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Maleš, Željani; Žuntar, Irena; Nigović, Biljana; Plazibat, Miško; Bilušić Vundać, Vjera

Source / Izvornik: **Acta Pharmaceutica, 2003, 53, 139 - 144**

Journal article, Published version

Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

Permanent link / Trajna poveznica: <https://um.nsk.hr/um:nbn:hr:163:301234>

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Download date / Datum preuzimanja: **2024-08-17**



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## Quantitative analysis of the polyphenols of the aerial parts of rock samphire – *Crithmum maritimum* L.\*

ŽELJAN MALEŠ<sup>1</sup>\*\*  
IRENA ŽUNTAR<sup>2</sup>  
BILJANA NIGOVIĆ<sup>3</sup>  
MIŠKO PLAZIBAT<sup>4</sup>  
VJERA BILUŠIĆ VUNDAČ<sup>1</sup>

<sup>1</sup> Department of Pharmaceutical Botany  
Faculty of Pharmacy and Biochemistry  
University of Zagreb, 10000 Zagreb,  
Croatia

<sup>2</sup> Faculty of Pharmacy and Biochemistry  
University of Zagreb, 10000 Zagreb  
Croatia

<sup>3</sup> Department of Analytics and Medicines  
Control, Faculty of Pharmacy and  
Biochemistry, University of Zagreb  
10000 Zagreb, Croatia

<sup>4</sup> Department of Botany, Faculty of  
Science, University of Zagreb  
10000 Zagreb, Croatia

Quantitative spectrometric analyses of the contents of flavonoids, tannins and total polyphenols in the aerial parts of rock samphire – *Crithmum maritimum* L., collected from three separated geographic locations along the Adriatic coast of Croatia in different growth stages, are reported. The results point to variability of the analyte content as a function of the growing site and growth stage. The content of flavonoids was found to be 0.08–0.42%. The highest content of flavonoids was found in the samples collected before flowering. The content of tannins ranged from 0.10 to 2.65%, while the content of total polyphenols varied from 4.72 to 9.48%. The highest contents of tannins and total polyphenols were found in the samples collected before flowering and at the beginning of flowering.

**Keywords:** *Crithmum maritimum* L. (*Apiaceae*), flavonoids, tannins, total polyphenols

Received February 20, 2003

Accepted May 8, 2003

Rock samphire, *Crithmum maritimum* L. (*Apiaceae*), is a fleshy, glabrous, very branched aromatic perennial, woody at base, up to 50 cm high. Its leaves are deltate, bitermately or tritermately compound, segments 1–4 cm, terete, subulate or subfusiform, acute. The flowers are in compound umbels, very small, petals are obcordate with a long

\* A part of this work was presented at the XVIII<sup>th</sup> Croatian Meeting of Chemists and Chemical Engineers, Zagreb, February 16–19, 2003

\*\* Correspondence; e-mail: zmales@pharma.hr

inflexed point, yellowish-green. The fruit is ovoid-oblong, ribbed, approximately 6 mm long, yellowish or purplish.

This plant can be found on cliffs and rocks or, more rarely, in shingle or sand by the sea.

It grows on the Atlantic coasts of Europe from Britain southwards, the coasts of the Mediterranean, the Black Sea, and Macaronesia (1–3).

The fleshy leaves of rock samphire are used as a condiment and pickle, or as a salad ingredient and their nutrient composition has been recently investigated (4).

Chemical investigations of *C. maritimum* indicated the presence of essential oil (5–15), flavonoids (16) and amino acids (17, 18).

This paper represents the first investigation of the content of flavonoids, tannins and total polyphenols of *C. maritimum*.

## EXPERIMENTAL

### *Plant material and chemicals*

Aerial parts of *C. maritimum* were collected at the three separated geographic localities along the Adriatic coast of Croatia (Punat – 45° 01' 15" N, 14° 08' 20" E; Zadar – 44° 05' 30" N, 15° 13' 55" E; Korčula – 42° 57' 30" N, 17° 08' 30" E), in different growth stages: before flowering, at the beginning of flowering, in full flowering and in the fructification stage between June and September 2000 and 2001. Voucher specimens (No. 99330-99349) were deposited at the Department of Pharmaceutical Botany, Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia.

Growing sites, time of collecting and growth stages are presented in Table I.

All solvents used were of analytical grade (Merck, Germany).

### *Quantitative analysis*

The measurements were carried out using an Agilent 8453 UV-Visible spectrophotometer diode-array (Hewlett-Packard, Germany).

The content of flavonoids, calculated as quercetin in plant samples, was determined by the method of Christ and Müller (19). After acid hydrolysis (with 25% hydrochloric acid in acetone for 30 minutes at 100 °C), the formed aglycones were spectrometrically determined at 425 nm by creating a complex with AlCl<sub>3</sub> in a methanol-ethyl acetate-acetic acid medium (20).

The content of tannins and total polyphenols was determined by Schneider's method (21). Buffered plant extracts (pH 5.0) were shaken with casein for one hour. Casein adsorbed tannins, both condensed and hydrolyzable. The analysis was carried out spectrometrically at 720 nm after addition of Folin reagent.

### Statistical analysis

The content of flavonoids, tannins and total polyphenols was evaluated upon five independent analyses and the data were expressed as means  $\pm$  SD. The significance of the differences between-groups was determined by Student's *t*-test using the SigmaStat (version 2.0, Jandel Corporation) program.

### RESULTS AND DISCUSSION

Results of quantitative analyses of flavonoids, tannins and total polyphenols in the aerial parts of *C. maritimum* are given in Table I.

Table I. Dried basis content of flavonoids, tannins and total polyphenols in the aerial parts of *Crithmum maritimum* L.

Sample No.	Locality	Collection time	Growth stage	Flavonoids (%) <sup>a</sup>	Tannins (%) <sup>a</sup>	Total polyphenols (%) <sup>a</sup>
1	Punat	2001.	bef. fl.	0.37 $\pm$ 0.02 <sup>b</sup>	2.65 $\pm$ 0.03 <sup>b,f</sup>	8.26 $\pm$ 0.04 <sup>b,f</sup>
2			beg. fl.	0.21 $\pm$ 0.01 <sup>c</sup>	0.92 $\pm$ 0.03 <sup>c,g</sup>	5.54 $\pm$ 0.03 <sup>c,g</sup>
3			full fl.	0.27 $\pm$ 0.02 <sup>d</sup>	0.98 $\pm$ 0.03 <sup>d,h</sup>	7.27 $\pm$ 0.07 <sup>d,h</sup>
4			fruct.	0.11 $\pm$ 0.01	0.30 $\pm$ 0.05 <sup>e</sup>	5.08 $\pm$ 0.07 <sup>e</sup>
5	Zadar	2000.	bef. fl.	0.28 $\pm$ 0.02 <sup>e</sup>	0.59 $\pm$ 0.03 <sup>i</sup>	8.83 $\pm$ 0.05 <sup>i,m</sup>
6			beg. fl.	0.21 $\pm$ 0.04 <sup>f</sup>	1.94 $\pm$ 0.05 <sup>i,l</sup>	9.48 $\pm$ 0.06 <sup>i,n</sup>
7			full fl.	0.22 $\pm$ 0.04 <sup>g</sup>	0.84 $\pm$ 0.04 <sup>k,m</sup>	6.70 $\pm$ 0.04 <sup>k,o</sup>
8			fruct.	0.08 $\pm$ 0.03	0.28 $\pm$ 0.04 <sup>n</sup>	5.10 $\pm$ 0.04 <sup>l,p</sup>
9	Zadar	2001.	bef. fl.	0.31 $\pm$ 0.06 <sup>h</sup>	0.78 $\pm$ 0.02 <sup>f,i,o</sup>	7.82 $\pm$ 0.07 <sup>f,i,r</sup>
10			beg. fl.	0.21 $\pm$ 0.03 <sup>i</sup>	1.08 $\pm$ 0.03 <sup>g,j,p</sup>	7.94 $\pm$ 0.05 <sup>g,i,s</sup>
11			full fl.	0.27 $\pm$ 0.05 <sup>j</sup>	0.33 $\pm$ 0.03 <sup>h,k</sup>	6.18 $\pm$ 0.07 <sup>h,k,t</sup>
12			fruct.	0.09 $\pm$ 0.03	0.25 $\pm$ 0.02 <sup>r</sup>	4.85 $\pm$ 0.04 <sup>l,u</sup>
13	Korčula	2000.	bef. fl.	0.31 $\pm$ 0.01 <sup>e,k</sup>	0.59 $\pm$ 0.02 <sup>s</sup>	6.30 $\pm$ 0.04 <sup>m,v</sup>
14			beg. fl.	0.14 $\pm$ 0.04 <sup>f,l</sup>	0.60 $\pm$ 0.03 <sup>l,t</sup>	7.48 $\pm$ 0.05 <sup>n,w</sup>
15			full fl.	0.16 $\pm$ 0.02 <sup>g,m</sup>	0.40 $\pm$ 0.03 <sup>m,u</sup>	6.24 $\pm$ 0.07 <sup>o,x</sup>
16			fruct.	0.10 $\pm$ 0.03	0.10 $\pm$ 0.02 <sup>n</sup>	5.46 $\pm$ 0.04 <sup>p,y</sup>
17	Korčula	2001.	bef. fl.	0.42 $\pm$ 0.02 <sup>b,h,k</sup>	0.39 $\pm$ 0.04 <sup>b,o,s</sup>	6.44 $\pm$ 0.05 <sup>b,r,v</sup>
18			beg. fl.	0.33 $\pm$ 0.04 <sup>c,i,l</sup>	0.27 $\pm$ 0.03 <sup>c,p,t</sup>	5.42 $\pm$ 0.07 <sup>c,s,w</sup>
19			full fl.	0.38 $\pm$ 0.03 <sup>d,j,m</sup>	0.30 $\pm$ 0.04 <sup>d,u</sup>	5.84 $\pm$ 0.06 <sup>d,t,x</sup>
20			fruct.	0.12 $\pm$ 0.03	0.12 $\pm$ 0.03 <sup>e,r</sup>	4.72 $\pm$ 0.06 <sup>e,u,y</sup>

bef. fl. – before flowering, beg. fl. – beginning of flowering, full fl. – full flowering, fruct. – fructification

<sup>a</sup>  $\bar{x} \pm$  SD, *n* = 5

<sup>b-y</sup> Statistically significant difference (*p* < 0.05) at the same growth stage at:

- different collection times at the same location
- different locations at the same collection time

The content of total flavonoids ranged from 0.08 to 0.42%. Significant differences in the content might be due to the growth stage and locality. It is evident that the samples collected at the same locality before flowering contained the highest content of flavonoids. A consistent decrease in the content of flavonoids at the beginning of flowering is also evident. The quantity of flavonoids in the samples collected during full flowering is somewhat higher. The lowest content of flavonoids was found in the samples collected in the fructification stage. Statistical analysis of the content for the samples collected at the same location but at different time of collection showed a significant difference only for the Korčula locality in all growth stages, except the fructification stage. A significant difference in the content of the samples collected at different locations was recorded between the Punat/Korčula samples collected in 2001 and Zadar/Korčula samples collected in 2000 and 2001 for the following growth stages: before flowering, beginning of flowering and full flowering.

The content of tannins (0.12–2.65%) and the content of total polyphenols (4.72–9.48%) in the samples collected at different localities showed significant differences, *e.g.*, in the content of flavonoids, which points to a certain degree of regularity, depending on geographic coordinates of the collecting sites, only for the quantity of tannins, which decreased from the north (Punat) to the south (Korčula). The highest quantity of tannins and polyphenols at the same locality was found in the samples collected before flowering or at the beginning of flowering, whereas the samples collected in the fructification stage contained the lowest content of tannins and polyphenols.

Significant differences in the content of tannins for the samples collected at the same location but at different time of collection were determined for the Zadar and Korčula localities in all cases, except the fructification stage. Statistical analysis of the content of tannins for the samples collected at different locations showed the following results: the samples collected at Punat/Korčula localities in 2001 showed statistically significant differences ( $p < 0.05$ ) in all growth stages; there was no significant difference between Punat/Zadar localities only for the fructification stage; the samples collected at Zadar/Korčula localities before flowering in 2000 showed no difference, while the significant differences between samples collected at Zadar/Korčula localities in 2001 were observed in all growth stages except the stage of full flowering.

Significant differences in the content of total polyphenols for the samples collected at the same location but at different time of collection were recorded for the Zadar and Korčula localities in all growth stages. Statistical analysis of the content of total polyphenols for the samples collected at different locations showed the following results: the samples collected at Zadar/Korčula localities in 2000, Zadar/Korčula in 2001 and Punat/Korčula in 2001 showed significant differences in all growth stages, while significant differences between samples collected at Punat/Zadar localities in 2001 were observed in all growth stages except the stage of fructification.

The above results indicate high variability in the content of the investigated biologically active compounds. The aerial parts collected before flowering and at the beginning of flowering were the richest in flavonoids, tannins and total polyphenols.

*Acknowledgements.* – This work was supported by the Ministry of Science and Technology of the Republic of Croatia (Project No. 0006521).

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## S A Ž E T A K

### Kvantitativna analiza polifenola nadzemnih dijelova petrovca – *Crithmum maritimum* L.

ŽELJAN MALEŠ, IRENA ŽUNTAR, BILJANA NIGOVIĆ, MIŠKO PLAZIBAT I VJERA BILUŠIĆ VUNDAČ

Količina flavonoida, trjeslovina i ukupnih polifenola u nadzemnim dijelovima petrovca – *Crithmum maritimum* L. skupljenim u različitim vegetativnim razdobljima na tri nalazišta u Hrvatskoj (uz obalu Jadranskog mora) određena je spektrometrijskim postupcima.

Rezultati kvantitativne analize pokazali su razlike u količini istraživanih, biološki aktivnih spojeva, koje su bile uvjetovane vegetativnim razdobljem u kojem se biljka nalazi te zemljopisnim položajem mjesta sabiranja. Ispitivani uzorci sadržavali su 0,08–0,42% flavonoida. Najveća količina flavonoida nađena je u uzorcima skupljenim prije cvjetanja. Sadržaj trjeslovina kretao se od 0,10 do 2,65%, dok je količina ukupnih polifenola bila promjenljiva (4,72 do 9,48%). Uzorci skupljeni prije cvjetanja i na početku cvjetanja sadržavali su najveću količinu trjeslovina i ukupnih polifenola.

*Ključne riječi:* *Crithmum maritimum* L. (*Apiaceae*), flavonoidi, trjeslovine, ukupni polifenoli

*Farmaceutsko-biokemijski fakultet Sveučilišta u Zagrebu, Zagreb,*

*Botanički zavod, Prirodoslovno-matematički fakultet Sveučilišta u Zagrebu, Zagreb*