Seasonal Variations in the Harpagoside Content of *Scrophularia scorodonia* L.

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Seasonal variations on the content of harpagoside in *Scrophularia scorodonia* L. (Scrophulariaceae) were investigated using plants collected monthly from January to December in 1995. During growth of this species the percentage of harpagoside was the highest during the maximum development of the plant, specially in July. Harpagoside levels differed among leaves, stems and flowers of *S. scorodonia.* Leaves were distinguished from other plant parts by higher levels of harpagoside. Drying at ambient temperature influenced the yield of harpagoside compared with the results of plant drying by microwave.

In recent years, the use of medicinal plants and crude extracts has widely progressed. It can be assumed that this trend will not continue unless standardization methods for these plant materials become available. In a previous paper we have reported that *S. scorodonia* is a potential new source of harpagoside (Díaz *et al.*, 1998). This compound is the main iridoid glycoside of *Harpagophytum procumbens* D. C. and appears to be implicated in its pharmacological properties. The secondary roots of the Devil's claw (*H. procumbens*) is registered in the French Pharmacopoeia (Xth edition).

Extracts of the secondary roots of Devil's claw are gaining growing interest due to their therapeutic use in rheumatic disorders (Wenzel and Wegener, 1995). Its use in the treatments of headaches and allergic reactions has also been reported. In Europe, *H. procumbens* is used for painful arthrosis, tendinitis, loss of appetite and dyspeptic complaints (Baghdikian *et al.*, 1997).

On the other hand, *Scrophularia ningpoensis* Hemsl. is an officially indexed drug in the Chinese Pharmacopoeia, the roots of this plant have been used for treatment of fever, swelling, constipation, pharyngitis, neuritis, and laryngitis (Miyazawa *et al.*, 1998). This species contains iridoids (harpagoside, aucuboside and 6-O-methyl-catalpol) and phenylpropanoid glycosides (acteoside and angoroside A) (Qian *et al.*, 1992; Kajimoto *et al.*, 1989) that are also present in European *Scrophularia* species, such as *S. scorodonia* (Fernández Matellano *et al.*, 1995; De Santos *et al.*, 1998). Although this species has not been used in folk medicine, it includes similar compounds as *H. procumbens* and *S. ningpoensis.* Accordingly, the presence of harpagoside, increases the possible pharmacological value of this species. Consequently, it is of interest to investigate seasonal variations on the content of harpagoside in *S. scorodonia.*

We have previously reported a reverse-phase high performance liquid chromatographic method for the determination of harpagoside, the main biologically active constituent according to current pharmacological knowledge, in *S. scorodonia.* The chromatographic method was validated and applied for quantitative determination of harpagoside, due to the medicinal values of this compound (Díaz *et al.*, 1998).

The objective of this investigation was to get more insight into the variation of the harpagoside content in different genotypes and during the ontogenesis. Such knowledge may lead to recommendations concerning the best harvest time of the plant. Moreover the drying technique may also be important.

Materials and Methods

Plant material

Flowers, stems and leaves of *S. scorodonia* were collected from the vicinity of Jaén (Spain) between January/December 1995. Plants were cut off just above the soil surface and transported immediately to the laboratory. The plant material was separated into flowers, stems and leaves. Their voucher specimens [(F. S.S 95), (S. S.S 95), (L. S.S 95)] were deposited at the Laboratory of Pharmacognosy, University of Alcalá de Henares. Plant

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parts were dried in a microwave oven for 3 min, and subsequently pulverized.

Compounds

Authentic samples of harpagoside were isolated from *Scrophularia scorodonia* in our laboratory in earlier studies.

Sample extraction for HPLC analysis

Ten grams of leaves, stems and flowers were extracted with 100 ml 80% MeOH for 12 h.

Apparatus and conditions

HPLC analysis was carried out with a Perkin Elmer chromatograph with a photodiode-array detector at λ=278 nm. Chromatographic runs were performed on a prepacked analytical column (300 × 3.9 mm) Phenomenex Bondclone Interchim (Montluçon, France) C_{18} guard column (Waters). The eluents were water from pump A and methanol from pump B according to the following profile: 0–7 min, 93–60% A, 7–40% B; 7–12 min, 60%A, 40% B; 12–17 min, 60–50% A, 40–50% B; 17–27 min, 50% A, 50% B; 27–32 min, 50–0% A, 50–100% B; 32–45 min, 0% A, 100% B; 45–48 min, 0–93% A, 100–7% B; 48–63 min, 93% A, 7% B. The flow rate was kept constant at 1.5 ml/min.

Generally aliquots of 20 μl of these extracts were used for analyses: stems extracts were injected directly, leaves and flower extracts diluted to 1/10 and 1/2, respectively. The standard compound (harpagoside) was dissolved in HPLC-grade methanol.

Data in histograms are presented as a mean ± SD. At least two separate experiments were performed in duplicate. Difference between treatments were analyzed by Student T-test and the minimum level of significance was defined at $P < 0.05$.

Validation

The quantitative determination was carried out using an external standard for calibration. The linearity of the HPLC method was observed in the range of 0.05–0.4 mg/ml. Data for least-squares regression analysis of the calibration graph were $y = 20+20\ x$ ($r = 0.98$) where y = peak area, x = concentration in mg/ml and r = correlation coefficient. At a 2:1 signal-to-noise ratio the detection of harpagoside was 0.625 μg/ml. The reproducibility of the method was calculated by assaying ten replicates of the same 80% methanol extracts. The relative standard deviation for stem extracts was estimated to be 1.4%. The reproducibility of harpagoside standard preparation was tested by assaying ten preparations at a concentration of 0.2 mg/ml. The relative standard deviation was estimated to be 0.60%. Four stem, flower and leaf extractions with 80% MeOH were tested for the reproducibility of the extraction method. The relative standard deviations were 2.6%, 3.5% and 3.1%, respectively. Quantification limit was 25 μg/ml for harpagoside, for ten injections, coefficient of variation was 2.2%. Concentration of samples was determined directly from the calibration graphs.

Results and Discussion

The final content of harpagoside for different months and different plant parts expressed in% of plant dry weight, is shown in Fig. 1. It can be seen that the content of harpagoside in leaves extract is the highest (1.9%) at July. This is followed by a decrease during September, October and December. The quantity of harpagoside, however, gradually increases from January to July (1.15- 1.9%). On the other hand harpagoside was detectable in all stem extracts (Fig. 1), the highest total harpagoside content was found at July (0.65%).The concentration of harpagoside observed in flower samples was generally low, but in April considerably higher amount of this compound was found (0.63%).

It was observed that the maximum harpagoside percentage is obtained just before and during the development of the plant. These data indicate that July is the most suitable month for harvesting.

The content of harpagoside is highest as a result of microwave drying. Compared with plant drying at ambient temperature, drying in the air may influence the harpagoside content, especially in leaf extracts where there is a variability in the total harpagoside content, from 1.9% to 1.2% in leaf extracts tested from July. In contrast, there is no considerable variation in levels of the total harpagoside content in stem and flower extracts from different months.

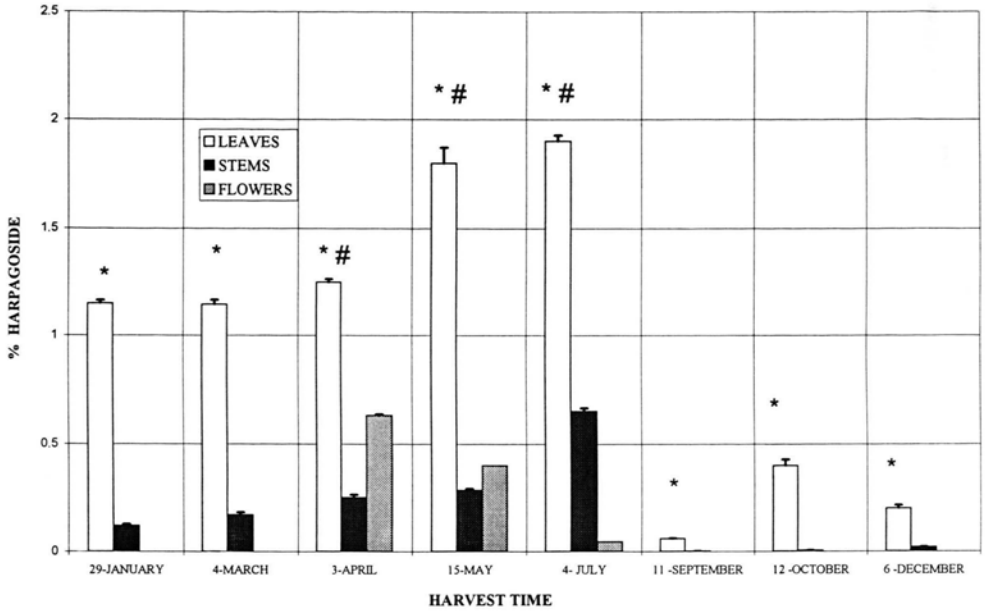

Fig. 1. Content of harpagoside in different plant parts of *S. scorodonia.* Values significantly different.
* $P < 0.01$ leaves versus stems in the same harvest time. # $P < 0.01$ leaves versus flowers in the same harvest time.

Harpagoside content was always highest in leaves and decreased in the order stems > flowers ($P< 0.05$ leaves *versus* stems, $P< 0.05$ leaves *versus* flowers), the content of harpagoside was highest at the maximun development of the plant. Within the growing season (Apr.-Sept.), the iridoid content of the aerial parts reached a maximum in July, after which it declined.

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