Content of trace metals in medicinal plants and their extracts

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Abstract

The heavy metals (Fe, Cu, Zn and Mn) contents of selected plant species, grown in Southeast region of Serbia, that are traditionally used in alternative medicine were determined. Among the considered metals, iron content was the highest one and varied from 137.53 up to 423.32 mg/kg, while the contents of Cu, Zn and Mn were remarkably lower, and ranged from 8.91 to 62.20 mg/kg. In addition, an analysis of plants extracts showed a significant transfer of heavy metals during extraction procedure; therefore, the corresponding extraction coefficients reached values up to 88.8%. Those were especially high in the ethanol based extracts. Moreover, it is was established that such coefficients mostly depend on the solvent nature and also on the treated plant species. The obtained results impose that medicinal plants from Southeast region of Serbia due to rather low content of heavy metals are appropriate for preparation of teas and medicinal extracts.

Keywords: Medicinal plants • Extracts • Heavy metals • AAS

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Medicinal plants and their extracts deserve special attention because of the important influence they have on human health. For the majority of the world population, medicinal plants represent the primary source of the health care. According to the World Health Organization (WHO) report, almost 80% of people in marginal communities use only medicinal plants for the treatment of various diseases [1–2]. Although the effectiveness of medicinal plants is mainly associated with their constituents such as are essential oils, vitamins, glycosides, etc., it was found that prolonged intake can cause health problems due to the possible presence of heavy metals [3].

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 heavy metals accumilated is of high importance. The human body requires both the metallic and the non-metallic elements within certain permissible limits for growth and good health. Therefore, the determination of element compositions in food and related products is essential for understanding their nutritive importance. Accordingly, the presence of some heavy metals in large quantities in the body may have a toxic effect [3-6].

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 [7].

In this work, heavy metals content in the following plants and their extracts: *Calendula officinalis* L., *Primula officinalis* L., *Origanum vulgare* L., *Cichorium intybus* L., *Saturea montana* L., *Delphinum consolida* L., *Papaver rhoeas* L., *Crataegus oxyacantha* L., *Prunus spinosa* L., was determined. These plants have been used in traditional Serbian medicines for the treatment of many diseases.

Papaver rhoeas L. is a plant from the Papaveraceae family. It has antiseptic and antibacterial effects, and as such is very effective against various inflammations, fungal infections, and skin diseases. For centuries, it has been used in traditional medicine for healing of wounds, furuncles, warts, etc. [8].

Origanum vulgare L. is a plant from the Lamiaceae family. It has antispasmodic, bronchodilating, and diuretic effects [9,10].

Primula officinalis L. is a plant from the Primulaceae family. It is used as a traditional medicine for the treatment of lung diseases, dizziness, and tachycardia [11–12].

Saturea montana L. is a plant from the Lamiaceae family. It has an extremely strong antiseptic effect, and as such is used for the treatment of respiratory and digestive organs ilnesses, and the inflammations of skin and mucosa.

Calendula officinalis L. is a plant from the Asteraceae family. It has antibacterial and bactericidal effects, therefore, it is used for the treatment of wounds, psoriasis, etc. [13].

Prunus spinosa L. is a plant from the Rosaceae family. It is used for the treatment of skin problems, to alleviate stomach colic, etc. [14].

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Crataegus oxyacantha L. is a plant from the Rosaceae family. It is used for the treatment of arteriosclerosis, heart diseases, and mild nervous disorders [15].

The aim of this investigation was to determine the concentrations of heavy metals in the above mentioned plants and their extracts and to determine the coefficient of extraction of metals in different solvents and their mixtures.

EXPERIMENTAL

Reagents

All the reagents used were of the analytical purity (Merck, Germany). The working solutions were prepared immediately before the analysis from the basic solution with 1000 mg/l concentration 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 over the night in 6 M HNO_3 solution and rinsed several times with ultra pure water to eliminate absorbance due to detergent.

Sample preparation

The plant material was collected in the flowering phase from the natural habitats of the plants *Calendula officinalis* L. (flower), *Primula officinalis* L. (flower), *Origanum vulgare* L. (whole part of plant), *Cichorium intybus* L. (whole part of plant), *Saturea montana* L. (whole part of plant), *Delphinium consolida* L. (whole part of plant), *Papaver rhoeas* L. (flower), *Crataegus oxyacantha* L. (fruit), and *Prunus spinosa* L. (fruit) in the stage of ful maturity, in the region of Southeast Serbia in July 2009. The study area is located in the surroundings of the city of Niš. Niš has about 300.000 inhabitants and it is the third-largest city in the country after Belgrade and Novi Sad; however, the industry in this area is poorly developed.

Sample sites were selected in accordance with the methods used in the European moss monitoring project [16]. A minimum distance of 300 m to major roads and larger settlements was required, a minimum distance of 100 m to minor roads and houses and a minimum distance of 5 m to forest roads.

The standard procedure described by Association of Official Analytical Chemists (AOAC) (2000) was followed for the preparation of the 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 ^{o}C for 3 h and then 5 ml of 6 M HCl was added to the crucible. Care was taken to ensure that all the ash came into contact with acid. Further, the crucible containing acid solution was kept on a hot plate and digested to obtain a clean 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 for checking the linearity.

Three samples (2 g in weight each) were separated from the previously homogenized material and extracted first with 30 ml of solvent, then several times with 20 ml of solvent. The solvents used were ethanol, water, and ethanol–water mixture (1:1). During the extraction, the samples were mixed in an ultrasonic bath. Each step of the extraction lasted for 15 min. The samples prepared in this manner were filtered through a Büchner funnel using Whatman No. 1 filter paper. The solid residue was washed several times to obtain the transparent extract. The final extract volume was 100 ml.

Apparatus

Atomic absorption measurements were made using a Varian SpectraAA 10 with background correction and hollow cathode lamps. Air–acetylene flame was used for determination of all the elements. The calibration interval, wavelength, slit, and detection level are given in Table 1.

RESULTS AND DISCUSSION

In our work the content of heavy elements, Fe, Zn, Cu, and Mn, Ni, Pb, and Cd was determined in the investigated plants from the region of Southeast Serbia and their extracts (Table 2). The presence of Fe, Zn, Cu, and Mn was confirmed, and their concentration was determined, while Ni, Pb, and Cd were not detected.

The highest concentration of metal in the medicinal plants and their extracts was that of iron. It ranged from 137.53 to 423.32 mg/kg in the plants, and from 16 to 182 mg/kg in the extracts.

Table 1. Analytical characteristics of the AAS determination

Metal	Working range, mg/l	Detection limit, mg/l	Wavelength, nm	Slit
Iron (Fe)	0.00-10.00	0.015	248.3	0.2
Copper (Cu)	0.00-1.00	0.007	213.9	1.0
Zinc (Zn)	0.00-5.00	0.021	324.8	0.5
Lead(Pb)	0.00-1.00	0.002	217.0	1.0
Cadmium (Cd)	0.00-1.00	0.003	228.8	0.5
Manganese (Mn)	0.00-2.00	0.005	279.5	0.2
Nickel (Ni)	0.00-1.00	0.002	232.0	0.2

Table 2. The content of heavy metals in medicinal plants and their extracts

Species	Traditional name	Extract	Content, mg/kg			
			Fe	Zn	Cu	Mn
Calendula officinalis L.	Marigold	Plant	137.53±2.75	18.15±0.36	12.82±0.26	24.38±0.48
		Water	16±0.32	4.46±0.09	1.50±0.03	5.40±0.11
		Ethanol	76±1.57	5.28±0.10	2.40±0.05	13.71±0.27
		Ethanol/water (1:1)	17±0.34	4.42±0.09	1.10±0.02	3.60±0.07
Primula officinalis L.	Herb Peter	Plant	303.61±6.07	22.36±0.45	20.35±0.41	36.60±0.73
		Water	–	3.50±0.07	–	–
		Ethanol	182±3.64	13.90±0.28	6.90±0.14	28.70±0.57
		Ethanol/water (1:1)	–	7.70±0.15	2.70±0.05	–
Origanum vulgare L.	Wild marjoram	Plant	152±3.04	49.65±0.99	23.95±0.48	21.80±0.44
		Water	–	29.10±0.58	4.53±0.09	–
		Ethanol	112.69±2.25	22.30±0.45	3.85±0.08	16.3±0.33
		Ethanol/water (1:1)	–	22.00±0.45	0.40±0.08	–
Cichorium intybus L.	Blue sailors	Plant	402.14±8.04	32.40±0.65	20.50±0.41	49.39±0.98
		Water	–	–	1.10±0.02	–
		Ethanol	68±0.58	28.77±0.57	8.50±0.17	7.21±0.14
		Ethanol/water (1:1)	29±0.58	3.70±0.07	3.20±0.06	4.80±0.09
Saturea montana L.	Winter savory	Plant	265.24±5.30	25.12±0.50	15.77±0.31	40.65±0.81
		Water	–	21.50±0.43	–	9.10±0.18
		Ethanol	160±3.20	3.10±0.06	3.17±0.06	30.80±0.62
		Ethanol/water (1:1)	–	6.90±0.14	0.22±0.01	2.60±0.05
Delphi–nidum consolida L.	Forking Larkspur	Plant	286.50±5.73	39.84±0.79	23.06±0.46	13.84±0.28
		Water	14±0.28	11.00±0.22	1.60±0.03	–
		Ethanol	176±3.52	14.00±0.28	18.80±0.37	3.32±0.07
		Ethanol/water (1:1)	–	12.20±0.24	3.60±0.07	–
Papaver rhoeas L.	Corn poppy	Plant	423.32±8.47	31.80±0.64	35.50±0.71	20.61±0.41
		Water	–	2.20±0.04	–	–
		Ethanol	78±1.56	3.50±0.07	22.10±0.44	16.4±0.33
		Ethanol/water (1:1)	–	7.60±0.52	5.30±0.10	–
Prunus spinosa L.	Blackthorn	Plant	261.60±5.23	62.20±1.24	8.91±0.18	9.15±0.18
		Water	–	12.70±0.25	–	–
		Ethanol	91±1.82	7.10±0.14	2.33±0.05	2.14±0.04
		Ethanol/water (1:1)	–	5.20±0.10	–	–
Crataegus oxyacanth L.	Hawthorn	Plant	172.16±3.44	50.80±1.02	9.97±0.19	16.02±0.30
		Water	29±0.58	5.60±0.11	3.26±0.06	2.70±0.05
		Ethanol	118±2.36	20.10±0.40	2.85±0.06	7.20±0.14
		Ethanol/water (1:1)	–	35.70±0.71	0.52±0.01	–

The concentration of zinc was the lowest in the plant *Calendula officinalis* L. and it amounted 18.15 mg/kg, while the highest content (62.2 mg/kg) was found in the plant *Prunus spinosa* L. In the extracts, the zinc concentration was in the range from 3.1 to 35.7 mg/kg.

Copper content in the medicinal plants ranged from 8.91 mg/kg in *Prunus spinosa* L. to 35.50 mg/kg in *Papaver rhoeas* L., and in the extracts it ranged from 0.40 to 22.10 mg/kg.

Prunus spinosa L. also had the lowest content of manganese (9.15 mg/kg). The highest concentration of manganese was found in the plant *Cichorium intybus* L. The extracts of the investigated plants contained manganese from 2.14 to 30.80 mg/kg.

The comparison of our results regarding the heavy metals content in the plants from the region of Southeast Serbia with the results of other authors showed considerable agreement. In the plant *Cressa cretica* L., the content of copper was between 12.2 and 14.3 mg/kg, of iron between 125.2 and 151.1 mg/kg, and that of zinc between 55.3 and 70.2 mg/kg [1]. Radanović *et al*. reported that the concentration of copper in the plant *Gentiana lutea* L. varied from 19 to 22 mg/kg, and that of zinc from 31 to 34 mg/kg [18]. The content of copper in Palestinian plants varied from 7.06 to 19.19 mg/kg, and of zinc from 17.38 to 65.85 mg/kg [3]. The copper content in the black tea originating from the region of south India varies between 15.9 and 32.2 mg/kg.

In the plants from the region of Southeast Serbia, the highest content is that of iron, while the contents of Zn, Cu, and Mn are significantly lower. Certain quantities of heavy metals in plants are below the limit acceptable for the medicinal plants. Heavy metals, such as Ni, Cd and Pb, were not detected in the investigated plants. This can be attributed, among other factors, to the fact that these were autochthonous plants growing in an non-polluted region of Southeast Serbia. Such medicinal plants can safely be used in form of herbal infusions and extracts for the treatment of various diseases.

In the black tea samples originating from the region of Iran, the copper concentration is within the range from 17.59 to 32.80 mg/kg, and in water extracts from 1.15 to 1.65 mg/kg [20].

The iron concentration in medicinal plant from region Turkey ranges from 2.45 to 107.4 mg/kg, of zinc from 3.90 to 18.00 mg/kg, and of copper from 2.45 to 8.10 mg/kg [21].

In the investigated samples of water extracts of black and green tea used in Egypt, the iron concentration was found to be between 30.56 and 122.45 mg/kg, that of zinc from 30.0 to 43.5 mg/kg, copper from 3.34 to 20.12 mg/kg, and of manganese from 130.77 to 220.0 mg/kg. [22].

Comparing our results with results of authors from other countries show that the heavy metals content is similar or smaller.

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

$$EC = 100\frac{c_{\mathrm{M(extract)}}}{c_{\mathrm{M(plant)}}} \quad (1)$$

The extraction coefficients *EC* obtained in this study varied markedly, from 0 to 88.8% (Table 3). Based on the results, the analyzed elements can be classified into three groups: elements with the low extraction coeffi-

Tabela 3. Extraction coefficients, EC, of heavy metals from the analysed medicinal plants by different extractants

Plant	Extractant	*EC* / %			
		Fe	Zn	Cu	Mn
Calendula officinalis L.	Water	11.6	24.6	11.7	22.1
	Ethanol	55.2	29.1	18.7	56.2
	Ethanol/water(1:1)	12.4	24.3	8.6	14.7
Primula officinalis L.	Water	–	15.6	–	–
	Ethanol	59.9	62.2	33.9	78.4
	Ethanol/water(1:1)	–	34.4	13.3	–
Origanum vulgare L.	Water	–	58.6	18.9	–
	Ethanol	74.1	44.9	16.1	74.8
	Ethanol/water(1:1)	–	44.3	1.7	–
Cichorium intybus L.	Water	–	–	5.4	–
	Ethanol	16.9	88.8	41.5	14.9
	Ethanol/water(1:1)	7.2	11.4	15.6	9.7
Saturea montana L.	Water	–	85.6	–	22.4
	Ethanol	60.3	12.3	20.1	75.8
	Ethanol/water(1:1)	–	27.5	1.4	6.4
Delphinum consolida L.	Water	4.9	27.6	6.9	–
	Ethanol	61.4	35.1	81.5	23.9
	Ethanol/water(1:1)	–	30.6	15.6	–
Papaver rhoeas L.	Water	–	6.9	–	–
	Ethanol	18.4	11.0	62.3	79.6
	Ethanol/water(1:1)	–	23.9	14.9	–
Prunus spinosa L.	Water	–	20.4	–	–
	Ethanol	34.8	11.4	26.1	23.4
	Ethanol/water(1:1)	–	8.4	–	–
Crataegus oxyacantha L.	Water	16.8	11.0	32.7	16.8
	Ethanol	68.5	39.6	28.6	44.9
	Ethanol/water(1:1)	–	70.3	5.2	–

cient (less than 10%); elements with the medium extraction coefficient (10–30%), and elements with the high extraction coefficient (more than 30%). The extraction coefficient depends mostly on the extraction medium. The lowest transfer of heavy metals is in the water extract, and the highest in the ethanol extract. The extraction coefficient also depends on the plant species that is being extracted. In water extracts of some plants certain metals have been detected, while the concentration of other metals remained below the detection limits of the apparatus. The water and water/ethanol extracts had a low efficiency of Fe extraction; medium efficiency of Mn and Cu extraction, and high efficiency of Zn extraction. The ethanol extracts of all plants had medium and high efficiencies of extraction of the investigated elements. Based on these results, we recommend the use of water extracts of lower abundance for the heavy metals extraction.

CONCLUSIONS

The analysis of chosen medicinal plants and their extracts from the region of Southeast Serbia showed the presence of Fe, Cu, Zn and Mn. The iron concentration in the plants was rather high and varied from 137.53 to 423.32 mg/kg. Cu, Zn and Mn concentrations were significantly lower and varied from 8.91 to 62.20 mg/kg.

By analyzing the extracts of medicinal plants the transfer of heavy metals from the plants to the extracts was found. The extraction coefficients varied in the interval from 0.0 to 88.8%. The extraction coefficient depends mainly on the extraction medium. The lowest transfer of heavy metals was found in water extract, and the highest in ethanol extract. The extraction coefficient also depends on the plant species that is being extracted. In water extracts of some plants only some metals were detected, while the concentration of other metals was less than the detection limit of the apparatus. Since the selected medicinal plants and their extracts are used in traditional medicine, there is a possible danger of heavy metal poisoning, if they come from the polluted areas. Therefore, the medicinal plants should be collected in non-polluted regions and they should be tested for the presence of heavy metals. These investigations are obligatory and they are recommended by the European standards in order to prevent poisoning by heavy metals. The obtained results show that medicinal plants from the region of Southeast Serbia are suitable for use and preparation of extracts because of the low content of Fe, Zn, Cu and Mn.

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IZVOD

SADRŽAJ TRAGOVA METALA U LEKOVITOM BILJU I NJIHOVIM EKSTRAKTIMA

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Atomskom absorpcionom spektrometrijom određen je sadržaj teških metala (Fe, Cu, Zn i Mn) u izabranim biljnim vrstama sa područja jugoistočne Srbije, koje se koriste u tradicionalnoj medicine. Utvrđeno je da je sadržaj gvožđa najveći i da varira u opsegu od 137,53 do 423,32 mg/kg. Koncentracije Cu, Zn i Mn znatno su niže i kreću se u intervalu od 8,91 do 62,20 mg/kg. Pored toga, analiza biljnih ekstrakata je pokazala da postoji značajan transfer teških metala tokom procesa ekstrakcije, tako da odgovarajući ekstrakcioni koeficijenti variraju u opsegu od 1,4 do 88,8%. Oni su posebno visoki u etanolnom ekstraktu. Utvrđeno je da ekstrakcioni koeficijenti najviše zavise od vrste rastvarača i tretirane biljne vrste. Dobijeni rezultati ukazuju da se lekovito bilje sa područja jugoistočne Srbije zbog niskog sadržaja teških metala može koristiti za pripremu čajeva i lekovitih ekstrakata.

Ključne reči: Biljne vrste • Ekstrakti • Teški metali • AAS