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## Determination of the content of the polyphenols of *Vitex agnus-castus* L. f. *rosea*

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Quantitative spectrometric analyses of flavonoids, tannins and total polyphenols in leaves, flowers and fruits of *Vitex agnus-castus* L. f. *rosea* from three locations in Croatia are reported. The results point to high variability of analytes content not only as a function of plant part but also sampling site and sampling stage. Content of flavonoids in leaves, flowers and fruits was found to be 0.18–0.32, 0.09–0.14 and 0.05–0.08%, respectively. The tannins content in leaves was ranging from 1.12% to 2.68%, in flowers from 0.88% to 2.28% and in fruits from 0.80% to 1.16%, whereas the content of total polyphenols varied in leaves (7.12–10.76%) and fruits (4.88–8.72%), but not in flowers (8.08–8.48%).

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**Keywords:** *Vitex agnus-castus* L. f. *rosea*, flavonoids, tannins, total polyphenols

*Vitex agnus-castus* L. (Chaste tree) is 2–4 m high shrub belonging to the family *Verbenaceae* of widespread distribution in the Mediterranean coastal region and central Asia (1, 2). It grows wild along the coastal sand shores of Adriatic Sea and river banks in Istria and Dalmatia (3, 4). *Vitex agnus-castus* contains iridoids, flavonoids,  $\Delta^4$ -3-ketosteroid hormones and an essential oil (5, 6). This plant has been used in herbal medicine since the ancient age. In the Middle Ages it was highly valued as an anaphrodisiac (4). *Vitex agnus-castus* is used as a folk remedy for disorders of the menstrual cycle (7, 8).

*Vitex agnus-castus* L. f. *rosea* is also found growing wild in Dalmatia (9). The first report dealing with the chemism of this plant has recently proved the presence of an essential oil in leaves and flowers (10). This work represents the first investigation of the content of flavonoids, tannins and total polyphenols of *Vitex agnus-castus* f. *rosea*.

### EXPERIMENTAL

#### *Plant material*

In 1996 leaves, flowers and fruits of *Vitex agnus-castus* f. *rosea* were collected from wild populations at three locations in Dalmatia: Medići, Pisak (near Omiš) and Makarska, with comparable climatic conditions. Voucher specimens have been deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia.

Collection sites and dates are presented in Table I.

Table 1. Dried basis content of flavonoids, tannins and total polyphenols in leaves, flowers and fruits of *Vitex agnus-castus* L. f. *rosea*

Plant part	Collection site	Collection date	Flavonoids (%) <sup>a</sup>	Tannins (%) <sup>a</sup>	Total polyphenols (%) <sup>a</sup>
Leaves	Medići	17.07.1996.	0.19 ± 0.03 <sup>b,c</sup>	1.12 ± 0.04 <sup>f,i,j</sup>	9.60 ± 0.15 <sup>f,h,i</sup>
	Medići	02.11.1996.	0.18 ± 0.03 <sup>e</sup>	2.68 ± 0.06 <sup>f,l</sup>	10.64 ± 0.18 <sup>f,k</sup>
	Pisak	17.07.1996.	0.32 ± 0.06 <sup>b,d</sup>	1.44 ± 0.04 <sup>g,i,k</sup>	7.60 ± 0.11 <sup>c,h,j</sup>
	Pisak	02.11.1996.	0.25 ± 0.06 <sup>e</sup>	2.60 ± 0.08 <sup>g,m</sup>	7.80 ± 0.09 <sup>c,k,l</sup>
	Makarska	17.07.1996.	0.25 ± 0.03 <sup>c,d</sup>	1.28 ± 0.04 <sup>h,j,k</sup>	7.12 ± 0.05 <sup>g,i,j</sup>
	Makarska	02.11.1996.	0.21 ± 0.04	1.84 ± 0.07 <sup>h,l,m</sup>	10.76 ± 0.13 <sup>g,l</sup>
Flowers	Medići	17.07.1996.	0.09 ± 0.03	0.88 ± 0.04 <sup>f,g</sup>	8.48 ± 0.15 <sup>b</sup>
	Pisak	17.07.1996.	0.14 ± 0.04	1.68 ± 0.07 <sup>f,h</sup>	8.08 ± 0.11 <sup>b,n</sup>
	Makarska	17.07.1996.	0.12 ± 0.03	2.28 ± 0.09 <sup>g,h</sup>	8.32 ± 0.09 <sup>n</sup>
Fruits	Medići	02.11.1996.	0.05 ± 0.03	1.16 ± 0.04 <sup>f,g</sup>	8.72 ± 0.12 <sup>f,g</sup>
	Pisak	02.11.1996.	0.08 ± 0.03	0.92 ± 0.04 <sup>f,b</sup>	5.44 ± 0.08 <sup>f,h</sup>
	Makarska	02.11.1996.	0.07 ± 0.03	0.80 ± 0.04 <sup>g,b</sup>	4.88 ± 0.09 <sup>g,h</sup>

<sup>a</sup>  $\bar{x} \pm SD$

Statistically significant difference between the sampling locations or sampling periods at significance level of:

<sup>b</sup>  $p < 0.005$ , <sup>c-e</sup>  $p < 0.05$ , <sup>f-m</sup>  $p < 0.001$ , <sup>n</sup>  $p < 0.01$ .

### Quantitative analysis

The measurements were carried out using spectrophotometer UV-1601 (Shimadzu, Japan).

The content of flavonoids calculated as quercetin in the plant samples was determined by method of Christ and Müller (11), official in *DAB 10* (12). After acid hydrolysis (with 25% hydrochloric acid in acetone for 30 minutes at 100 °C) the arisen aglycones have been spectrometrically determined at 425 nm by creating a complex with  $AlCl_3$  in medium methanol-ethyl acetate-acetic acid.

The content of tannins and total polyphenols was determined by Schneider's method (13). Buffered plant extracts (pH = 5.0) were shaken with casein for one hour. Casein adsorbed tannins, both condensed and hydrolizable. The analysis was carried out spectrometrically at 720 nm after an addition of Folin reagent.

The content of flavonoids, tannins and total polyphenols was evaluated upon five independent analyses. Statistical analysis was assessed by *F*-test and Student's *t*-test.

### RESULTS AND DISCUSSION

Results of quantitative analyses of flavonoids, tannins and total polyphenols in *Vitex agnus-castus* f. *rosea* are given in Table I.

## Flavonoids

The leaves were collected in summer and autumn, at three locations. The samples of young leaves contained 0.19%, 0.32% and 0.25% flavonoids, whereas the leaves of the same origin collected on branches with fruits contained 0.18%, 0.25% and 0.21% flavonoids. A consistent decrease in flavonoids content with ageing of leaves is evident. It may be ascribed to the distribution of flavonoids between different plant parts during vegetation. Significant difference in the content has been found among the samples of young leaves collected at different locations, and in one case only among the samples of leaves collected in November.

The total flavonoid content in flowers was ranging from 0.09% to 0.14%, whereas the samples of fruits contained 0.05–0.08% flavonoids. There was no significant difference in the flavonoid content between sampling locations.

Proportionality of flavonoid content of different parts of plant for each sampling site was also observed stressing out that the samples collected in Pisak were those richest in flavonoids. This might be ascribed to the most convenient pedological factors.

## Tannins

The samples of young leaves contained significantly smaller quantities of tannins (1.12%, 1.44%, 1.28%) in comparison with the leaves collected in a fruit period (2.68%, 2.60%, 1.84%). This might be due to the vegetation stage factors. Moreover, significant differences were found in the content of the samples of young leaves from different locations. Significant difference in the content of tannins in leaves collected in November was found for two pairs of locations.

The tannin content in the samples of flowers varied greatly (0.88%, 1.68%, 2.28%), whereas tannins in fruits were present within a range of 0.80%–1.16%. Statistical analysis showed significant differences in the content among all the locations.

## Total polyphenols

Analogously to the content of tannins, the leaves collected in the fruit period contained significantly larger quantities of total polyphenols (10.64%, 7.80%, 10.76%) in comparison with the young leaves (9.60%, 7.60%, 7.12%). Moreover, significant differences among all the locations have been found for young leaves, whereas the leaves collected in November showed differences in the content only for two pairs of locations.

The content of total polyphenols in the samples of flowers was ranging from 8.08% to 8.48%. Significant difference in the content was obtained for two pairs of locations.

The total polyphenol content in the samples of fruits collected at different locations (8.72%, 5.44%, 4.88%) also showed significant differences.

The above results indicate high variability in the content of investigated biologically active compounds in various plant parts. Significant differences in their content might be due to pedological and/or vegetation stage factors. The investigations in this direction are already under way.

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## S A Ž E T A K

### Određivanje sadržaja polifenola ružičaste konopljike – – *Vitex agnus-castus* L. f. *rosea*

ŽELJAN MALEŠ

Količina flavonoida, trjeslovina i ukupnih polifenola u listovima, cvjetovima i plodovima ružičaste konopljike (*Vitex agnus-castus* L. f. *rosea*) skupljenima na tri nalazišta u Hrvatskoj određena je spektrometrijskim metodama. Rezultati kvantitativne analize pokazali su razlike u količini istraživanih spojeva koje su bile uvjetovane biljnim organom, nalazištem i vegetacijskim čimbenicima. Količina flavonoida bila je različita u pojedinim biljnim organima (listovi 0,18–0,32%, cvjetovi 0,09–0,14%, plodovi 0,05–0,08%). Sadržaj trjeslovina u listovima kretao se od 1,12 do 2,68%, u cvjetovima od 0,88–2,28%, a u plodovima od 0,80–1,16%. Količina ukupnih polifenola bila je promjenljiva u listovima (7,12–10,76%) i plodovima (4,88–8,72%), a slična u cvjetovima (8,08–8,48%).

*Ključne riječi:* *Vitex agnus-castus* L. f. *rosea*, flavonoidi, trjeslovine, ukupni polifenoli

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