Chemical Constituents of *Urospermum picroides*

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Seven phenolic compounds were isolated from the aerial parts of *U. picroides*. They were identified by UV spectra, TLC and HPLC-DAD as luteolin, luteolin-7-glucoside, quercetin, quercetin-3-galactoside, kaempferol-3-galactoside, chlorogenic and isochlorogenic acids. Other phenolics were characterized by HPLC-DAD analysis: gallic, protocatechuic, caffeic, ferulic and isoferulic acids, quercetin-3-glucoside and luteolin-4′-glucoside.

Introduction

Urospermum picroides is a species which belongs to the Leontodontinae, one of the eight subtribes, that comprise the tribe Lactuceae. Previous phytochemical studies of species of this subtribe have revealed the presence of flavonoids in *Hypochoeris* [1–3], sesquiterpene lactones in *Hypochoeris* [4, 5], *Urospermum* [6, 7] and *Picris* [8], and triterpenoids and steroids in *Hypochoeris* [4, 5] and *Picris* genera [9, 10]. Within the group of flavonoids, isoetin and luteolin glycosides are the compounds most frequently isolated in different species of *Hypochoeris*. However, no studies on isolation of flavonoids from the *Urospermum* genus are contained in the literature. Since these compounds are good taxonomic markers, we have studied the occurrence of phenolics in the aerial part of *U. picroides* in order to obtain a better chemotaxonomic description of this genus.

Results

Seven phenolic compounds were isolated from MeOH extract of *U. picroides* by standard chromatography techniques. Their structures were identified on the basis of the UV spectral data, TLC and HPLC-DAD comparison with authentic samples. Aglycones and sugars were also identified by co-TLC against standards after acid hydrolysis of glycosides. Peak wavelengths of the spectral measurements with the usual shifts are shown in Table I. R_f values in different TLC conditions and retention times (t_r) on HPLC-DAD are given in Table II. See also Figs. 1 and 2.

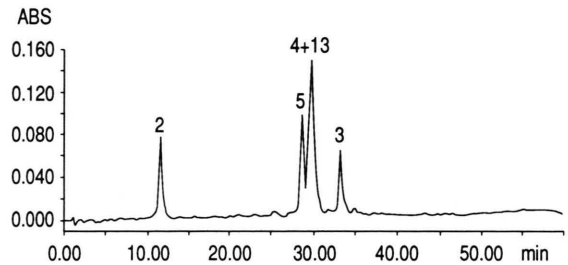

Fig. 1. HPLC-DAD chromatogram of the BuOH extract of *Urospermum picroides* (360 nm). **2,** chlorogenic acid; **3,** kaempferol-3-galactoside; **4,** quercetin-3-galactoside; **5,** luteolin-7-glucoside; **13,** quercetin-3-glucoside.

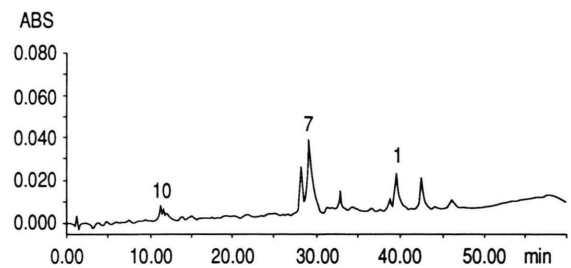

Fig. 2. HPLC-DAD chromatogram of the Et_2O of *Urospermum picroides* (360 nm). **1,** Luteolin; **7,** isochlorogenic acid; **10,** caffeic acid.

Compounds **1–7** were identified as luteolin, chlorogenic acid, kaempferol-3-galactoside, quercetin-3-galactoside, luteolin-7-glucoside, quercetin and isochlorogenic acid, respectively. In addition to these compounds, the following substances

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Table I. UV spectral data of the isolated compounds. Maxima in nm, values in brackets correspond to shoulders.

	1	**2**	**3**	**4**	**5**	**6**	**7**
MeOH	348	327	348	357	348	370	327
	(292)	299	(281)	(305)	(264)	(295)	302
	268	243	265	266	256	(263)	243
	253	217	224	255		254	218
NaOMe	405	376	402	408	405	330	371
	(325)	308	326	328	272	(293)	308
	270	263	374	271		(243)	262
		214	222				
$AlCl_3$	424		396	436	429	460	
	(326)		350	(342)	(297)	(326)	
	(294)		272	273	271	270	
	272		223				
$AlCl_3$/HCl	388		395	402	(387)	427	
	357		343	362	355	360	
	(297)		273	298	(293)	(298)	
	273		223	268	270	267	
NaOAc	360	(383)	370	374	393	377	327
	(327)	328	272	325	265	327	302
	268	300	222	272		(271)	243
		244				256	
		218					

Table II. TLC of the isolated compounds. A: cellulose layer, BuOH : AcOH : H_2O (4 : 1 : 5) u.p.; B: cellulose layer, $CHCl_3$: AcOH : H_2O (25 : 23 : 2.5); C: silica gel layer, AcOEt : AcOH : H_2O (100 : 11 : 27). t_r data of HPLC-DAD.

Compound	R_f A	R_f B	R_f C	t_r [min]
1	85	86	92	42.4
2	61	31	12	11.8
3	74	53	36	32.4
4	63	38	29	29.2
5	48	52	26	28.7
6	73	77	84	39.5
7	77	52	49	29.0
8	60	53	76	2.8
9	79	55	81	6.3
10	79	88	91	11.6
11	83	95	86	20.4
12	82	96	81	24.9
13	61	44	24	29.6
14	62	56	24	35.5

were characterized by applying Diode Array technology: gallic (**8**), protocatechuic (**9**), caffeic (**10**), ferulic (**11**) and isoferulic (**12**) acids, quercetin-3-glucoside (**13**) and luteolin-4′-glucoside (**14**). They were identified, not only by retention times but also by comparison of the UV spectra of the HPLC-separated compounds with the UV data of the authentic samples, stored by a computer system.

Discussion

Quercetin and luteolin have been found in the free state and as glycosides. The presence of these free aglycones could be attributed to enzymatic hydrolysis during the air-drying process, but we think that, in fact, they are originally in the plant, because our earlier study on fresh and dried organs of the genus *Lactuca* showed that free aglycones did not arise from spontaneous hydrolysis [12].

Flavonols are the major compounds and clearly predominate over flavones. Within the Lactuceae, this special situation is typical of the closely related subtribe Scorzonerinae, and is also observed in genera like *Scolymus, Lactuca* or *Rhagadiolus* (the last of which belongs to Leontodontinae subtribe [6]). In a large-scale screening of flavonoid aglycones in herbarium samples quercetin was the only flavonoid detected in *Urospermum* [13] and in the present study it is also found to be the main flavonol, in 3-galactosyl, 3-glucosyl and free forms. On the other hand, kaempferol appears as 3-galactosyl derivative in smaller amounts. The only flavone present in this species is luteolin, in free and

glucosylated forms in 7 and in 4′ positions, whereas apigenin derivatives are absent. The great variety of phenolic acids identified in this species, with both phenylpropanoid and benzoic structures is noteworthy. Within the second group of this type of compounds the presence of gallic acid, which has not been found in other Lactuceae species surveyed in our previous studies, should be pointed out.

Materials and Methods

Plant material and extraction

The aerial part of *Urospermum picroides* (L.) Scop. ex F. W. Schmidt was collected in Torrent (Valencia, Spain) in April 1990 and a voucher specimen was deposited in the Herbarium of the Faculty of Pharmacy (Valencia, Spain). Air-dried plant material (194.4 g) was extracted in a soxhlet with CH_2Cl_2 and the marc was extracted with MeOH at room temperature. The MeOH extract was evaporated under reduced pressure, dissolved in water and fractionated with Et_2O and *n*-BuOH to yield Et_2O (2.4 g) and *n*-BuOH (18.4 g) extracts.

Separation and identification

Et_2O extract was chromatographed on a Sephadex LH-20 column with MeOH and compound **1** was obtained directly.

n-BuOH extract was also fractionated by CC on a Sephadex LH-20 eluting with MeOH and eight fractions were obtained (B_1-B_8). Fraction B_6 was re-chromatographed in identical conditions and eight fractions were obtained (C_1-C_8). Fraction C_2 was re-chromatographed on a Lobar Lichroprep RP-8 column with MeOH : H_2O (4 : 6), this mixture was progressively enriched in MeOH and compound **2** was obtained. Fraction C_4 was fractionated on a silica gel 60 (Merck) column with EtOAc : MeOH : H_2O (120 : 5 : 5) yielding compound **3.** Fraction C_5 afforded a ppt. which was purified by washing with MeOH and compound **4** was obtained. Compounds **5** and **6** were isolated from fraction B_7 by PTLC on cellulose with *n*-BuOH : AcOH : H_2O (4 : 1 : 5) u.p. Compound **7** was obtained by precipitation from fraction B_8 in the same way as **4.**

Phenolic compounds were detected and quantified by TLC and HPLC-Diode Array Detector (HPLC-DAD). TLC was performed using silica gel 60 G microcrystalline cellulose (Merck) on aluminium sheets. Spots were detected under UV light (365 nm) and by spraying with aminoethyl ester of diphenylboric acid 1% in MeOH (Neu's reagent). R_f values were compared with authentic samples and literature data. HPLC-DAD was carried out on a Merck-Hitachi HPLC system (L-6200 pump) equipped with a L-3000 Photodiode Array Detector and a prepacked analytical column (12.5 × 0.7 mm) of Lichrospher RP-18 (5 μm). The following conditions were used: Eluents: H_2O + TFA 0.05% (A), MeOH + TFA 0.05% (B). Elution profile: 0 min, 90% A; 0–5 min, 80% A; 5–45 min, 50% A; 45–55 min, 20% A; 55–59 min, 80% A. Flow rate was 1 ml/min, column pressure 60–80 bar and UV detector was set at 255 nm. Samples of extracts (10 mg/ml) in MeOH were applied to the column by means of a 20 μl loop valve. Data were compared with authentic samples. Identification was carried out by UV spectra on a Perkin Elmer Lambda 15 UV/VIS spectrophotometer.

Acid hydrolysis of glycosides was carried out with 2 N HCl. Aglycones were separated by repeated extraction with EtOAc and identified by co-chromatography. TLC of the sugars was performed with silica gel (EtOAc : AcOH : MeOH : H_2O, 65 : 20 : 15 : 15) spraying with 0.5% thymol in H_2SO_4–EtOH (5 : 95) and heating at 120 °C for 15 min.

Authentic samples

Authentic samples were purchased from C. Roth (Karlsruhe, F.R.G.), Merck (Darmstadt, F.R.G.), Apin Chemicals (Abingdon, U.K.) and Sigma Chemical Co. (St. Louis, U.S.A.).

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