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LEVELS OF SOME MICROELEMENTS AND ESSENTIAL HEAVY METALS IN HERBAL TEAS IN SERBIA

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Abstract: Levels of Fe, Mn, Cu, Zn, Mo, Co, Ni, Se, Sn and Al were determined in 14 medicinal plants from Serbia, which are widely used in phytopharmacy as herbal teas. The following plants were investigated: yarrow (*Achillea millefolium* L.*)*, basil (*Ocimum basilicum* L.*)*, St. John's wort (*Hypericum perforatum* L.*)*, peppermint (*Mentha x piperita* L.), field horsetail (*Equisetum arvense* L.), stinging nettle (*Urtica dioica* L.), thyme (*Thymus serpyllum* L.), maize silk (*Zea mays* L. – *Maydis stigma*), hibiscus (*Hibiscus sabdariffa* L.), marshmallow (*Althaea officinalis* L.), chamomile (*Matricaria chamomilla* L.), rosehip/dog rose (*Rosa canina* L.), winter savory (*Satureja montana* L.) and spearmint (*Mentha spicata* L.). A total of 16 samples of different parts of medicinal plants (root, leaf, flower, herba) were examined, whereby 13 samples were delivered in original package and three samples were loose leaf herbs. Samples were prepared using the microwave digestion technique, and measurements were performed applying the atomic absorption spectrometry and mass spectrometry with inductively coupled plasma. Contents of microelements in the examined samples were in the range: Mn (23.86 – 453.71 mg/kg); Fe (61.87 – 673.0 mg/kg); Cu (6.68 – 24.46 mg/kg); Zn (16.11 – 113.81 mg/kg); Mo (0.576 – 4.265 mg/kg); Co (0.039 – 0.532 mg/kg); Se (0.036 – 0.146 mg/kg); Ni (0.738 – 6.034 mg/kg); Al (154.0 – 3015.0 mg/kg) and Sn (2.68 – 10.22 mg/kg). According to determined amounts of microelements, the investigated samples of herbal teas are considered safe for human consumption.

Keywords: herbal teas, microelements, AAS, ICP- MS

Mineral elements are of a unique and manifold importance for both the plant world and human beings. Minerals are found in plants as ions, inorganic and organic salts or are incorporated into diverse organic compounds (1). Mineral elements are involved in a range of chemical reactions in plants. According to their proportional representation in the plant composition, they are grouped into macro, micro and ultra microelements. The content of mineral elements in plants may vary significantly. Variations in mineral matter content in plants are due to a variety of factors, including plant species, plant age, pedological features of soil, climate and implementation of agrotechnical measures (2–5). The same plant species differ in microelement content under different ecological conditions, while diverse species in the same biotope accumulate different amounts of microelements (6).

Human body needs appropriate concentrations of various minerals to maintain the normal function and sustain life. Deficiency or excess of essential heavy metals in the diet can induce various health disorders. Medicinal plants are either direct or indirect source of minerals in human diet and regular consumption of tea can contribute to the dietary requirements of these elements (5). Species used for obtaining a range of phytopreparations in pharmaceutical industry, supplied as monocomponent teas or tea blends that are widely applied in traditional medicine in Serbia and thus are of particular importance. Sources for obtaining medicinal raw material are wild crafted plants (over 200 species) or cultivated plants (ca. 30 species) (7). Chemical composition of teas is very complex, encompassing flavonoids, alkaloids, enzymes, minerals and trace elements (8). In Serbia, herbal teas are mainly prepared from aromatic herbal species that contain etheric oils, which are not only remedial but also have a very pleasant scent and aroma. Phytotherapy has a very long tradition and became popular in modern medicine, since medicinal herbs are not aggressive and do not have severe side effects.

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Intensive agrotechnical measures in modern agriculture, vicinity of industrial plants, mines, traffic roads inevitably lead to contamination of the soil and plants with pesticides and heavy metals (9). Plants are a link for the transfer of trace elements from soil to man. Thus, the content of heavy metals is an important criterion when using plant material in the production of traditional remedies and herbal infusions (10). In that respect, continuous and planned monitoring of hygienic safety of plants used as raw material in pharmaceutical industry is important. The determination of microelements content is important in view of plant, animal and human health and environmental aspect as well (4). However, there are no standards for medicinal raw plant materials, which establish a permissible level of microelements except for heavy metals: lead, cadmium (11) and mercury (12). Content and bioavailability of potentially harmful microelements as well as their interaction with the plant constituents need to be established prior to potential toxicity assessment.

Owing to the importance of minerals present in teas, in the present study, some microelements (Mo, Co, Ni, Se, Sn, Al) including essential heavy metals (Fe, Cu, Zn, Mn), were determined in order to update and evaluate the knowledge on the presence and to determine the correlation between the selected elements in different Serbian tea plant samples. The aim was also to establish the health-safety of these phyto products, having in mind their wide application in folk medicine.

EXPERIMENTAL

Materials

All samples used in this research are herbal teas (Table 1), widely applied and popular among Serbian population and in folk medicine practices. Cultivated herbal tea samples in original package (samples No. 1–13) were collected from the retail shops in the territory of Novi Sad (Vojvodina, North Serbia region, locality I), whereas three samples of wild growing medicinal plants (samples No. 14, 15 and 16) were collected directly from the natural habitat (East Serbia region, locality II).

The following reagents were used: standard solutions of Mn, Fe, Cu, Zn, Mo, Co, Ni, Se, Sn and Al at a concentration of 1 mg/kg (Accu Trace™ Reference Standard, USA), nitric acid and H_2O_2 (Merck, Germany), deionized water with conductivity < 0.2 µS/cm.

Apparatus

The mineralization was carried out in a microwave digestion system (Ethos Microwave Labstation, Milestone, Italy).

Table 1. Herbal teas and plant parts used in research.

No	Plant	Latin name (family)	Parts with medicinal properties
1	Yarrow	*Achillea millefolium L.* (Asteraceae)	herba
2	Basil	*Ocimum basilicum L* (Lamiaceae)	herba
3	St. John's wort	*Hypericum perforatum L.* (Hypericaceae)	herba
4	Peppermint	*Mentha x piperita L.* (Lamiaceae)	leaf
5	Field horsetail	*Equisetum arvense L.* (Equisetaceae)	herba
6	Stinging nettle	*Urtica dioica L.* (Urticaceae)	root
7	Stinging nettle	*Urtica dioica L.* (Urticaceae)	leaf
8	Thyme	*Thymus serpyllum L.* (Lamiaceae)	herba
9	Maize	*Zea mays, Maydis stigma* (Poaceae)	silk
10	Hibiscus	*Hibiscus sabdariffa L.* (Malvaceae)	flower
11	Marshmallow	*Althaea officinalis L.* (Malvaceae)	root
12	Chamomile	*Matricaria chamomilla L.* (Asteraceae)	flower
13	Rosehip, dog rose	*Rosa canina L.* (Rosaceae)	fruit
14	Winter savory	*Satureja montana L.* (Lamiaceae)	herba
15	St. John's wort	*Hypericum perforatum L.* (Hypericaceae)	herba
16	Spearmint	*Mentha spicata L.* (Lamiaceae)	herba

The measurements were performed using atomic absorption spectrophotometer SpectrAA-10 (Varian, USA) and inductively coupled plasma mass spectrometer Agilent ICP-MS 7700x.

Procedures

The measurement of the content of elements in herbal tea samples was performed subsequent to determining the loss of mass after drying according to the procedure given in the European Pharmacopoeia 6.0 (13).

The samples were prepared applying the microwave digestion method (14) with the use of the mixture H_2O_2/HNO_3 (1 : 4, v/v). After this process, the samples were transferred to 50 mL volumetric flasks and diluted with deionized water.

Determination of Mn, Fe, Cu, Zn by AAS method

The concentrations of manganese (λ = 279.5 nm), iron (λ = 248.3 nm), copper (λ = 324.7 nm) and zinc (λ = 213.9 nm) were determined by atomic absorption spectrophotometry applying Varian SpectrAA-10 apparatus. The measurements were performed in triplicates for each particular sample.

Determination of Mo, Co, Ni, Se, Al, Sn by ICP-MS method

Determination of cobalt (He-M, IT 0.1 s/P), nickel (He-M, IT 1 s/P), selenium (He-M, IT 5 s/P), molybdenum (He-M, IT 0.1 s/P), tin (NoG-M, IT 0.1 s/P) and aluminium (He-M, IT 0.3 s/P) was performed using Agilent ICP-MS 7700 mass spectrometer under common operating conditions with an excitation power of plasma of 1550 w, flow-rate of carrier gas 1.01–1.11 L/min, flow rate of He gas 3.2–4.0 mL/min. Peristaltic pump program: uptake speed 0.50 rps; uptake time 25 s and stabilization time 20 s in spectrum multi tune mode. Determination was done using isotopes: ^{59}Co, ^{60}Ni, ^{78}Se, ^{95}Mo, ^{118}Sn and ^{27}Al.

Statistical analysis:

The results were expressed as the means and standard deviations. Pearson correlation coefficient was used for comparing the results between microelements. Significance level was determined as $p < 0.01$. The analysis was performed using a software package IBM SPSS Statistics 20.

Table 2. Content of microelements determined by AAS in the herbal tea samples, mg/kg dry weight, n = 3.

Sample no.	**Mn** [mg/kg]	**Fe** [mg/kg]	**Cu** [mg/kg]	**Zn** [mg/kg]
1.	77.90	67.24	15.56	28.48
2.	71.98	539.87	24.46	38.09
3.	80.86	63.71	10.93	22.18
4.	111.97	443.90	17.15	26.86
5.	254.70	617.46	7.76	27.9
6.	28.18	673.00	12.71	22.75
7.	57.84	303.00	13.23	29.14
8.	127.06	445.78	8.94	44.26
9.	31.60	193.21	8.10	59.80
10.	453.71	219.82	7.80	46.24
11.	23.86	114.16	15.69	23.59
12.	76.14	130.26	14.25	34.67
13.	70.99	61.87	6.68	16.11
14.	54.22	155.83	10.76	51.11
15.	170.78	217.32	19.86	113.81
16.	37.24	150.85	15.87	31.43
Mean	108.06	274.83	13.11	38.53
min	23.86	61.87	6.68	16.11
max	453.71	673	24.46	113.81
CV [%]	101	74	38	60

RESULTS AND DISCUSSION

In Table 2, the results of contents of Mn, Fe, Cu and Zn determined by AAS in examined tea samples are displayed.

The contents of Mn in herbal teas ranged from 23.86 ± 0.43 mg/kg (marshmallow root) to 453.71 ± 14.07 mg/kg (hibiscus flower), with an average value of 108.06 mg/kg and CV 101.48%. Mn contents in herbal teas: peppermint, chamomile, stinging nettle (leaf), stinging nettle (root), St. John's wort, yarrow and basil correspond with the results of other authors, including low content in the marshmallow (15–17). Concentration of Mn in the peppermint and chamomile was also in accordance with recent data from the literature (111.97 in relation to 120.4 mg/kg and 76.14 in relation to 69.3 mg/kg), but Mn content obtained in rosehip tea was multifold lower (70.99 in relation to 1585.9 mg/kg) (8). The cited authors themselves pointed out the high manganese levels in herbal teas obtained in their research. It should be emphasized that content of Mn in hibiscus tea (453.71 ± 14.07 mg/kg) was elevated as compared to other samples. Similar results revealing high Mn content in hibiscus (717 ± 15 mg/kg), but also in chamomile (137 ± 5 mg/kg) as compared to other plants are pointed out in the researches from Bulgaria and Macedonia (18). Manganese content in stinging nettle leaf sample is in correlation with the result obtained for nettle (herba) collected from natural habitats from Transylvania: 52.73 ± 0.21 mg/kg (4). The obtained levels of Mn in chamomile, St. John's wort, stinging nettle, peppermint, rosehip, yarrow and thyme were somewhat higher than levels measured in Romanian plants, especially for field horsetail: 254.70 in relation to 18 mg/kg (19).

The levels of Fe established in herbal teas ranged from 61.87 ± 1.11 mg/kg (rosehip) to 673 ± 18.18 mg/kg (stinging nettle – root). High Fe content in the following herbal teas should be pointed out: basil, peppermint, field horsetail, stinging nettle – root and thyme. Higher Fe concentration was observed in the peppermint leaf as compared to the samples of spearmint (herba). This could be explained by its function in chlorophyll synthesis in the leaf. Wild growing St. John's wort obtained from locality II revealed higher content of Fe as compared to the cultivated St. John's wort (locality I). Fe levels established in the following herbal teas: peppermint, stinging nettle, St. John's wort, marshmallow, yarrow and basil compare well with some minor differences with previous results for Serbian samples (15).

Maximum Cu level was established in basil, being 24.46 ± 0.19 mg/kg, while minimum Cu content (6.68 ± 0.06 mg/kg) was determined in rosehip fruit. Copper concentrations in chamomile, stinging nettle, St. John's wort and marshmallow are in accordance with the results of other authors (15), whereas Cu levels obtained in peppermint, yarrow and basil were somewhat higher than those reported by aforementioned authors. Content of Cu measured in stinging nettle is the same as recorded in Transylvanian nettle herba (4). On the other hand, this content is lower than in Romanian sample (20). Obtained levels in samples of peppermint, chamomile, and thyme are higher than in Romanian plants (20).

The concentrations of zinc were fairly consistent among the tea samples, with the exception of St. John's wort from the locality II that revealed maximum content of Zn of 113.81 ± 4.89 mg/kg. This value was five times higher than that measured in St. John's wort sample originating from the locality I (22.18 ± 1.10 mg/kg). Potential reason for such difference is most probably the soil type. Results on Zn content in peppermint, chamomile, stinging nettle, St. John's wort, marshmallow, yarrow and basil correspond with the results of other authors (15). Furthermore, measured Zn concentrations in peppermint and rosehip well correspond with recent result, but levels obtained for chamomile were higher (8). The obtained Zn content in samples of chamomile and hibiscus were similar as determined in Bulgarian and Macedonian plants, being 45 ± 2 and 33 ± 2 mg/kg, respectively (18).

As it can be seen from Table 3, Mo content in tea samples was low. Maximum Mo content was measured in a sample of nettle leaf: 4.265 ± 0.034 mg/kg and the minimum content was determined in rosehip 0.576 ± 0.011 mg/kg. Mo content in nettle root (0.733 ± 0.011 mg/kg) was somewhat lower than in Hungarian research for nettle (herba): 1.14 ± 0.77 mg/kg (4). In nettle leaf, Mo content was significantly higher: 4.265 ± 0.034 mg/kg, which probably can be explained by its increased presence in the composition of nitrate reductase in the nettle leaf.

Maximum Co level was established in field horsetail being 0.532 ± 0.002 mg/kg, while minimum Co content (0.039 ± 0.001 mg/kg) was determined in rosehip fruit. In nettle sample from Transylvania, Co was not detected (4). Co content was below the detection limit in samples of peppermint, chamomile, nettle and thyme from Romania (20). Contrary to this, higher Co content was determined in chamomile and hibiscus samples in

Table 3. Content of microelements determined by ICP-MS in the herbal tea samples, mg/kg dry weight, n = 3.

Sample No.	**Mo** [mg/kg]	**Co** [mg/kg]	**Ni** [mg/kg]	**Se** [mg/kg]	**Al** [mg/kg]	**Sn** [mg/kg]
1.	2.267	0.130	2.401	0.055	3015	3.01
2.	2.720	0.310	1.037	0.084	808	6.09
3.	2.212	0.102	2.567	0.085	1340	5.87
4.	2.695	0.161	1.882	0.107	554	3.66
5.	0.972	0.532	2.763	0.063	1702	2.77
6.	0.733	0.485	6.034	0.047	585	2.77
7.	4.265	0.084	0.738	0.074	222	10.22
8.	0.747	0.199	1.938	0.086	903	3.85
9.	2.369	0.079	1.012	0.047	438	3.89
10.	1.123	0.314	3.481	0.104	1185	2.68
11.	0.680	0.100	1.362	0.102	1383	2.79
12.	1.940	0.104	2.518	0.146	1354	3.99
13.	0.576	0.039	1.262	0.080	351	3.01
14.	0.778	0.074	0.892	0.036	225	6.05
15.	1.707	0.350	2.645	0.078	154	5.37
16.	1.507	0.060	0.816	0.062	622	6.91
Mean	1.706	0.195	2.084	0.078	928	4.56
min	0.576	0.039	0.738	0.036	154	2.68
max	4.265	0.532	6.034	0.146	3015	10.22
CV [%]	60	80	65	35	79	45

Bulgaria: 0.30 ± 0.01 and 1.00 ± 0.03 mg/kg, respectively (18).

Maximum Ni content was measured in a sample of nettle root and the minimum content was recorded in nettle leaf. Similar results were obtained for Co content suggesting similar mechanisms of its uptake from the soil. Ni content in St. John's wort samples originating from two different locations was almost the same. In rosehip, peppermint, chamomile, St. Jon's wort and nettle leaf, Ni contents were similar to Romanian results: 0.9; 1.8; 3.9; 2.1; and 0.6 ppm, respectively (19). When comparing the results with Romanian ones and taking into consideration the type of mint and plant part, peppermint and nettle leaf samples were comparable, while results for nettle root indicated 10 times higher content of Ni. Results for chamomile and yarrow were similar to those obtained in previous investigations for these plants in Serbia (3.14 and 3.27 mg Ni/kg) (17).

Maximum Se content was measured in a sample of chamomile flower 0.146 ± 0.004 mg/kg, while the minimum content was determined in winter savory: 0.036 ± 0.001 mg/kg. Similar to Ni, Se content in samples of St. John's wort from two localities were comparable. Contents of Se in St. Jon's wort and nettle were higher than those obtained in Romanian samples (21). This can be explained by the soil type and fertilizer application in cultivated conditions.

In the analyzed herbal teas, unexpectedly high values for Al were obtained, especially in yarrow. The lowest content of Al was determined in St. John's wort from the locality II – natural habitat, while cultivated St. John's wort from locality I revealed 10 times higher Al content. On the other hand, Al content in cultivated peppermint leaf was similar to those in spearmint from locality II. Analogous conclusions about the effects of plant species and locality on the mineral composition are confirmed in the literature (6). Content of Al in root nettle sample (585 ± 11 mg/kg) is similar to the results obtained in Transylvanian samples of nettle (herba): 476.8 ± 6.38 mg/kg (4). Result 222 ± 22 mg/kg in leaf nettle sample corresponds to previous Serbian result, while Al content in other tea samples is different (15).

Maximum Sn level was established in nettle leaf, being 10.22 ± 0.09 mg/kg, while minimum Sn content (2.68 ± 0.02 mg/kg) was determined in hibiscus flower. As visible from Table 3, Sn content in St. John's wort from locality I is equal to that at locality II. Concentration of Sn determined in peppermint and chamomile sample was similar to previous results (8), while content measured in rosehip was lower.

Results of microelements determination in some Polish herbs showed lower mean concentrations of Cu, higher levels of Zn and Ni and similar content of Fe in comparison to Serbian tea herbs (22); while another research demonstrated similar content of Ni and lower of Fe and Cu (23). Results on Mn, Fe, Cu and Zn levels in Spanish peppermint and chamomile are very similar to our results, while Al content was different (24).

In order to estimate possible correlation between microelements, the obtained results are analyzed using statistical software. Results demonstrated significant Pearson correlations (at the 0.01 level) between Fe-Co, Co-Ni, Mo-Sn and Ni-Sn: 0.78; 0.70; 0.66 and –0.50, respectively. Contrary to the literature data (25), our results did not show any significant correlation between Zn-Cu and Mn-Zn, but the investigated herbal teas were different. Correlations are comparable to those reported for Romanian herbal teas with respect to significant Fe-Co correlation and without correlations between Zn-Cu-Mn (19).

CONCLUSION

Sixteen samples of 14 different herbal teas widely consumed among Serbian population were analyzed for concentration of microelements, with an aim of establishing the mineral status and hence the health safety of medicinal plants used for very popular herbal teas. The contents of the examined elements were within the ranges reported in the literature, with some variations associated with plant species, applied agricultural measures and pedological features of soil. Large scale variability in the concentration of microelements was observed according to the species of medicinal plants and the availability of microelements in the soil. The highest contents of essential heavy metals were established for Fe (61.87–673.0 mg/kg). Maximum Fe level was observed in stinging nettle root (673 ± 18.18 mg/kg). The research revealed that basil, peppermint, stinging nettle (root), field horsetail and thyme are rich in iron. Thus, these plants are beneficial Fe source for humans. The highest variations are established for Mn content (CV = 101.48%) and the lowest for selenium (CV = 35.67%). The examined plant species may be considered as non-toxic natural source of essential microelements.

This paper is one of the rare comprehensive examinations of mineral composition of plants from different geographical regions of Serbia and represents a basis for further research on availability and interactions with other tea constituents. However, continuous monitoring of products for human consumption conducted by relevant institutions is indispensible. Thus, there is an increasing demand for even more reliable and rapid methods of quality control of medicinal plants. Cultivation of medicinal plants and good agricultural practice are the first step in quality and health safety assurance of plant-based medicinal products.

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