



Determination of the content of total polyphenols, non-tannin polyphenols and tannins in five species of the genus *Teucrium* L.

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Abstract

Background and Purpose: Many species of the genus *Teucrium* L. are used in folk medicine. These species contain various chemical compounds that contribute to its biological activities. No literature could be found in respect of the determination of total polyphenols, non-tannin polyphenols and tannins in the species *T. halacsyanum* Heldr., *T. hyrcanicum* L. and *T. scorodonia* L. Quantitative analysis of polyphenols and tannins was therefore performed.

Materials and Methods: Plant material was collected over two consecutive seasons in the Pharmaceutical Botanical Garden “Fran Kušan” of the Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia. The content of total polyphenols, non-tannin polyphenols and tannins was determined by combination of spectrophotometric and hide powder method.

Results: The quantity of total polyphenols of investigated species ranged from 0.87 to 4.1%. The content of polyphenols unadsorbed on hide powder (non-tannin polyphenols) was between 0.83 and 3.4%, while the quantity of tannins ranged from 0.04 to 1.4%. The sample of *T. scorodonia* that grows along the hedge contained the highest content of total polyphenols (4.1%) and tannins (1.4%), while the highest quantity of non-tannin polyphenols was found in the sample of *T. chamaedrys* (3.4%) collected in 2013. The sample of *T. botrys* collected in 2012 contained the lowest amounts of all investigated compounds.

Conclusion: For the first time the content of total polyphenols, non-tannin polyphenols and tannins in the species *T. halacsyanum*, *T. hyrcanicum* and *T. scorodonia* is reported. The content of all investigated compounds depended on the plant species investigated and year of collection.

INTRODUCTION

Teucrium L. is a large genus of about 200 species of flowering plants in the family Lamiaceae (1). The species *T. botrys* L., *T. chamaedrys* L. and *T. scorodonia* L. are widespread in Croatia (2). *T. halacsyanum* Heldr. grows in the Eastern Mediterranean (Greece and Egypt) (3), while *T. hyrcanicum* L. is distributed in the region of Caucasus, Iran and Turkey (4).

The species of the genus *Teucrium* contain various secondary metabolites such as flavonoids, phenolic acids, tannins, bitter principles, essential oil, saponins, sterols, sugars and microelements that contribute

to its biological activities (4-8). Many species of the genus are used in folk medicine as antidiabetics, diuretics, diaphoretics, expectorants, spasmolytics, antioxidants, antiseptics and stomachics (7, 9).

Polyphenols are known as antioxidants, while tannins comprise a large group of complex substances that are widely distributed in the plant kingdom and have anti-diarrhoeal, antioxidant and antiseptic properties (10).

As no literature data could be found in respect of the determination of total polyphenols, non-tannin polyphenols and tannins in the species *T. halacsyanum*, *T. hyrcanicum* and *T. scorodonia*, quantitative analysis of total polyphenols, non-tannin polyphenols and tannins in five cultivated species of the genus *Teucrium* was conducted.

MATERIALS AND METHODS

Plant material and chemicals

Five species of the genus *Teucrium* (*T. botrys*, *T. chamaedrys*, *T. halacsyanum*, *T. hyrcanicum* and *T. scorodonia*) were cultivated in the Pharmaceutical Botanical Garden "Fran Kušan" of the Faculty of Pharmacy and Biochemistry University of Zagreb. The garden is located in the northern part of Zagreb - 45° 50' N, 15° 59' E, at the 195 m height above sea-level, with prevailing pseudo clay and western exposition. Climate is temperate with moderate changes in temperature and rainfall distributed throughout the year without extreme dry periods. The greatest amount of precipitation falls in late spring and autumn, and between them is a moderate dry period. The average monthly temperature in the coldest month (January) is not less than -0.4 °C, while the warmest month (July) does not exceed 22.2 °C.

Voucher specimens (No. 125000-125011) were deposited in the Herbarium of the Department of Pharmaceu-

tical Botany of the Faculty of Pharmacy and Biochemistry, University of Zagreb. The aerial parts were collected during flowering period in July 2012 and 2013 and dried at room temperature.

All solvents (Kemika, Zagreb) and reagents (Merck, Germany) used were of analytical grade.

Quantitative analysis

The measurements were carried out using spectrophotometer Varian Cary 50 Bio (Varian Inc., USA).

The determination of total polyphenols, polyphenols unadsorbed on hide powder (non-tannin polyphenols) and tannins was performed by the spectrophotometric method with phosphorous - tungsten acid and hide powder (11, 12).

Statistical analysis

The content of total polyphenols, non-tannin polyphenols and tannins was evaluated upon five independent analyses and data were expressed as means \pm SD. The statistical analysis between samples collected in 2012 and 2013 was performed by Student's *t*-test ($\alpha = 0.05$) using R program (www.r-project.org).

RESULTS AND DISCUSSION

The results of quantitative analysis of total polyphenols, non-tannin polyphenols and tannins are presented in Table 1.

The quantity of total polyphenols of investigated samples ranged from 0.87 to 4.1%. The content of total polyphenols in the sample of the species *T. scorodonia* that grows along the hedge was found to be approximately

TABLE 1. Dried basis content of total polyphenols (w, %) in the investigated species of the genus *Teucrium*.

Sample	Species	Year of collection	Total polyphenols (%)	Non-tannin polyphenols (%)	Tannins (%)
1	<i>T. botrys</i>	2012	0.87 \pm 0.04	0.83 \pm 0.01	0.04 \pm 0.03
2	<i>T. botrys</i>	2013	1.68 \pm 0.04**	1.15 \pm 0.01**	0.53 \pm 0.03**
3	<i>T. chamaedrys</i>	2012	3.13 \pm 0.01	3.02 \pm 0.06	0.11 \pm 0.03
4	<i>T. chamaedrys</i>	2013	3.5 \pm 0.2	3.4 \pm 0.2	0.17 \pm 0.04*
5	<i>T. halacsyanum</i>	2012	2.2 \pm 0.1	1.14 \pm 0.01	1.06 \pm 0.09
6	<i>T. halacsyanum</i>	2013	4.0 \pm 0.1**	3.0 \pm 0.2*	0.99 \pm 0.03
7	<i>T. hyrcanicum</i>	2012	2.8 \pm 0.1	2.2 \pm 0.09	0.61 \pm 0.03
8	<i>T. hyrcanicum</i>	2013	2.11 \pm 0.03	1.60 \pm 0.03	0.51 \pm 0.01
9 ho	<i>T. scorodonia</i>	2012	2.4 \pm 0.01	2.24 \pm 0.07	0.19 \pm 0.04
10 ho	<i>T. scorodonia</i>	2013	1.83 \pm 0.08*	1.5 \pm 0.2	0.4 \pm 0.1
11 he	<i>T. scorodonia</i>	2013	4.1 \pm 0.1	2.7 \pm 0.1	1.4 \pm 0.2

% = Mean \pm SD, n = 5, ho = hotbed, he = hedge, * p < 0.05, ** p < 0.01.

twice higher, than the content in the sample of *T. scorodonia* from the hotbed.

The content of polyphenols unadsorbed on hide powder (non-tannin polyphenols) was between 0.83 and 3.4%. The quantity of non-tannin polyphenols in the sample of the species *T. scorodonia* that grows along the hedge was found to be higher in comparison with the content in the sample of *T. scorodonia* from the hotbed.

The investigated samples contained 0.04 to 1.4% tannins. The content of tannins in the sample of the species *T. scorodonia* that grows along the hedge was found to be approximately five times higher than the content in the sample of *T. scorodonia* from the hotbed (Table 1).

The content of non-tannin polyphenols in comparison with content of tannins was greater in all investigated *Teucrium* species.

The above results indicate high variability in the content of total polyphenols, non-tannin polyphenols and tannins that depended on the plant species investigated and the slight weather conditions differences between two compared sampling years (13). Meteorological conditions for July 2012 and 2013 in Zagreb were described as extremely warm, but an average rainfall in July 2012 was slightly higher.

The investigated samples contained lower quantities of total polyphenols, non-tannin polyphenols and tannins in comparison with some other native *Teucrium* species (6). The chemical variability in *Teucrium* species may reflect environmental influence on the content of investigated compounds (14). The content of total polyphenols and tannins in the samples of the species *T. botrys* and *T. chamaedrys* is significantly lower in comparison to those reported Jurišić Grubešić *et al.* (5). However, this can be attributed to the different method used for quantitative analysis of polyphenols and tannins.

To the authors' knowledge, based on literature search this is the first time that the content of total polyphenols, non-tannin polyphenols and tannins in the species *T. halacsyanum*, *T. hyrcanicum* and *T. scorodonia* is reported. Polyphenols represent most abundant and widely distributed antioxidants that have beneficial health promoting

effects. Our results confirm that *Teucrium* species are good source of polyphenols and can be basis for further biological studies.

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