POLYPHENOLIC PROFILES OF SELECTED MEDICINAL HERBS

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Abstract: The total phenolic contents and contents of main individual phenolics in plant extracts of bilberry, willow gentian, wild raspberry, spearmint and peppermint were determined. The above-ground parts of plants were subjected to the exhaustive extraction with ethanol using Soxhlet apparatus. The total phenolic content for the medicinal herbs, ranged from 2.8 to 15.2 mg GAE/g dm, were as follows: spearmint > peppermint > willow gentian > wild raspberry > bilberry. Using high-performance liquid chromatography, an extremely high content of rosmarinic acid was revealed in peppermint sample (849 mg/100 g dm). The plant of bilberry was characterized by greater amounts of chlorogenic acid, rutin and sinapinic acid (367, 248, and 229 mg/100 g dm, respectively) than the above-ground parts of other analyzed species.

Key words: Soxhlet extraction, rosmarinic acid, *Rubus*, *Lamiaceae*, liquid chromatography

Introduction

Medicinal herbs have attracted the scientific interest of the biotechnology, pharmaceuticals and food industry due to their sourceness of phytochemicals with potential antioxidative properties. These effects are mainly addressed to biologically active components that are naturally present in plants, spices and herbs, the most important of which being the phenolic compounds, carotenoids, vitamins, etc. (Embuscado, 2015).

Mentha belongs to *Lamiaceae* family and includes about 40 species. Spearmint and peppermint are commonly produced as a crop for their essential oils for food products, cosmetics and pharmaceuticals. It is also being utilized in folk medicine, due to its ability of suppressing allergies, asthma and atherosclerosis. It was actually revealed that rosmarinic acid, a naturally occurring compound of peppermint and spearmint and potent polyphenolic antioxidant, is responsible for these health benefits (Fletcher et al., 2009). The genus *Rubus* comprises around 700 species, with only ten being used commercially. Apart from cultivation of raspberries and blackberries as costly edible fruits, leaf extracts of *Rubus* species have been used in traditional medicine as antidiarrhoeics, hypoglycemic, choleretic and anti-inflammatory agents, as well as menstrual and colic pain-killer (Gudej et al., 2004; Patel et al., 2004).

Willow gentian belongs to the genus *Gentiana* that comprises about 400 species, with 29 species recorded in Europe, and eleven of them being distributed in Serbia (Mihailović et al., 2013). Willow gentian is used in Serbia as traditional medicine for hepatitis infections. The main secondary metabolites found in *Gentiana asclepiadea* L. are iridoids, secoiridoides, xanthones and flavonoids (Krstić et al., 2004), but with no

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correlation proved between the biochemical composition of this plant and its biological activity or therapeutic uses.

The objective of work herein was to reveal the polyphenolic fingerprints of selected medicinal herbs, highly used in folk medicine, and by that to justify the usage of these herbs in traditional medicine.

Material and methods

Plants of bilberry (*Vaccinium myrtillus* L), willow gentian (*Gentiana asclepiadea* L.), wild raspberry (*Rubus idaeus* L.) and spearmint (*Mentha spicata* L.) were collected from a mountain of Čemerno (43°34'57″N 20°25′33″E), Serbia, while peppermint (*Mentha piperita* L.) was collected from Vranovo (44°36′02″N 20°59′24″E), the village situated in the Southern Serbia. Plant samples were collected in June 2016, and only the above-ground parts of the plants were utilized for analyses (Figure 1).

Фигура 1. Надземни део тестираних биљака: дивља боровница (a); плава линцура (b); дивља малина (c); дивља нана (d); питома нана (e).
Figure 1. Above-ground parts of tested plants: bilberry (a); willow gentian (b); wild raspberry (c); spearmint (d); peppermint (e).

The dry matter content was determined by drying fresh plant samples at 105ºC until constant mass.

Exhaustive extraction with ethanol was performed in Soxhlet apparatus, to which exactly weighted (20 g) and grounded portions of plant materials were placed in a thimble-holder. Continuous extraction was performed for 6 h. The obtained extracts were evaporated to dryness, and transferred into the oven at 50°C until the constant weight. Extraction yield was measured by evaporation of ethanol after the extraction until the constant mass.

Total phenolic content was determined using a modified Folin-Ciocalteu colorimetric method (Singleton et al., 1999), with results expressed as milligrams of gallic acid equivalents per 100 g dry matter (mg GAE/g dm).

Samples were analyzed using an Agilent 1200 series HPLC (Agilent Technologies, Santa Clara, CA, US), linked to a linked to a ChemStation data handling system, using a Eclipse XDBC-18 (4.6×150 mm). Injection volume was 5 μL and the temperature was set at 25ºC. Solvent A was formic acid (2%) in water and solvent B was acetonitrile (80%) and formic acid (2%) in water. The gradient used was as follows: 0─10 min, pure A; 10─28 min, 0─25% of B in A; 28─30 min, 25% of B in A; 30─35 min, 25-50% of B in A; 35-40 min, 50-80% of B in A, and 40-45 min, 80-0% of B in A. A good purity and separation was achieved in all tested samples. Phenolic compounds were identified according to peak retention time and UV/Vis spectra by comparing them with those of the standards. The quantities of the different phenolic compounds were based on peak areas, and expressed as mg/100 g dm.

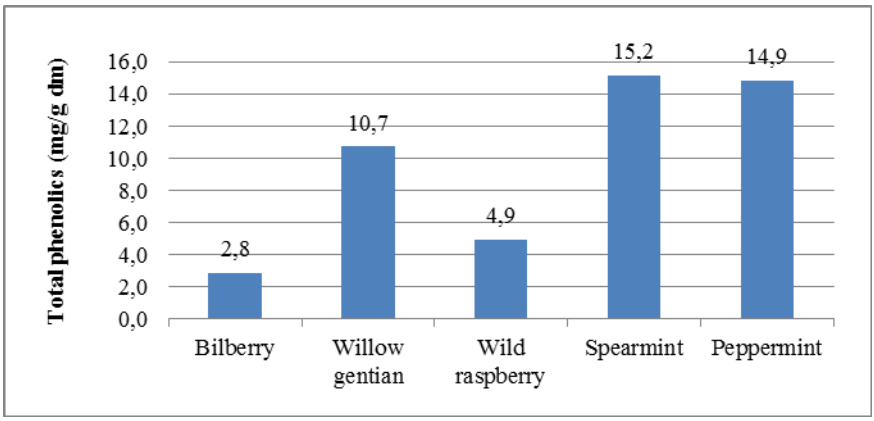
Results and discussion

Dry matter content of fresh herbs and extraction yields were calculated and given in Table 1. One can notice that the lowest extraction yield was obtained for peppermint (8.6%), while the highest extraction content was achieved for willow gentian (29.5%). All tested herbs have similar dry matter content.

Табела 1. Сува материја и екстракциони принос анализираних биљака.
Table 1. Dry matter content and extraction yield of tested herbs.

	Bilberry	Willow gentian	Wild raspberry	Spearmint	Peppermint
Dry matter content (%)	91.6	93.3	89.3	90.6	94.7
Extraction yield (%)	26.5	29.5	30.1	14.9	8.6

Total phenolic content was measured for all tested samples and the results are presented in Figure 2. The total phenolic contents showed great variation in different herbs. As can be noticed, the highest level of total phenolics was obtained for spearmint and peppermint, followed by willow gentian, while the lowest level was determined in wild raspberry and bilberry. The results obtained for spearmint are in great agreement with results by other researchers (Fadel et al., 2010; Orphanides et al., 2013). Capecka et al. (2005) found in fresh and dried peppermint that total phenolic contents were 19.54 and 25.80 mg/g of fresh weight, respectively. Nevertheless, Huang et al. (2013) determined quite higher polyphenolic content of different G*entiana* species (ranging from 42.28 to 102.24 mg GAE/g dm) compared to the same content of willow gentian in our work (10.7 mg GAE/g dm).



Фигура 2. Садржај полифенола (mg ГКЕ/g см) анализираних биљака.
Figure 2. Total phenolic content (mg GAE/g dm) of tested herbs.

Analyzing HPLC-DAD chromatograms of aqueous ethanol extracts of medicinal herbs, plenty of peaks were clearly identified. Identified compounds were quantified and summarized in Table 2. Great variations in different herb samples can be clearly observed. The highest content of polyphenolic of the above-ground parts of the plants, analyzed by HPLC, were determined in peppermint, followed by bilberry and spearmint, wild raspberry and willow gentian. The most abundant compounds in bilberry were chlorogenic acid, sinapinic acid and rutin. Rosmarinic acid was the most dominant compound in peppermint. In our previous work, it was published that dried fruits of bilberry posses very high content of polyphenolics, particularly in quercetin, gallic acid, chlorogenic acid and *p*-coumaric acid (Miletić et al., 2014). Therefore it is not surprising that above-ground parts of the bilberry are also rich in certain phenolic compounds.

Табела 2. Садржај најзаступљених полифенола (mg/100 g см) у биљним узорцима
Table 2. Content of main phenolics (mg/100 g dm) in selected medicinal herbs

	Bilberry	Willow gentian	Wild raspberry	Spearmint	Peppermint
p-Hydroxybenzoic acid	1.05			1.21	3.71
Caffeic acid				0.80	
Chlorogenic acid	367.0		6.41	1.21	28.2
p-Coumaric acid	50.6			1.40	2.86
Ferulic acid	3.37		3.38	0.54	5.14
Sinapinic acid	229.0		1.51	2.23	
Rutin	248.0	6.00		6.87	31.6
Luteolin	32.4		0.71		
Apigenin		0.160			4.18
Rosmarinic acid			12.5	59.4	849.0
Quercetin	56.8	0.42	6.11	16.1	17.9
Kaempferol	3.45		3.25	13.6	21.5

Areias et al. (2001) found very high content of rosmarinic acid in fourteen samples of peppermint (from 759 to 4429 mg/kg, dry basis), but being quite lower compared to our peppermint sample (8490 mg/kg, dry basis). It was reported that accumulation of rosmarinic acid in spearmint and peppermint is strongly influenced by the physiological age of the plant, with the highest level present in vegetative state prior to flowering, and soil type, with clay soil base being the best due to the water holding capacity which favors rosmarinic acid production (Fletcher et al., 2009). There are very few publications regarding the polyphenolic profiles of willow gentian. As reported by Gudej and Tomczyk (2004), the contents of quercetin and kaempferol in leaves of wild raspberry (*Rubus idaeus* L.) are 0.21 and 0.18 % of dry weight, respectively, which is quite higher compared to our corresponding samples (6.11 and 3.25 mg/100 g dm).

Conclusion

Extracts of five medicinal herbs were analyzed for total phenolic content and contents of main individual phenolics. Regarding the highest levels of phenolic contents, plants of peppermint and spearmint are distinguishable from other plant species analyzed. High performance liquid chromatography revealed significant contents of selected flavonols and phenolic acids, present in analyzed plant extracts. Extremely high content of rosmarinic acid was determined in peppermint plant, while bilberry plant sample showed quite greater amounts of chlorogenic acid, rutin and sinapinic acid. All these analytical results justified the long tradition of usage of tested plants in folk medicine.

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