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DETERMINATION OF SELECTED ELEMENTS IN DIFFERENT PHARMACEUTICAL FORMS OF SOME POLISH HERBAL MEDICINAL PRODUCTS

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Abstract: In the present work, the following microelements and heavy metals were determined in the Willow bark (Cortex Salicis), in the St. John's wort (Herba Hyperici), in the infusions from these raw materials and in the tablets containing these herbs: Cr, Cu, Fe, Ni, Zn and Ba, Cd, Pb. The concentration of the metals were determined by means of the GFAAS and ICP-MS methods. Considering the examined two herbs as a source of toxic metals, the occurrence of high levels (exceeding the European Pharmacopoeia limits) of Cd should be emphasized. The level of cadmium was low in the infusions because of a low extraction efficiency of this metal, so that the infusions can be found as a rather safe form of herbal medicine. In the tablets, the Cd contents were similar to the levels found in the raw materials. Because the total heavy metal content can be accessible, it may cause a potential health risk for human.

Keywords: St. John's wort, herbal medicine, metal content, atomic spectrometry, cadmium, infusions

Phytotherapy is a very old branch of medicine and despite of the large development of synthetic drugs, it still enjoys vast popularity. According to the World Health Organization (WHO) about 70–80% of the world population relies on a non-conventional medicine and still uses many herbal products in the primary healthcare (1). Even the developed countries spend a significant amount of their incomes on the OTC (over-the-counter) herbal products. However, despite the fact that the herbal drugs are very often considered as a natural and therefore harmless, they are not free from some adverse effects (2). They can be contaminated with undesirable, harmful substances like pesticides, microbial contaminants, heavy metals and chemical toxins. The sources of these contaminations would be: contaminated environment, collecting of the plant materials, inappropriate storage conditions or mistakes made during the production processes.

Until recently, there have been no uniform standards for the herbal drugs concerning a permissible level of toxic metals. The WHO mention the maximum permissible levels in the herbal drugs only for cadmium and lead (0.3, 10 mg/kg) (3). In 2008, Pharmeuropa reported the newly proposed standards for cadmium, lead and mercury (0.5, 5.0, 0.1 mg/kg) (4). The European Pharmacopoeia 7.0 published new standards for these three heavy metals. They are 1.0 mg/kg for cadmium, 5.0 mg/kg for lead and 0.1 mg/kg for mercury (5).

In the present work, some essential trace elements such as Ba, Cr, Cu, Fe, Ni, Zn and heavy metals such as Cd and Pb were determined in raw herbs (willow bark – Cortex Salicis; St. John's wort – Herba Hyperici), in the infusions and in the tablets. The concentration of the metals was determined by the GFAAS and ICP-MS methods.

MATERIALS AND METHODS

Materials

Samples of Cortex Salicis (*Salix sp*.) and Herba Hyperici (*Hypericum perforatum*) in different pharmaceutical forms were supplied from retail pharmacies located in Warszawa. Apart from the crude herbs there were also analyzed the infusions and the tablets such as Salicortex (Salicis Cortex – 330 mg), Rutinosal C (Salicis Cortex – 300 mg, Rutosidum – 20 mg, Vitaminum C – 40 mg) and Hyperherba (Hyperici Herba – 330 mg).

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Analysis of the certified reference material Mixed Polish Herbs (INCT-MPH-2) was also performed to assure a proper quality of our results. The following reagents were used: standard solutions of Cr, Cu, Fe, Ni, Zn and Ba, Cd, Pb at a concentration of 1 mg/mL (Merck), 0.1 M HCl prepared from 37% HCl (Merck), concentrated nitric acid (65%) of purity suitable for ICP-MS (Merck), redistilled water was additionally purified in the Nanopure Deionization system (Barnstead) and argon 99.99% (BOC Gazy).

Apparatus

The measurements were performed with a VG PlasmaQuad 3 inductively coupled plasma mass spectrometer, an Avanta Ultra Z atomic absorption spectrometer equipped with the graphite furnace (GBC

Table 1. Parameters of mineralization

Stage	Microwave power		Minimum pressure [atm]	Maximum pressure [atm]	Heating time [min]	Cooling time [min]
	[%]	[W]				
I	25	87.5	22	25	2	2
	45	157.5	42	45	2	10
II	60	210.0	22	25	2	2
	80	280.0	42	45	2	10
III	90	315.0	22	25	2	2
	95	350.0	42	45	2	10

Table 2. Parameters for the determination of some elements by the GFAAS method.

Element	Wavelength [nm]	Slit width [nm]	Lamp current [mA]	Pyrolysis [^{o}C]	Atomization [^{o}C]	Modifier
Ba	553.6	0.5	8	1400	2700	1% $(NH_4)HPO_4$ + 0.06% $Mg(NO_3)_2$
Cd	228.8	0.5	3	300	2050	–
Cr	357.9	0.2	6	1200	2500	–
Cu	324.7	0.5	3	800	2100	–
Fe	248.3	0.2	10	800	2300	–
Ni	232.0	0.2	8	1000	2600	0.01% $Pd(NO_3)_2$
Pb	217.0	1.0	5	1500	1800	1% $(NH_4)HPO_4$ + 0.06% $Mg(NO_3)_2$
Zn	213.9	0.2	8	1000	1800	–

Table 3. Results of the determination of some elements by GFAAS in the certified reference material (n = 6).

Element	Mixed Polish Herbs (INCT-MPH-2)	
	Certified value [mg/kg]	Determined value [mg/kg]
Ba	32.5 ± 2.5	32.5 ± 1.2
Cd	0.199 ± 0.015	0.184 ± 0.008
Cr	1.69 ± 0.13	1.49 ± 0.11
Cu	7.77 ± 0.53	8.03 ± 1.13
Fe	460	486 ± 20
Ni	1.57 ± 0.16	1.68 ± 0.30
Pb	2.16 ± 0.23	1.86 ± 0.11
Zn	33.5 ± 2.1	35.0 ± 1.8

Scientific Equipment Pty, Ltd., Dandenong, Australia). The mineralization was carried out in a microwave digestion system (Plazmatronika, Wrocław, Poland).

Procedures

The samples of 1.0 g of the analyzed material were placed in teflon crucibles. Next, 5 mL of concentrated nitric acid was added. After 24 h, the mineralization was carried out in a closed system with the use of microwave energy (Table 1). After cooling, the sample solutions were transferred into volumetric flasks and were made up to 100 mL with deionized water.

The loss of mass after drying (moisture content) was also determined according to the procedure given in the Polish Pharmacopoeia VIII.

The infusions of analyzed herbs were prepared according to the indications given by the producers. About 2.0 g of Herba Hyperici was placed in a glass beaker, 150 mL of boiling deionized water was added and the sample was left for 60 min under cover. About 3.0 g of Cortex Salicis were placed in a glass beaker, 150 mL of deionized water was added and the sample was warmed under a cover on a water bath at 95°C for 15 min. After cooling, the extracts of the both herbs were filtered through a sieve and the volume of infusion was filled up to 200 mL with deionized water.

The herbal material remaining after the infusion was left for 24 h and then dried at 110°C to a constant weight. About 1.0 g of dried material was placed in teflon digestion vessels. The mineralization was performed according to the procedure given for the raw material.

Determination of the metal release from tablets

The determination of the metals release was performed according to standards from the monograph for the tablets from the Polish Pharmacopoeia VIII. Five tablets of the examined products were put into the beakers with 500 mL of 0.1 M HCl at 37.0 ± 0.5°C. The release of the elements from this pharmaceutical form was carried out during 30 min. After this time, the solutions from beakers were filtered to 100 mL flasks where was added earlier 0.1 mL (10 g/L) indium, and the flasks were made up to the mark with hydrochloric acid. These solutions were analyzed (without mineralization) for the elements content by the GFAAS and ICP-MS methods.

Determination of Cd, Co, Cr, Ni, Pd by ICP-MS method

Parameters used during the analyses were as follows: the excitation power of plasma: 1380 W; the flow rate for: plasma gas – 12.5–12.7 L/min, nebulization gas – 0.74–0.80 L/min; auxiliary gas – 0.7–0.9 L/min; the base line of the background – below 10 cps; the amount of doubly charged ions 70/140 Ce^{2+}/Ce and 69/138 Ba^{2+}/Ba – below 3%; the amount of oxide ions 156/140 CeO/Ce – below 3% and that of 154/138 BaO/Ba – below 0.2%; the aspiration time of a sample was 180 s and the measurement time was 15 s, in threefold repetitions.

Determination of Ba, Cd, Cr, Cu, Fe, Ni, Pb, Zn by GFAAS method

The determination of studied elements was performed in a graphite tube pyrolytically coated. During the determination, the Zeeman-effect background correction was applied. The determination parameters of the elements by the GFAAS method are presented in Table 2.

RESULTS AND DISCUSSION

Accuracy of the data quality has been checked by an analysis of the certified reference material Mixed Polish Herbs (INCT-MPH-2). The obtained results are in a good agreement with the certified values, as it is presented in Table 3.

The obtained results show that studied medicinal herbs contain significant amounts of the elements and their content is strongly variable. Microelements are considered as a factor indispensable for the proper functioning of living organisms, therefore, it is essential to estimate their amounts in water, food and also in the medicinal plants. This is particularly important now, when using of supplements is very popular. This creates a risk of exceeding the daily demands for different elements.

According to the literature, many medicinal plants can present a health risk due to the presence of various toxic elements. Our studies have demonstrated that in the Cortex Salicis – one of the most popular herbs in Poland, the content of cadmium exceeds the limit of European Pharmacopoeia (maximum permissible level of cadmium 1.0 mg/kg dry weight) (Table 4 and 5). This problem was mentioned earlier by Chizzola (7).

Raising the standard for cadmium doesn't seem to solve the problem of high amounts of this metal in herbal products or plants. According to Schneider (6), Chizzola (7) and Vargas (8), the content of cadmium exceed the new limit, sometimes even several times. It refers mainly to some plant species like Willow and St. John's wort, which are known to be able to accumulate a high content of cadmium.

Table 4. Results of the determination of some elements by GFAAS in infusions and remainders after leaching (n = 6).

Element	Cortex Salicis			Herba Hyperici		
	Raw material [mg/kg dry weight]	Infusion [%]	Remainder [%]	Raw material [mg/kg dry weight]	Infusion [%]	Remainder [%]
Ba	23.6 ± 3.2	10.5 ± 3.2	84.0 ± 5.5	25.3 ± 3.8	56.6 ± 0.2	60.2 ± 8.7
Cd	2.07 ± 0.56	2.1 ± 0.3	85.6 ± 1.4	0.81 ± 0.7	8.1 ± 0.3	93.2 ± 4.7
Cr	0.62 ± 0.23	ND	74 ± 15	0.39 ± 0.13	ND	ND
Cu	29.5 ± 0.94	23.6 ± 3.2	91 ± 16	7.68 ± 0.21	54.0 ± 6.5	41.8 ± 7.7
Fe	116 ± 18	4.6 ± 1.4	86 ± 15	152 ± 35	17.4 ± 6.2	65.5 ± 9.1
Ni	3.1 ± 1.2	14.7 ± 6.2	78 ± 11	2.15 ± 0.65	73.9 ± 7.3	29.4 ± 9.7
Pb	1.33 ± 0.65	ND	74 ± 13	1.58 ± 0.20	ND	89.0 ± 7.5
Zn	640 ± 110	47 ± 11	48.8 ± 8.0	75 ± 13	66 ± 15	42.1 ± 8.3

ND – below the limit of detection.

Table 5. Results of the determination of some elements in the analyzed tablets and herbs (GFAAS and ICP-MS), (mg/kg dry weight), n = 5 (Salicortex, Rutinosal C, Hyperherba), n = 7 (Salicis Cortex, Hyperici Herba).

Metal	Method	Salicortex	Rutinosal C	Hyperherba	Salicis Cortex	Hyperici Herba
Ba	GFAAS	20.9 ± 5.0	15.5 ± 3.2	3.21 ± 0.67	23.6 ± 1.2	25.3 ± 1.8
Cd	GFAAS	1.67 ± 0.12	1.73 ± 0.14	0.739 ± 0.075	1.966 ± 0.090	0.800 ± 0.052
	ICP-MS	1.986 ± 0.071	2.190 ± 0.035	0.769 ± 0.013	2.018 ± 0.092	0.803 ± 0.013
Co	ICP-MS	0.1862 ± 0.0078	0.174 ± 0.014	0.3504 ± 0.0081	0.3148 ± 0.0088	0.1982 ± 0.0089
Cr	GFAAS	4.3 ± 1.3	3.83 ± 0.67	2.64 ± 0.49	0.596 ± 0.062	0.380 ± 0.054
	ICP-MS	3.24 ± 0.16	2.636 ± 0.056	1.322 ± 0.059	0.63 ± 0.10	0.407 ± 0.041
Cu	GFAAS	3.16 ± 0.55	3.03 ± 0.23	4.74 ± 0.61	2.95 ± 0.29	7.68 ± 0.91
Fe	GFAAS	131 ± 25	176 ± 29	162 ± 35	115.9 ± 6.4	152 ± 18
Ni	GFAAS	2.55 ± 0.31	2.84 ± 0.66	2.17 ± 0.64	2.86 ± 0.26	2.58 ± 0.40
	ICP-MS	2.65 ± 0.14	2.15 ± 0.11	1.956 ± 0.078	3.48 ± 0.44	2.79 ± 0.23
Pb	GFAAS	1.29 ± 0.18	2.83 ± 0.29	2.59 ± 0.26	1.41 ± 0.15	1.68 ± 0.28
	ICP-MS	1.350 ± 0.096	3.56 ± 0.22	2.59 ± 0.14	1.252 ± 0.095	0.99 ± 0.11
Zn	GFAAS	289 ± 52	179 ± 18	194 ± 36	638 ± 77	75.3 ± 8.3

Table 6. The release of some metals from the tablets (%); x min. (the smallest release), x max. (the highest release), x (medium relase), SD (standard deviation).

Metal	Release [%]			
	x min.	x max.	x	SD
Ba	27.5	66.0	54.5	17.6
Cd	76.0	109.8	94.1	16.5
Co	51.9	102.1	78.6	14.2
Cr	7.6	49.4	24.1	10.4
Cu	60.8	104.8	88.6	13.0
Fe	11.4	59.4	34.8	14.0
Ni	21.7	91.6	59.7	18.4
Pb	34.7	90.7	67.4	17.5
Zn	37.3	58.9	45.0	7.7

The analysis of infusions have revealed that the percentage of elements in infusion is very different. When it comes to toxic heavy metals like Cd and Pb, the percentage of release from the herbs to the infusions is low. This fact suggests that this is a safe form of using herbs by patients. Our results are compatibile with those published in recent years (9, 10). Tablets are very popular pharmaceutical forms because of easiness to application and the void of any disagreable smell and taste during the use. The content of studied elements in the tablets was similar to herbs and also exceeded the proposed limits (Table 5). There has also been estimated the availability of various elements (releasing the elements from the tablets to the solutions of hydrochloric acid). The results indicated on the high degree of release of metals such as Cd and Pb and different microelements from this pharmaceutical form (Table 6).

The present results indicate two major problems. First of all, the herbs like St. John's wort and willow may present a health risk for people. Secondly, the incompability with the aforementioned standards can cause serious problems with marketing of these herbs and exporting them to the other countries in the European Union.

CONCLUSIONS

The obtained results show that some of the examined herbal medicines contain a high amount of cadmium which exceeds the European Pharmacopoeia limits. It shows the necessity of complex studies to check the level of this element in herbs and soil in Poland and a constant monitoring the cadmium concentration in pharmaceutical forms of herbal medicines.

The low extracion efficiency of heavy metals to infusions can be an argument to change some law regulations. It seems to be desirable to evaluate not only the content of heavy metals in herbs, but also to estimate the amount which is available for a patient after dosage of herbal pharmaceutical forms. These facts suggest that maybe it would be advisable to create the relevant standards for different pharmaceutical forms.

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