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Antiprotozoal Activity of Flavonoids Isolated from *Mimosa tenuiflora* (Fabaceae-Mimosoideae)

Elihú Bautista,[1] Fernando Calzada,[2] Alfredo Ortega,[1*] and Lilian Yépez-Mulia[3]

[1] Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, México D.F. México.
[2] Unidad de Investigación Médica en Farmacología de Productos Naturales, UMAE Hospital de Pediatría, 2º Piso, CORCE, Centro Médico Nacional Siglo XXI, IMSS, Av. Cuauhtémoc 330, Col. Doctores, CP 06725, México D.F., México.
[3] Unidad de Investigación Médica en Enfermedades Infecciosas y Parasitarias, UMAE Hospital de Pediatría, 2º Piso, Centro Médico Nacional Siglo XXI, IMSS, Av. Cuauhtémoc 330, Col. Doctores, CP 06725, México D.F., México.
hormigaqfb@hotmail.com

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Abstract. As result of the chemical study of the leaves and flowers of *Mimosa tenuiflora* (Willd.) Poir. (Fabaceae-Mimosoideae) eigth flavonoids were isolated: 6-methoxy-4'-*O*-methylnaringenin (**1**), santin (**2**), 6-methoxynaringenin (**3**), tenuiflorin A (**4**), 5, 7, 4'-trihydroxy-3, 6-dimethoxyflavone (**5**), 6-demethoxy-4'-*O*-methylcapilarisine (**6**), 6-methoxykaempferol (**7**) and tenuiflorin C (**8**). Antiprotozoal activity of these compounds as well as the tenuiflorina B (**9**) and 6-desmethoxycapilarisine (**10**), isolated in a previous study was assessed against *Entamoeba histolytica* and *Giardia lamblia* trophozoites.
Keywords: *Mimosa tenuiflora,* Fabaceae, flavonoids, 2-phenoxycromones, antiprotozoal activity, tepescohuite.

Resumen. Como resultado del estudio químico de las hojas y flores de *Mimosa tenuiflora* (Willd.) Poir. (Fabaceae-Mimosoideae) se aislaron ocho flavonoides: 6-metoxi-4'-*O*-metilnaringenina (**1**), santina (**2**), 6-metoxinaringenina (**3**), tenuiflorina A (**4**), 5, 7, 4'-trihidroxi-3,6-dimetoxiflavona (**5**), 6-desmetoxi-4´-*O*-metilcapilarisina (**6**), 6-metoxicamperol (**7**) y tenuiflorina C (**8**). La actividad antiprotozoaria de los compuestos aislados, así como la de la tenuiflorina B (**9**) y de la 6-desmetoxicapilarisina (**10**), aisladas en un estudio previo fue determinada contra trofozoitos de *Entamoeba histolytica* and *Giardia lamblia.*
Palabras clave: *Mimosa tenuiflora,* Fabaceae, flavonoides, 2-fenoxicromonas, actividad antiprotozoaria, tepescohuite.

Introduction

Parasitic infections constitute one of the most widespread human health problems, and most of them occur through contaminated food or water, these infections are more prevalent in developing countries. The human intestine is a major target of these ingested pathogenic microorganisms, resulting in severe infections, such as dysentery and diarrhea [1]. Two of the most common causes of such symptoms are intestinal protozoa *Entamoeba histolytica* and *Giardia lamblia*, the first cause amoebic dysentery, the second cause giardiasis [2].

Metronidazole is a classical and effective treatment for both diseases; however, resistance to drug as well as the risk of potential mutagenicity and carcinogenicity has been described. In prolonged treatment or high doses often cause side effects such as headache, dry mouth, metallic taste, glossitis and urticaria [1, 2]. Owing to these undesired side effects and taking into account the possibility of the development of resistant strains of the *E. histolytica* and *G. lamblia* against metronidazole, there is a clear need for new, effective, and safer antiprotozoal agents [1]. Recently different natural products that possessed antiprotozoal activity, has been reported, they are alkaloids [3], terpenoids [4], and flavonoids [5] among other. We are particularly interested in flavonoids since they are present in several of the plants used in traditional medicine to treat amoebic dysentery and severe diarrhea [6].

Mimosa tenuiflora (Willd.) Poir (Fabaceae), is known by the common name of "tepescohuite" [7]. The bark of this tree is used in Mexican traditional medicine to treat wounds caused by burns, prevention of inflammation and as an antimicrobial agent [8]. In Brazil the bark from *Mimosa tenuiflira* is used to prepare a sacramental drink called "jurema wine" [9]. The species has been chemically studied several times; triterpene saponins [10], indole alkaloids [9] and polysaccharides [11], have been isolated from its bark; while chalcones [12], flavanones [13], phenoxicromones [14] and diterpene rhamnosides [13] have been obtained from its leaves and flowers.

In this study, we present the isolation and antiprotozoal activity of ten flavonoids isolated from the leaves of *Mimosa tenuiflora*, including five 2-phenoxychromones, that are rare natural products whose biosynthesis has been associated with flavonoids [15].

Results and Discussion

The chemical study of leaves and flowers from *Mimosa tenuiflora* lead us to obtain two flavanones: 6-methoxy-4'-*O*-methylnaringenin (**1**) [15] and 6-methoxynaringenin (**3**) [17]; three flavonols: santin (**2**) [16], 5,7,4'-Trihydroxy-3,6-dimethoxyflavone (**5**) [18] and 6-methoxykaempferol (**7**) [19], and three 2-phenoxycromones: tenuiflorin A (**4**) [14], 6-demethoxy-4'-*O*-methylcapillarisin (**6**) [14] and tenuiflorin C (**8**) [14]. It is the first time that flavonols are isolated from this species.

The flavanones and flavonols showed oxygenated functions at positions 5, 6, 7 and 4'; meanwhile, the 2-phenoxychromones showed a 5, 7 and 4' oxygenated pattern.

In a previous study the antiamoebic and antigiardial activity of some flavanones, flavones, flavonols and catechins were evaluated [14]. Those compounds with hydroxy groups at C3, C5 and C7 resulted more active, the flavonoids with a substituent in C6 were inactive and when the degree of oxygenation increased in the B-ring the antiprotozoal activity decreased [23]. The biological activity of 2-phenoxychromones has been little studied; therefore we decided to evaluate their antiprotozoal activity against *Entamoeba histolytica* and *Giardia lamblia.* In this study we evaluated how the presence of an oxygen bridge between rings B and C affects the antiprotozoal activity. Results of this assay are shown in Table 1 [14].

Among the tested compounds, **4** (IC_{50} = 41.1 μg/mL) was the most active against *E. histolytica* and **2** (IC_{50} = 75.3 μg/mL) against *G. lamblia*, unfortunately, these values are higher than those presented by kaempferol against both protozoa (*E. histolytica:* IC_{50} = 7.9 μg/mL and *G. lamblia:* IC_{50} = 8.1 μg/mL).

Conclusions

The antiprotozoal activity of the extracts and pure flavonoids were moderate. These results were consistent with the reported structural requirements of flavonoids for antiprotozoal activity [23]. Compounds with a C6 substituent showed IC_{50} higher than kaempferol and the results with 2-phenoxychromones indicate that the presence of an oxygen atom between rings B and C of the flavonoid framework has no significant effect on the antiprotozoal activity.

General experiments procedures. Melting points were determined on a Fisher Jones Apparatus and are uncorrected. EIMS data were determined on a JEOL JMS-AX505HA mass spectrometer at 70 eV. ^{1}H NMR and ^{13}C NMR data were obtained on a Varian Unity 300 or on a Varian Unity Plus 500 instrument. Chemical shifts were referred to TMS (δ 0). Column chromatography (VCCs) was performed under vacuum using Silica gel 60 G (Merck, Darmstadt, Germany). TLC was carried out on Silica gel 60 GF254 (Macherey-Nagel).

Plant material. *Mimosa tenuiflora* (Willd.) Poir. (Fabaceae-Mimosoideae) was collected 15 km from Salina Cruz, Oaxaca on the vicinity of the road to Huatulco in March 2007. A voucher specimen, identified by Dr. Oswaldo Téllez Valdés (MEXU-417862) has been deposited at the National Herbarium of México, Instituto de Biología, Universidad Nacional Autónoma de México.

Extraction and isolation. The dried leaves (1 kg) from *M. tenuiflora* were successively extracted by percolation with hexane (10 L), acetone (8 L) and methanol (8 L). Solvents were evaporated under vacuum to obtain the respective extracts. The acetone extract (51.42 g) was fractionated in a vacuum column chromatography (VCC) using $CHCl_3$ (fraction A) and acetone (fraction B). Fraction A (11.30 g) was purified by successives VCC eluted with hexane-EtOAc 9:1 and CH_2Cl_2-acetone 95:5 mixtures to obtain: 6-methoxy-4′-*O*-methylnaringenin (**1**, 35.7 mg, 0.004%), mp 183-183 °C (lit. 169-174 °C) [16]. Fraction B (40.0 g) was purified by VCC eluted with $CHCl_3$-acetone

Table 1. Antiprotozoal activity of flavonoids against *E. histolytica* and *G. lamblia* IC_{50} μg/mL.

Plant material	Sample	IC_{50} μg/mL (CI)[a]	
		E. histolytica	*G. lamblia*
Extracts	Hexane	65.9	80.2
	Acetone	80.7	116.8
	Methanol	73.5	95.5
Compounds	6-Methoxy-4'-*O*-metylnaringenin (**1**)	72.7	82.9
	Santin (**2**)	69.7	75.3
	6-Methoxynaringenin (**3**)	76.4	84.1
	Tenuiflorin A (**4**)	41.1	108.6
	4',5,7-Trihydroxy-3,6-dimethoxyflavone (**5**)	69.8	77.1
	6-Demethoxy-4'-*O*-methylcapillarisin (**6**)	80.7	91.8
	6-Methoxykaempferol (**7**)	71.6	77.8
	Tenuiflorin C (**8**)	82.8	92.8
	Tenuiflorin B (**9**)	89.9	100.9
	6-Demethoxycapillarisin (**10**)	78.7	86.6
	Kaempferol[b]	7.9	8.1
	Emetine[c]	2.2	0.8
	Metronidazole[c]	0.23	1.22

Results are expressed as mean (n = 6) IC_{50} (Concentration that inhibited the growth of trophozoites in 50 %).
[a]Results are expressed as mean (n = 6), CI = 95% confidence intervals.
[b]See ref. [23].
[c]Positive controls.

95:5 (fr. 1), acetone (fr. 2) and MeOH (fr.3). Fraction 1 (13.34 g) was purified by VCC eluted with CH_2Cl_2 (fr. 1A), CH_2Cl_2-acetone 99.5:0.5 (fr. 1B), CH_2Cl_2-acetone 99:1 (fr. 1C) and CH_2Cl_2-acetone 98:2 (fr. 1D). In turn, crystallization of fraction 1A with acetone-hexane yielded: santin (**2**, 375.2 mg, 0.038%), mp 161-163 °C [17]. Fraction 1B was purified by VCC with CH_2Cl_2 and CH_2Cl_2-acetone 99.5:0.5, the fractions eluted with CH_2Cl_2 crystallized tenuiflorin A (**4**, 323 mg, 0.032%), mp 186-188 °C (lit. 185-187 °C) [14] and from fractions eluted with CH_2Cl_2-acetone 99.5:0.5 6-methoxynaringenin (**3**, 132.4 mg, 0.013%) mp 215-217 °C [18] was isolated. Fraction 1C was purified by VCC developed with hexane-EtOAc-MeOH mixtures to obtain, from the fractions eluted with hexane-EtOAc-MeOH 80:20:1.5, 5,7,4'-trihydroxy-3,6-dimethoxyflavone (**5**, 1.32 g, 0.13 %) mp 230-233 °C [19]. Fractions eluted with hexane-EtOAc-MeOH 70:30:1.5 yield 6-demethoxy-4'-*O*-methylcapillarisin (**6**, 27 mg, 0.0027%), mp 235-238 °C (lit. 221-225 °C) [14] and from fractions eluted with hexane-EtOAc-MeOH 70:30:5, 6-methoxykaempferol (**7**, 323 mg, 0.032%), mp 278-280 °C [20] was isolated. Crystallization with EtOAc-hexane of fraction 1D gave: tenuiflorin C (**8**, 37 mg, 0.0037%), mp 285-287 °C (lit. 283-285 °C) [14]. Tenuiflorin B (**9**) and 6-demethoxycapillarisin (**10**) were isolated in a previous study of the same plant [14].

Evaluation of antiprotozoal activity. Antiprotozoal assays. *Entamoeba histolytica* strain HM1-IMSS used in all experiments was grown axenically at 37 ºC in TYI-S-33 medium supplemented with 10% heat inactivated bovine serum. In the case of *Giardia lamblia*, strain IMSS: 8909:1 was grown in TYI-S-33 modified medium supplemented with 10% calf serum and bovine bile. The trophozoites were axenically maintained and for assays were employed in the log phase of growth. *In vitro* susceptibility tests were performed using a subculture method described previously [21]. Briefly, *E. histolytica* (6×10^3) or *G. lamblia* (5×10^4) trophozoites were incubated for 48 h at 37 ºC in the presence of different concentrations (2.5-200 µg/mL) of pure compounds in dimethyl sulfoxide (DMSO). Each test included metronidazole and emetine as standard amoebicidal and giardicidal drugs, a control (culture medium plus trophozoites and DMSO) and a blank (culture medium). After incubation, the trophozoites were detached by chilling and 50 µL samples of each tube were subcultured in fresh medium for another 48 h, without antiprotozoal samples. The final number of parasites was determined with a hemocytometer and the percentages of trophozoites growth inhibition were calculated by comparison with the control culture. The results were confirmed by a colorimetric method: the trophozoites were washed and incubated for 45 min at 37 ºC in phosphate buffer saline with MTT (3-[4,5-dimethylhiazol-2-il]-2,5-diphenyl tetrazolium bromide) and phenazine methosulfate. The dye produced (formazan) was extracted and the absorbance was determined at 570 nm. The experiments were performed in duplicate for each protozoan and repeated at least three times.

Statistical analysis. Data were analysed using probit analysis. The percentage of trophozoites surviving was calculated by comparison with the growth in the control group. The plot of probit against log concentration was made; the best straight line was determined by regression analysis and the 50% inhibitory concentration (IC_{50}) values was calculated together with the 95% confidence limits [22].

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