Iridoid glucoside and antifungal phenolic compounds from *Spathodea campanulata* roots

Iridóide glicosilado e derivados fenólicos antifúngicos isolados das raízes de *Spathodea campanulata*

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Abstract

The chemical study of *Spathodea campanulata* (Bignoniaceae) roots peels afforded an iridoid glucoside (ajugol) and two phenolic derivatives (*p*-hydroxy-benzoic acid and methyl *p*-hydroxy-benzoate). The compounds were characterized upon spectral data interpretation. Bioactivities of the constituents were evaluated against fungus *Cladosporium herbarum*.
Key words: *Spathodea campanulata,* ajugol, phenolic compounds, *Cladosporium herbarum*

Resumo

O estudo químico das cascas das raízes de *Spathodea campanulata* (Bignoniaceae) conduziu ao isolamento de um irióide glicosilado (ajugol) e dois derivados fenólicos (ácido *p*-hidroxi-benzóico e *p*-hidroxi-benzoato de metila). Os compostos foram identificados com base na interpretação dos seus dados espectrais. A atividade biológica destes constituintes foi avaliada contra o fungo *Cladosporium herbarum*.
Palavras-chave: *Spathodea campanulata,* ajugol, compostos fenólicos, *Cladosporium herbarum*

Introduction

Spathodea campanulata P. Beauv. is a species belonging to the Bignoniaceae family, native from equatorial Africa. It is a medium-size tree (15-25 m high), characterized by red garish flowers. It is often employed in gardening in tropical and subtropical areas, including South America (JOLY, 1985).

This species has many uses in folk medicine for example, the flowers are employed as diuretic and anti-inflammatory, while the leaves are used against kidney diseases, urethra inflammations and as an antidote against animal poisons. The stem bark preparations are employed against enemas, fungus skin diseases, herpes, stomachaches and diarrhea (JARDIM; MENDONÇA; FERREIRA, 2003; MENDES et al., 1986).

The widespread use of *S. campanulata* in folk medicine has stimulated more accurate pharmacological studies. Flowers and stem bark extracts have shown molluscicidal activity (MENDES

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et al., 1986). Hypoglycemic, anti-HIV and anti-malarial activities were also observed in stem bark extracts (NIYONZIMA et al., 1999; MAKINDE; AMUSAN; ADESOGAN, 1988).

Several phytochemical studies were performed with different parts of *S. campanulata*, including stem barks, leaves, flowers and fruits. Spathodic acid, steroids, saponins, ursolic acid, tomentosolic acid and pectic substances have ever been isolated from the stem bark (NIYONZIMA et al., 1999; NGOUELA et al., 1990; NGOUELA; TSAMO; SONDENGAM, 1988; AMUSAN; MSONTHI; MAKHUBU, 1995; AMUSAN; ADESOGAN; MAKINDE, 1996). The leaves have furnished spathodol, caffeic acid, other phenolic acids and flavonoids (NGOUELA et al., 1991; SUBRAMANIAN; SULOCHANA; NAGARAJAN, 1973; EL-HELA, 2001a; EL-HELA, 2001b), while the fruits contain poliphenols, tanins, saponins and glucosides (AMUSAN; MSONTHI; MAKHUBU, 1995). Banerjee and De (2001) showed the presence of anthocyanins in flowers of *S. campanulata* and Petacci et al.(1998) related that the floral nectar contains a complex mixture of triterpenoids and steroids. No chemical investigations on the roots of this plant were found in the literature. Thus, this work aimed to isolate and characterize constituents from the external part of the roots of *S. campanulata*, and evaluate their qualitative fungitoxic activity against *Cladosporium herbarum* CCT 0279.

Materials and Methods

Equipments. Gas Chromatography-Mass Spectrometry (GC-MS) analysis were carried out on a 17-A Shimadzu equipment, column DB1 30 m x 0.25 mm x 0.25 mm film, detector operating in electron impact mode at 70 eV. IR spectra were obtained from a Shimadzu FTIR 8300 spectrometer using KBr (Merck) as sample support. NMR spectral data were recorded on Jeol spectrometer (400 MHz – ^{1}H and 100 MHz – ^{13}C) using MeOH-d4 or $CDCl_3$ as solvents. TMS (*d* 0.0 ppm) or residual solvent signals were used as reference peaks. Analysis and purification steps were carried out on High Performance Liquid Chromatography (HPLC) Shimadzu LC-6AD, UV-VIS 254nm detector model SPD-10A, coupled to a preparative column Keystone Scientific, 250 x 20mm (silica), and ethyl acetate as mobile phase.

Biological Material. *S. campanulata* roots were collected at the campus of State University of Londrina {UEL), Londrina, Paraná, Brazil, 2002. A voucher specimen was deposited at UEL herbarium. *Cladosporium herbarum* CCT 0279 was maintained in solid malt extract medium, under refrigeration (5ºC).

Isolation of Ajugol (1). External membranes taken from the roots (262.0 g) were dried at 50 ºC, under air circulation. The dried material was exhaustively extracted with ethanol and the crude ethanolic extract was partitioned with methanol, yielding 26.2 g. The methanolic partition was purified in silica gel (189 g) column chromatography, with a mixture of dichloromethane: ethyl acetate: methanol (50 : 25 : 5) as solvent. 127 fractions were obtained, with 125 ml each one. Fractions 36-84 (5.32 g) were grouped and purified by column chromatography using Sephadex LH-20 as stationary phase and methanol as solvent, followed by microcrystalline cellulose column chromatography, with solvents dichloromethane, acetone, methanol and glacial acetic acid in increasing polarity. Fractions were grouped by relatedness in Thin-layer chromatography (TLC) analysis. Further purification was carried out using semi-preparative HPLC (conditions described above).

Isolation of Phenolic Constituents (2) and (3). Fresh root peels (922.05 g) were added to a saturated Na_2CO_3 aqueous solution (1800 mL) and remained without stirring for 24 hours at room temperature, followed by paper filtration. Aqueous HCl (6 M) was added to the filtered solution, until pH 1. A brown precipitate was formed. The solution was filtered and the supernatant was extracted with ethyl acetate (1000 mL). The organic layer was dried over anhydrous sodium sulfate, followed by filtration

and concentration under reduced pressure. The crude material was partitioned with ethanol (10 x 20 mL) and the ethanolic partition was purified by preparative TLC (ethyl acetate/methanol 9/1), followed by Sephadex LH-20 column chromatography using methanol as mobile phase. The compounds were isolated as crystalline solids.

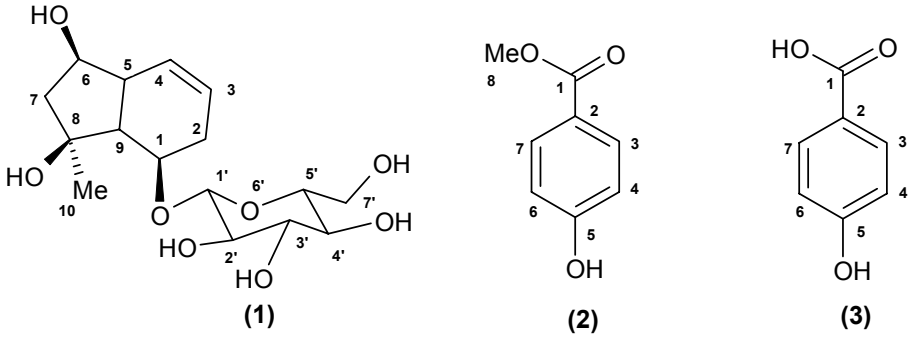
Methyl *p*-hydroxybenzoate (2). GC-MS (IE, 70 eV) *m/z*: 152 (M^+), 121, 93, 65. IR (KBr): 3600-3000 (large, n_{O-H}), 3027 (n_{C-H} sp^2), 2950 (n_{C-H} sp^3), 1686 ($n_{C=O}$), 1635, 1616 and 1585 ($n_{C=C}$), 1439 (d_{CH3}), 1289 (n_{C-O}, phenol), 1233, 1164 and 1103 cm^{-1} (n_{C-O}, ester). ^{1}H-NMR (400 MHz, $CDCl_3$, TMS): 6.85 (d, H-4 and H-6, 2H, *J* 8.4 Hz), 7.95 (d, H-3 and H-7, 2H, *J* 8.4 Hz), 5.30 ppm (s, OH), 3.88 ppm (s, 3H, –OMe).

***p*-hydroxybenzoic acid (3)**. ^{13}C-NMR (100 MHz, MeOH-d4): 170.07 (C-1), 163.36 (C-5), 132.99 (C-3 and C-7), 122.73 (C-2), 116.03 (C-4 and C-6) ppm. ^{1}H-NMR (400 HMz, MeOH-d4): 7.86 (H-3 and H-7, m), 6.80 (H-4 and H-6, m) 5.48 (H-1, s), 4.87 (H-5, s).

Fungitoxic Activity Evaluation. Solutions of compounds (**1**), (**2**) and (**3**) were applied alone in TLC plate (Merck aluminum TLC silica gel sheets, F_{254}), corresponding to the amount of 100 μg/spot. The fungitoxic ethyl acetate extract of *Flammulina velutipes* micellium was used as positive control (ISHIKAWA et al., 2001). The plate was then developed with CH_2Cl_2:MeOH 4:1 and the spots were visualized under ultraviolet light (λ = 254nm). Then, a spore suspension of *C. herbarum* CCT 0279 in a glucose-mineral salts medium was sprayed over the developed TLC plate, which was incubated at 28ºC under humid conditions. After 5 days incubation time, the fungal growth was evaluated over the plate (POMINI et al., 2006; HOMANS; FUCHS, 1970).

Results and Discussion

From dried extracts of *S. campanulata* root peels, a highly polar compound (2.00 mg) was isolated and identified as ajugol (**1**), an iridoid glucoside. The identification was carried out by NMR ^{1}H/^{13}C spectroscopic data (Table 1) interpretation, including bi-dimensional experiments as ^{1}H-^{1}H correlation (COSY), long-range ^{1}H-^{13}C correlation (HMQC) and NOESY, allowing also relative stereochemistry characterization by literature comparison (NISHIMURA et al., 1988) (Figure 1).



(1) (2) (3)

Figure 1. Compounds isolated from *S. campanulata* root peels.

Table 1. ^{1}H and ^{13}C-NMR chemical shift assignments for compound (**1**) (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR, MeOH-d4, TMS).*

C	δ_C	δ_H	C	δ_C	δ_H
1	93.73	5.45 (d, *J* 2.2)	**10**	25.24	1,31 (s)
3	140.44	6.15 (dd, *J* 6.4, 2.2)	**1'**	99.43	4.63 (d, *J* 8.0)
4	105.92	4.82 (m)	**2'**	74.80	3.19 (dd, *J* 9.2, 8.0)
5	41.27	2.72 (m)	**3'**	77.80	3.34 (m)
6	77.78	3.91 (ddd, *J* 5.6, 4.8, 2.9)	**4'**	71.70	3.27 (m)
7	50.04	1.78 (dd, *J* 13.4, 4.8); 2.03 (dd, *J* 13.4, 5.6)	**5'**	78.00	3.30 (m)
8	79.45	-	**7'**	62.90	3.65 (dd, *J* 11.8, 2.2) 3.91 (ddd, *J* 5.6, 4.8, 2.9).
9	51.81	2.54 (dd, *J* 9.4, 1.9)			

* δ in ppm and *J* in Hertz. Splitting pattern is as follow: d – dublet; dd – double-dublet, m – multiplet, ddd – double-double-doublet.

The iridoid ajugol (**1**) has been isolated from plants belonging to the Bignoniaceae family, as *Crescentia cujete* L. and *Kigela pinnata* DC., both employed in folk medicine against stomach disorders (GOUDA et al., 2003; KANEKO et al., 1997).

Acid-base extraction of fresh *S. campanulata* root peels and exhaustive purification yielded two phenolic compounds, identified as methyl *p*-hydroxy-benzoate (**2**) and *p*-hydroxy-benzoic acid (**3**). Both phenolic constituents exhibited an evident aromatic 1,4 substitution pattern in ^{1}H-NMR spectra. The presence of compound (**3**) was already reported in the leaves of *S. campanulata* (EL-HELA, 2001a).

The biological profile of compounds (**1**), (**2**) and (**3**) against the fungus *Cladosporium herbarum* CCT 0279 was evaluated by bioautography on thin layer chromatoplate (HOMANS; FUCHS, 1970; POMINI et al., 2006). The biological activity was qualitatively accessed by clear zones over active substances, without fungal development. The compound (**1**) did not exhibit any mortality against *C. herbarum*, while phenolic constituents (**2**) and (**3**) displayed biological activity.

The antibacterial properties of *p*-hydroxy-benzoic acid (**3**) against Gram-positive bacteria were previously reported (GARCIA; MARHUENDA, 1985). Esters of *p*-hydroxy-benzoic acid (parabenes) are widely used as antimicrobial agents, employed in food and drug preservation in several countries, mainly against fungi (DAVIDSON; BRANEN, 1993). Probably, the phenolic derivatives produced in *S. campanulata* roots are defensive substances of this plant against fungi, similar fungitoxic properties that were observed in vitro.

In conclusion, this work described the first phytochemical survey on the roots of *S. campanulata.* Three substances were identified, two of them never isolated from other parts of the plant (**1** and **2**). We believe that the phenolic derivatives could play an important role in root tissues in defensive mechanisms against fungi and other organisms in tropical soil, however, this hypothesis deserves further studies.

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