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AN HPLC STUDY OF FLAVONES FROM SOME SPANISH *SIDERITIS* SPECIES

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Key Word Index—*Sideritis*; Labiatae; flavones; HPLC; chemotaxonomy.

Abstract—Nine flavone aglycones were identified from the ether extracts of fifteen *Sideritis* species by means of HPLC techniques.

INTRODUCTION

The genus *Sideritis* of which more than 30 species grow in Spain, is difficult to classify because of a strong tendency of a number of species to hybridize. A chemotaxonomic study of the essential oils of six *Sideritis* species was carried out previously by Mateo *et al.* [1].

Flavonoids are well known to be useful as taxonomic markers in many plants [2]. In this work we report an analysis of the flavone aglycones present in the ether extracts of fifteen *Sideritis* species. All flowers were collected at flowering and authenticated by Dr. D. Rivera. The analysis of flavonoids by high performance liquid chromatography (HPLC) [3–8] offers an accurate sensitive technique which yields results in minutes compared to the more classical procedures which require days or weeks for analysis and use much larger amounts of plant material.

RESULTS AND DISCUSSION

The species analysed are listed in Table 1. The available authentic samples: luteolin, apigenin, chrysoeriol, cirsimaritin, cirsilineol, cirsiliol, xanthomicrol, 5,3′,4′-trihydroxy-6,7,8-trimethoxyflavone (sideritoflavone) and 5,4′-dihydroxy-6,7,8,3′-tetramethoxyflavone were obtained from *Sideritis leucantha* and *S. angustifolia* [9–11], and identified by UV, MS and NMR techniques. These flavonoids were then analysed by HPLC and BD-TLC and their R_t and R_f values calculated (Table 2).

The ether extracts obtained from the species studied were BD-TLC developed on silica gel, and revealed by spraying with Naturstoffreagenz-A. The colours visualized under UV-light (360 nm) gave interesting structural information. A dark colour indicated a flavone which lacked an *ortho*-dihydroxy system in the B-ring and substitution at C-6 and/or C-8. An orange fluorescent colour indicated an *ortho*-dihydroxy system in the B-ring in flavones with substituents at C-6 and/or C-8, or a phloroglucinol-based flavone (5,7-dihydroxyflavone).

The ether extracts were also analysed by HPLC, and nine different peaks were observed (Table 3). It is remarkable that all the *Sideritis* species studied gave qualitatively similar flavone patterns but showed marked quantitative differences. Thus, 5,3′,4′-trihydroxy-6,7,8-trimethoxy flavone is the major component in eight of the fifteen species studied i.e. *S. leucantha*, *S. flavovirens*, *S. pusilla*, *S. mugronensis*, *S. angustifolia*, *S. carthaginensis*, *S. arbo-*

Table 1. *Sideritis* species studied

Species	Herbarium No.	Place of collection	Date	Abbreviation
S. leucantha Cav.	7244	Santomera (Murcia)	4-82	leu
S. flavovirens Rouy	9504	Puerto Lumbreras (Murcia)	4-83	fla
S. pusilla Lge. (Pau)	9813	Uleila del Campo (Almeria)	6-83	pus
S. mugronensis Borja	—	Bonete (Albacete)	7-83	mug
S. angustifolia Lag.	6981	Ayora (Valencia)	7-81	ang
S. pusilla carthaginensis (Lge.) Pau, F. Q.	3336	La Manga (Murcia)	8-83	car
S. granatensis (Pau) Rivas Goday	9601	Gata (Almeria)	6-83	gra
S. Glauca Cav.	6984	Abanilla (Murcia)	4-82	gla
S. incana subsp. *virgata* L.	1786	El Carche (Murcia)	6-78	inc
S. hirsuta L.	2072	Valladolid	6-78	hir
S. montana L. subsp. *ebracteata* (Asso) Murb.	8324	La Celia (Murcia)	5-82	mon
S. arborescens subsp. *arborescens* Salzm. ex Bentham	9824	—	5-83	arb
S. tragoriganum Lag. × *S. leucantha* Cav.	1774	El Carche (Murcia)	5-80	X
S. spinulosa Barnades ex Asso	5556	Alba de Cerrato (Palencia)	7-80	spi
S. scordioides L. subsp. *scordioides*	4300	S. Mauricio (Gerona)	7-80	sco

Table 2. HPLC and TLC data for some flavonoid components of *Sideritis* species

Flavonoids	HPLC R_t	TLC R_f*	TLC R_f†	TLC UV‡
5,7,3',4'-Tetrahydroxyflavone (luteolin)	2.67	0.12	0.06	orange
5,7,4'-Trihydroxyflavone (apigenin)	4.70	0.17	0.07	lemon
5,7,4'-Trihydroxy-3'-methoxyflavone (chrysoeriol)	5.47	0.18	0.09	lemon
5,3',4'-Trihydroxy-6,7-dimethoxyflavone (cirsiliol)	6.27	0.25	0.17	orange
5,3',4'-Trihydroxy-6,7,8-trimethoxyflavone (sideritoflavone)	8.75	0.40	0.30	orange
5,4'-Dihydroxy-6,7-dimethoxyflavone (cirsimaritin)	11.89	0.52	0.29	dark
5,4'-Dihydroxy-6,7,3'-trimethoxyflavone (cirsilineol)	14.54	0.59	0.18	dark
5,4'-Dihydroxy-6,7,8-trimethoxyflavone (xanthomicrol)	15.67	0.59	0.47	dark
5,4'-Dihydroxy-6,7,8,3'-tetramethoxyflavone	17.38	0.68	0.35	dark

*TLC on silica gel with C_6H_6–$C_4H_8O_2$–HOAc (90:25:4).
†TLC on silica gel with *n*-BuOH–C_6H_{14} (3:17).
‡Flavonoid colours observed under UV light (360 nm) after spraying with Naturstoffreagenz-A.

rescens and the hybrid *S. leucantha* × *S. tragoriganum*. Xanthomicrol is the most variable flavone, being the principal component in *S. glauca*, an important component in *S. arborescens* and *S. spinulosa*, and is found only in trace amounts in *S. angustifolia* and *S. montana*. Cirsiliol is another frequent and abundant flavone in these species. The phloroglucinol-based flavones: luteolin, apigenin and chrysoeriol, are important in *S. granatensis*, *S. glauca*, *S. incana*, *S. hirsuta*, *S. montana* and *S. spinulosa*, but only occur in trace amounts in *S. leucantha*, *S. flavovirens*, *S. pusilla*, *S. mugronensis*, *S. angustifolia* and *S. arborescens*. Apigenin is the major component in *S. scordioides*.

The identification of flavones in the ether extracts was carried out by the complementary analyses of BD-TLC and HPLC and confirmed in some cases by UV and MS techniques as described previously [9–11]. However, it is of interest that depending on the extraction technique, different flavone aglycones have been found in different studies of the same *Sideritis* species. Thus, extraction with non-polar solvents such as petrol (60–80°), has shown that the highly methoxylated flavones, 5-desmethylnobiletin, gardenin D, 5,4'-dihydroxy-6,7,8,3'-tetramethoxyflavone and cirsilineol are the principal components [12], while extraction with ethanol yielded both highly methoxylated flavones and other less methoxylated constituents such as xanthomicrol, cirsimaritin, pectolinarigenin, salvigenin, artemetin and eupatorin [13]. On the other hand, aqueous alcoholic mixtures (the system used in this work) favoured the more polar flavones: luteolin, apigenin and chrysoeriol, and others of intermediate and high methoxylation such as xanthomicrol, cirsimaritin, cirsiliol, 5,3',4'-trihydroxy-6,7,8-trimethoxyflavone and 5,4'-dihydroxy-6,7,8,3'-tetramethoxyflavone [9, 10]. Thus, qualitative differences in the flavone aglycones obtained from *Sideritis* plants does depend on the solvent used in extraction.

However, from the findings of the present investigation it is possible to divide the *Sideritis* species into two groups: those with trace quantities of phloroglucinol-based flavones and 5,3',4'-trihydroxy-6,7,8-trimethoxyflavone as the principal components (group 1), and those with phloroglucinol-based flavones as the major constituents (group 2) (Table 3). With regard to the evolutionary status of the flavonoid characters [2, 14] group 2 species appear to be more primitive than group 1. Apart from *S. granatensis*, *S. spinulosa* and *S. scordioides*, group 1 species are placed in subsection Carpostegiatae, and group 2 in subsection Gymnocarpae [15]. However, no correlation between flavonoid pattern and plant geography has been found.

EXPERIMENTAL

Plant material. Voucher specimens of the *Sideritis* species were deposited in the herbarium of the Botany Department of the Facultad de Ciencias de Murcia. Dried and powdered aerial parts were extracted with EtOH–H_2O (7:3). The aq. ethanolic extracts were concd under red. pres. until only H_2O remained, which was extracted with Et_2O.

BD-TLC. The Et_2O extracts were developed by BD-TLC on silica gel plates (10 × 10 × 0.01 cm, Carlo Erba) with C_6H_6–$C_4H_8O_2$–HOAc (90:25:4) and *n*-BuOH–C_6H_{14} (3:17). Flavonoids were visualized under UV-light (360 nm) and after spraying with Naturstoffreagenz A.

HPLC. Analysis by HPLC was carried out with a Perkin–Elmer liquid chromatograph HPLC, equipped with a pump module 2/2, a model LC85B Vis-UV variable wavelength detector, and a data treatment station Sigma 15. A C 18 reversed phase column with 3 μm particle was used (10 cm × 2.7 mm). Working solns containing 1–5 mg of extract per 2 ml of MeOH were filtered through a swinny stainless-steel unit with a 0.45 μm filter. Runs were carried out for 20 min. The elution solvents were H_2O–HCOOH (19:1) from pump B and acetonitrile (ACN) from pump A. Flow-rate was 1.5 ml/min (about 3500 psi), with pump A providing 23% and pump B 77%, isocratic during 10 min. A gradient increasing 2% ACN/min was then installed until it reached 28% ACN. At this moment the gradient was stopped and the system became isocratic up to 20 min. Samples of 6 μl were injected in each assay, and peaks were detected at 340 nm.

Elucidation of structures. Structures were established as reported previously by UV, NMR and MS procedures. Authentic samples were obtained from *S. leucantha* and *S. angustifolia* [9–11].

Acknowledgement—The authors are grateful to Dr. Diego Rivera for supplying and identifying the plant material used in this study.

Table 3. HPLC analysis of the ether extracts obtained from the *Sideritis* species studied

	Sideritis species*														
	Group 1								Group 2						
	leu	fla	pus	mug	ang	car	arb	X	gra	gla	inc	hir	mon	spi	sco
Luteolin	+	tr	tr	+	+	+++	tr	tr	tr	+	+	+++	++	tr	+
Apigenin	tr	tr	+	+	tr	+	tr	+	++	++	+++	+++	++	++	+++++
Chrysoeriol	tr	tr	+	tr	tr	tr	++	++	++++	++	+++	+++++	++++	++	+
Cirsiliol	+++	+++	+++	++++	++++	+++	+++	+++	++++	+++	++++	+	tr	+++	+
5,3',4'-OH-6,7,8-OMe-flavone	++++	+++++	+++++	+++++	+++++	+++++	+++++	+++++	+++	+++	+	+++	+	+++	+
Cirsimaritin	+	tr	tr	tr	+	tr	+	++	tr	++	+++	tr	++	+	tr
Cirsilineol	+	+	+	+	++	tr	+	++	tr	tr	++	tr	++++	tr	++
Xanthomicrol	+	+	+	+	tr	++	++++	+	+++	++++	+	+	tr	++++	+
5,4'-OH-6,7,8,3'-OMe-flavone	++	+++	++	+++	++	++	++	++	+	+++	tr	++	tr	++++	++

The same response factor (at 340 nm) is considered for each flavonoid. Concentration (%): (tr) trace quantity, lower than 1%; (+) 1–5%; (++) 5–10%; (+++) 10–20%; (++++) 20–40%; (+++++) higher than 40% (area normalization method was used).

*For key to abbreviations of species names see Table 1.

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