IOŚ-PIB OCHRONA ŚRODOWISKA I ZASOBÓW NATURALNYCH
ENVIRONMENTAL PROTECTION AND NATURAL RESOURCES 2015
DE GRUYTER OPEN
VOL. 26 NO 4(66): 26-29
DOI 10.1515 /OSZN-2015-0024

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Phosphorus, iron, manganese, zinc and copper in relation to total flavonoids in medicinal herbs and their infusions originating from Poland, Lithuania and Ukraine

Fosfor, żelazo, mangan, cynk i miedź w relacji do sumy flawonoidów w ziołach leczniczych i naparach pochodzących z Polski, Litwy i Ukrainy

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Keywords: medicinal plants, essential elements, total flavonoids, harvest in Poland, Lithuania and Ukraine, statistical methods
Słowa kluczowe: rośliny lecznicze, pierwiastki niezbędne, suma flawonoidów, zbiór w Polsce, Litwie i Ukrainie, metody statystyczne

Abstract

The aim of the investigation was is to identify differences in an elemental composition (P, Fe, Mn, Zn and Cu) and their total flavonoids contents of medicinal herbs originating from 9 botanical species, harvested in Poland, Lithuania and Ukraine. Metallic elements were determined by FAAS technique in mg·kg^{-1} of dry weight (d. wt) in the order: Fe > Mn > Zn > Cu in plant materials, and in infusions: Mn > Zn > Fe > Cu. The ratio of water-soluble form to total amount of a metal was as follows: 66.2% for Cu, 22.4% for Mn, 19.7% for Zn and 3.8% for Fe. The contents of P total, P inorganic and total flavonoids were determined by UV/Vis spectroscopic methods. By using of analysis of variance, correlation and cluster analyses it has been shown that a significant impact on the diversity of samples has the genetic factor – belonging to a particular botanical species of medicinal plant. The origin of investigated plants proved to be statistically significant only in the case of total Fe concentration, which was higher ($\alpha < 0.05$) for the samples grown in Ukraine. A number of significant correlations was also obtained ($r > 0.7$) between: P total – P inorganic, Mn total – Mn extractable and Cu total – Cu extractable, also ($r > 0.5$) between: total flavonoids – Cu extractable, P total – Cu total, P total – Cu extractable, P inorganic – Zn total. Moreover, comparison of the results of P, Fe, Mn, Zn and Cu determination in infusions of medicinal plants with the norms of RDA has shown that a significant amount (several percentage) of Mn and Cu can be supplemented to human organism with 2 cups of infusions prepared from *Helichrysi inforescentia* and *Hyperici herba*.

Streszczenie

Celem badań była identyfikacja różnic w składzie pierwiastkowym (P, Fe, Mn, Zn i Cu) oraz w zawartości sumy flawonoidów w ziołach leczniczych pochodzących od 9 gatunków botanicznych, zebranych w Polsce, Litwie i Ukrainie. Pierwiastki metaliczne oznaczono techniką FAAS (mg·kg^{-1} s.m.) w następującym porządku: Fe > Mn > Zn > Cu w suchej masie surowców, oraz w naparach: Mn > Zn > Fe > Cu. Stosunek form rozpuszczalnych w wodzie do całkowitej zawartości pierwiastków był następujący: 66,2% dla Cu, 22,4% dla Mn, 19,7% dla Zn i 3,8% dla Fe. Zawartość P całkowitego, nieorganicznego oraz sumę flawonoidów oznaczono techniką spektrofotometrii UV/Vis. Dzięki użyciu analizy wariancji, korelacji i skupień wykazano, że istotny wpływ na zróżnicowanie próbek wywierał czynnik genetyczny – przynależność do poszczególnych gatunków botanicznych roślin leczniczych. Natomiast oznaczono statystycznie wyższy ($\alpha < 0.05$) poziom Fe całkowitego w próbkach rosnących na Ukrainie. Otrzymano też szereg istotnych statystycznie korelacji ($r > 0.7$) pomiędzy: P całkowitym – P nieorganicznym, Mn całkowitym – Mn rozpuszczalnym oraz Cu całkowitą – Cu rozpuszczalną, również ($r > 0.5$) pomiędzy: sumą flawonoidów – Cu rozpuszczalną, P całkowitym – Cu całkowitą, P całkowitą – Cu rozpuszczalną, P nieorganicznym – Zn całkowitym. Ponadto, porównanie wyników oznaczenia P, Fe, Mn, Zn i Cu w naparach z roślin leczniczych z normami RDA wykazało, że istotne ilości (kilkanaście procent) Mn i Cu mogą być dostarczane do organizmu ludzkiego poprzez wypijanie 2 filiżanek naparu otrzymanego z *Helichrysi inforescentia* i *Hyperici herba*.

1. INTRODUCTION

Herbal drugs are very popular among populations of many European countries. The herbal enterprises settled in countries of Central-Eastern Europe deliver plant materials for medicinal purposes for their own societies, and also for export. It is also known that medicinal plants can be cultivated or are being collected from natural areas, and their quality control has received growing attention [World Health Organization report 2003, Schippmann *et al.* 2006].

Medicinal plant composition has been studied for numerous purposes. Secondary metabolites were analysed to select the plants with the optimum concentration of biologically active substances, for instance, flavonoids [Rohman *et al.* 2010, Marinova *et al.* 2005, Barros *et al.* 2012], also elemental contents have been characterised in several studies [Tokalioglu 2012, Karadas and Kara 2012, Arceusz *et al.* 2011, Razic *et al.* 2006, Mohanta *et al.* 2003]. The problem of influence of plant species and origin of herbs from various geographical location on the contents of essential elements in medicinal plants was studied, too [Konieczynski and Wesolowski 2007, Konieczynski *et al.* 2011].

Therefore the aim of this research is to identify differences in an elemental composition (P, Fe, Mn, Zn and Cu) and total flavonoids contents in medicinal herbs originating from nine different botanical species, harvested in Poland, Lithuania and Ukraine.

2. MATERIAL AND METHODS OF STUDY

Within the study 53 samples of medicinal herbs harvested in Poland, Lithuania and Ukraine, originating from 9 botanical plant species were analysed. They are listed below (sample numbers are given in parentheses): *Crataegi inflorescentia* (1-4), *Crataegi fructus* (5-8), *Equiseti herba* (9-15), *Helichrysi inflorescentia* (16-21), *Violae herba* (22-25), *Hyperici herba* (26-31), *Tiliae inflorescentia* (32-37), *Sambuci flos* (38-43), *Polygoni avicularis herba* (44-49) and *Matricariae flos* (50-53).

The plant samples were ground in a Knifetec sample mill 1095 (Höganäs, Sweden) and were kept in polyethylene containers up to the analyses. In order to prepare the plant materials for determination of total concentrations of the studied elements, the herbal samples were digested by microwave-assisted digestion procedure (UniClever BM-1z, Plazmatronika Wrocław, Poland). For carrying out the analysis of water-soluble forms of the elements, as well as for determination of the sum of flavonoids, single extraction by hot distilled water was used. Distilled water obtained in double-distillation system (Heraeus Quarzglas, Germany) was used throughout.

Spectrophotometric UV/Visible method based on phosphomolybdate blue was applied for total P and water-extractable P determinations using SP-870 spectrophotometer (Metertek, South Korea), whereas flame-atomic absorption spectroscopy applying A250 plus spectrometer (Varian, Australia) was used to determine Fe, Mn, Zn and Cu concentrations both in digests obtained from plant samples, and in aqueous extracts (teas) prepared from the studied herbal materials [Konieczynski and Wesolowski 2007]. Total flavonoids content was determined by spectrophotometric method with $AlCl_3$ at the analytical wavelength 510 nm using the same apparatus as for P determinations [Zhishen *et al.*, 1999].

3. RESULTS AND DISCUSSION

Based on the results it was shown that mean total flavonoids content in all studied samples was equal to 50.07 $mg \cdot g^{-1}$ of dry plant weight (d. wt). The lowest amount, 22.43 $mg \cdot g^{-1}$ d. wt was determined in *V. herba* (sample 25), and the highest, 128.33 $mg \cdot g^{-1}$ d. wt in *H. herba* (sample 30). The mean values of total flavonoids contents in particular botanical plant species are presented in Fig. 1.

The infusions prepared from *H. herba* can be characterised as having the highest level of total flavonoids – 90.84 $mg \cdot g^{-1}$ d. wt, and the lowest amount – 16.06 $mg \cdot g^{-1}$ d.wt was found in infusions from *V. herba*. The results of ANOVA calculations confirm statistically significant differences ($\alpha < 0{,}05$) in mentioned above

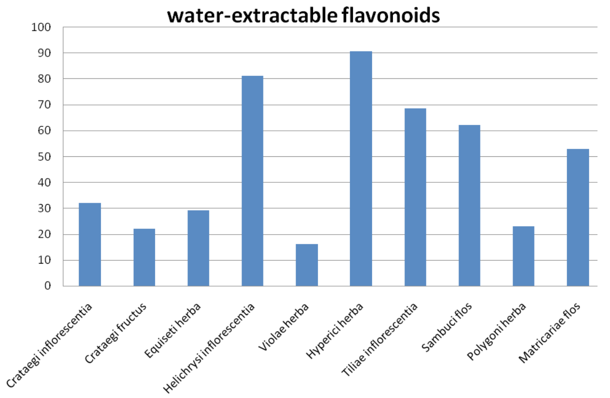

Fig. 1. Total flavonoids contents [$mg \cdot g^{-1}$ d. wt] determined in various botanical plant species

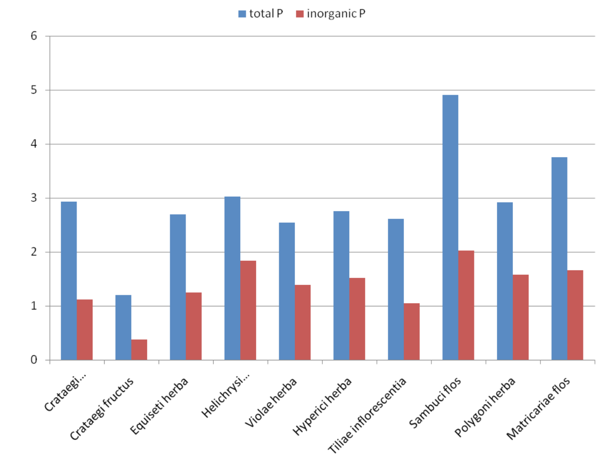

Fig. 2. Total and water-extractable phosphorus [$mg \cdot g^{-1}$ d. wt] in the samples of medicinal herbs.

levels among herbs from various plant species. Moreover, Tukey test indicated the significant difference occurring among plant samples from the following plant species: *Polygonum, Helichrysum, Hypericum, Tilia* and *Sambucus*.

Total phosphorus (total P) mean concentration was determined in all samples as 2.98 $mg \cdot g^{-1}$ d. wt, whereas water-extractable form of P ($P\text{-}PO_4$) as 1.42 $mg \cdot g^{-1}$ d. wt. The level of phosphorus forms is differentiated taking into account the origin of the studied samples from different plant species, as shown in Fig. 2. The highest mean concentration of total P – 4.90 $mg \cdot g^{-1}$ d. wt was found in *S. flos*, and the lowest – 1.20 $mg \cdot g^{-1}$ d. wt in *C. fructus*. Similarly, in the same plant materials water-extractable inorganic P was determined, 2.02 $mg \cdot g^{-1}$ d. wt in *S. flos*, and 0.38 $mg \cdot g^{-1}$ d. wt in *C. fructus*, respectively. On average, water-extractable inorganic P constitute 48% of total level of that element in all studied samples. This is compatible with the previous studies of water-extractable inorganic P in medicinal plant infusions [Konieczynski *et al.* 2011]. The results of ANOVA test revealed that the difference among plant samples are statistically significant ($\alpha < 0{,}05$), and the use of Tukey's test has proven that significant differences in total and inorganic P levels occur among the plants from *Crataegus, Equisetum, Viola, Polygonum, Helichrysum, Hypericum, Tilia, Chamomilla* and *Sambucus* botanical species.

Table 1. Results of correlation analysis. Correlation coefficients (r) statistically significant (α < 0,05) are printed in bold

	Total flav.	**P**	**$P\text{-}PO_4$**	**Zn**	**Zn-ext**	**Mn**	**Cu**
$P\text{-}PO_4$	**0.34**	**0.78**	1.00				
Fe	**-0.37**	0.15	0.15				
Fe-ext	-0.04	0.14	0.15				
Zn	0.19	**0.41**	**0.63**	1.00			
Zn-ext	**0.48**	-0.12	0.24	**0.61**	1.00		
Mn	**0.36**	0.03	0.17	**0.29**	**0.53**	1.00	
Mn-ext	**0.42**	0.01	**0.28**	**0.50**	**0.68**	**0.84**	
Cu	**0.37**	**0.65**	**0.57**	0.13	-0.08	0.01	1.00
Cu-ext	**0.56**	**0.57**	**0.55**	0.19	0.32	0.11	**0.80**

Total flav. = total flavonoids contents; ext = water-extractable form of an element

Table 2. Contribution to the RDA norms after drinking 2 cups of herbal infusion (500 mL)

Herbal remedy	**P**	**Mn**	**Cu**	**Fe**	**Zn**
	[% RDA]				
Crataegi inflorescentia	2.76	5.07	7.99	0.82	1.54
Crataegi fructus	0.96	0.69	4.19	1.41	nd
Equiseti herba	3.11	9.63	5.58	1.08	0.38
Helichrysi inflorescentia	4.64	18.57	12.54	1.39	3.05
Violae herba	3.27	14.27	9.74	1.74	2.2
Hyperici herba	3.83	12.13	13.08	1.03	2.2
Tiliae inflorescentia	2.57	8.88	12.92	1.07	nd
Sambuci flos	5.08	5.27	15.62	1.5	0.9
Polygoni avicularis herba	3.86	4.61	12.12	1.16	0.31
Matricariae flos	4.06	3.45	14.19	1.4	nd

nd – not detected

In the case of studied metallic elements, it can be stated that the analysed plant samples contained them in the following order: Fe > Mn > Zn > Cu in mg/kg^{-1} d. wt, and in infusions: Mn > Zn > Fe > Cu mg/kg^{-1} d. wt. The ratio of water-soluble form to total level of a metal was as follows: 66.2% for Cu, 22.4% for Mn, 19.7% for Zn and 3.8% for Fe. These results are concurrent with those reported by others [Tokalioglu 2012, Karadas and Kara 2012, Razic *et al.* 2006, Mohanta *et al.* 2003]. ANOVA calculation allows to state that the differences in metallic elements level in the studied plant samples are statistically significant (α < 0,05), and application of Tukey's test enabled to reveal that these differences are important for all botanical plant species. However, taking into consideration the origin of studied medicinal plants from various countries, it was found that only in the case of total Fe content, it was statistically higher (α < 0.05) for the samples grown in Ukraine. The reason for this difference is not easy to explain, perhaps the level of Fe in Ukrainian soils was higher than in other locations, but this metal was not assayed in soil samples. The results of correlation analysis are presented in Table 1. Among 55 calculated correlations, there are 22 statistically significant relations (α < 0.05). It is possible to indicate high correlations (r > 0.7) between P total – P inorganic, Mn total – Mn extractable and Cu total – Cu extractable, also (r > 0.5) between: total flavonoids – Cu extractable, P total – Cu total, P total – Cu extractable, P inorganic – Zn total. High correlation between total P and inorganic P was found in our earlier studies [Konieczynski and Wesolowski 2007, Konieczynski *et al.* 2011], and the relation between flavonoid contents and metallic elements can indicate on their participation in the same metabolic pathways in medicinal plants, moreover Cu as electro-active metal can interact with flavonoid compounds [Konieczynski, 2015].

Analysing the obtained results in context of delivery of essential elements to human organism, it was assumed that a consumer drinks daily two cups of herbal infusion (500 ml). The results of the calculation are shown in Table 2. On average, 2 cups of herbal infusions from the studied medicinal plants deliver 3.4 % of the recommended dietary allowances [National Academy of Sciences, 2004] for P. Taking into account the bioavailable level of essential metallic elements, it is possible to notice that a significant amount (several percentage) of Mn and Cu can be supplemented to human organism with two cups of infusions prepared from *H. inforescentia* and *H. herba*. The infusions prepared from other plant species deliver rather lower amounts (several percents) of metallic elements in comparison with dietary norms.

4. CONCLUSIONS

By analysis of total flavonoids together with metallic elements and P contents it was possible to detect the differences occurring among medicinal plant species originating from Poland, Lithuania and Ukraine. Due to the application of ANOVA statistically significant differences among the studied plant samples were revealed, and the main reason for their differentiation appeared to be the botanical plant species. The origin of investigated plants proved to be statistically significant only in the case of total Fe concentration, which was higher ($\alpha < 0.05$) for the samples grown in Ukraine.

ACKNOWLEDGEMENTS

This paper was supported by the Ministry of Science and Higher Education of the Republic of Poland from the quality-promoting subsidy programme (ST-15). The Authors wishes to thank dr Viktorija Vastakaite for kind delivery of the plant samples from Lithuania, and dr Oleg M. Koshoviy for the samples from Ukraine.

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