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Comparative analysis of phenolic and mineral composition of traditionally used wild medicinal plants from Southeast Serbia

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In this study a comparative analysis was carried out of the contents of total phenols, flavonoids and metals in different extracts of wild winter savory (*Satureja montana* L.) and herb peter (*Primula vulgaris* L.*)* plants.

The results showed that the content of total phenols in the investigated extracts is very high. The difference in the total content of phenolic compounds depends on the extraction solution. The content of the metal is higher in the extracts of the *Primula vulgaris* L. plant compared to the extracts of the plant *Satureja Montana* L. There is a high correlation coefficient between total phenols and flavonoids in both plants. Also, there is a high correlation coefficient between total content of phenol and flavonoids and the contents of Mn and Cu in the plant extracts of *Satureja montana* L., and the content of Mn in the plant extracts of *Primula vulgaris* L. The two cultivars studied: wild winter savory and herb peter plant from Southeast Serbia are suitable for the preparation of teas and herbal extracts because they are an excellent source of polyphenol compounds and a good source of the minerals Fe, Cu, Zn and Mn.

Keywords: total phenols, flavonoids, metals, *Satureja montana* L., *Primula vulgaris* L.

INTRODUCTION

The potency of different medicinal plants is related to their individual mechanisms of action in different disorders. Humans consume and use a variety of medicinal plants in the form of teas, extracts, tinctures and other. Although medicinal plants are widely considered to be of lower risk compared with synthetic drugs, they are not completely free from the possibility of toxicity or other adverse effects [1], therefore, analysis of the plants used in the treatment is necessary. Phenolic compounds from plants belong to a class of bioactive components with antioxidant activities [2, 3]. Flavonoids represent one of the most studied classes of phenolic compounds containing carbohydrate units important for their biological activities [4]. Flavonoids exhibit a wide range of biological effects (antibacterial, antiviral, anti-inflammatory and anti-allergic) by reducing low-density lipoproteins in plasma, inhibiting platelet aggregation, scavenging free radicals, and preventing cell proliferation [5]. However, there is considerable interest in identifying natural antioxidants from plants that protect against free radical damage as an alternative to synthetic medicines.

Winter savory (*Satureja montana* L.) belongs to the *Lamiaceae* family native to the Mediterranean regions. It is a perennial herb (20–30 cm tall) with white flowers and small rough leaves[6]. Winter savory is a well-known aromatic and medicinal plant which contains various biologically active constituents such as essential oil, triterpenes [7], flavonoids [8], and rosmarinic acid [9]. The whole herb is mildly antiseptic, aromatic, carminative, digestive, mildly expectorant and stomachic, while its essential oil is used in the food industry, liqueurs and in perfumery. The positive effects of savory on human health are attributed to its active constituents which show a high antioxidant effect [10]. The typical phenolic compound of *Satureja montana* L. is carvacrol, and the prevailing carvacrol chemotype occurs also in Italy and the former Yugoslavia [11].

Herb peter (*Primula vulgaris* L.), *Primula* is a plant genus including about 400 species. Some of them are popular garden plants because of their colourful blossoms. Efficacy of primrose extracts, which are rich in saponins, has been demonstrated in a number of pharmacological studies, as potent anti-asthmatic, anti-inflammatory and antiviral properties. Phenolic glycosides and saponins are characteristic compounds for the genus *Primula* [12]. Flavonoids may have existed in nature for over one billion years. Methoxyflavones have important effects in plant biochemistry and physiology, acting as antioxidants, enzyme inhibitors, precursors of toxic substances and have long been recognized to possess anti-allergic, anti-inflammatory, antiviral, anti-proliferative and anti-carcinogenic activities,

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as well as to affect some aspects of mammalian metabolism [13]. Ten lipophilic flavones were isolated from *Primula veris* L. *in vitro* cultures. Two new flavonol glycosides have been identified and isolated from Italian *Primula* species [14]. *Primula* species can also contain allergens and some species are used traditionally to treat epilepsy and convulsions.

Although the effectiveness of medicinal plants is mainly associated with their constituents such as essential oils, vitamins, glycosides, etc., it was found that prolonged intake can cause health problems due to the possible presence of heavy metals [15]. Plants can easily be contaminated by heavy metals in the course of cultivation or later during the processing stage and, therefore, determining the content of the accumulated heavy metals is of high importance. The content of heavy metals is one of the criteria for the use of plant material in the production of traditional medicines and herbal infusions. Therefore, control of heavy metals in medicinal plants and their products should be made such to ensure safety and efficacy of herbal products [16].

However, there is no information about comparative analysis of contents of total phenols, flavonoids, and metals in the different extracts of the herbs peter plant (*Primula vulgaris* L.) and winter savory (*Satureja montana* L.).

The present study investigated the contents of total phenols, flavonoids, and heavy metals of herb peter and winter savory in order to evaluate their medicinal value and to point to an easily accessible source of natural antioxidants that could be used as a possible food supplement or in the pharmaceutical industry. Therefore, this work represents the first report on phenolic content and contents of heavy metals in different extracts of two plant cultivars of wild winter savory and herb peter plant from Southeast Serbia.

Statistical analysis was used to evaluate possible correlations among metal ions amount, and the amounts of phenolic compounds and flavonoids in the investigated extracts.

EXPERIMENTAL

Preparation of materials

Fully dry samples of winter savory *(Satureja montana* L.*)* and herb peter (*Primula vulgaris* L.), collected throughout the months of April and May 2017, were used for the investigation. This region of Serbia (Soko Banja) at the foothills of the Rtanj mountain is said to be free of negative environmental influences, as it is largely devoid of industries and major highways.

Determination of selected metals

The standard procedure for the determination of selected metals described by the Association of Official Analytical Chemists (AOAC) was followed for the preparation of samples for the analysis of heavy metals [17]. Accurately weighed (2 g) sample was transferred into a silica crucible and kept in a muffle furnace for ashing at 450°C for 3 h and then 5 ml of 6 M HCl was added to the crucible. Care was taken to ensure that all ash came into contact with the acid. Further, the crucible containing acid solution was kept on a hot plate and digested to obtain a clear solution. The final residue was dissolved in 0.1 M HNO_3 solution and made up to 50 ml. Working standard solutions were prepared by diluting the stock solution with 0.1 M nitric acid in order to check the linearity.

Preparation of herbal extracts

The dry samples of winter savory *(Satureja montana* L.*)* and herb peter (*Primula vulgaris* L.*)* were ground in a blender, and 2 g samples were extracted by either of the following solvents: ethanol and ethanol-water (50/50, v/v%). Extraction was carried out in an ultrasonic bath for 15 min three times in succession with 30, 20, and 20 ml of the solvent, respectively. The extract was filtered through a Büchner funnel and filter paper (blue collar) (CHEMLAB, Spain), transferred into a 100 ml flask and made up to the mark with the same solvent.

Chemicals and reagents

Quercetin and $AlCl_3$ were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Folin-Ciocalteu's phenol reagent and sodium carbonate were purchased from Merck Chemical Suppliers (Darmstadt, Germany). Sodium chlorate buffer (pH 1.0) and acetate buffer (pH 4.5) were purchased from the same producer. All other chemicals used, including solvents, were of analytical grade Sigma-Aldrich (GmbH, Steinheim, Germany). The working solutions were prepared immediately before the analysis from the basic solution (concentration 1000 mg l^{-1} for all metals). For the preparation of standard solutions high-purity Milli-Q water was used. The glassware and polyethylene containers used for analysis were washed with tap water, then soaked overnight in 6 M HNO_3 solution and rinsed several times with ultra-pure water to eliminate absorbance due to detergent.

Determination of the total phenolics

Total phenol contents in the extracts were determined by the modified Folin-Ciocalteu method

[18]. An aliquot of the extracts (1 ml) was mixed with 0.5 ml of Folin-Ciocalteu reagent and 1.5 ml of sodium carbonate solution (20 %). Tubes were vortexed for 15 s and allowed to stand at 40°C for 30 min in order to develop color. Absorbance was then measured at 765 nm using Hewlett Packard UV-VIS spectrophotometer. Total phenol content was expressed as mg g^{-1} of gallic acid equivalent (GAE). The result of each assay was obtained from three parallel determinations.

Determination of total flavonoid content

Total flavonoid contents were determined using the spectrophotometric method based on the formation of flavonoid complex with aluminum [19]. A volume of 0.5 ml of 2 % $AlCl_3$ methanol solution was added to 0.5 ml of sample solution. After one hour-standing at room temperature, the absorbance was measured at 420 nm. The yellow color indicated that the extracts contained flavonoids. Total flavonoid content was calculated as concentration of quercetin (mg g^{-1}) using the equation based on the calibration curve.

Statistical analysis

The experimental results were expressed as mean value ± standard error of the mean value of three replicates. In order to estimate statistically any significant differences among mean values, where applicable, the data were subjected to a one-way analysis of variance (ANOVA) test [20].

RESULTS AND DISCUSSION

In our work the content of total phenols was determined in the investigated extracts of dry samples of the plants winter savory (*Satureja montana* L.) and herb peter (*Primula vulgaris* L.) from the region of Southeast Serbia. The content of total phenols in the extracts of the investigated dry samples was determined using Folin-Ciocalteu method and was expressed as GAE g^{-1} of the dry sample (Fig. 1).

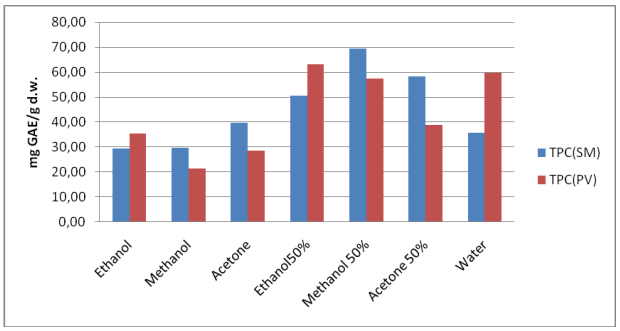

Fig. 1. Comparative study of total phenols content (mg GAE/g d.w.) in the investigated extracts of *Satureja montana* L. and *Primula vulgaris* L.

The results showed that the content of total phenols in the investigated extracts is very high. The highest content of total phenols was in methanol-water (50/50, v/v (%)) extract of winter savory (69.5 mg GAE g^{-1} d.w.) and in ethanol-water (50/50, v/v (%)) extract of peter herb (63.1 mg GAE g^{-1} d.w.). The difference in the total content of phenolic compounds depends on the extraction solution. Ethanol-water (50/50, v/v (%)) and (methanol-water (50/50, v/v (%)) extracts of winter savory have a higher content of total phenolics in relation to the water extract. Ethanol, methanol and acetone extracts of herb peter have a lower content of total phenolics in relation to the corresponding extracts which contain 50% of water.

The content of phenols represents a pharmacological characteristic of the plant. On the basis of numerous studies it is known that the content of polyphenol compounds depends on genotype, soil conditions and difference in plant ripening [21-23]. Also, outdoor conditions, like altitude, light, temperature, content of feeding material in the soil can have an effect on the phenylpropanoid metabolism [24]. Study allowed evidencing that *S. montana* L. growing in Egypt has a high content of phenolic and flavonoid compounds which could be responsible for the remarkable radical scavenging and antioxidant properties observed. This promising efficacy suggests its possible role as antioxidant agent, in order to improve antioxidant status and counteract the risk of diseases associated with oxidative stress [25]. It has been reported that the content of total phenols in the water extract of winter savory is 27.1 mg GAE g^{-1} d.w. [26]. The results of this study agree with those of our work with respect to total phenols. The differences in total phenol compounds content depended on the extraction medium used and are a consequence of the different polarity of the organic solvents used and their mixtures, which selectively extract individual phenol compounds.

On the basis of the experimental results shown in Fig. 2, the content of total flavonoids in the extracts of the investigated plants is much lower than the phenol content. The content of total flavonoids is expressed in mg of quercetin equivalent (QE g^{-1} d.w.).

The content of total flavonoids is very low and ranges from 2.3 to 8.5 mg QE g^{-1} d.w. (ethanol and ethanol-water (50/50, v/v (%))) extract of winter savory and from 5.7 to 21.2 mg QE g^{-1} d.w. extract of peter herb. The highest content of total flavonoids has the water extract of herb peter. On the basis of the experimental results the content of flavonoids in

the extracts of the investigated herb peter is almost uniform.

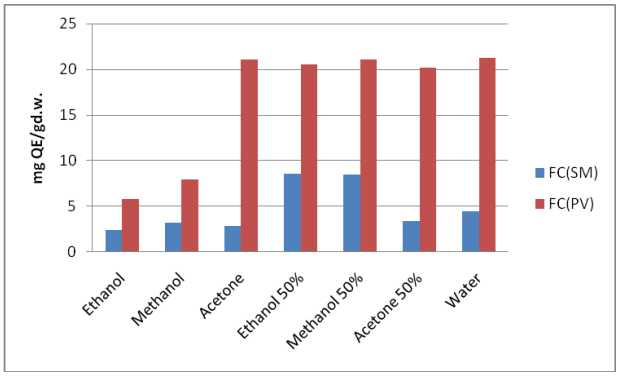

Fig. 2. Comparative study of flavonoids content (mg QE/g d.w.) in the investigated extracts of *Satureja montana* L. and *Primula vulgaris* L.

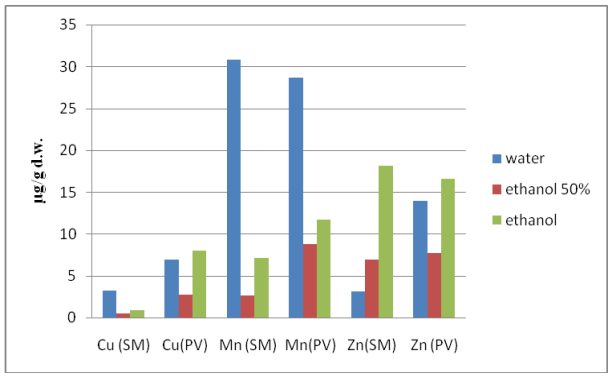

Fig. 3. Comparative survey of metal content in the extracts of *Satureja montana* L. and *Primula vulgaris* L.

In their study, Nayan *et al.* investigated the phenolic composition, antioxidant and radical scavenging activities of *Primrose* (*Primula vulgaris* L.) [27]. Comparing our results for the total content of phenol and flavonoids of the investigated extracts of plants from the Southeast area of Serbia with the results of other authors, we can see a firm agreement.

The presence of heavy metals in the extracts can be explained by possible complex formation that occurs between metal and organic compounds in the plants. The presence of metals in plants is a result of the transfer of metals from the soil, water and atmospheric precipitation during growing. Content of metals in the investigated plants *Satureja montana* L. and *Primula vulgaris* L. is presented in Table 1.

Ions of Cd, Pb, and Cr were not detected in the plants. Contents of other metals are generally low in the extracts using different solvents. Iron content is the highest in all plant extracts. The content of the metal is higher in the extracts of the *Primula vulgaris* L. plant compared to the extracts of the plant *Satureja Montana* L. (Fig. 3).

The highest content of Fe is in water (160.0<182.0 µg/g), 50% ethanol (110.28>65.33 µg/g) and ethanolic (12.40>8.40 µg/g) extract of both plants.

The content of the metal is higher in the extracts of the *Primula vulgaris* L. plant compared to the extracts of the plant *Satureja montana* L. The content of Cu and Zn is higher in ethanolic than in ethanol-water extracts of both plants. Water extracts have a higher content of Cu and Mn than ethanolic and ethanol-water extracts of both plants.

Minerals are inorganic substances present in all body tissues and fluids and their presence is necessary for the maintenance of certain physicochemical processes which are essential to life [15,28, 29]. These include calcification of bone, blood coagulation, neuromuscular activity, acid-base equilibrium, enzyme activity, osmotic regulation.

Analyzed was the correlation between the contents of total phenols, flavonoid compounds, and heavy metals of the investigated extracts, which is presented in Table 2. This work represents the first report on the correlation between the contents of total phenols, flavonoid compounds, and heavy metals of the investigated extracts of selected medicinal plants from Southeast Serbia.

There is a high correlation coefficient between total phenols and flavonoids in both plants. Also, there is a high correlation coefficient between total content of phenol and flavonoids and the content of Mn and Cu in the plant extracts of *Satureja montana* L, and the content of Mn in the plant extracts of *Primula vulgaris* L. There is a high correlation coefficient between the contents of Fe and Zn, Cu and Mn in the extracts of *Satureja montana* L., and between Fe and Mn, Zn and Cu in the extracts of *Primula vulgaris* L.

Table 1. Content of metals in the investigated plants *Satureja montana* L. and *Primula vulgaris* L.

Plant	Fe (µg/g)	Cu (µg/g)	Zn (µg/g)	Mn (µg/g)
Satureja montana L.	265.24 ±4.70	28.75±0.57	31.00±0.62	40.65 ±0.85
Primula vulgaris L.	303.61±3.16	35.70±0.71	22.90±0.45	38.20±1.15

These differences are a result of phylogenetic characterization of plants since they originate from the same field and the use of different extraction solvents.

Our study confirms the results of many authors who have established that the selected medicinal plants are an excellent source of polyphenol compounds, which together with other biologically active compounds as vitamin C, vitamin E and carotenoids can be regarded as a source of natural antioxidants. They may also be a good source of the minerals Fe, Cu, Zn and Mn. Based on these results we recommend the use of the investigated extracts because they are an excellent source of polyphenol compounds and a good source of minerals Fe, Cu, Zn and Mn, aspresented in Table 3.

Extraction coefficient, *EC*, is defined by Eq. (1):

$$EC=\frac{CM(extract)\times 100}{CM(plant)} \quad (1)$$

The extraction coefficients *EC* obtained in this study varied markedly. Based on the results, the analyzed elements can be classified into three groups: elements with a low extraction coefficient (less than 10%); elements with a medium extraction coefficient (10–30%), and elements with a high extraction coefficient (more than 30%).

Table 2. Correlation coefficients of total phenolic, flavonoids contents, and metal content in the investigated extracts *Satureja montana* L.and *Primula vulgaris* L.

Plant	-	Total phenols content[a]	Flavonoid content[b]	Fe mg/kg	Cu mg/kg	Zn mg/kg	Mn mg/kg
Satureja montana L.	Total phenols content[a]	1.0	0.97	0.01	0.90	0.03	0.80
	Flavonoid content[b]		1.0	0.08	0.89	0.05	0.94
	Fe (mg/kg)			1.0	0.4	0.98	0.25
	Cu (mg/kg)				1.0	0.16	0.98
	Zn (mg/kg)					1.0	0.15
	Mn (mg/kg)						1.0
Primula vulgaris L,	Total phenols content[a]	1.0	0.97	0.83	0.12	0.04	0.98
	Flavonoid content[b]		1.0	0.94	0.01	0.00	0.91
	Fe (mg/kg)			1.0	0.02	0.04	0.78
	Cu (mg/kg)				1.0	0.98	0.18
	Zn (mg/kg)					1.0	0.05
	Mn (mg/kg)						1.0

[a]Expressed as mg GAE/g d.w.
[b]Expressed as mg QE/g d.w.

Table 3. Coefficients of extraction of metals with different solvents

Plant	Extract	*EC* (%)			
		Fe	Cu	Zn	Mn
Satureja montana L.	Water	60.32	11.02	10.10	75.76
	Ethanol 50%	41.57	0.76	22.25	6.39
	Ethanol	4.67	3.90	58.38	17.46
Primula vulgaris L.	Water	59.94	19.32	60.69	75.13
	Ethanol 50%	21.51	7.65	33.62	22.98
	Ethanol	2.76	22.4	72.48	30.62

The extraction coefficient mostly depends on the extraction medium. The highest transfer of heavy metals is in the aqueous extract. The extraction coefficient also depends on the plant species that is being extracted. In aqueous extracts of the investigated plants certain metals were detected while the concentration of other metals remained below the detection limits of the apparatus. The aqueous and aqueous/ ethanolic extracts had a low efficiency of Fe extraction; medium efficiency of Mn and Cu extraction, and high efficiency of Zn extraction. The ethanolic extracts of all plants had medium and high efficiencies of extraction of the investigated elements. Based on these results, we recommend the use of aqueous extracts of lower abundance for the heavy metals extraction.

CONCLUSIONS

All investigated extracts of the selected medicinal plants, winter savory (*Satureja montana* L.) and herb peter (*Primula vulgaris* L.*)* contain high polyphenol concentration. The contents of heavy metals in their extracts are low. The investigated plants from Southeastern Serbia are suitable for the preparation of teas and herbal extracts due to the low content of toxic metals (Zn, Fe, Cu, Mn) and the high content of phenolic compounds.

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