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

Cláudia Sampaio de Andrade Lima[1], Elba Lúcia Cavalcanti de Amorim[2], Kêsia Xisto da Fonseca Ribeiro de Sena[3], Alda de Andrade Chiappeta[3], Xirley Pereira Nunes[4], Maria de Fátima Agra[4], Emidio Vasconcelos Leitão da-Cunha[4,5], Marcelo Sobral da Silva[4] and José Maria Barbosa-Filho[4]*

[1]Departamento de Biofísica, Universidade Federal de Pernambuco, [2]Departamento de Ciências Farmacêuticas, Universidade Federal de Pernambuco, [3]Departamento de Antibióticos, Universidade Federal de Pernambuco, [4]Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba, [5]Departamento de Farmácia e Biologia, Universidade Estadual da Paraíba

***Correspondence:**
J. M. Barbosa-Filho
Laboratório de Tecnologia Farmacêutica
Universidade Federal da Paraíba
Caixa Postal 5009
58051-970 - João Pessoa - PB
Brasil
E-mail: jbarbosa@ltf.ufpb.br

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, Enterecoccus faecalis, Escherichia coli, Serratia marcensis *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. pulchera* 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 Piaui 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 Paraiba. A voucher specimen is deposited in the Herbarium of the Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraiba, under the nº 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 H_2O:MeOH (7:3 v/v) and submitted to a partition between hexane (9.0 g), $CHCl_3$ (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 F_{254} (Merck) developed with I_2 or Neu/Peg reagent, some fractions were reunited yielding 19 fractions. The main fraction (2.0 g) was an amorphous pale yellow powder. Its ^{13}C-APT NMR ($CDCl_3$, 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 10^7 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 nº 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 ºC 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 cephalexin 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 ºC, as showed in Table 1 (Méllo *et al*., 1988).

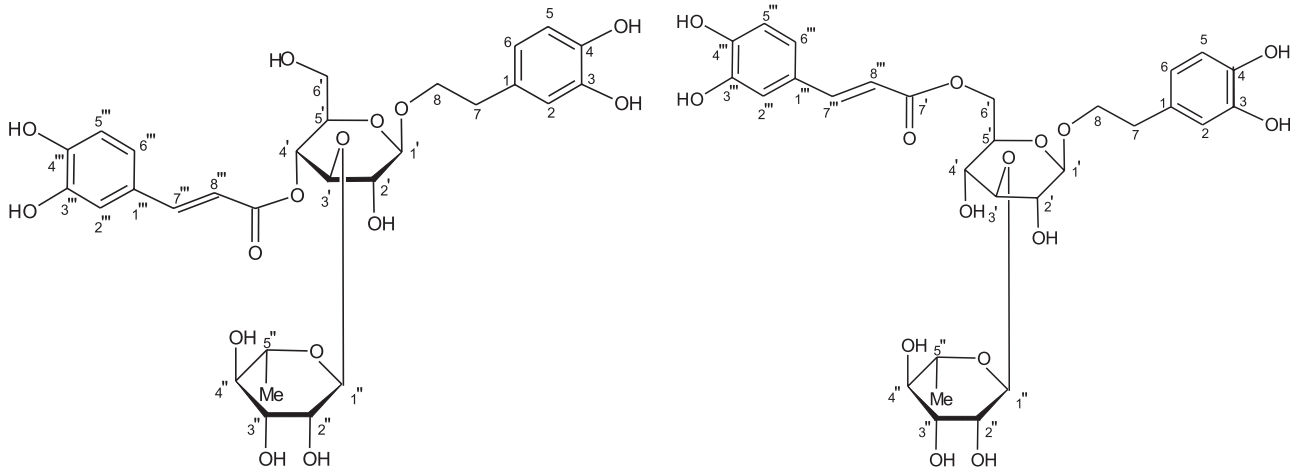
RESULTS AND DISCUSSION

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 I_2 vapor showed only one spot. Analysis of the 300 MHz ^{1}H NMR spectrum of this material, showed the signals characteristic of aromatic compounds linked to a carbohydrate group. The analysis of the ^{13}C-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 isoverbascoside 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 antineoplasic action of verbascoside and isoverbascoside 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 a 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 marcensis*), 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.



A **B**

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

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