Triterpenic Acids and Flavonoids from *Satureja parvifolia*. Evaluation of their Antiprotozoal Activity

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Bioassay-guided fractionation of a *Satureja parvifolia* MeOH extract led to the isolation of eriodictyol, luteolin and ursolic and oleanolic acids as its active components against *Plasmodium falciparum* K1. This is the first time these compounds are reported as constituents of *S. parvifolia*. Ursolic acid showed an IC_{50} of 4.9 μg/ml, luteolin 6.4 μg/ml, oleanolic acid 9.3 μg/ml and eriodictyol 17.2 μg/ml. Antiplasmodial activity of eriodictyol and luteolin is reported here for the first time.

Besides, the four compounds showed activity against *P. falciparum* 3D7 strain and *Trypanosoma brucei rhodesiense*. Eriodictyol showed moderate activity on all the parasites but was the most selective compound as a result of its rather low cytotoxicity (IC_{50} 174.2 μg/ml) on the mammalian KB cell line.

Key words: *Satureja parvifolia*, Antiprotozoal Compounds

Introduction

Satureja parvifolia (Philippi) Epling (Lamiaceae) grows at the verge of rivers descending from the hills in the northwestern provinces of Argentina (Salta, Jujuy, Córdoba, Catamarca, Tucumán) and is known with the common name of "muña-muña". Its traditional uses are as digestive, emmenagogue, aphrodisiac (Bandoni *et al.*, 1972) and against altitude sickness (Orfila, 1972). Previous investigations on this plant deal with the chemical composition and the antifungal activity of its essential oil (Zygadlo and Grosso, 1995; Muschietti *et al.*, 1996; Viturro *et al.*, 2000). The brine shrimp cytotoxicity (Mongelli *et al.*, 1996), smooth muscle relaxant activity on the Guinea pig corpus cavernosum (Hnatyszyn *et al.*, 2003) and antimicrobial activity have been reported for *S. parvifolia* extracts (Hernández *et al.*, 2000).

In the course of an ongoing screening of Argentine medicinal plants for antiplasmodial activity, MeOH and water extracts of *S. parvifolia* leaves showed significant activity against *Plasmodium falciparum* K1 with IC_{50} of 3 and 8.5 μg/ml, respectively (Debenedetti *et al.*, 2002). Bioassay-guided fractionation of the MeOH extract was encouraged in the search for the bioactive compounds. The ability to inhibit *Plasmodium falciparum* 3D7, a chloroquine sensitive strain, and *Trypanosoma brucei rhodesiense* was further tested for the isolated compounds.

Material and Methods

Plant material

The aerial parts of *S. parvifolia* were collected in Camino al Infiernillo, km 77, Tucumán Province, Argentina, in December 2002. A voucher specimen (A. Slanis 551) is kept at Instituto Miguel Lillo Herbarium, Tucumán, Argentina.

Extraction and isolation

The active MeOH extract was obtained as reported by Debenedetti *et al.* (2002) and fractionated by column chromatography (CC) on Se-

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phadex LH20 eluted with MeOH. Seven fractions were obtained. Fractions 3 and 4 (F3, F4) proved to be the most active ones in the antiplasmodial assay. F3 was submitted to CC on Sephadex LH20 eluted with a solvent gradient from 100% EtOAc to 100% MeOH. Eleven fractions were obtained. Fractions F5 to F7 were purified by paper chromatography (PC) in 40% AcOH. Two bands were eluted. F4 was submitted to CC using the same gradient as described for F3. A white amorphous powder precipitated from fractions F7 to F8. This was analyzed by GC-MS.

GC-MS analysis

GC-MS analysis was performed on a Perkin Elmer Clarus 500 GC-FID-MS instrument using a DB-5 fused-silica column (60 m × 0.25 mm, film thickness 0.25 μm; J&W Scientific Inc.); injector (split) temperature: 300 °C; splitting ratio: 1:60; oven temperature: 300 °C (isothermic); He: 1.8 ml/min. Using a vent system (MSVent™) at the end of the column the flow was splitted into two detectors: a) FID detector maintained at 310 °C; b) MS quadrupolar detector (70 eV). Transfer line temperature: 180 °C; source temperature: 150 °C; scan range: 40–600 Da.

Antiplasmodial assay

Antiplasmodial assay technique was performed as described in a previous paper (Debenedetti *et al.*, 2002), measuring the uptake of [3H]-hypoxanthine by *P. falciparum*. The initial concentration of the fractions was 8 mg/ml diluted in medium to give a final concentration of 40 μg/ml. The percentage growth inhibition on *Plasmodium falciparum* K1 at this concentration was measured. Pure compounds and chloroquine (standard antiplasmodial drug) were dissolved in DMSO to give stock solutions of 20 mg/ml and diluted with culture medium to give final concentrations of 100 to 0.41 μg/ml against K1 (chloroquine-resistant strain), 60 to 0.25 μg/ml against 3D7 (chloroquine-sensitive strain) and 30 μg/ml to 0.2 ng/ml for chloroquine against both strains of *P. falciparum*. Threefold serial dilutions of the test compounds were made and they were performed in triplicate. The highest content of DMSO in the assay was 0.5%. Each experiment was repeated twice. Statistical analysis was done using the non-paired heteroscedastic Student's test.

Trypanocidal assay

In vitro cultivation of bloodstream trypomastigote forms of *Trypanosoma brucei rhodesiense* STIB 900 (susceptible to melarsoprol, pentamidine and suramin) and determination of the trypanocidal activity of the test compounds was carried out as described by Asres *et al.* (2001). Each assay was done in triplicate and repeated at least once. Test compounds and pentamidine (as a standard antitrypanosomal drug) were dissolved in DMSO to give stock solutions of 20 mg/ml and diluted with culture medium to give final concentrations of 60 to 0.25 μg/ml and 1 μg/ml to 5.6 pg/ml, respectively. The highest content of DMSO in the assay was 0.3%.

Cytotoxicity assay

The cytotoxic properties of the test compounds were determined as described by Asres *et al.* (2001). The mammalian oral epidermoid carcinoma cell line KB cultivated *in vitro* in 10% HIFCS in RPMI-1640 was used. Test compounds and podophyllotoxin (standard cytotoxic drug) were dissolved in DMSO to give stock solutions of 20 mg/ml and diluted with culture medium to give final concentrations of 300 to 0.3 μg/ml and 300 to 0.003 pg/ml, respectively. The highest content of DMSO was 1.5%.

Results and Discussion

The bioassay-guided fractionation of a *Satureja parvifolia* MeOH extract afforded two major active fractions (F3 and F4) which showed inhibition of *Plasmodium falciparum* K1 growth (Table I). Phytochemical examination of these two fractions resulted in the isolation of two flavonoid and two triterpenoid compounds.

Table I. *P. falciparum* K1 inhibition by fractions from *Satureja parvifolia* MeOH extract.

Fraction	% Inhibition [40 μg/ml]
F 1	16
F 2	1
F 3	61
F 4	63
F 5	37
F 6	22
F 7	0
0.5% DMSO control	0

Luteolin and eriodictyol were isolated from F3. These compounds were also present in F4. They were identified by comparison of their spectroscopic data with literature values (Mabry *et al.*, 1970; Gujer *et al.*, 1986) and by co-chromatography with authentic samples. Ursolic and oleanolic acids were identified from F4 by GC-MS analysis. The retention times and mass spectra of their methyl derivatives were compared with those obtained with authentic samples. The four of them inhibited the growth of *P. falciparum* K1, a chloroquine-resistant strain, with IC_{50} values ranging from 4.9 to 17.2 μg/ml. The isolated compounds were further tested for their ability to inhibit the *in vitro* growth of *P. falciparum* 3D7, a chloroquine-sensitive strain, and against *T. brucei rhodesiense*. All compounds showed activity against this *P. falciparum* strain with IC_{50} values ranging from 6.3 to 34.5 μg/ml and against *T. brucei rhodesiense* (1.5 to 14.4 μg/ml). Cytotoxicity on mammalian KB cell line for these compounds was also assessed in order to determine their selectivity against the parasites (Tables II and III).

It is worth to point out that eriodictyol showed rather weak cytotoxicity to KB cells (IC_{50} 174.2 μg/ml), whilst retaining its antiprotozoal activity, it showed a higher selectivity than luteolin for the parasite. Luteolin had been previously reported as cytotoxic to other cell lines (Galvez *et al.*, 2003).

Trypanocidal activity for eriodictyol on *T. brucei brucei* (Salem and Werbovetz, 2005) and for this compound and luteolin on *T. cruzi* (Grael *et al.*, 2000, 2005) has already been reported. In our investigation, trypanocidal activity against *T. brucei rhodesiense* was also found for both compounds (IC_{50} 14.4 μg/ml and 2.3 μg/ml, respectively) thus broadening their antiparasitic activity spectra.

As results of the bioguided fractionation of the active extracts of many medicinal plants, ursolic and oleanolic acids have been repeatedly reported in the literature as the antiparasitic principles (Steele *et al.*, 1999; Abe *et al.*, 2002; Suksamrarn *et al.*, 2003; Cunha *et al.*, 2003; Taketa *et al.*, 2004). The results obtained herein showed activity for these two compounds against both *P. falciparum* strains and against *T. brucei rhodesiense*, consistent with the data found in the literature.

In conclusion, four antiprotozoal compounds: ursolic and oleanolic acids, eriodictyol and luteolin have been isolated from the MeOH extract of *S. parvifolia* by bioassay-guided fractionation. To the best of our knowledge, their presence in this species and the antiplasmodial activity of eriodictyol and luteolin are reported here for the first time. Eriodictyol showed a high selectivity for the parasites. This facts makes it an interesting lead structure for the development of new antiparasitic drugs.

Table II. Antiprotozoal activity and cytotoxicity of ursolic acid, oleanolic acid and eriodictyol from *Satureja parvifolia*.

	IC_{50} [μg/ml] (average ± std)			
Test compound	*P. falciparum* K1	*P. falciparum* 3D7	*T. brucei*	Mammalian KB cell line
Ursolic acid	4.9 ± 0.1	12.7 ± 4.6	1.5 ± 0.7	14.1 ± 7.7
Oleanolic acid	9.3 ± 0.2	34.5 ± 1.5	5.1 ± 0.2	77.7 ± 10.7
Eriodictyol	17.2 ± 0.5	27.1 ± 1.7	14.4 ± 1.6	174.2 ± 18.1
Chloroquine	0.17 ± 0.01	0.015 ± 0.005	–	–
Pentamidine	–	–	0.0008 ± 0.0002	–
Podophyllotoxin	–	–	–	0.001 ± 0.001

Table III. Antiprotozoal activity and cytotoxicity of luteolin from *Satureja parvifolia*.

	IC_{50} [μg/ml] (average ± std)			
Test compound	*P. falciparum* K1	*P. falciparum* 3D7	*T. brucei*	Mammalian KB cell line
Luteolin	6.4 ± 0.2	6.3 ± 0.8	2.3 ± 1.7	13.3 ± 1.0
Chloroquine	0.09 ± 0.06	0.0075 ± 0.0040	–	–
Pentamidine	–	–	0.0004 ± 0.0001	–
Podophyllotoxin	–	–	–	0.010 ± 0.001

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