Seasonal Variation of Free Flavone Aglycones from *Sideritis leucantha* (Lamiaceae)

F. A. T. Barberán, F. Tomás, and J. M. Núñez

Laboratorio de Flavonoides, Centro de Edafología y Biología Aplicada del Segura, C.S.I.C., Apdo. 195, Murcia, 30003, Spain

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A quantitative and qualitative study of the seasonal variation of free flavone aglycones from *Sideritis leucantha* (Lamiaceae) has been carried out by means of HPLC. The flavonoid pattern remained unvariable with seasonal variation supporting flavonoid aglycones as idoneous taxonomic markers. Quantitative variations were observed, being the summer months which yielded a higher content in these compounds. These highly methylated flavone aglycones are located in the leaves surface as part of the excretion material.

Introduction

Infusions and decoctions of *Sideritis* species are used in Spanish folk-medicine as antiinflammatory, antirheumatic and digestive drugs [1]. Recently, the new flavonoid compound hypolaetin-8-β-D-glucoside has been isolated from *Sideritis leucantha* [2] and *Sideritis mugronensis* [3], and the antiinflammatory activity of this compound was evidenced [4]. These pharmacological actions encouraged us to study the flavonoid compounds from *Sideritis* species and in the last few years a lot of work has been carried out on the flavonoid aglycones [5, 6] and glycosides [2, 7–9]. The free flavone aglycones xanthomicrol, cirsimaritin, cirsiliol, cirsilineol, 8-methoxycirsilineol and sideritoflavone (5,3′,4′-trihydroxy-6,7,8-trimethoxyflavone) have been isolated and identified from *Sideritis leucantha* [5, 6]. These flavone aglycones exhibited also interesting pharmacological actions. Thus, 8-methoxycirsilineol and cirsilineol showed spasmolytic activity higher than thymol and carbacrol [10, 11], cirsimaritin exhibited antibacterial activity [12] and all these flavones showed an inhibitory effect on lense aldose reductase from rat [13].

In this work we have studied the quantitative and qualitative seasonal variation of those pharmacologically active flavone aglycones from naturally occurring *Sideritis leucantha* plants, in order to establish the idoneous month for collection of plants to yield a higher level of each flavone, and total free flavone aglycones as a whole.

The work has been carried out by means of High Performance Liquid Chromatography (HPLC), that offers an accurate sensitive technique which yields quantitative and qualitative results in minutes and with very small samples [14].

Results and Discussion

The HPLC analysis of flavonoid compounds from the ether extracts of *Sideritis leucantha*, allows a quantitative survey of the free flavone aglycones on the basis of their absorbance at 340 nm [14] (Fig. 1). Thus, the flavone aglycones cirsiliol, sideritoflavone, cirsimaritin, cirsilineol, xanthomicrol and 8-methoxycirsilineol have been evaluated from samples of these plants collected monthly. Results are shown in Table I. The flavonoid pattern remained invariable with seasonal variation. These results support previous reports in which flavonoid compounds, and flavonoid aglycones in particular, were presented as the most favoured of all plant constituents as taxonomic markers, since they are chemically stable and very little affected by physiological conditions [15–16]. In all samples studied, sideritoflavone was the principal flavonoid (63–75%) and cirsiliol was rather constant (8–11%). The most variable

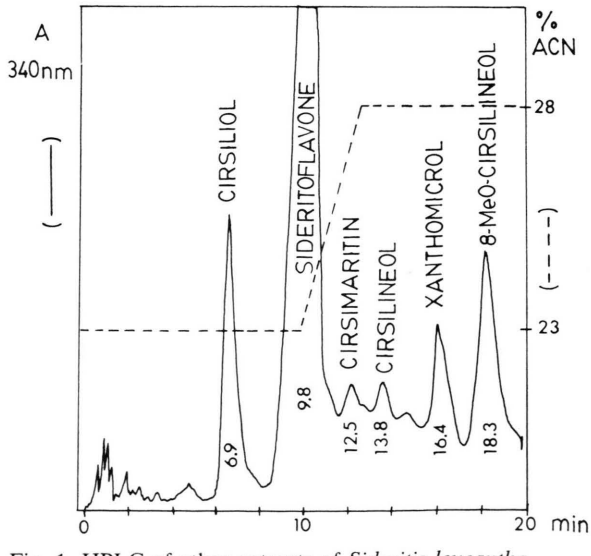

Fig. 1. HPLC of ether extracts of *Sideritis leucantha*.

Reprint requests to F. A. T. Barberán.

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Table I. Seasonal variation of free flavone aglycones.

	J	F	M	A	M	J	J	A	S	O	N	D
Chlorophylls (A 663 nm)	0.415	0.810	0.795	0.755	0.745	0.200	0.350	0.120	0.225	0.180	0.625	0.550
free flavone aglycones*	6.2	6.0	6.6	9.0	13.0	14.0	16.4	13.8	8.2	7.4	4.6	4.6
Total Absorbance (340 nm) ($\times 10^6$)**	3.75	3.52	3.86	4.45	7.04	7.04	8.00	6.62	3.89	3.47	2.80	2.80
Cirsiliol (5,3',4'-OH-6,7-OMe)	8.30	8.56	9.40	9.95	9.75	10.25	9.04	9.58	10.40	10.90	8.22	9.73
Sideritoflavone (5,3',4'-OH-6,7,8-OMe)	67.90	72.15	70.60	73.14	75.55	72.84	63.66	65.48	67.19	66.26	69.58	67.49
Cirsimaritin (5,4'-OH-6,7-OMe)	4.60	2.54	2.60	3.85	1.12	1.67	6.32	5.90	3.94	3.01	3.01	4.41
Cirsilineol (5,4'-OH-6,7,3'-OMe)	6.20	4.47	4.40	2.52	0.95	1.02	5.20	4.40	4.85	4.00	4.20	5.42
Xanthomicrol (5,4'-OH-6,7,8-OMe)	7.80	8.14	4.50	3.38	4.84	4.89	6.18	5.71	4.69	5.79	8.34	8.21
8-Methoxycirsilineol (5,4'-OH-6,7,8,3'-OMe)	5.20	4.14	8.50	7.16	7.79	9.33	9.60	8.93	8.94	10.04	6.65	4.74

* Free flavone aglycones evaluated as mg of sideritoflavone/g of dried plant.
** Total absorbance (total counts at 340 nm) of complete chromatograms, calculated by data treatment station Sigma-15. The same response factor at 340 nm is considered for each flavonoid.

flavones were cirsimaritin and cirsilineol (1–6%), that showed values of about 1% during May and June and higher levels the rest of the year. Xanthomicrol (3–9%) and 8-methoxycirsilineol (4–10%) exhibited intermedial quantities.

When total free flavone aglycones were analysed (as mg of sideritoflavone/g of dried plant), significative quantitative variations were found. Thus, from May to August were the months which yielded a higher content in these flavonoids (higher than 10 mg/g dried plant), meanwhile November and December showed the lowest levels (lower than 5 mg/g of dried plant). These quantitative results were calculated by two methods based on the spectrophotometry, the classic technique reported by Lebreton *et al.* [17], and an evaluation of total absorbance at 340 nm of the complete chromatogram, carried out by the Sigma-15 data treatment station, and very good correlations were found between the two methods (Table I).

Plants growing in xeric habitats usually show high levels of excretion products (terpenoids and flavonoids) [18, 19]. The physiological actions of these excretion products include the reduction of cuticular transpiration, the protection of microbial attacks (antifungal, antibacterial, antiviral agents), the insects deterrency, the reflection of irradiations and the absorption of excessive UV light (Wollenweber, personal communication). All these possible functions would be of special importance for plants that, as *Sideritis leucantha*, fight for survival in extreme climatic conditions.

The location of the flavonoid aglycones from *Sideritis leucantha*, was assayed by rinsing a twig of this plant in $CHCl_3$ for a minute, and all the methylated flavones found in the plant were also found in this extract suggesting an external localization for these products as components of the excretion matter.

The fact that these excretion flavones are increased in summer months is in agreement with the physiological functions mentioned above.

In the obtaining of flavonoid aglycones from vegetal sources, high levels of chlorophylls are undesirable, since they make difficult the subsequent flavonoid purification from these extracts. A seasonal evaluation of chlorophylls has been carried out in parallel, being the summer months which showed the lower levels. The coincidence of high levels of flavonoid aglycones and low levels of chlorophylls from June to August, make these three months the idoneous to extract flavonoid aglycones. *Sideritis leucantha* during these three months showed dried flowers (June) and seeds (August).

Experimental

The monthly sampling was carried out during 1984 in the Experimental Field of the Centro de Edafología y Biología Aplicada del Segura, near Santomera (Murcia, Spain) where these plants grow spontaneously. A voucher specimen was deposited in the Herbarium of the University of Murcia, being identified as *Sideritis leucantha* Cav. The samples

were air-dried and stored in a dried room until analysis.

Extraction of flavonoids

Approximately 1 g of dried aerial parts were extracted during 18 h with 10 ml of cold EtOH-H_2O (7:3) with frequent shaking. Extracts were taken to dryness and suspended in 5 ml H_2O. These aqueous extracts were extracted with 10 ml Et_2O (5 × 2 ml). The Et_2O was removed and the concentrates were redissolved in 1 ml MeOH. These MeOH extracts were HPLC analysed.

HPLC analysis

This was achieved as described previously [14], with a Perkin-Elmer liquid chromatograph HPLC, equiped 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). Samples were filtered through a swinny stainless-steel unit with a 0.45 μm filter. Runs were carried out for 20 minutes. 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 (3000 psi), with pump A providing 23% and pump B 77% isocratic during 10 minutes. 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 minutes (Fig. 1). Samples of 6 μl were injected in each assay and peaks were detected at 340 nm.

Quantitative analysis of total free flavone aglycones

The total free flavone aglycones from the Et_2O extracts were analysed by the spectrophotometric method described by Lebreton *et al.* (1967) [17], and were evaluated as mg of sideritoflavone/g of dried plant. Sideritoflavone showed a molar absorption of 18.000 ($\varepsilon = 18.000$). These results were corroborated by the total counts registered in the HPLC chromatograms (340 nm) by the data treatment station Sigma-15.

Chlorophyll estimation

The chlorophyll degradation products were estimated by spectrophotometry at 663 nm as described Harborne (1973) [20].

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