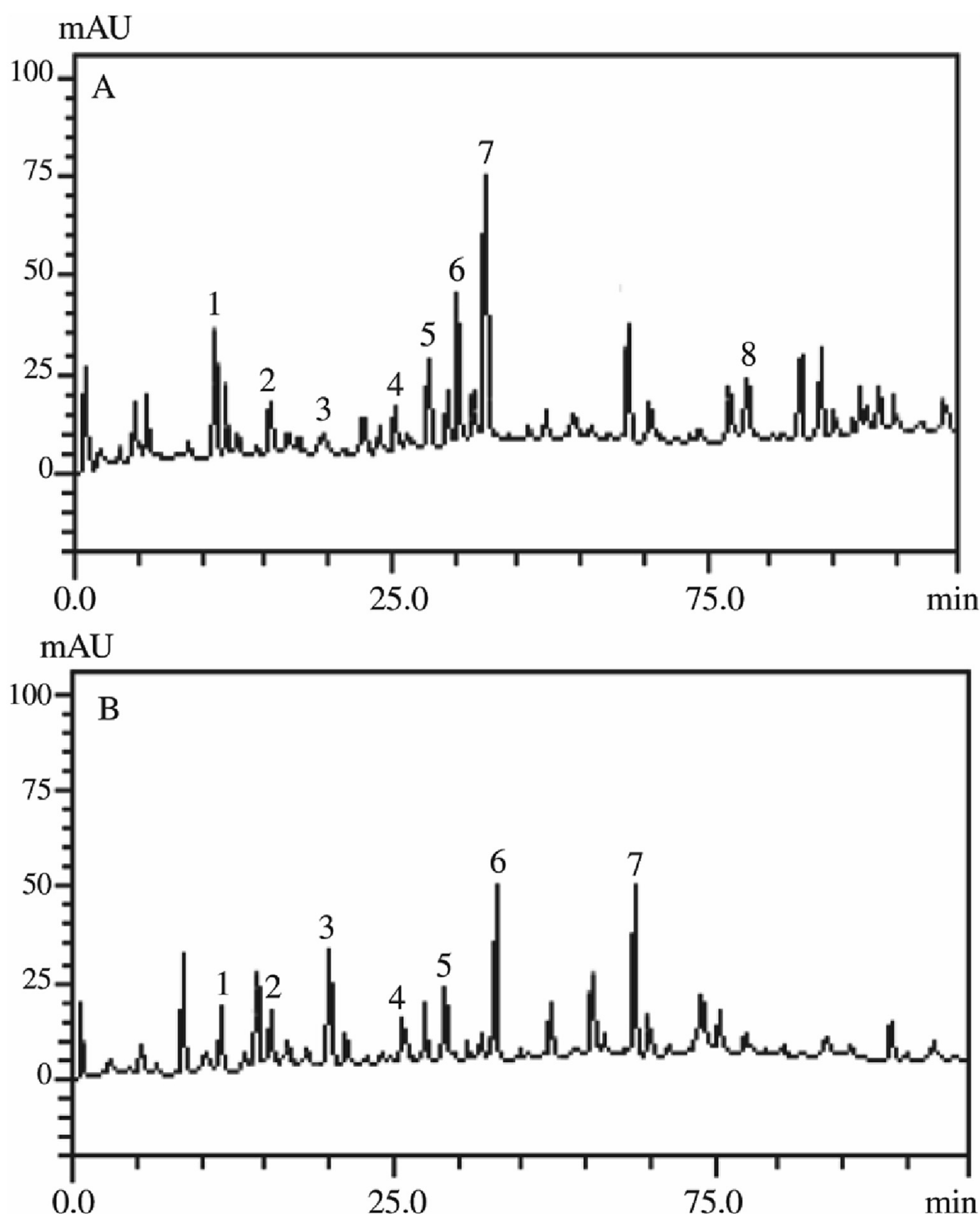

Figure 1. Representation of high-performance liquid chromatography profile of *S. floribunda* stalk's inner bark (A) and heartwood (B).
Gallic acid (peak 1), cyanidin (peak 2), catechin (3 peak), chlorogenic acid (4 peak), caffeic acid (peak 5), cinchonain (peak 6), quercetin (peak 7) and apigenin (peak 8).

Table 2

Phenolic and flavonoids composition in ethanolic extracts of the stalk's inner bark and heartwood of *S. floribunda*.

Compounds	Inner bark (mg/g)	Heartwood (mg/g)	LOD (μg/mL)	LDQ (μg/mL)
Gallic acid	3.27 ± 0.02[a]	1.38 ± 0.03[a]	0.008	0.027
Cyanidin	1.48 ± 0.01[b]	1.34 ± 0.01[a]	0.019	0.063
Catechin	0.61 ± 0.03[c]	3.29 ± 0.02[b]	0.025	0.081
Chlorogenic acid	1.39 ± 0.01[b]	1.18 ± 0.01[c]	0.007	0.024
Caffeic acid	3.05 ± 0.02[d]	1.40 ± 0.03[a]	0.034	0.113
Cinchonain	10.17 ± 0.01[e]	5.06 ± 0.02[d]	0.023	0.069
Quercetin	3.26 ± 0.01[a]	5.03 ± 0.01[d]	0.016	0.048
Apigenin	1.49 ± 0.04[b]	–	0.030	0.091

Results are expressed as mean ± standard deviation (SD) of three determinations. Means followed by different letters differ by Tukey test $P < 0.05$.

superior to that presented by the stalk's inner bark extract [(5.03 ± 0.01) mg/g and (3.26 ± 0.01) mg/g, respectively]. With next values, cyanidin presented (1.48 ± 0.01) mg/g for the internal husk extract of the stem and (1.34 ± 0.01) mg/g for the heartwood extract.

The most abundant phenolic acid was the gallic acid having (3.27 ± 0.02) mg/g for the stalk's inner bark and (1.38 ± 0.03) mg/g for the heartwood. In the stalk's inner bark extract, phenolic acids (gallic acid, caffeic acid and chlorogenic acid) stand out with considerable amounts, with the exception of chlorogenic acid that presented similar quantities between the distinct parts of the plant (1.39 ± 0.01) and (1.18 ± 0.01) mg/g, respectively.

3.4. Infrared spectroscopy

Figure 3A and 3B showed spectra in the infrared region for the ethanolic extracts of *S. floribunda* obtained by transmittance mode with very similar characteristics. Specific absorptions corroborate the identification of present flavonoids and phenolic compounds, wherein the spectroscopic profile obtained for the extract of the stalk's inner bark (Figure 3A) showed among the main absorptions: broadband related to the axial deformation of O−H at 3 376 cm^{-1}, in the region of 2 929 and 2 852 cm^{-1}, bands relating to the axial deformation of the CH_2 and CH_3 groups, C = C axial deformation of aromatic ring at 1 612, 1 520 and 1 447 cm^{-1}.

The infrared spectrum of the heartwood extract (Figure 3B) presents the following absorption bands: broadband relating to the O−H group at 3365 cm^{-1}, C−H axial deformation at 2 925 cm^{-1} relating to CH_2 group, C−H axial deformation at 2 854 cm^{-1} relating to CH_3 group, axial deformation band of the C = O group at 1 733 cm^{-1}, and angular deformation of the C−H plane at 1 055 cm^{-1}. Between the major bands, OH deformation from phenolic groups was observed in Figure 3.

3.5. Antibacterial activity

In the evaluation of the antibacterial activity by microdilution, it was observed that the extract of the stalk's inner bark showed a minimum inhibitory concentration of 512 μg/mL in all bacterial strains, except for strains of *S. aureus* ATCC 12692 with MIC values of 128 μg/mL and *E. coli* ATCC 25922 with MIC of 64 μg/mL. The heartwood extract showed an MIC of 512 μg/mL for *E. coli*, *S. aureus* ATCC 12692 and *S. aureus*

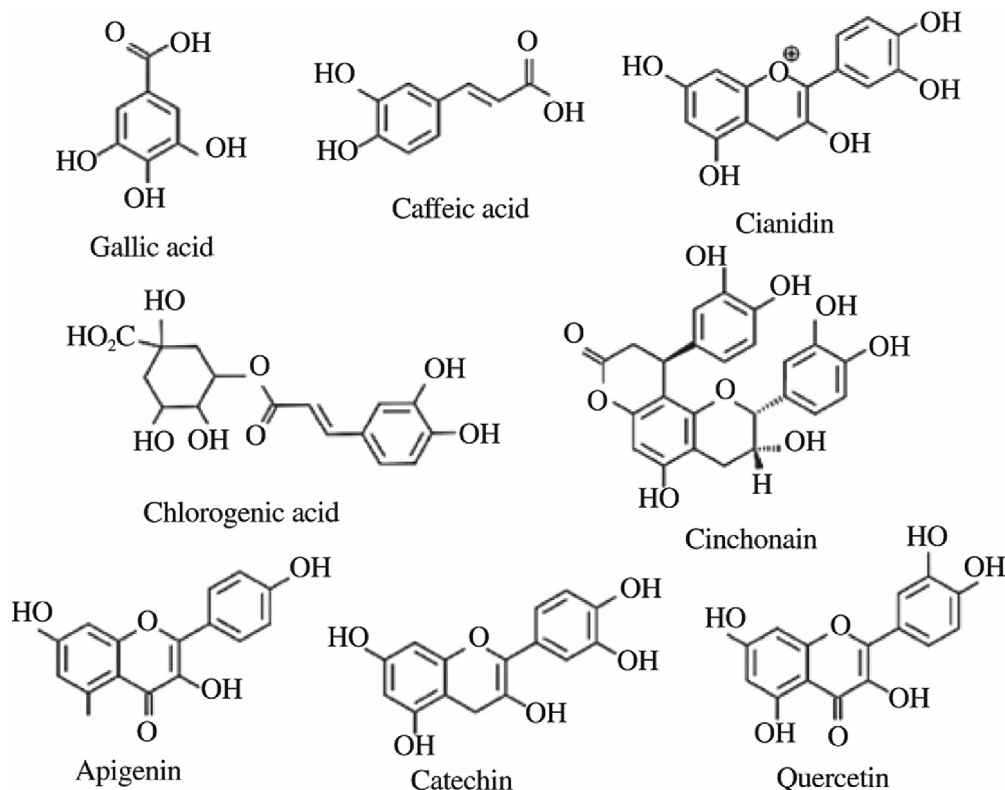

Figure 2. Structural representation of the phenolic compounds detected in the ethanolic extract of the stalk's inner bark and heartwood of *Secondatia floribunda*.

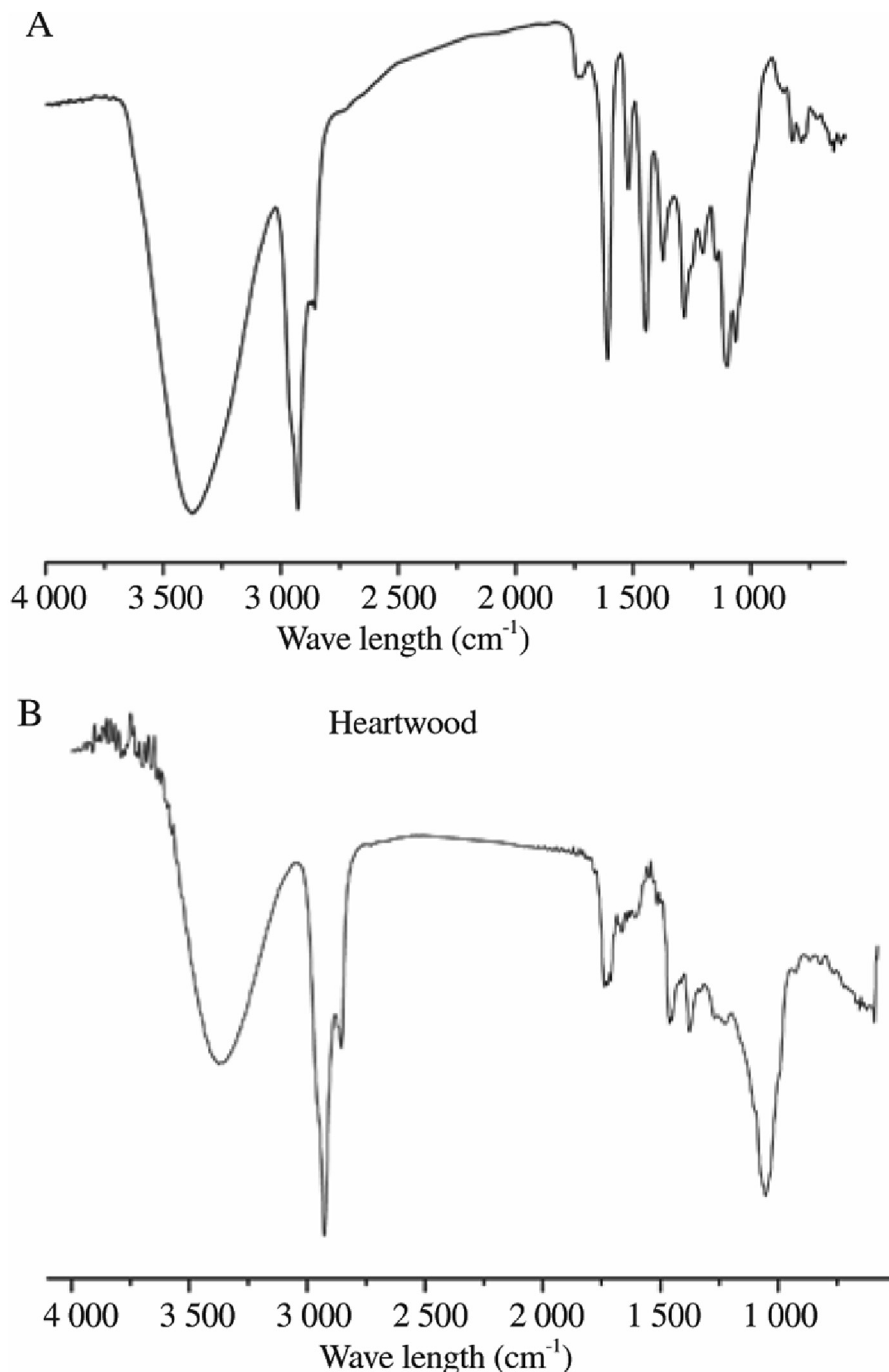

Figure 3. Spectrum in the infrared region of extract of *S. floribunda*. (A) stalk's inner bark extract and (B) heartwood extract.

358 and a MIC ≥ 1 024 μg/mL for *E. faecalis* (ATCC 4083), *P. vulgaris* (ATCC 13315) and *E. coli* (27), with no substantial effect considered.

3.6. Modulatory activity to aminoglycosides

The modulatory activity of the extracts was performed with a Gram-positive bacterial strain and Gram-negative strain, which obtained results with MIC ≤ 512 μg/mL. In the association of the extracts with the aminoglycoside antibiotics in subinhibitory concentrations (MIC 1/8), significant changes were observed in the interaction between the two strains tested, with synergistic and antagonistic activity, and in some cases where it did not influence the action of the antibiotic (Figure 4). The most representative value for synergetic activity was observed for the heartwood extract against the *S. aureus* strain, reducing the MIC of neomycin to less than 20%. Regarding the antagonistic effect, an increase in MIC was noted when both extracts were combined with gentamicin against *E. coli*.

For the stalk's inner bark extract, a synergistic effect was observed when combined with neomycin against the two standard strains tested (*S. aureus* and *E. coli*), reducing the MIC of the antibiotic by 63% in both strains. In the standard strain of *E. coli*, extract interaction with gentamicin generated an increase in the antibiotic's MIC (antagonistic effect). The heartwood extract showed significant synergism with two of the antibiotics

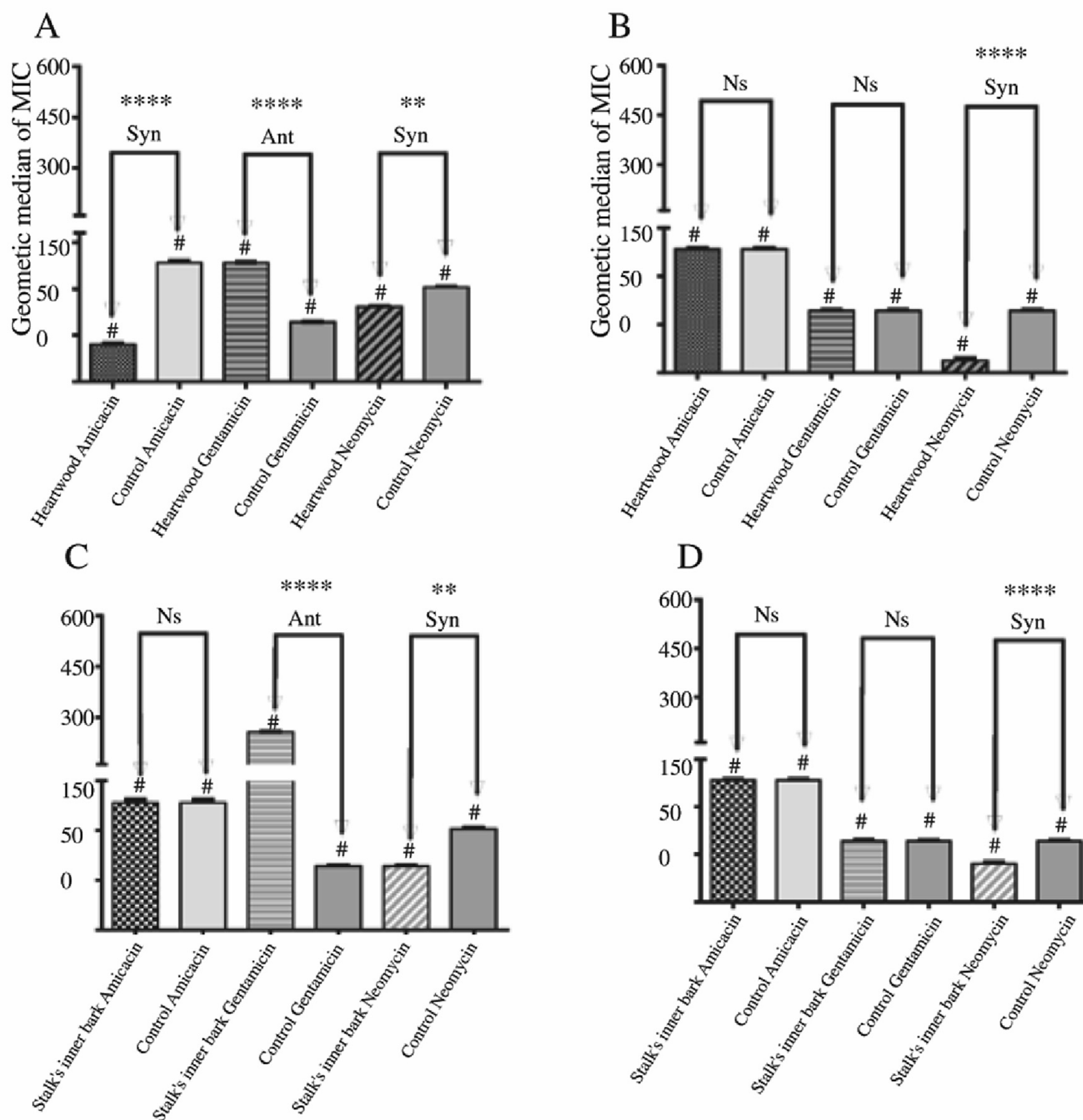

Figure 4. Minimum inhibitory concentration (MIC) of aminoglycosides (Amikacin, Gentamicin or Neomicin) in the absence and presence of stalk's inner bark and heartwood extracts of *Secundatia floribunda*.
(A) Heartwood of *S. floribunda* towards *E. coli* (ATCC 25922). (B) Heartwood of *S. floribunda* towards *S. aureus* (ATCC 12692). (C) Stalk's inner bark of *S. floribunda* towards *E. coli* (ATCC 25922). (D) Stalk's inner bark of *S. floribunda* towards *S. aureus* (ATCC 12692). ***P* < 0.01 compared with control group, *****P* < 0.000 1 compared with control group; #*P* < 0.05 compared with MIC. Ns = Non-significant; Syn = Synergism and Ant = Antagonism.

tested. When associated with neomycin, it reduced the MIC by 19.8% and 79.3% against the *S. aureus* and *E. coli* standard strains, respectively. When in combination with amikacin against *E. coli*, it reduced the MIC of the antibiotic by 31.4% ($P < 0.000\ 1$). In addition, the extract presented antagonism when associated with gentamicin against the same bacterial strain. In the remaining tested interactions, no significant modulatory activity was observed for both extracts evaluated.

4. Discussion

The identified phenolic compounds are widely found in the vegetable kingdom and used in popular medicine for different therapies, playing a key role in the benefit to human health. The great interest in this medicinal use has intensified studies to evaluate the potential of these substances, some of which already present recognized therapeutic activities, such as the flavonoids that among its numerous activities, we can mention its anti-cancer, anti-inflammatory and antioxidant activity and tannins which have an antimicrobial action, antiviral, antitumor and accelerates the healing process [23–27].

Phenolic compounds including flavonoids are among the natural constituents that exhibit physicochemical properties and structural characteristics capable of acting and being efficient in different biological activities [28,29]. The determination of these bioactive substances are important because their presence is attributed to several mechanisms including antibacterial capacity, where they can aid in inhibiting the growth of microorganisms, including enzymes, toxins and signaling receptors and act as modulating agents in the treatment of infectious diseases [30,31]. Furthermore, they are able to forming complexes with soluble extracellular proteins that bind to the cell wall of the bacteria or breach of its cell membrane, exerting antimicrobial action [32].

Among the tested compounds by HPLC, the analysis revealed a greater quantity for flavonoids in both parts of the plant. Some authors have reported the prioritized presence of this class in other species of the family Apocynaceae [19,33,34]. Presenting quantitative variability, Cinchonain followed by quercetin and gallic acid were the major compounds found in *S. floribunda*.

Many factors are responsible for the qualitative and quantitative change in the concentration of compounds. This variation may occur between species or in the same individual and is constantly caused by environmental conditions [35]. Knowledge of these factors is important as it can help in achieving higher concentrations of target chemical compounds by establishing a specific period of collection [36]. Among the factors that interfere are seasonality [37], water stress [38], soil composition [39], solar radiation and temperature [40], phenological stage [41] among others.

Structurally, phenolic compounds represent a wide variety of substances which have one or more aromatic rings linked to at least one hydroxyl group and/or other substituents [28,29] (Figure 2). The determination of their biological activity is directly linked to the vast structural diversity of these compounds [42] that can be well recognized by infrared spectroscopy, according to Schulz and Baranska [43]. Due to the hydroxyl group (OH), phenolic compounds have hydrogen bonding potential and can interact with proteins and enzymes, as well as chelate metal ions [8,18,43].

Cinchonain is a flavonolignan, which has a phenylpropanoid substituent, this class was first detected in the bark of a species of the genus *Cinchona* sp. (Rubiaceae) [44] and later was observed in families of different species, among them is the family Apocynaceae [45]. This compound became a chemical marker of the species *Trichilia catigua* Adr. Juss (Meliaceae) for being majority in the extract [46]. Besides, it has promising biological activities, some already reported, such as antioxidant [47–49] and antibacterial [46,50].

Phenolic acids present a carboxyl functional group in their basic structures, that although it remains the same, the numbers and positions of the OH group on the aromatic ring vary [29]. The chlorogenic acid has greater hydroxylation capacity, followed by gallic acid and caffeic acid, which is the most representative compound of the class [51]. In biological systems, these acids have a considerable factor that refers to the ability to interact in lipophilic and hydrophilic environments [52], thereby these acids have an important biological capability that includes minimizing oxidative processes [53,54].

The results against Gram-positive and Gram-negative bacteria show that both extracts had inhibitory effects, since a MIC $\leq$ 256 μg/mL exhibit clinically relevant level [32], however, effect with MIC $\geq$ 1 000 μg/mL according to Houghton *et al.* [55], does not show a confidence level from the clinical point of view, since it would require large quantities of the natural product to reach a concentration in plasmatic levels. The inhibitory effects observed may be related to an interaction between phenolic acids and flavonoids since these classes of secondary metabolites have well-recognized antibacterial activity even in low content concentrations [56,57]. In addition, the significant activity demonstrated by the stalk's inner bark extract can be attributed to the considerable quantity of the cinchonaine compound, which has a reported antibacterial action [50], and/or the presence of apigenin identified in its composition, which has an antimicrobial effect described in the literature [30,58].

Evaluating the antimicrobial properties of the crude extract and fractions (dichloromethane, ethyl acetate and butanol) from the leaves and barks of *Tabernaemontana catharinensis* A. DC (Apocynaceae) Boligon *et al.* [19] described that dichloromethane and butanol fractions showed better results against gram-positive bacteria; this activity is related to the synergistic action of different compounds present in the plant, particularly phenolic acids, flavonoids, alkaloids and terpenes, in combination.

Pallant *et al.* [59] which attributed good activity for the of the root extract of *Tabernaemontana elegans* (Stapf.), against gram-positive bacteria, including *S. aureus* and *E. faecalis*. Similarly, Assis *et al.* [60] testing the stem's antibacterial activity the of *Tabernaemontana angulata* Mart. ex Müell. Arg. (Apocynaceae) by the broth dilution method, observed that the extract possess significant activity against *S. aureus*. However, Suffredini *et al.* [61] show that the stem's extract of same specie showed no activity against *S. aureus*. Additionally, methanolic extract of *Aspidosperma ramiflorum* (Apocynaceae) was inactive against *E. coli* and *Pseudomonas aeruginosa* at concentrations $\leq$1000 μg/mL [62].

It is noted that works regarding the antibacterial action for species of the Apocynaceae family often diverge. This variation may be related to the chemical composition of the extract, which while mixing has several substances derived from both primary and secondary metabolism, that together can enhance the activity or mask its effects [19]. Furthermore, variations concerning the activity may relate to the different techniques for each test or even through the different mechanisms that vegetable substances can act [63].

The search for natural products with modulatory property has been widely reported. Many of these plant products have considerable synergic effects, positively altering the effect of antibiotics and constituting an important alternative to combating the increase of microbial resistance [64]. Antibiotics of the aminoglycosides class act in the inhibition of protein synthesis [65] such that bacterial resistance mechanisms mainly involve the activation of efflux and target modification systems [66]. Thus, the synergistic effects that are generally obtained by the association of natural products with antibiotics are related to an increase in the influx of the drug, which alter the permeability of the cell membrane, favoring the penetration of antibiotics and potentializing its effect [30]. Thus, studies on the association of natural products and synthetic drugs are still promising in an attempt to discover new chemical classes with antibiotic potential [67,68].

It is known that phenolic compounds, including tannins and flavonoids, due to their constitution and concentration may prove potentially active, affecting the growth and metabolism of bacteria and inhibition of nucleic acid synthesis [19,69]. Some researchers have reported potentiation of antibiotic action when combined with extracts containing phenolic compounds against resistant bacterial strains [10]. This association mainly affects the cellular membrane fluidity [70] and interacts with bacterial enzymes responsible for mechanisms involved in the replication, transcription and repair of DNA-topoisomerase, causing a significant inhibitory effect [71]. Many of these studies emphasize the isolation of these compounds to more effectively assess their potential [8,30].

Analyzing the antibacterial activity of seven pure phenolic compounds against *Serratia marcescens*, *Proteus mirabilis*, *E. coli*, *Klebsiella pneumoniae* and *Flavobacterium* sp. Vaquero *et al.* [69] found that caffeic acid had an effective antibacterial

activity and quercetin was the strongest inhibitor against all bacteria investigated. Likewise, Fattouch *et al.* [72] evaluating an extract and pure phenolic compounds found that chlorogenic acid exhibited a higher antibacterial activity, particularly against *S. aureus*.

In a study by Lima *et al.,* [57] evaluating the effects of the antibiotic activity of gallic acid, caffeic acid and pyrogallol, they verified that all the phenolic compounds tested were effective in the antibacterial activity against *S. aureus*, with caffeic acid potentiating activity against all tested bacterial strains, among them *E. coli*. Parveen *et al.* [73] described that an isolated flavonoid from the leaves of *Alstonia macrophylla* (Apocynaceae) caused the significant growth inhibition for *S. aureus*, *E. coli* and *Salmonella typhimurium*. In another study conducted by Camargo and Raddi [74] as observed the antibacterial action of quercetin against *S. aureus*.

Among the phenolic compounds identified by HPLC-DAD, most are related to antibacterial activity in other studies. Apigenin and quercetin were previously reported to have antibacterial activity acting through various targets, including enzymatic inhibition in *E. coli* [58]. Even though apigenin is not present in the heartwood composition and quercetin is not predominant in either extracts, the synergic actions demonstrated by these extracts indicate the contribution of these compounds even in smaller quantities. In addition, this synergistic action also suggests the participation of other phenolics such as cinchonaine, predominant in the extracts evaluated, in the activity and in possible combinations with the antibiotics. In this manner, the non-significant activities reported are possibly linked to the various types and concentrations of non-phenolic substances present in the extract that may reflect on the modulating effect.

The data obtained in this study is reported for the first time for *S. floribunda* and suggests that the ethanolic extracts of its stalk's inner bark and heartwood, which are clinically relevant source of new antibacterial therapy, due to the presence of significant levels of compounds with activity already recognized, such as phenols and flavonoids. The individual content of phenolic compounds by HPLC were higher in the stalk's inner bark's extract, however, the heartwood extract showed greater synergetic activity, indicating the possible use of these components associated with the usual antibiotics against gram-positive and Gram negative strains. In addition, the results observed in this work for *S. floribunda* underscore its therapeutic potential and the need for studies evaluating plant species that are used as a source of biologically active natural products, paving the way for contributions in the search and development of new medicines.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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