

Antimicrobial activity of a mixture of two isomeric phenylpropanoid glycosides from *Arrabidaea harleyi* A.H. Gentry (Bignoniaceae)

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Arrabidaea harleyi A.H. Gentry (Bignoniaceae) is an ornamental plant found in some regions of the Atlantic forest in Brazil. From its bark a mixture of verbascoside and isoverbascoside was isolated. This mixture was shown to be active against *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus subtilis*, *Bacillus mycoides*, *Enterococcus faecalis*, *Escherichia coli*, *Serratia marcescens* and *Candida albicans*. The minimal inhibitory concentration (MIC) was established by diffusion method.

Uniterms:

- *Arrabidaea harleyi*
- Bignoniaceae
- Verbascoside
- Isoverbascoside
- Antimicrobial activity

INTRODUCTION

The family Bignoniaceae is essentially tropical, with more than 100 genera and nearly 800 species encountered mainly in the North part of South America. The plants are mostly trees and lianas, rarely as herbs (Brummitt, 1992). Only a few Brazilian species have been investigated so far. The genus *Arrabidaea* occurs in tropical America, from Mexico to Argentina. In Brazil most of species of *Arrabidaea* as *A. triplinervia* and *A. pulchra* are found in the cerrado region. Other species e.g. *A. agnus-castus* have been found in the semi-desert regions of Northeastern Brazil (Noronha, 1964), whereas the others e.g. *A. bilabiata* and *A. chica* have been found in Amazonas, (Takemura *et al.*, 1995; Zorn *et al.*, 2001).

Arrabidaea harleyi known as “cipó-do-mato” is a ligneous vine, used in folk medicine as a fungicide, especially in the treatment of dandruff. It occurs along the margins of the Atlantic Forest, from the Brazilian state of

Piauí to Minas Gerais. This is the first report of the chemical and biological studies of this species and the first report of the title compounds in plants of this genus.

MATERIAL AND METHODS

Plant material

The bark of *Arrabidaea harleyi* A.H. Gentry was collected in March 2001 in a forest reserve in the city of João Pessoa, state of Paraíba. A voucher specimen is deposited in the Herbarium of the Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba, under the n° Agra 0364 (JPB).

Extraction and Isolation

Air dried ground bark of *A. harleyi* (3.5 Kg) was exhaustively extracted with 5.0 L of 95% ethanol. The

resulting extract (120.0 g) was dissolved in H₂O:MeOH (7:3 v/v) and submitted to a partition between hexane (9.0 g), CHCl₃ (6.0 g), AcOEt (10.0 g) and *n*-BuOH (20.0 g). The AcOEt fraction was subjected to column chromatography using silica gel (Merck) 70-230 mesh. The solvents were P.A. Merck: chloroform - methanol gradient (5 - 20%). 93 fractions of 100 mL were collected. After analysis by TLC aluminium sheets silica gel-60 F₂₅₄ (Merck) developed with I₂ or Neu/Peg reagent, some fractions were reunited yielding 19 fractions. The main fraction (2.0 g) was an amorphous pale yellow powder. Its ¹³C-APT NMR (CDCl₃, 75 MHz) showed pairs of signals with the same amplitude and these were denominated **A** and **B**. (**A**): 131.1 (C1), 117.0 (C2), 144.0 (C3), 145.6 (C4), 116.3 (C5), 121.0 (C6), 36.1 (C7), 71.5 (C8), 103.7 (C1'), 76.0 (C2'), 81.0 (C3'), 70.1 (C4'), 75.6 (C5'), 62.1 (C6'), 102.0 (C1''), 72.0 (C2''), 71.8 (C3''), 73.5 (C4''), 70.0 (C5''), 18.4 (C6''), 127.3 (C1'''), 115.9 (C2'''), 146.1 (C3'''), 149.0 (C4'''), 116.8 (C5'''), 123.0 (C6'''), 147.0 (C7'''), 115.1 (C8'''), 167.6 (C=O). (**B**): 131.0 (C1), 117.0 (C2), 144.0 (C3), 145.6 (C4), 116.3 (C5), 121.0 (C6), 36.0 (C7), 71.4 (C8), 103.5 (C1'), 74.9 (C2'), 83.9 (C3'), 69.5 (C4'), 74.7 (C5'), 64.2 (C6'), 101.9 (C1''), 72.0 (C2''), 71.8 (C3''), 73.4 (C4''), 69.5 (C5''), 18.0 (C6''), 127.4 (C1'''), 114.8 (C2'''), 146.1 (C3'''), 148.8 (C4'''), 115.9 (C5'''), 122.7 (C6'''), 146.1 (C7'''), 115.0 (C8'''), 167.3 (C=O).

Antimicrobial qualitative assay

A modified diffusion test (Bauer *et al.*, 1966) was used to determine the antimicrobial activity. The isomer mixture was dissolved in DMSO and tested against *Staphylococcus aureus* DAUFPE 01, *Bacillus subtilis* DAUFPE 16, *Bacillus mycoides* DAUFPE 14, *Micrococcus luteus* DAUFPE 06, *Enterococcus faecalis* DAUFPE 138, *Escherichia coli* DAUFPE 224, *Pseudomonas aeruginosa* DAUFPE 39, *Serratia marcescens* DAUFPE 398, *Mycobacterium smegmatis* DAUFPE 71, and *Candida albicans* DAUFPE 1007. These microorganisms were obtained from the Culture Collection of the Departamento de Antibióticos (Méllo, 1988). From the fresh cultures of the microorganisms standardized suspensions were prepared in physiological solutions to comparison of 0.5 to MacFarland scale, equivalent at 10⁷ UFC (Murray, 1995; Washington, 1972). Antimicrobial activities were evaluated by the diffusion test on paper discs over Müller Hinton agar, glucose-yeast extract agar and sabouraud-dextrose media. The suspensions were spread on a surface of the medium in Petri dishes with Drigalsky's loop (100 µL per dish). Paper

discs at Whatman n° 2 (6.0 mm diameter) were moistened with 20 µL of the mixture of isomers at the concentration of 30 mg/mL, which is equivalent to 600 µg/disc. After a proper incubation at 30 or 35 °C for 24 or 48 h, the inhibition zones around the discs were measured (Bauer *et al.*, 1966). The tests were performed in triplicate and the results were expressed in mm as the arithmetic media of diameters of the inhibition zones. The blank control was performed with DMSO. A standard solution of cephalixin and cetoconazol were used as a positive control to bacteria and yeast, respectively.

Minimal Inhibitory Concentration (MIC)

A solution of 6 000 µg/mL of the mixture of A and B, was prepared and distributed in Petri dishes in different volumes (1.0, 0.5, 0.25, 0.125, 0.06, 0.03, 0.015 and 0.007 mL), which contained 10 mL of the proper culture medium. The microorganisms were spread and the dishes were incubated for 24 or 48 hours at 30 or 35 °C, as showed in Table 1 (Méllo *et al.*, 1988).

RESULTS AND DISCUSSION

Phytochemical analysis of EtOAc extract of *Arrabidaea harleyi* yielded a chromatographic fraction (F6), which when eluted in a TLC chromatogram and sprayed with Neu/Peg reagent or in the presence of I₂ vapor showed only one spot. Analysis of the 300 MHz ¹H NMR spectrum of this material, showed the signals characteristic of aromatic compounds linked to a carbohydrate group. The analysis of the ¹³C-APT NMR spectrum showed that all the signals were present in pairs, which suggested that the fraction was a mixture of two isomers. A detailed spectral analysis and also a comparison with the literature data (Krebs, Habermehl, 1992), showed that these compounds were verbascoside (acteoside) (**A**), a glycosylated phenylpropanoid, and isoverbacoside (isoacteoside) (**B**). Microbiological assays with this mixture showed that it possesses a wide spectrum of activity against Gram positive and Gram negative bacteria and also against yeast (Table I). The minimal inhibition concentrations (MIC) are also showed in Table I. Phytochemical studies of the family Bignoniaceae show the presence of verbascoside and isoverbacoside in some genera such as *Deplanchea* (Davioud *et al.*, 1989), *Jacaranda*, *Mussatia* (Jimenez *et al.*, 1987, 1988, 1989) *Tecoma* (Guiso, 1997) and *Newboldia* (Gafner, 1997), but this is the first report of these compounds in the genus *Arrabidaea*. Earlier reports describe the antineoplastic action of verbascoside and isoverbacoside against

murine-388 (PS) lymphocytic leukemia (Pettit *et al.*, 1990). It was determined the antiviral properties of these compounds obtained from *Markhamia lutea* (Kernan *et al.*, 1998). The pharmacological effects of these compounds obtained from *Mussatia* was described by Cano *et al.* (1990).

CONCLUSIONS

On an inseparable mixture of two isomeric compounds was isolated. These compounds were

identified as verbascoside (A) and isoverbascoside (B). Five Gram positive bacteria (*Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus subtilis*, *Bacillus mycoides*, *Enterococcus faecalis*), two Gram negative bacteria (*Escherichia coli* and *Serratia marcescens*), and one yeast (*Candida albicans*) were shown to be sensitive to this mixture (A/B), whereas *Pseudomonas aeruginosa* (Gram negative bacterium) and *Mycobacterium smegmatis* (alcohol-acid resistant bacterium) were found to be resistant. These results may support some traditional uses of this plant by local population.

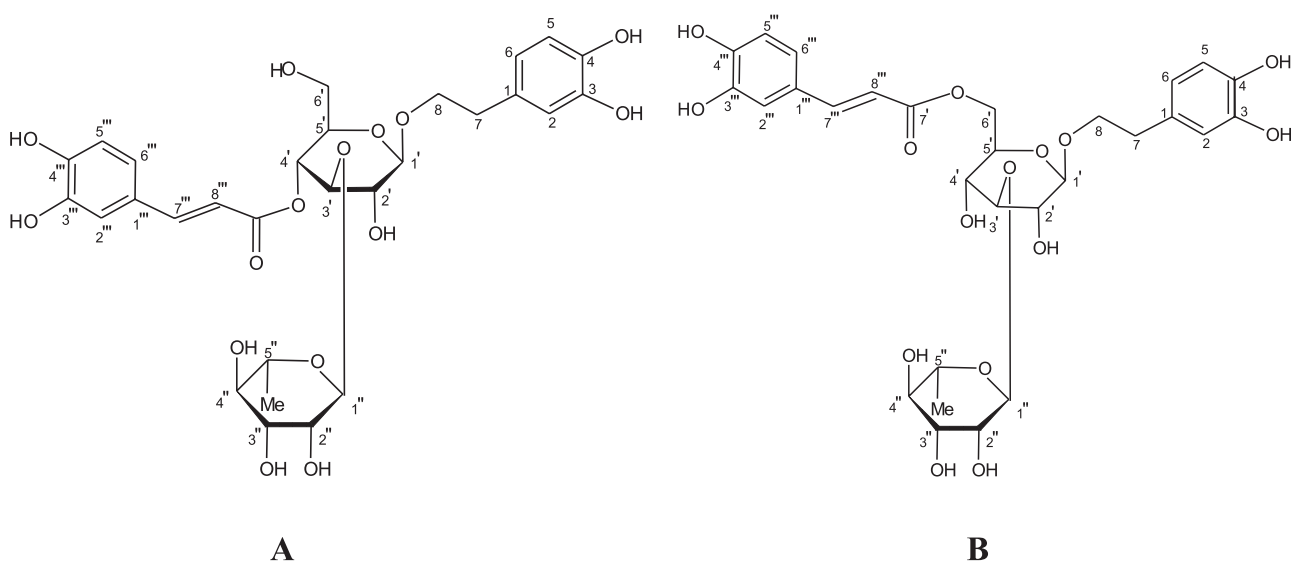


TABLE I - Inhibition zones and MIC of a mixture of verbascoside and isoverbascoside (A/B) against Gram positive, Gram negative, alcohol-acid bacteria and yeast

Microorganisms	Inhibition zone (mm) 600 µg/disc			MIC (µg/mL)
	A/B	Cephalexin (30 µg)	DMSO	
Bacteria				
<i>Staphylococcus aureus</i> DAUFPE 01 (G +)	15	26	0	600
<i>Micrococcus luteus</i> DAUFPE 06 (G +)	30	47	0	300
<i>Bacillus subtilis</i> DAUFPE 16 (G +)	19	34	0	600
<i>Bacillus mycoides</i> DAUFPE 14 (G +)	11	24	0	600
<i>Pseudomonas aeruginosa</i> DAUFPE 39 (G -)	0	0	0	>600
<i>Mycobacterium smegmatis</i> DAUFPE 71 (AAR)	0	0	0	>600
<i>Enterococcus faecalis</i> DAUFPE 138 (G +)	15	11	0	600
<i>Escherichia coli</i> DAUFPE 224 (G -)	15	18	0	600
<i>Serratia marcescens</i> DAUFPE 398 (G -)	9	0	0	600
Yeast		Cetoconazol (300 µg)		
<i>Candida albicans</i> DAUFPE 1007	18	24	0	300

(A/B) – not separated isomers mixture; (G +) - Gram positive bacterium; (G -) - Gram negative bacterium; (AAR) - Alcohol-acid resistant bacterium; (MIC) Minimal Inhibitory Concentration.

RESUMO

Atividade antimicrobiana de uma mistura de dois isômeros de glicosídeos fenilpropanóides de *Arrabidaea harleyi* A.H. Gentry (Bignoniaceae)

Arrabidaea harleyi A.H. Gentry (Bignoniaceae) é uma planta ornamental, encontrada em algumas regiões da Mata Atlântica do Brasil. A partir das cascas do caule foi isolada a mistura dos isômeros verbascosídeo e isoverbascosídeo. A mistura mostrou-se ativa frente a *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus subtilis*, *Bacillus mycoides*, *Enterococcus faecalis*, *Escherichia coli*, *Serratia marcescens* e *Candida albicans*. Foi estabelecida a concentração mínima inibitória (CMI) através do método de difusão em meio sólido.

UNITERMOS: *Arrabidaea harleyi*. Bignoniaceae. Verbascosídeo. Isoverbascosídeo. Atividade antimicrobiana.

REFERENCES

- BAUER, A. W.; KIRBY, W. M. M.; SHERRIS, J. C.; TURC, M. Antibiotic susceptibility testing by a standardized single disk method. *Amer. J. Clin. Pathol.*, Philadelphia, v. 45, p. 493-496, 1966.
- BRUMMITT, R. K. *Vascular plant families and genera*. London: Royal Botanic Garden, 1992. p. 505-506.
- CANO, E.; VEIGA, M.; JIMENEZ, C.; RIGUERA, R. Pharmacological effects of three phenylpropanoid glycosides from *Mussatia*. *Planta Med.*, Stuttgart, v. 56, n. 1, p. 24-26, 1990.
- DAVIOUD, E.; BAILLEUL, F.; DELAVEAU, P.; DEBRAY, M. M. Iridoid glycosides and phenylpropanoid glycosides from *Deplanchea speciosa*. *Planta Med.*, Stuttgart, v. 55, n. 1, p. 87, 1989.
- GAFNER, S.; WOLFENDER, J. L.; NIANGA, M.; HOSTETTMANN, K. Phenylpropanoid glycosides from *Newbouldia laevis* roots. *Phytochemistry*, New York, v. 44, n. 4, p. 687-690, 1997.
- GUISO, M.; MARRA, C.; PICCIONI, F.; NICOLETTI, M. Iridoid and phenylpropanoid glycosides from *Tecoma capensis*. *Phytochemistry*, New York, v. 45, n. 1, p. 193-194, 1997.
- JIMENEZ, C.; VILLAVERDE, M. C.; RIGUERA, R.; CASTEDO, L.; STERMITZ, F. R. Three phenylpropanoid glycosides from *Mussatia*. *Phytochemistry*, New York, v. 26, n. 6, p. 1805-1810, 1987.
- JIMENEZ, C.; VILLAVERDE, M. C.; RIGUERA, R.; CASTEDO, L.; STERMITZ, F. R. Five phenylpropanoid glycosides from *Mussatia*. *Phytochemistry*, New York, v. 27, n. 9, p. 2947-2951, 1988.
- JIMENEZ, C.; VILLAVERDE, M. C.; RIGUERA, R.; CASTEDO, L.; STERMITZ, F. R. Phenylpropanoid glycosides from *Mussatia hyacinthina*. *J. Nat. Prod.*, Columbus, v. 52, n. 2, p. 408-410, 1989.
- KERNAN, M. R.; AMARQUAVE, A.; CHEN, J. L.; CHAN, J.; SESIN, D. F.; PARKINSON, N.; YE, Z. J.; BARRETT, M.; BALES, C.; STODDART, C. A.; SLOAN, B.; BLANC, P.; LIMBACH, C.; MRISHO, S.; ROZHON, E. J. Antiviral phenylpropanoid glycosides from the medicinal plant *Markhamia lutea*. *J. Nat. Prod.*, Columbus, v. 61, n. 5, p. 564-570, 1998.
- KREBS, H. C.; HABERMEHL, G. G. Two dimensional NMR spectroscopy of glycosides. *Magn. Reson. Chem.*, Chichester, v. 30, p. 556-559, 1992.
- MÉLLO, B. R. *Catálogo da coleção de microrganismos*. 2.ed. Recife: UFPE/Departamento de Antibióticos, 1988. p. 45.
- MURRAY, P. R.; BARON, E. J.; PFALLER, M. A.; TENOVER, F. C.; YOLKEN, R. H. *Manual of clinical microbiology*. 6. ed. Washington: American Society for Microbiology, 1995. p.165.
- NORONHA, H. A propósito dos cipós medicinais do Brasil. *Rev. Bras. Farm.*, Rio de Janeiro, v. 45, n. 3, p. 151-159, 1964.
- PETTIT, G. R.; NUMATA, A.; TAKEMURA, T.; ODE, R. H.; NARULA, A. S.; CRAGG, G. M.; PASE, C. P. Antineoplastic agents 107. Isolation of acteoside and isoacteoside from *Castilleja linariaefolia*. *J. Nat. Prod.*, Columbus, v. 53, n. 2, p. 456-458, 1990.
- TAKEMURA, O. S.; IINUMA, M.; TOSA, H.; MIGUEL, O. G.; MOREIRA, E. A.; NOZAWA, Y. A. Flavone from leaves of *Arrabidaea chica* f. *cuprea*. *Phytochemistry*, New York, v. 38, n. 5, p. 1299-1300, 1995.

- WASHINGTON, J. A.; WARREN, E.; KARLSON, A. G. Stability of barium sulfate turbidity standards. *Appl. Microbiol.*, Washington, v. 24, p. 1013, 1972.
- ZORN, B.; GARCÍA-PIÑERES, A. J.; CASTRO, V.; MURILLO, R.; MORA, G.; MERFORT, I. 3-Desoxyanthocyanidins from *Arrabidaea chica*. *Phytochemistry*, New York, v. 56, n. 8, p. 831-835, 2001.

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