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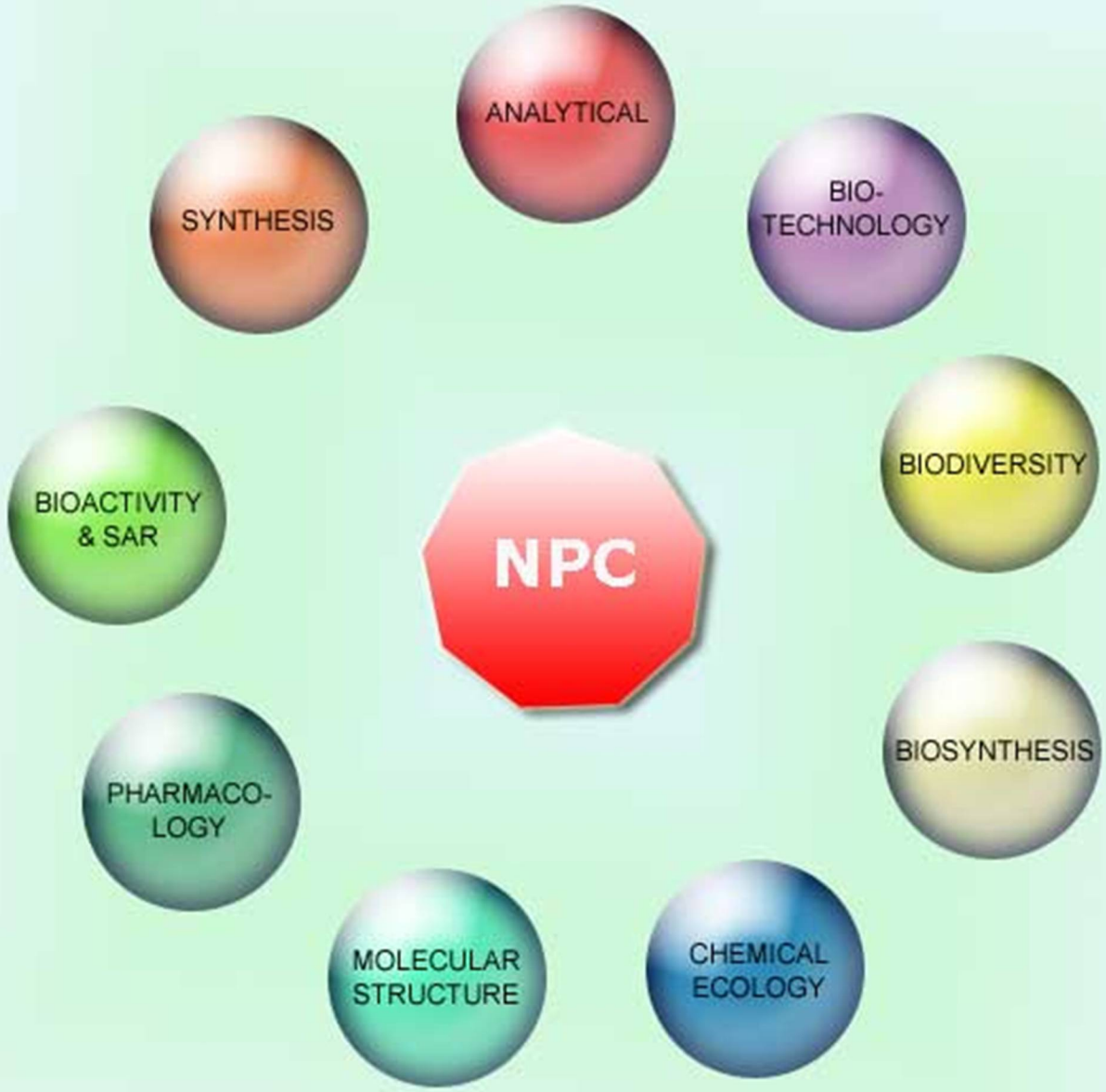

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Diuretic Activity of *Lophophytum leandri*

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A bioassay-oriented study was carried-out in order to validate the traditional uses of *Lophophytum leandri*, a parasitic plant used as a diuretic in traditional medical practices of Argentina. Four known flavonoids have been isolated from the active fraction.. Quercetin-3-*O*-β-D-glucopyranoside was identified as the active principle of the fraction. However, the diuretic activity of the extract and of the most active fraction had greater activity than that of the pure isolated compounds.

Keywords: *Lophophytum leandri,* Diuretic activity, Flavonoids, Quercetin-3-*O*-β-D-glucopyranoside, Traditional plant uses.

Lophophytum leandri Eichl. (Balanophoraceae), a parasitic plant distributed in tropical and subtropical regions of north eastern Argentina and south eastern Brazil, grows on some Fabaceae species {*Anadenthera macrocarpa* (Benth.) Brenan, *Apuleia* sp. and *Piptadenia rigida* Benth.} [1]. In Argentina this plant is known by the vernacular name *Flor de piedra* and in the regional traditional medical practices the corm of the plant is used, in decoction or infusion, as a diuretic and a urolythic ethnotherapeutical agent [2,3]. The presence of flavonoids has been reported in the rhizomes of *L. leandri* [4,5]. The present paper reports a bioassay-oriented study carried out to evaluate the potential diuretic activity on male Wistar rats of an ethanol extract of the plant, a chromatographic fraction, and pure isolated compounds.

Table 1 reports the diuretic activity of the methanol fraction of an ethanol extract and its chromatographic fractions from *L. leandri.* The whole extract showed, at the tested dose of 300 mg/kg, an increase in the volume of urine of about 76% in comparison with the control. The extract was administered at a dose of 300 mg/kg, based on the dose employed in traditional medicine. Fractionation of this extract, by gel-permeation chromatography, resulted in 8 fractions. Of these, only one, fraction IV, which represented about one third of the weight of the extract, demonstrated an appreciable diuretic activity, with an increase in the volume of urine of 62%. Purification of this fraction permitted the isolation of four known flavonoids, whose diuretic activity is presented in Table 1.

Only quercetin-3-*O*-glucoside (synonym isoquercitrin) showed an increase of diuretic activity at all the doses tested. For this compound, the diuretic activity was significant and dose-dependent, although not comparable with the activity of hydrochlorothiazide. Among the other isolated flavonoids, only (+)-catechin showed an appreciable diuretic activity, at the maximum dose tested. Even if the biological activity of the crude extract seemed to be greater than that of its chromatographic fractions, the activity was greater for compounds isolated from fraction IV. This allowed the identification of quercetin-3-*O*-glucoside as the main active principle in this fraction.

Table 1: Diuretic activity of the methanol extract, chromatographic fraction IV and pure compounds isolated from *Lophophytum leandri.*

Compound	Dose (mg/kg)	Urine excretion (mL24 h)
Control	-	4.2±0.4
Methanol extract	300	7.4±0.3***
Fraction IV	100	6.8±0.4**
Quercetin-3-*O*-glucoside	25	5.5±0.3*
	50	6.9±0.4**
	100	7.7±0.4***
Rutin	25	3.8±0.5
	50	4.0±0.6
	100	4.2±0.6
(+)-Catechin	25	4.3±0.6
	50	4.9±0.8
	100	7.0±0.3***
Naringenin	25	4.0±0.4
	50	4.1±0.5
	100	3.9±0.3
Hydrochlorothiazide	10	9.5±0.5***

*significant at : *P<0.05; ** P<0.01 ; ***P<0.001 Vs Control

Our results agree with the scarce literature data that identified flavonoid compounds as responsible for diuretic activity in some vegetal drugs. Quercetin-3-*O*-glucoside has been reported as the diuretic principle in *Tropaeolum majus* L. [6] and *Achyranthes bidentata* DC. [7]. Rutin and some related flavonols, found in *Sambucus nigra* L. flowers, also exerted diuretic activity [8].

Mpalatinos and co-workers [9] isolated (+) catechin as one of the hypotensive and diuretic components of *Alpinia zerumbet* (Pers.) B. L. Burtt & R.M. Sm. Naringenin and silymarin have been reported for their effects on urinary excretion of water and electrolytes in rats [10]. Other flavonoids have also been proved to possess diuretic activity: 7-methoxy flavonoids [11], hesperidin [12], sinensetin and 3-hydroxy-5,6,7,4'-tetramethoxyflavone [13]. The flavonoids isolated from *Spergularia purpurea* Pers. showed diuretic and

antihypertensive activity in both normotensive and spontaneously hypertensive rats [14]. Recently, the anti-inflammatory and diuretic activity of *Juniperus oxycedrus* L. was attributed to its flavonoid fraction, which includes luteolin, kaemferol, quercetin, isoquercitrin and rutin [15].

Different mechanisms have been suggested for the diuretic activity of the flavonoids. Oral administration for 1 week produced significant increase in water and solute renal excretion, revealing reduction in tubular reabsorption of water and accompanying anions [16]. Recently, 7-methoxy flavonoids have been reported as active ligands of the adenosine A1 receptor. Among the different roles of adenosine A1 receptor antagonists in renal protection, many studies have shown that they can induce diuresis and sodium excretion [11]. Although our results provide support for the traditional use of *L. leandri* as a diuretic, further studies are necessary to evaluate its safety and modes of action.

Experimental

Plant material: *L. leandri* was collected in June 2008 in the Department of Candelaria (Misiones). The plant was identified by one of us (Dr A. Amat). A voucher specimen was deposited at the Herbarium, Department of Pharmacy, Facultad de Ciencias Exactas, Químicas y Naturales of the National University of Misiones.

Extraction and isolation of pure compounds: The rhizome-like corms were dried in an oven at 40°C and powdered. Five hundred g of the powder obtained were extracted in a glass column with ethanol at room temperature for 2 days. The extract was concentrated *in vacuo,* giving 18 g of residue. This extract (9 g), dissolved in methanol, was purified on a Sephadex LH-20 column in 3 g aliquots, giving 96 fractions, pooled into 8 main fractions (I-VIII) on the basis of their chemical similarity as deduced by TLC in BAW (*n*-butanol-acetic acid-water, 60:25:15) and $CHCl_3$-MeOH-H_2O (80:18:2). Fraction IV, the more active in biological assays, was purified by RP-HPLC, using a μ-Bondapack C_{18} column. From the active fraction, eluted with a mixture of MeOH-H_2O (1:1), pure rutin (57 mg) was isolated. The same fraction, eluted with a mixture of MeOH-H_2O (55:45), permitted the isolation of quercetin-3-*O*-β-D-glucopyranoside (32 mg), (+)-catechin (12 mg), and naringenin (21 mg). The compounds were identified on the basis of their ^1H and ^{13}C NMR spectroscopic data [17]. The extraction process described above was repeated several times in order to obtain an adequate quantity of compounds for carrying out the *in vivo* animal experiments.

Animals: All animal experiments complied with the Italian legislative decree (D.L.) no. 116 of January 27, 1992 and associated guidelines in the European Communities Council Directive of November 24, 1986 (86/609/ECC). The diuretic activity was studied in male Wistar rats (180-220 g). The animals, provided by Harlan Nossan (Chieti, Italia), were housed for 7 days under controlled temperature (23±2°C, humidity 50±2%, and 12 h light/dark cycles), and fed with standard pellets produced by Mucedola Mangimi (Settimo Milanese, Italia); water *at libitum*. The animals were randomly divided into groups of 6 each. One control group was given saline (50 mL/kg) by the intraperitoneal route. The reference drug (hydrochlorothiazide, 10 mg/kg) and the drugs under trial were administered only once by gavage at 25, 50 and 100 mg/kg in 5 mL/kg of 10% gum arabic at T_0. The animals were kept in single metabolic cages for urine collection for 24 h following drug administration.

Data analysis and statistics: All data are expressed as mean ± ES and analyzed using one-way analysis of variance (ANOVA) followed by either Bonferroni's test for multiple comparisons or by t-test when the comparison was between two means. A value of $p<0.05$ was considered statistically significant.

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