

**ANTIMICROBIAL ACTIVITY OF CRUDE EXTRACTS AND FLAVONOIDS
FROM LEAVES OF *PLUCHEA CAROLINENSIS* (JACQ.) G. DON.**

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Summary

We evaluated the antibacterial and antifungal activity of five crude extracts obtained by fractionating the leaves of *Pluchea carolinensis* (Jacq.) G. Don and two flavonoids isolated from AcOEt crude extracts. The evaluation was made against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Candida albicans*, *Aspergillus niger*, *Trichophyton mentagrophytes* and *Fusarium oxysporum* microorganisms. The crude extracts CHCl₃, AcOEt y n-BuOH showed positive antibacterial results, while the flavonols isolated and identified as eupalitin and isorhamnetin are not the responsible for the biological effect found in the AcOEt crude extract.

Keywords: *Pluchea carolinensis*, antibacterial activity, 3,5,7,4'-tetrahydroxy-3'methylflavone, 3,5,4'-trihydroxy-6,7-dimethylflavone.

Introduction

Flavonoids are a group of secondary metabolites with a wide range of biological properties such as antimicrobial (1), antioxidant (2) and anti-inflammatory (3) among others. Flavonoids occupy the second group of metabolites of most distribution(4) in genera *Pluchea* Cass. The relationships between the biological properties of some plant species of the genera and the presence of this kind of metabolites have been reported. Knowing that one of the pharmacological target studies in the *Pluchea* genera are the antibacterial and antifungal activities, we have proposed to evaluate the biological properties of the crude extracts and those of the flavonoids obtained from the leaves from *Pluchea carolinensis* (Jacq.) G. Don.

Materials and methods

The specie *P. carolinensis* was collected in Sierra del Rosario location, Pinar del Río province in March 2003. A Voucher specimen is kept in the herbarium of the Institute of Ecology and Systematic (HAC 41725). The leaves were dried and turned into powder.

1,7 Kg of dried and turned into powder leaves were extracted in EtOH:H₂O (7:3 v/v) at room temperature and reflux. All the crude extracts were unified and reduced in volume at low pressure. The resulting aqueous crude extract was subjected to extraction with n-hexane, CHCl₃, AcOEt y n-BuOH.

The evaluation of five crude extracts was made against *Escherichia coli* (A), *Staphylococcus aureus* (B), *Bacillus subtilis* (C), *Candida albicans* (D), *Aspergillus Níger* (E), *Trichophyton mentagrophytes* (F) and *Fusarium oxysporum* (G) microorganisms(5).

Flavonoid portions from AcOEt crude extract were subjected to column chromatography on silica and we used as eluted CHCl₃ and mixture of CHCl₃: EtOH increasing amounts of EtOH.

Fractions eluted with CHCl₃:EtOH (4%) were monitored and comparisons with markers were made by TLC on silica gel with the solvents CHCl₃:AcOEt:acid formic (9:2:1 v/v/v). Such fraction (CHCl₃:EtOH (4%)) was subjected to a column flash chromatography on silica gel and eluted with n-hexane, n-hexano: CHCl₃ and CHCl₃: EtOH, increasing the amount of CHCl₃ and EtOH respectively. The compound (**1**) eluted in CHCl₃:EtOH (2%), while (**2**) made it in CHCl₃:EtOH (5 %). Both fractions were subjected to recrystallisation with EtOH (98 %).

The spectra were recorded in the UV-Visible range in a Spectrophotometer (Ultraspec 2100. Amersham Biosciens), while NMR-¹H and NMR-¹³C were recorded in a spectrometer Varian Unity with a frequency of 500 MHz and 75 MHz respectively, using TMS as internal reference.

Results

From AcOEt crude extract obtained by fractionating the leaves of *P. carolinensis* we isolated two aglycones flavonols. The following compounds were identified using UV-Visible, NMR-¹H and NMR-¹³C spectroscopic techniques.

(1) UV-Visible: MeOH (nm): 257, 271, 346, 364; NaOAc (nm): 256, 270, 346, 367; NaOAc/H₃BO₃ (nm): 267 (h), 346, 365; AlCl₃ (nm): 267, 304 (h), 349 (h), 364 (h), 418; AlCl₃/HCl (nm): 264, 305 (h), 346 (h), 364, 418. RMN ¹H en DMSO-d₆: 6,88 s (1H, H-8), 8,10 d (2H, H 2' - 6'), 6,92 d (1H, H 3' - 5'), 3,73 s (3H, 6-OCH₃), 3,91 s (3H, 7-OCH₃), 9,5 s (1H, 3-OH), 12,42 s (1H, 5-OH), 10,12 s (1H, 4'-OH) RMN ¹³C en DMSO-d₆: C-2 (147,3); C-3 135,7; C-4 (176,1); C-5 (151,0); C-6 131,2; C-7 (158,5); C-8 (91,2); C-9 (151,5); C-10 (104,3); C-1' (121,6); C₂₋₆' (129,5); C₃₋₅' (155,4); C-4' (159,3); 60,1 (OCH₃); 56,4 (OCH₃)

(2) Pf 305 °C, UV-Visible: MeOH (nm): 254, 305 (h), 370; NaOAc (nm): 275, 318, 385; NaOAc/H₃BO₃ (nm): 254, 372; AlCl₃ (nm): 262, 303, 362, 428; AlCl₃/HCl (nm): 261, 302, 360, 422. RMN ¹H en DMSO-d₆: 6,18 d (1H, H-8) y 6,46 d (1H, H-6); 6,93 d (1H, H-6'); 7,68 dd (1H, H-5'); 7,74 d (1H, H-6'); 3,83 s (3H, 3'-OCH₃); 9,4-10,8 3s (3 H; 3, 7, 3'-OH); 12,45 s (1H, 5-OH) RMN ¹³C en DMSO-d₆: C-2 (156,1); C-3 (135,8); C-4 (175,8); C-5 (160,6); C-6 (98,2); C-7 (163,9); C-8 (93,5); C-9 (148,7); C-10 (102,9); C-1' (121,6); C-2' (115,5); C-3' (146,5); C-4' (147,3); C-5' (111,6); C-6' (121,9); OCH₃ (55,7).

The results of the antibacterial and antifungal activities of the crude extracts and pure flavonols are shown in the table 1

Table 1. Antibacterial and antifungal evaluation of crude extracts and pure compounds.

Sample	Microorganisms tested (CMI, µg/mL)						
	A	B	C	D	E	F	G
n-hexane	-	-	-	-	-	-	-
CHCl ₃	-	-	1000	-	-	-	-
AcOEt	-	1000	1000	-	-	-	-
n-BuOH	-	1000	1000	-	-	-	-
aqueous	-	-	-	-	-	-	-
(1)		-	-				
(2)		-	-				

Escherichia coli (A), *Staphylococcus aureus* (B), *Bacillus subtilis* (C), *Candida albicans* (D), *Aspergillus Níger* (E), *Trichophyton mentagrophytes* (F) and *Fusarium oxysporum* (G) microorganisms.

Discussion

The compound commonly known as isorhamnetin or isorhamnetol, is found widely distributed in the vegetal kingdom and was isolated from *Cheiranthus cheiri* L. in 1896 for first time. Isorhamnetin was isolated only from *P. symphytifolia* W. T. Gillis by Ahmad(6) in the *Pluchea* genera, the isolation of this flavonol constitutes the second one reported in the genera. However, eupalitin has been isolated from *Ipomopsis aggregata* (Pursh) V. Grant, *Eupatorium spp*, *Artemisia* and others Asteraceae' species⁴, but in the *Pluchea* genera it is the first report of this flavonol. The chemical structures of both flavonoids are shown in the Figure 1.

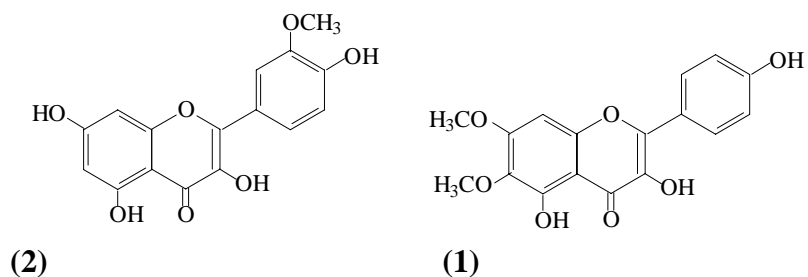


Fig 1. Structure of the flavonols

The five crude extracts were evaluated against seven microorganisms: three bacteria, three fungus and one yeast. The AcOEt and n-BuOH crude extracts inhibited the grow of the microorganisms *Staphylococcus aureus* and *Bacillus subtilis* provoking inhibition halos of 10 mm and 9 mm respectively, while $CHCl_3$ crude extract showed positive results against *Bacillus subtilis* (7 mm). The pure flavonols were evaluated against *Staphylococcus aureus* and *Bacillus subtilis* but we obtained negative results so these metabolites are not the responsible for the biological effect found in AcOEt crude extract.

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References

- (1) Russo A, Acquaviva R, Campisi A, et al. Bioflavonoids as antiradicals, antioxidants and DNA cleavage protectors. *Cell. Biol. Toxicol* 2000;16:91-98.
- (2) Bohm H, Boeing H, Hempel J, et al. Flavonols, flavone and anthocyanins as natural antioxidants of foods and their possible role in the prevention of chronic diseases. *Ernahrungswiss* 1998;2:147-163.
- (3) Rotelli AE, Guardia T, Juárez A, et al. Compative Study of flavonoids in experimental models of inflammation. *Pharm. Res* 2003;48:601-606.
- (4) Napralert (TM) (1975-2003), Base de Datos del College of Pharmacy of the University of Illinois at Chicago, E.U.
- (5) Jones D, Pell PA, Sneath PHA. Maintenance of bacteria on glass beads at -600C to -760C. In *Maintenance of Microorganisms. A Manual of Laboratory Methods*. eds. B. E. Kirsoy and J. J S. Snell: Academic Press London, 1984:35-40.
- (6) Ahmad VU, Farooqui TA, Sultana A, Fizza K, Khatoon R. Caffeoylequinic acids and some biological activities of *Pluchea symphytifolia*. *Phytochemistry* 1992;31(8):2888-2890.