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Article

Phenolic compounds and antioxidant, antimicrobial and antimycobacterial activities of *Serjania erecta* Radlk. (Sapindaceae)

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Serjania erecta Radlk.(Sapindaceae) is a medicinal plant traditionally used in Brazil. We assayed the ethanolic extract of leaves and roots against seven microorganisms. The REMA (Resazurin Microtiter Assay) assay was used to measure the biological activity *in vitro* against *Mycobacterium tuberculosis* and our results showed moderate activity of the ethanolic extract. On the other hand *S. aureus, P. aeruginosa, S. setubal, C. albicans, S. cerevisiae* and *E. coli* revealed that the leaves and roots of *S. erecta* inhibited the growth of all microorganisms. The ethanolic extracts of leaves and roots showed low values of antioxidant activities. The ethanolic extracts of leaves and roots were analyzed by chromatographic and spectrometric methods. (-)-Epicatechin, kaempferol aglycone and five glycoside derivates were isolated: kaempferol-3-O-α-L-rhamnopyranoside, kaempferol-3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside from the roots and kaempferol, kaempferol 3,7-di-O-α-L-rhamnopyranoside, vitexin, isovitexin and (-)-epicatechin in the leaves. This is the first chemical study reported in the literature about this specie.

Uniterms: *Serjania erecta* Radlk/pharmacognosy. *Serjania erecta* Radlk/ethanolic extract/antioxidant activity. *Serjania erecta* Radlk/ethanolic extract/antimicrobial activity. Sapindaceae/pharmacognosy. Cipó-cinco-folhas/pharmacognosy. Flavonoids. Medicinal plants.

Serjania erecta Radlk. (Sapindaceae) é uma planta medicinal utilizada no Brasil. Analisamos o extrato etanólico de folhas e raízes para sete microorganismos. O REMA (Ensaio de Microtitulação Resazurina) foi utilizado para medir a atividade biológica *in vitro* contra o *Mycobacterium tuberculosis* e nossos resultados mostraram atividade moderada do extrato etanólico. Por outro lado, ensaios com *S. aureus, P. aeruginosa, S. setubal, C. albicans, S. cerevisiae* e *E. coli* revelaram que as folhas e raízes de *S. erecta* inibiram crescimento em todos os microorganismos. Os extratos etanólicos de folhas e raízes apresentaram valores baixos de atividade antioxidante. Os extratos etanólicos de folhas e raízes foram analisados por métodos cromatográficos e espectroscópicos. (-)-Epicatequina, canferol aglicona e cinco derivados glicosídicos foram isolados: canferol -3-O-α-L-ramnopiranosídeo, canferol-3-O-α-L-ramnopiranosil-(1→6)-β-D-glucopiranosídeo a partir das raízes e canferol, canferol 3,7-di-O-α-L-ramnopiranosídeo, vitexina, isovitexina e (-) epicatequina nas folhas. Este é o primeiro estudo químico na literatura sobre esta espécie.

Unitermos: *Serjania erecta* Radlk/farmacognosia. *Serjania erecta* Radlk/extrato etanólico/atividade antioxidante. *Serjania erecta* Radlk/extrato etanólico/atividade antimicrobiana. Sapindaceae/farmacognosia. Cipó-cinco-folhas/farmacognosia. Flavonóides. Plantas medicinais.

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INTRODUCTION

The tribe *Paullinieae* belongs to the Sapindaceae and shows neotropical distribution and comprises approximately 450 species in seven genera: *Cardiospermum, Houssayanthus, Lophostigma, Paullinia, Serjania, Urvillea and Thinouia* (Ferrucci, 2000) . The *genus Serjania* with about 231 species is the largest one of *Paullinieae* and occurs in drier areas, along forest edges and in weedy and disturbed areas (Ferrucci, 2000). *Serjania erecta* Radlk. is known in Brazil as "cinco-folhas" or "cipó-cinco-folhas" (Guarim Neto *et al*., 2000; Pott *et al.,* 2004). Its leaves are used in folk medicine against inflammation, stomach ache, ulcerative diseases, and the roots to treat hypertension (Guarim Neto *et al*., 2000; Pott *et al.,* 2004). From aerial parts of the medicinal plant *S. triquetra* 11α-hydroperoxy-hederagenin, a sapogenin, stigmasterol, oleanolic acid, morolic acid, hederagenin were isolated (Chávez, Delgado, 1994).

Chemical investigations on *S. salzmanniana* have revealed the presence of antifungal and molluscicidal saponins in the roots (Ekabo *et al*., 1993, 1996). Leaf extracts of *S. yucatanensis* have *in vitro* activity against epimastigotes and trypomastigotes of *Trypanosoma cruzi* (Hernández *et al*., 2012). Some species, such as *S. lethalis* inhibited the production of NO by J774 macrophages showing an important anti-inflammatory activity (Mesquita *et al*., 2007).

In the present communication, our interest in identification of compounds from native plants in Cerrado motivated us to conduct a chemical investigation on *Serjania erecta* Radlk. and to evaluate antimicrobial, antimycobacterial and antioxidant activities and phenolic and flavonoids contents of polar extract of leaves and roots of this specie.

MATERIAL AND METHODS

Plant material

S. erecta was collected in the municipality of Aquidauana, MS, Brazil, in November 2009, and identified by A. Pott. A voucher specimen (HMS 8355) is deposited in the Herbarium of the Embrapa Gado de Corte in Mato Grosso do Sul, Brazil.

Extraction, isolation and identification of compounds

The leaves (600 g) and roots (400 g) of *S. erecta* were extracted successively with hexane, ethyl acetate and ethanol at room temperature. Extracts were filtered and concentrated under vacuum. For this work we used only the ethanol extract (12.0 g and 3.4 g) of leaves and roots, respectively. Both extracts were fractionated by XAD-2 (Supelco, Bellefonte, PA, USA) resin column chromatography (30 cm × 3 cm). The leaves extract was eluted with 1.0 L of water, followed by 0.8 L of methanol and finally with 0.2 L of acetone. Roots extract was eluted with 0.5 L of water, followed by 0.5 L of methanol and finally with 0.2 L of acetone. An aliquot of 1.2 g and 0.95 g of the methanolic fraction (FRMeOH) of leaves and roots extracts were dissolved in 10 mL and 5 mL of methanol, respectively, and fractionated by Sephadex LH-20 (Amersham Pharmacia Biotech, Uppsala, Sweden), column chromatography (70 cm × 3 cm) eluted with methanol at a flow rate of 0.3 mL min^{-1}. We collected 40 fractions of 8 mL from ethanolic extract of leaves and 28 fractions from the root extracts. The fractions were combined according to their behavior by thin-layer chromatography (silica gel plates, ethyl acetate/*n*-propanol/water, 120:8:70 by volume, upper phase). The substances obtained were further purified by repeated column chromatography either on polyvinylpolypyrrolidone (Sigma, eluted with MeOH) eluted with methanol. NMR spectra were recorded on a Bruker DPX 300 spectrometer. IR spectrum were performed in a FT-IR-Nicolet Impact IMACT-400, KBr and UV spectrum were performed in a Hitachi 110 spectrophotometer.

Spectral data

- kaempferol (1):

^{1}H NMR [300 MHz, DMSO d_6, J (Hz)]: δ 6.21 (*d*, J=2.0 Hz, H-6); 6.42 (*d*, J=2.0 Hz, H-8); 6. 95 (*d*, J=8.5 Hz, H-3'); 6.95 (*d*, J=8.5 Hz, H-5'); 8.00 (*d*, J=8.5 Hz, H-2', H-6'). RMN ^{13}C (70 MHz, DMSO-d6): δ 146.9 (C-2); 98.9 (C-6); 164.1 (C-7); 93.9 (C-8); 156.3 (C-9); 103.1 (C-10); 122.1 (C-1'); 130.1 (C-2'); 115.1 (C-3'); 159.0 (C-4'); 115.1 (C-5'); 130.1 (C-6'). UV λ_{max}: 266 nm e 368 nm. IV υ_{max}: 3320, 1610 cm^{-1}.

- kaempferol-3,7-di-O-α-L-rhamnopyranoside (2):

^{1}H NMR [(300 MHz, DMSO d_6, J (Hz)]: δ 6.43 (*ls*, H-6), 6.68 (*ls*, H-8), 7.76 (*d,* J=8.0 Hz, H-2', H6'), 6.92 (*d*, J=8.0 Hz, H-3',5'), 5.54 (*ls,* H-1''), 5.29 (*ls*, H-1'''), 1.26 (*d*, J=6.0 Hz, H-6''), 0.92 (*d,* J=5.6 Hz, H-6'''), 3.1-4.0 (*m,* sugar). RMN ^{13}C (70 MHz, DMSO d_6): δ 158.0 (C-2), 134.5 (C-3), 178.0 (C-4), 160.9 (C-5), 99.8 (C-6), 161.9 (C-7), 95.0 (C-8), 157.0 (C-9), 105.5 (C-10), 120.4 (C-1'), 132.0 (C2'), 116.0 (C-3'), 161.0 (C-4'), 116.0 (C-5'), 132.0 (C-6'), Rha'': 98.5 (C-1''), 70.2 (C-2''), 69.8 (C-3''), 71.6 (C-4''), 70.0 (C-5''), 17.9 (C-6''), Rha''':

101.9 (C-1'''), 70.3 (C-2'''), 70.6 (C-3'''), 72.3 (C-4'''), 70.0 (C-5'''), 17.4 (C-6'''). UV λ_{max}: 260 nm e 345 nm. IV υ_{max}: 3340, 1650, 1592 cm^{-1}.

- (-)-epicatechin (3):

^{1}H NMR [300 MHz, acetona, *J* (Hz)]: δ 4.88 (*ls*, H2), 4.20 (*m*, H-3), 2.86 (*dd*, J= 16.5; 4.5, H-4), 2.74 (*dd*, ***J***= 16.5; 3.0, H-4), 6.02 (*d,* J= 2.5, H-6), 5.92 (*d,* J= 2.0, H-8), 7.05 (*d,* J=2.0, H-2'), 6.78 (*d,* J= 8.0, H-5'), 6.84 (*dd,* J =8.0; 2.0, H-6'). RMN ^{13}C (70 MHz, acetona): δ 79.5 (C-2), 67.0 (C-3), 29.0 (C-4), 157.0 (C-5), 96.2 (C-6), 157.2 (C-7), 95.8 (C-8), 157.7 (C-9), 99.9 (C-10), 132.4 (C-1'), 115.3 (C2'), 145.3 (C-3'), 145.4 (C-4'), 115.5 (C-5'), 119.5 (C-6'). UV λ_{max}: 280 nm. IV υ_{max}: 3300, 1640, 1150 cm^{-1}.

- apigenin-6-C-β-D-glucopyranoside (isovitexin) (4):

^{1}H NMR [(300 MHz, DMSO d_6, J (Hz)]: δ 6.72 (*s*, H-3), 6.47 (*s*, H-8), 7.89 (*d*, J= 8.5 Hz, H-2′, H- 6′), 6.89 (d, J = 8.6 Hz, H-3′, 5′), 4.58 (d, J = 10 Hz, H-1″), 3.2-4.3 (*m,* sugar). RMN ^{13}C (70 MHz, DMSO-d6): δ 164.6 (C-2), 102.8 (C-3), 181.7 (C-4), 156.3 (C-5), 108.9 (C-6), 163.3 (C-7), 95.7 (C-8), 161,3 (C-9), 102.3 (C-10), 120.9 (C-1'), 128.3 (C-2', C-6'), 116.0 (C-3', C-5'), 160.6 (C-4'), Glu'': 78.9 (C-1''), 73.1 (C-2''), 73.1 (C-3''), 70.6 (C-4''), 81.4 (C-5''), 61.4 (C-6''). UV λ_{max}: 272 nm e 335 nm. IV υ_{max}: 3417, 1643, 1600 cm^{-1}.

- apigenin-8-C-β-D-glucopyranoside (vitexin) (5):

^{1}H NMR [(300 MHz, DMSO d_6, J (Hz)]: δ 6.75 (*s*, H-3), 6.31 (*s*, H-6), 7.99 (*d*, J= 8.5 Hz, H-2′, H- 6′), 6.91 (d, J = 8.7 Hz, H-3′, 5′), 4.53 (d, J = 10 Hz, H-1″), 3.15-4.25 (*m,* sugar). RMN ^{13}C (70 MHz, DMSO-d6): δ 164.4 (C-2), 102.7 (C-3), 181.8 (C-4), 155.9 (C-5), 98.8 (C-6), 162.8 (C-7), 104.5 (C-8), 160.8 (C-9), 104.6 (C-10), 121.7 (C-1'), 128.7 (C-2', C-6'), 116.2 (C-3', C-5'), 160.8 (C-4'), Glu'': 78.9 (C-1''), 73.8 (C-2''), 71.5 (C-3''), 70.7 (C-4''), 81.6 (C-5''), 61.6 (C-6''). UV λ_{max}: 270 nm e 338 nm. IV υ_{max}: 3400, 1610, 1580 cm^{-1}.

- kaempferol-3-O-α-L-rhamnopyranoside (6):

^{1}H NMR [(300 MHz, CD_3OD, J (Hz)]: δ 6.19 (*s*, H-6), 6.39 (*s*, H-8), 7.76 (*d*, J= 8.4 Hz, H-2', H- 6'), 6.91 (*d*, J = 8.4 Hz, H-3', 5'), 5.36 (*d*, J = 1.8 Hz, H-1''), 0.90 (*d,* J=5.4 Hz, H-6''), 3.2-4.3 (*m,* sugar). RMN ^{13}C (70 MHz, DMSO-d6,): δ 157.8 (C-2), 134.0 (C-3), 177.6 (C-4), 161.9 (C-5), 98.5 (C-6), 164.1 (C-7), 93.5 (C-8), 156.8 (C-9), 103.9 (C-10), 121.8 (C-1'), 130.3 (C-2', C-6'), 115.3 (C-3', C-5'), 161.3 (C-4'), Rha'': 101.9 (C-1''), 70.4 (C-2''), 70,6 (C-3''), 70.8 (C-4''), 69,8 (C-5''), 16,6 (C-6''). UV λ_{max}: 264 nm e 345 nm. IV υ_{max}: 3242, 1655, 1607, 1505 cm^{-1}.

- kaempferol-3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (7):

^{1}H- NMR [(300 MHz, DMSO d6, J (Hz)]:): δ 6.24 (brs, H-6), 6.42 (brs, H-8), 7.97 (*d*, J = 8.6 Hz, H-2', H-6'), 6.89 (*d*, J = 8.6 Hz, H-3', H-5'), 5.30 (*d*, J = 7.6 Hz, H-1''), 4.40 (brs, H-1'''), 3.0-4.4 (*m*, sugar), 1.14 (*d*, J = 6.5 Hz, H6''). ^{13}C NMR (70 MHz, DMSO-d6): δ 156.9 (C-2), 133.4 (C-3), 177.2 (C-4), 161.4 (C-5), 99.2 (C-6), 164.4 (C-7), 93.9 (C-8), 156.4 (C-9), 103.2 (C-10), 121.9 (C-1'), 130.5 (C-2', C-6'), 115.2 (C-3', C-5'), 160.1 (C-4'), Glu'': 101.5 (C-1''), 74.3 (C-2''), 76.5 (C-3''), 70.0 (C-4''), 75.4 (C-5''), 66.4 (C-6'') Rha''': 100.7 (C-1'''), 70.2 (C-2'''), 70.6 (C-3'''), 71.7 (C-4'''), 68.1 (C 5'''), 17.5 (C-6'''). UV λ_{max}: 260 nm e 335 nm. IV υ_{max}: 3376, 1656, 1592, 1546, 1488 cm^{-1}.

Phenolic and flavonoid tests and antioxidant activity

The phenolic and flavonoid contents and antioxidant activity, in two different models (Coutinho *et al*., 2008; Lin, Tang, 2007; Blois, 1958), were measured in the leaves and roots. Total soluble phenolics in the leaves and root extract of *S. erecta* were determined with Folin-Ciocalteau reagent according to the method using gallic acid as a standard phenolic compound. Aliquots of 0.1 mL samples were mixed with 0.5 mL (1/10 dilution) of the Folin-Cicauteau reagent and 1 mL of ultrapurified water. The solutions were mixed and incubated at room temperature for 1 min. After 1 min, 1.5 mL of 20% sodium carbonate solution was added and incubated at room temperature for 40 min. The reaction mixture absorbance was measured at 760 nm on a spectrophotometer. The blanks were prepared with all the reagents except the sample. Gallic acid was chosen as a standard using 6 point standard curve (10, 50, 100, 150, 300 and 400 μg mL^{-1}). All the tests were performed in triplicate. The data were expressed as milligram gallic acid g extract $^{-1}$.

Flavonoids were determined in the leaf and root extracts of *S. erecta*, with 1.50 mL of ethanol 95%, 0.10 mL of $AlCl_3.6H_2O$ 10%, 0.10 mL $NaC_2H_3O_2.3H_2O$ 1 mol/L and 2.80 mL of ultrapurified water. The solutions were mixed and incubated at room temperature for 40 min. The reaction mixture absorbance was measured at 415 nm on a spectrophotometer. The blanks were prepared with all the reagents except the sample. Quercetin was chosen as a standard using 6 point standard curve (10, 50, 100, 150, 300 and 400 μg mL^{-1}). All the tests were conducted in triplicate. Data were expressed as milligram quercetin g extract^{-1}.

The antioxidant activity of ethanolic extract, based on free radical scavenging and β-carotene/linoleic acid

assays, was determined by the method described by Blois, 1958 and Coutinho *et al*., 2008, respectively. For these assays the extracts were evaluated at concentrations at 40, 80, 160, 320 and 640 μg mL^{-1}. The radical scavenging effect of each sample was calculated and compared with that of quercetin (Sigma) (2.5, 5, 10, 20, and 40 μg mL^{-1}), and the experimental values in β-carotene/linoleic acid assay were compared with those of *tert*-butylated hydroxytoluene. All the tests were conducted in triplicate.

Antimycobacterial and antimicrobial activities

The anti-*M. tuberculosis* activity of the ethanolic extract of leaves and roots was determined using the REMA (Resazurin Microtiter Assay) assay as analytical method (Collins, Franzblau, 1997). The minimal inhibitory concentration (MIC) values of these compounds necessary to inhibit 90% of growth of *M. tuberculosis* H_{37}Rv ATCC 27294 were determined in triplicate in sterile 96-well plates (Falcon 3072; Becton Dickinson, Franklin Lakes, NJ, USA), in a SPECTRAfluor Plus (Tecan, Männedorf**,** Switzerland) microfluorimeter. For the standard test, the MIC value of isoniazid was determined each time. The acceptable MIC of isoniazid ranges from 0.015 to 0.03 μg mL^{-1}.

The antimicrobial activity of the ethanolic extracts was assayed using the broth microdilution method (Silva *et al*., 2009). A collection of six microorganisms was used: two Gram-positive bacteria (*Staphylococcus aureus* [ATCC 6538p] and *Pseudomonas aeruginosa* [ATCC27853]), two Gram-negative bacteria (*Escherichia coli* [ATCC 11103] and *Salmonella setubal* [ATCC 19796]), and two yeasts (*Saccharomyces cerevisiae* [ATCC 2601] and *Candida albicans* [ATCC 10231]). Standard strains of microorganisms were obtained from the American Type Culture Collection (Manassas, VA, USA), and the standard antibiotics chloramphenicol and nystatin were used in order to control the sensitivity of the microbial test. The minimal inhibitory concentration (MIC) was determined in 96-well culture plates by a microdilution method using a microorganism suspension at a density of 10^5 colony-forming units/mL with soy casein broth incubated for 24 hours at 37 °C for bacteria and Sabouraud broth incubated for 72 hours at 25 °C for yeasts. Proper blanks were assayed simultaneously, and samples were tested in triplicate.

RESULTS AND DISCUSSION

The extract of leaves and roots led to the isolation of compounds **1-7** (Figure 1). The extract of leaves showed the presence of kaempferol **1** (6 mg) and one derivative kaempferol-3,7-di-*O*-α-L-rhamnopyranoside **2** (5 mg), (-)-epicatechin **3** (8 mg) and two apigenin derivatives: apigenin-6-*C*-β-D-glucopyranoside (isovitexin) **4** (5 mg) and apigenin-8-*C*-β-D-glucopyranoside (vitexin) **5** (6 mg). The extract of roots resulted in the isolation of the compounds: kaempferol-3-*O*-α-L-rhamnopyranoside **6** (2 mg) and kaempferol-3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside **7** (2 mg).

The identification of all compounds (Figure 1) was achieved by the experimental data (IV, NMR, and ultraviolet). We also confirmed their structures by comparing them with the previously reported respective literature data (Agrawal, 1989; Harborne, 1996).

The UV spectrum showed typical results for flavonoids for compounds **1**-**7**, with bands between 250-280 nm relative to ring A and 310-365 nm relative to ring B (Mabry, Markham, Thomas, 1970). The IV spectrum revealed absorption bands that range between 3242 and 3417 cm^{-1} that typically result from the OH link axial deformation. Bands close to 1650 cm^{-1} suggest the presence of carbonyl group. Furthermore, the presence of an aromatic ring may be observed, evidenced by a set of bands that range between 1600 and 1450 cm^{-1}, which denotes the axial deformation of the aromatic C=C. (Silverstein, Bassler, Morril, 1994).

The ^{1}H and ^{13}C NMR signals for compounds **1**, **2**, **6** and **7** were in good agreement with those of kaempferol, the difference between them was located at the glycoside linked to the C-3 and C-7 of the aglycone. These glycosides were identified by the signals at sugar region δ (16-18 and 60-102) in the spectrum by comparing against those described in the literature. For compound **1**, no signals were identified at this region. The signals for compound **2** indicated two rhamnopyranoside moieties, for compound **6** one rhamnopyranoside moiety and for the compound **7** one rhamnopyranoside and one glucoside moieties (Agrawal, 1989; Harborne, 1996). The ^{1}H NMR signals for these compounds were found in the aromatic region δ (6-8) and sugar region δ 3-4. For compound **2**, the sugars showed the anomeric hydrogen at δ 5.54 (*ls*, H1'') and δ 5.29 (*ls*, H1''') and signals at δ 1.26 and δ 0.92 of H6'' and H6''' indicating the presence of one unit of rhamnose at C-7 and C-3, respectively (Harborne, 1996). Compound **6** showed anomeric proton at δ 5.36 with J = 1.8 Hz, showing a α configuration for rhamnopyranoside and value for H6'' at δ 0.90. For compound **7**, the presence of two anomeric signals at δ 5.30 (*d*, J = 7.6 Hz) and δ 4.40 (brs)) in the ^{1}H NMR spectrum and two anomeric carbons at δ 101.5 and δ 100.7 in the ^{13}C NMR spectrum suggested that compound to be a disaccharide. Furthermore, the presence of one doublet with J = 6.5 Hz at δ 1.14 in the

^{1}H-NMR spectrum and at δ 17.5 in the ^{13}C NMR spectrum indicated the presence of one rhamnose moiety. The connecting position of the sugars was established using HMBC experiments. Correlations were observed between the anomeric signal of glucose (δ 5.30) and the C-3 of the kaempferol (δ 133.4) and the anomeric hydrogen signal of the rhamnose (δ 4.40, *d*, J = 1.8 Hz) and the C-6 of glucose (δ 66.4) (Agrawal, 1989; Harborne, 1996).

For compounds **4** and **5**, the ^{1}H and ^{13}C spectra presented characteristic resonance of a glycoside flavone. The H-3 exhibited as a singlet at δ 6.72 and 6.75, respectively. The two anomeric protons of glucose units showed a duplet at δ 4.58 (J = 10 Hz) for **4** and δ 4.53 (J = 10 Hz) for **5**. Characteristic ^{1}H and ^{13}C chemical shift values and coupling constant data indicated that the structure of **4** was based on that of apigenin-6-C-β-D-glucopyranoside (isovitexin) and **5** was based on that of apigenin-8-C-β-D-glucopyranoside (vitexin) (Agrawal, 1989; Harborne, 1996).

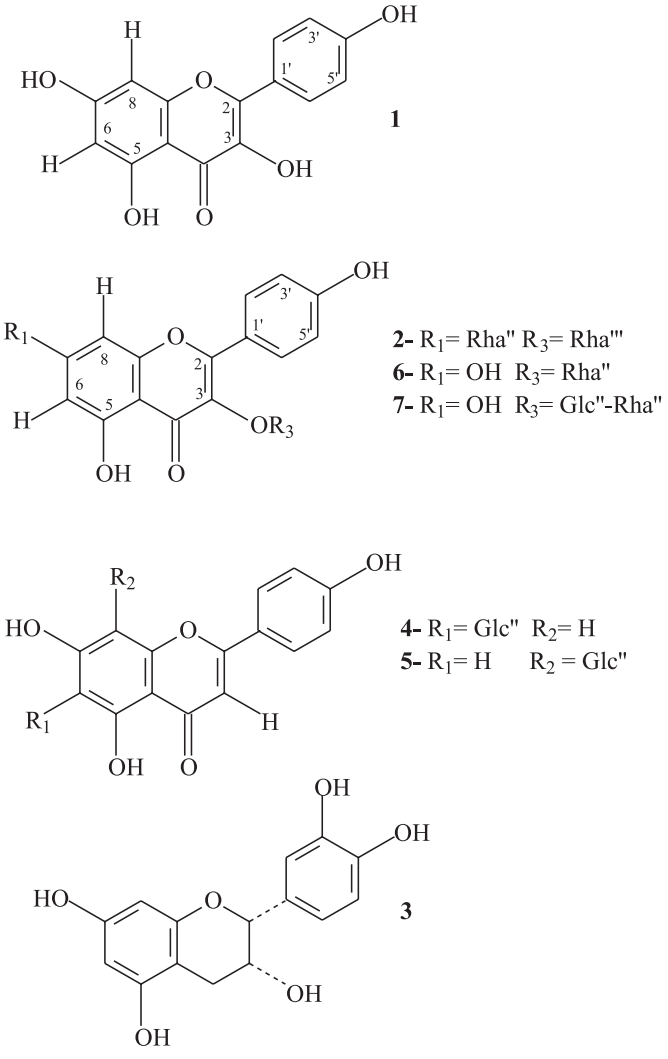

FIGURE 1 - Substances isolated from leaves and roots of *S. erecta.*

^{1}H NMR spectrum of **3** presented chemical shifts of characteristic patterns of catechins, with two double doublets at δ 2.86 (H-4α) and δ 2.74 (H-4β) bonded to C-4, a multiplet at δ 4.21 assigned to H-3 and a broad singlet at δ 4.88 referring to H-2. This set of signals is typical of the C ring of epicatechin. The ^{13}C spectrum showed 15 signals which could be assigned to the epicatechin (Harborne, 1996; Agrawal, 1989). Signals assigned to C-2 (δ 79.5) and C-3 (δ 67.04) characterizing the relative stereochemistry of epicatechin 3-OHα that differentiates of its catechin epimer.

Sapindaceae is a rich source of secondary metabolites and their studies have previously been identified in phytochemical analyses: isoprenoids and polyphenols, saponins, triterpenes, diterpenes, flavonols, flavones, tannins and catechins (Gomig *et al*., 2008; Albiero *et al*., 2002). Our phytochemical study led to the isolation of seven flavonoids (Figure 1). The isolation of C-glycoside flavonoids in *S. erecta* is reported for the first here for this species and also for the genus.

Flavonoids are widely present in the plant kingdom, being found in almost all fruits and plants as colouring pigments. Phenolic compounds, such as flavonoids, could also protect membrane lipids from oxidation acting as antioxidants due to their redox properties, allowing them to act as reducing agents, hydrogen donors, free radical quenchers and metal chelators (Shukla *et al.*, 2009).

The results showed ethanolic extract of leaves with high phenolic and flavonoid contents of 298.4±0.4 and 254.2 ± 0.5 mg g^{-1}, respectively, in comparison to roots (213.4 ± 0.6 mg g^{-1} and 193.2 ± 0.5 mg g^{-1}).

The ethanolic extract of leaves exhibited free radical scavenging activity ranging from 31.4 ± 0.2% to 87.2 ± 0.2%, compared with the quercetin standard having scavenging activity of 94.3 ± 0.1% (at 40 μg mL^{-1}). The inhibition of peroxidation values ranged from 17.1 ± 0.2% to 58.9 ± 0.1%, compared with the *tert*-butylated hydroxytoluene standard of 90.6 ± 0.3 % (at 40 μg mL^{-1}).

The ethanolic extract of roots showed free radical scavenging activity ranging from 25.5 ± 0.1% to 77.6 ± 0.3%, and the inhibition of peroxidation values ranging from 15.1 ± 0.1% to 58.5 ± 0.2%.

The results showed that the antioxidant activity measured for the free radical scavenging assay in the same concentrations was greater than that of the β-carotene/ linoleic acid assay. This might be attributed to the structures present in this extract as flavonoids, which exhibit their antioxidant activity by donating hydrogen (Rice-Evans *et al*., 1996).

Radical-scavenging activity of phenolics depends, among other factors, on the number and position of hydroxyl (-OH) group substituents in the molecules. The presence of the carboxyl, alkyl or other groups affects the antioxidant activity of phenol compounds. The maximum effectiveness for radical scavenging apparently requires the two hydroxyl groups in the ortho-diphenolic arrangement in the B ring and 3-OH group attached to the 2,3-double bond and adjacent to the 4-carbonyl in the C ring. The glycosylation of flavonoids reduces their activity when compared to the corresponding aglycones (Rice-Evans *et al*., 1996).

The substances **2**, **4**-**7** isolated from *S. erecta* have one hydroxyl group in the B ring and glycosylation in the rings A and C. The substances **1** and **3** are aglycones and they present one and two hydroxyl groups in the B ring, respectively.

Because *S. erecta* is a plant largely used as medicinal, we also decided to investigate the effect of ethanol extract against seven microorganisms: *Mycobacterium tuberculosis*, *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella setubal, Candida albicans, Saccharomyces cerevisiae* and *Escherichia coli*.

The ethanolic leaf extract showed an MIC value of 128.0 μg mL^{-1} and root extract MIC value of 256.0 μg mL^{-1} for the anti-*M. tuberculosis* activity. According to Gu *et al*., 2003, a sample with an MIC value of ≤128.0 μg mL^{-1} is defined as active. According to Pauli *et al.* (2005) the MIC of a crude natural extract may or may not be a reliable indicator of the chances for success in isolating a potent antimycobacterial agent from that extract.

The literature reports that flavonoids and saponins are inactive or weakly to moderately active against *M. tuberculosis*. And because of presence of polar substances in ethanolic extracts, the results led to a dramatic decrease or complete loss of activity, since the high lipophilicity is probably the main factor that allows penetration of the compounds through the mycobacterial cell wall (Lin *et al*., 2002), besides the synergistic effect among the compounds present in the extract could affect the final results of analysis (Inui *et al.*, 2007).

On the other hand, the antimicrobial activity of the ethanolic extracts examined against the other six microorganisms: *S. aureus, P. aeruginosa, S. setubal, C. albicans, S. cerevisiae e E. coli*, revealed that the leaves and roots of *S. erecta* inhibited the growth of all microorganisms and that *P. aeruginosa* with MICs of 5.0 μg mL^{-1} and 10.0 μg mL^{-1}, respectively, was the most sensitive. *C. albicans* was the most resistant microorganisms with an MIC of 25.0 μg mL^{-1} for ethanolic extracts leaves (Table I). Our results showed interesting antimicrobial activity of the ethanol extract from *S. erecta*. This is probably due to substances present in the extract, as flavonoids.

Studies of antimicrobial activity indicate that crude extracts containing high content of flavonoids have showed significant activity against various strains of *Staphylococcus aureus, Streptococcus faecalis* and *Escherichia coli* (Chattopadhay *et al*., 2001).

The crude extract and eight isolated compounds from *Castanea sativa* was evaluated against twenty strains of Gram-positive and Gram-negative bacteria. The bioactive compounds were mainly flavonoids (luteolin, kaempferol and apigenin) and triterpenoids (Schinor *et al.*, 2007).

TABLE I - Antimicrobial and antimicobacterial activities of the ethanolic extracts of leaves and roots of *S. erecta*

Microorganism[a]	MIC	
	Leaves	Roots
S. aureus	15.0±0.1	15.0±0.1
P. aeruginosa	5.0±0.1	10.0±0.1
C. albicans	25.0±0.2	15.0±0.1
S. Setubal	20.0±0.2	15.0±0.1
S. cerevisiae	15.0±0.1	10.0±0.1
E. coli	15.0±0.1	15.0±0.1
M. tuberculosis	128.0±0.7	256.0±0.8

Data are mean values of three replicates in μg mL^{-1} ± standard desviation. MIC, minimal inhibitory concentration. [a]Obtained from the American Type Culture Collection.

Serjania lethalis is yet another example. Stems and leaves of *S. lethalis* showed particularly strong inhibition of growth of strains of *Staphylococcus aureus* with minimum inhibitory concentration (MIC) values ≤100 μg mL^{-1}. *S. lethalis* showed good results for this activity, although the screened plant tissue can differ from that indicated by the traditional use of the plant (Lima *et al*., 2006). In northeastern Brazilian region, the leaves of this species are used after cooked against kidney problems (Guarim Neto *et al*., 2000). The same occurs with *S. erecta* that although people use the leaves and roots against a number of diseases (Guarim Neto *et al*., 2000; Pott *et al.,* 2004), there is no knowledge about the use of this species as antimicrobial.

CONCLUSION

In conclusion, in this study we report an investigation of the phytochemical composition of the polar ethanolic

extracts of leaves and roots from *S. erecta*, which is reported herein for the first time in the literature. We also report for the first time the antimicrobial and antimycobacterial activities, and the evaluation of antioxidant activity.

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