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## Quantitative analysis of the polyphenols and tannins of *Vitex agnus-castus* L.\*

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The content of total polyphenols and tannins in the leaves, flowers and fruits of *Vitex agnus-castus* L. collected from various parts of Croatia were determined by spectrometric method according to Schneider. The investigation results showed the variety of total polyphenol content between plant parts (leaves 7.36–13.20%, flowers 9.00–10.64% and fruits 6.92–13.24%). The highest tannin content was found in leaves (0.68–3.00%), but it was similar in flowers and fruits (0.24–2.00%, respectively 0.24–1.60%).

**Keywords:** *Vitex agnus-castus* L., polyphenols, tannins

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Chaste tree – *Vitex agnus-castus* L. is a species of the *Verbenaceae* family growing in the Mediterranean coastal region and in Central Asia. In Croatia this plant grows wildly, especially along the coastal sand of Adriatic Sea and river banks in Istria, Croatian Littoral and Dalmatia (1). The leaves, flowers and fruits of *Vitex agnus-castus* contain iridoids (2), flavonoids (casticin, isovitexin, isovitexin xylosid, orientin, isoorientin, penduletin and chryso-splenol D) (3),  $\Delta^4$ -3-ketosteroid hormones (4) and the essential oil, which are responsible for a number of hormonal activities in the human endocrine system (5). In the ancient times and in the Middle Ages this plant was highly valued as an antiaphrodisiacum and it was used in herbal medicine for curing many illnesses (6).

In our previous paper (7), we determined the flavonoid content in different plant parts of *Vitex agnus-castus*. No literature date could have been found concerning the determination of total polyphenol content and tannin content of *Vitex agnus-castus*. The polyphenols could be considered as antimicrobial agents (8). They have an influence on the antimicrobial effect of *Vitex agnus-castus* (9). Therefore, the content of total polyphenols and tannins in the leaves, flowers and fruits of this plant has been determined.

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## EXPERIMENTAL

*Plant material*

Leaves, flowers and fruits of *Vitex agnus-castus* were collected in the coastal region and on the islands of the Adriatic (1991 and 1993) as well as in the gardens of Zagreb during 1993. The collection locations and dates are presented in Tables I and II.

*Quantitative analysis*

The spectrophotometer SP 8-100 (Pye Unicam, Cambridge, UK) was used for the light absorption measurements. The content of total polyphenols and tannins in the plant samples was spectrometrically determined by Schneider's method (10). This procedure was repeated five times in all the samples, and from these results an average value ( $\bar{x}$ ) and standard error (SE) were calculated. The reproducibility of this determination was  $\pm 0.08\%$  ( $n = 5$ ).

## RESULTS AND DISCUSSION

Table I. Dried basis content of total polyphenols and tannins in leaves of *Vitex agnus-castus* L.

Location	Collection time	Total polyphenols $\bar{x} \pm \text{SE} (\%)$	Tannins $\bar{x} \pm \text{SE} (\%)$
Rabac	22.06.1991.	8.64 $\pm$ 0.08	1.32 $\pm$ 0.07
Rabac	26.07.1993.	9.52 $\pm$ 0.06	1.20 $\pm$ 0.03
Kraljevica	22.06.1991.	9.08 $\pm$ 0.11	2.16 $\pm$ 0.10
Kraljevica	26.07.1993.	10.08 $\pm$ 0.09	1.40 $\pm$ 0.08
Vir	23.06.1991.	7.36 $\pm$ 0.07	0.72 $\pm$ 0.05
Vir	25.07.1993.	9.36 $\pm$ 0.12	0.68 $\pm$ 0.11
Medulin	31.07.1993.	9.60 $\pm$ 0.09	1.52 $\pm$ 0.07
Makarska F	16.10.1993.	10.48 $\pm$ 0.07	1.24 $\pm$ 0.08
Makarska S	16.10.1993.	9.04 $\pm$ 0.09	1.24 $\pm$ 0.07
Bot. Garden »F. Kušan«	08.07.1993.	8.84 $\pm$ 0.11	1.52 $\pm$ 0.08
Bot. Garden »F. Kušan«	20.07.1993.	10.08 $\pm$ 0.05	0.96 $\pm$ 0.07
Bot. Garden »F. Kušan«	28.09.1993.	13.20 $\pm$ 0.04	3.00 $\pm$ 0.07
Garden »A«	08.07.1993.	10.00 $\pm$ 0.06	2.80 $\pm$ 0.08
Garden »A«	19.07.1993.	10.72 $\pm$ 0.08	2.40 $\pm$ 0.07
Garden »A«	05.10.1993.	8.28 $\pm$ 0.03	1.60 $\pm$ 0.06
Garden »B«	08.07.1993.	9.20 $\pm$ 0.10	2.40 $\pm$ 0.06
Garden »B«	19.07.1993.	12.00 $\pm$ 0.09	2.40 $\pm$ 0.05
Garden »B«	05.10.1993.	8.00 $\pm$ 0.06	1.28 $\pm$ 0.05

$\bar{x}$  = average value, SE = standard error

During two years (1991 and 1993) the leaves were collected in the coastal region (Rabac, Kraljevica, Medulin, Makarska) and on the island of Vir of the Adriatic and in the

gardens of Zagreb during 1993. The samples of leaves from Rabac, Kraljevica and Vir collected in 1991 contained lower quantities of total polyphenols (8.64%, 9.08%, 7.36%) in comparison with the samples of the same origin collected in 1993 (9.52%, 10.08%, 9.36%). The content of tannins in the same samples is higher than in the samples collected in 1993. The samples of leaves from Medulin contained 9.60% total polyphenols and 1.52% tannins. The sample of leaves marked with Makarska F was collected on blooming branches and contained 10.48% total polyphenols, and the sample of leaves marked with Makarska S was collected on branches with fruits and contained 9.04% total polyphenols. It was evident that the content of total polyphenols was higher in blooming branches. The samples collected on July 8, 1993 in the gardens A and B represented the leaves from the branches just before blossoming and contained 10.00% and 9.20% total polyphenols. The samples collected on July 19, 1993 were taken from the leaves of the branches in full blossom with total polyphenols content of 10.72% and 12.00%, respectively, whereas the samples from the leaves on the branches with fruits collected on October 5, 1993 contained 8.28% and 8.00% total polyphenols. The highest content was found in the samples of the leaves with blooming branches and the lowest in the leaves at the fruit time. The highest content of total polyphenols (13.20%) and tannins (3.00%) was found in the sample from the Botanical Garden »F. Kušan« collected during the fruit period on September 28, 1993.

The content of total polyphenols and tannins in flowers of Chaste tree collected at different locations along the Adriatic Coast and in the gardens of Zagreb is presented in Table IIa.

Table II. Dried basis content of total polyphenols and tannins in flowers and fruits of *Vitex agnus-castus* L.

Location	Collection date	Total polyphenols $\bar{x} \pm SE$ (%)	Tannins $\bar{x} \pm SE$ (%)
a) in the flowers			
Rabac	26.07.1993.	10.12 $\pm$ 0.08	1.44 $\pm$ 0.07
Kraljevica	26.07.1993.	10.20 $\pm$ 0.10	0.68 $\pm$ 0.04
Medulin	31.07.1993.	10.54 $\pm$ 0.02	0.24 $\pm$ 0.09
Makarska	16.10.1993.	10.12 $\pm$ 0.08	0.68 $\pm$ 0.10
Bot. Garden »F. Kušan«	20.07.1993.	9.00 $\pm$ 0.05	1.28 $\pm$ 0.06
Garden »A«	19.07.1993.	10.36 $\pm$ 0.12	1.80 $\pm$ 0.10
Garden »B«	19.07.1993.	9.60 $\pm$ 0.06	2.00 $\pm$ 0.08
b) in the fruits			
Medulin	31.07.1993.	8.52 $\pm$ 0.09	0.24 $\pm$ 0.12
Makarska	16.10.1993.	9.64 $\pm$ 0.07	0.72 $\pm$ 0.05
Bot. Garden »F. Kušan«	28.09.1993.	10.00 $\pm$ 0.06	1.60 $\pm$ 0.11
Bot. Garden »F. Kušan« I	26.10.1993.	7.84 $\pm$ 0.08	1.20 $\pm$ 0.04
Bot. Garden »F. Kušan« II	26.10.1993.	6.92 $\pm$ 0.06	1.00 $\pm$ 0.11
Garden »A«	05.10.1993.	8.88 $\pm$ 0.08	1.24 $\pm$ 0.06
Garden »B«	05.10.1993.	13.24 $\pm$ 0.13	0.88 $\pm$ 0.04

$\bar{x}$  = average value, SE = standard error

There is no significant difference in the total polyphenol content in the samples of flowers. The highest total polyphenol content (10.64%) and the lowest tannin content (0.24%) was found in the flowers sample from Medulin collected at the end of blooming. The lowest total polyphenol content (9.00%) was found in the sample from the Botanical Garden »F. Kušan«, and the highest tannin content (2.00%) was determined in the sample from the garden B. The flower sample from Makarska was collected in autumn *i.e.* on October 16, 1993 during ripening of fruits, however it showed no significant difference in total polyphenol (10.12%) and tannin content (0.68%) in comparison with other flower samples.

Table IIb represents the results of the assay of total polyphenol and tannin content in the fruits of Chaste tree collected in 1993. The sample from Medulin represents green fruits collected on July 31 at the end of blooming and at the beginning of fruit formation, respectively. The sample of green fruits had the lowest tannin content (0.24%). The highest tannin content was found in the fruits from the Botanical Garden »F. Kušan« (1.60%) collected on September 28, 1993.

Significant difference in total polyphenol content has been found in the samples of fruits. The lowest total polyphenol content (6.92%) was found in the fruits from the Botanical Garden »F. Kušan« II and the highest content in the fruits from the garden B (13.24%).

Summarizing the results it has been found that the total polyphenol content varied. In leaves it was ranging from 7.36 to 13.20%, in flowers from 9.00 to 10.64% and in fruits from 6.92 to 13.24%. The highest tannin content has been found in leaves (0.68–3.00%), however, in flowers (0.24–2.00%) and in fruits (0.24–1.60%) it was similar.

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S A Ž E T A K

**Kvantitativna analiza polifenola i treslovina konopljike *Vitex agnus-castus* L.**

ANKICA ANTOLIĆ i ŽELJAN MALEŠ

Istraživanja u ovom radu obuhvatila su određivanje količine ukupnih polifenola i treslovina u listovima, cvjetovima i plodovima vrste *Vitex agnus-castus* L. Biljni uzorci sakupljeni su 1991. i 1993. godine na različitim lokacijama u Hrvatskoj. Sadržaj ukupnih polifenola i treslovina odredivan je spektrometrijskom metodom prema Schneideru. Rezultati istraživanja pokazali su razliku u sadržaju ukupnih polifenola među biljnim organima (listovi 7,36–13,20%, cvjetovi 9,00–10,64%, plodovi 6,92–13,24%). Sadržaj treslovina bio je najviši u listovima (0,68–3,00%, a vrlo sličan u cvjetovima (0,24–2,00%) i plodovima (0,24–1,60%).

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