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NATURAL DRUGS

PHYTATE, INORGANIC AND TOTAL PHOSPHORUS AND THEIR RELATIONS TO SELECTED TRACE AND MAJOR ELEMENTS IN HERBAL TEAS

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Abstract: Phytate phosphorus in 59 samples of herbal teas was determined within the range of 2.44-36.90 μg/mL. Extraction yield was statistically higher in extracts from the leaves than that found in extracts from other plant organs. Average total level of trace elements determined in medicinal herbs follows the order: Fe > Mn > Zn > Cu, for major elements the order is: Ca > Mg > Na > K. Correlation analysis revealed that relations of phytate P to other phosphorus forms and metals were statistically insignificant. However, there were several characteristic relations of inorganic phosphorus to water-extractable K, Zn and total Zn. Furthermore, positive relations were found between total and water-extractable fractions of metals. Principal component analysis grouped the samples into separate clusters. It was also found that inorganic P, as well as water-extractable Zn and Na, had a huge impact on differentiation of the studied plant material.

Keywords: phytate phosphorus, anti-nutrient component, medicinal herbal teas, trace and major elements, inter-elemental relations

Phosphorus (P), among other major and trace elements, is one of the most important indispensable constituents of all living organisms. This non-metallic element contributes to the biochemical transitions, which are necessary for vital processes through creating highly energetic bonds in molecules of ATP and ADP. It is a constituent of DNA and RNA, amino acids and peptides. As phosphates, P plays a crucial role in acid-base homeostasis by regulating the buffer properties of blood (1).

Total phosphorus (total P), along with the trace and major elements level in medicinal herbs, has been reported in many studies, and it varied within the range of mg/g of dry plant tissue (2, 3). The concentration of inorganic phosphate (inorganic P) in aqueous extracts from medicinal herbs has also been studied. It was found that this fraction of P occurs over the range of 53 to 63% of the total P in the plant material (4, 5). Inorganic P has also a positive effect on the synthesis of secondary metabolites of medicinal plants (6).

However, the inorganic P is not only a single chemical species, in which this element occurs in a plant material. It has also been found that several plant materials and foods of plant origin, including cereals and legumes, contain P in a phytate form (7-9). The content of phytates in food of plant origin can vary depending on various processing and cooking procedures (10).

The phytate form of phosphorus consists of phytic acid (phytate; *myo*-inositol 1,2,3,4,5,6-hexakisphosphate) (Fig. 1), which is the primary source of inositol and the principal storage form of P in plant seeds contributing between 50 and 80% of total P (11). This is important for health, because phytate P can bind certain elements, such as Fe or Mg, making them less available for humans (11). In this context, the phytate P can be regarded as an anti-nutrient component. However, there is also information in the literature that phytates play a beneficial role in prevention of different types of cancer, as well as they have antioxidant properties (12, 13). It has also been reported that the diet high in phytic acid can have protective effect against heavy metals, like cadmium (14).

A previous study has shown that inorganic P is often correlated to total level of this element, and with several metallic elements occurring in medici-

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nal plants, e.g., with Mg (4). These relations can be explained by the fact that P, Mg and Fe have a synergistic effect in plant metabolic processes (15). A literature review has shown that phytate P level in medicinal herbs has not been widely studied. However, the contents of phytic acid was analyzed in several African medicinal plants and it was found within the range of 0.89 – 2.55 mg/g (16). The analysis of phytates is crucial for patients drinking herbal teas, because phytate P can chelate certain metals, such as Mg, Ca, K or Fe, thus lowering their bioavailability for humans. Therefore, the objective of this study was to determine the phytate P in herbal teas and to investigate its relation to total P, inorganic P, and selected trace and major elements in herbal teas prepared from different morphological parts of medicinal plants.

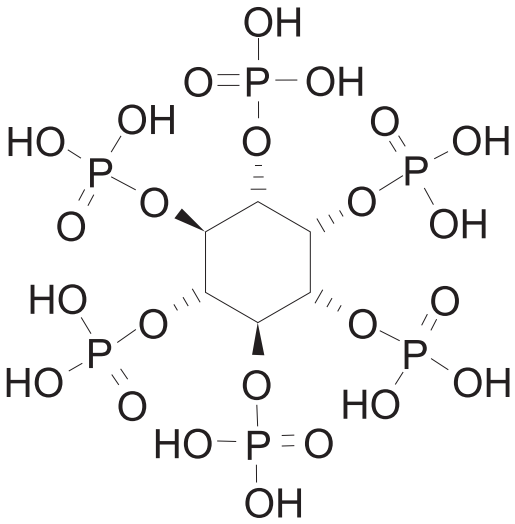

Figure 1. Chemical structure of phytic acid. From www.http://chemistry.about.com

EXPERIMENTAL

Plant material

The studied medicinal herbal materials comprised herbs (sample numbers are given in parenthesis): *Herba Euphrasiae* (*1* and *2*), *Millefolii* (*3* and *4*), *Equiseti* (*5-7*), *Hyperici* (*8-10*) and *Violae tricoloris* (*11* and *12*); leaves: *Folium Salviae* (*13-16*), *Sennae* (*17-19*) and *Urticae* (*20* and *21*); flowers: *Inflorescentia Tiliae* (*22-25*), *Crataegi* (*26-29*), *Flos Sambuci* (*30* and *31*), *Anthodium Calendulae* (*32-34*) and *Chamomillae* (*35-38*); fruits: *Fructus Anisi* (*39-41*), *Crataegi* (*42-45*), *Foeniculi* (*46-48*), *Rubi* (*49* and *50*), *Sorbi* (*51*), *Rosae* (*52*), *Aroniae* (*53*), *Sambuci* (*54*), *Myrtilli* (*55*) and *Pericarpium Phaseoli* (*56*); seeds: *Semen Sylibi mariani* (*57*, *58*) and *Psylli* (*59*).

All samples were taken from Polish herbal firms. Most of them originated from “Kawon”,

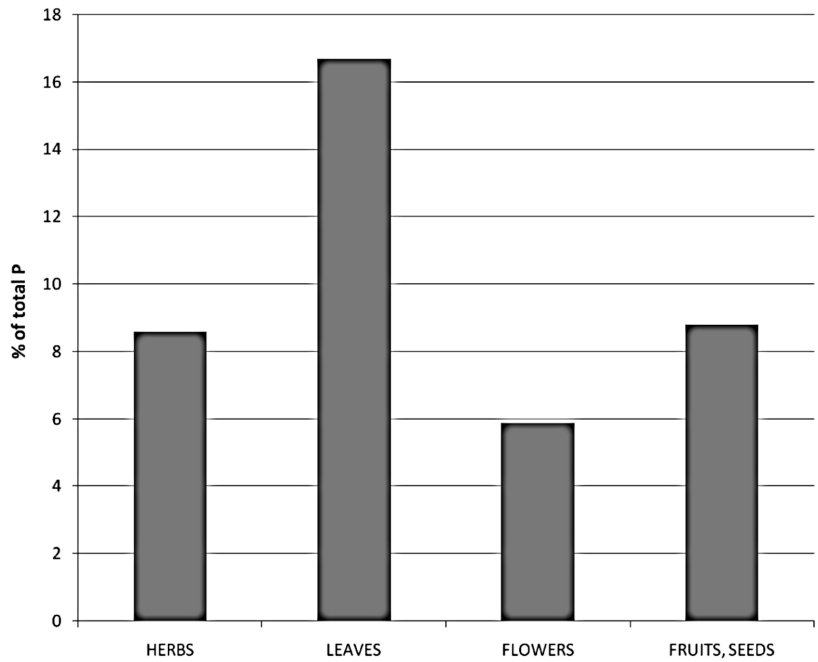

Figure 2. The average extraction yield of phytate P (% of total P) obtained for different morphological parts of medicinal plants

Table 1. Recovery and precision (n = 6) of the methods tested on the Certified Reference Material: Mixed Polish Herb (INCT-MPH-2).

Element (total concentration)	Declared contents (mg/kg d.w.)	Determined ± SD (mg/kg d.w.)	Recovery (%)	Precision as CV
P	2.50*	2.16 ± 0.06*	86.4	2.8
Fe	460.00	360.00 ± 4.41	78.3	1.2
Zn	33.50	37.72 ± 1.39	112.6	3.7
Mn	191.00	219.04 ± 8.56	117.5	3.8
Cu	7.77	7.59 ± 0.22	97.7	2.9
Mg	2.92*	3.32 ± 0.03*	113.7	0.9
Ca	10.8*	10.0 ± 0.01*	92.6	0.1
Na	350.00	359.90 ± 2.50	102.8	0.7
K	19.1*	16.8 ± 0.04*	88.0	0.2

* (mg/g d.w.), CV – coefficient of variation, d.w. – dry weight

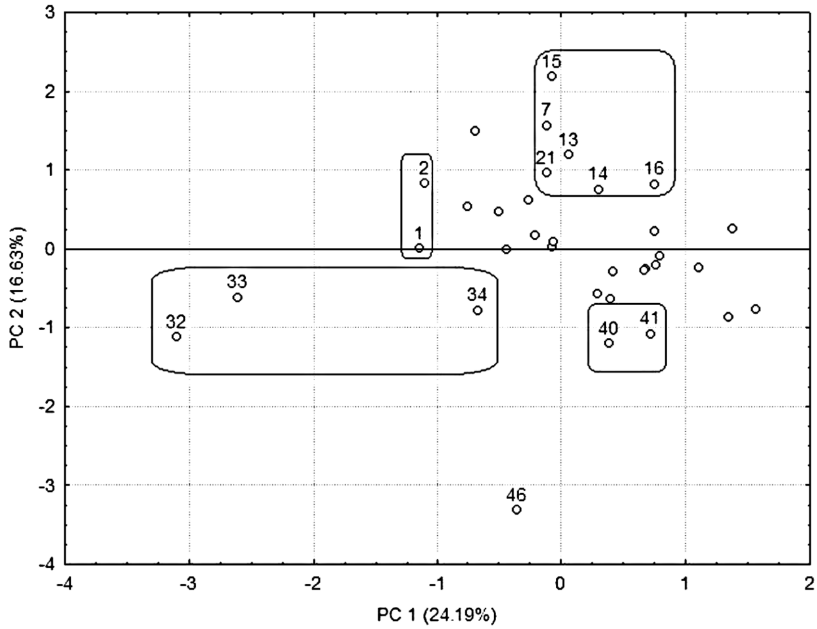

Figure 3. Distribution of the analyzed samples of medicinal herbal materials in two-dimensional plot of PC1 *vs.* PC2. Sample numbers correspond to herbal materials characterized in the Experimental part and in Table 2

samples No. *22, 43* and *59* were from "Herbapol", *49* and *51* from "Dary Natury", *57* from "Herbavita", and sample No. *58* from ZPH "ASZ" herbal firms.

Digestion of plant samples

The quantification of total level of P and metallic elements was proceeded by microwave digestion of the analyzed herbal material. The samples were placed in teflon vessels, and a mixture of HNO_3 (65% Selectipur solution) and H_2O_2 (30% solution) (3 + 5, v/v) (Merck, Germany) was added. Then, the samples were digested in a Uniclever BM-1z mineralization device (Plazmatronika, Poland). The following parameters of microwave digestion were applied: pressure over the range of 31 to 45 atm., temperature between 250 and 350°C, the power of microwaves set to 85% of the maximum value, time of digestion 7 min, time of cooling 5 min. After digestion, the samples were transferred into volu-

Table 2. Results of determination of phytate phosphorus, total phosphorus and inorganic phosphorus in the studied medicinal plant samples.

Sample No.	Phytate P (µg/mL)			Total P	$P\text{-}PO_4$	$P\text{-}PO_4$/ total P (%)
	Range	Mean ± SD	Phytate P/ total P (%)	mean (mg/g)		
Herbs *(Herba)*						
1	10.46 – 12.13	11.44 ± 0.87	3.9	2.71	2.28	84.1
2	28.06 – 29.46	28.95 ± 0.77	7.5	3.56	1.63	45.8
3	10.79 – 14.19	12.77 ± 1.76	3.9	2.86	1.86	65.0
4	13.73 – 15.33	14.37 ± 0.84	4.8	2.66	2.42	91.0
5	25.52 – 27.06	26.04 ± 0.88	12.6	1.79	1.24	69.3
6	21.59 – 23.66	22.64 ± 1.03	11.4	1.82	0.98	53.8
7	22.73 – 27.59	26.08 ± 2.09	10.1	2.15	1.62	75.3
8	n.d.	n.d.	n.d.	2.37	1.72	72.6
9	20.72 – 23.06	21.50 ± 1.30	8.3	2.48	2.05	82.7
10	24.39 – 25.19	24.77 ± 0.40	10.4	2.20	1.16	52.7
11	19.66 – 21.59	20.75 ± 0.99	8.5	2.34	1.19	50.9
12	23.79 – 25.39	24.61 ± 0.80	12.9	1.88	1.51	80.3
Leaves (*Folium)*						
13	10.86 – 12.13	11.68 ± 0.71	6.5	2.17	1.46	67.3
14	15.13 – 17.19	16.37 ± 1.09	13.0	2.11	1.43	67.8
15	19.13 – 20.13	19.71 ± 0.51	18.5	1.25	1.01	81.5
16	22.26 – 23.79	23.13 ± 0.78	17.6	1.72	1.25	72.7
17	36.26 – 37.66	36.90 ± 0.70	48.3	0.73	0.17	23.3
18	34.93 – 35.06	35.02 ± 0.07	19.9	1.55	0.74	47.7
19	19.33 – 23.19	20.62 ± 2.23	18.8	1.75	0.42	24.0
20	22.33 – 24.79	23.22 ± 1.36	4.1	4.58	0.79	17.2
21	15.13 – 17.13	16.26 ± 1.02	3.5	4.22	0.73	17.3
Flowers (*Flos, Inflorescentia, Anthodium)*						
22	12.19 – 14.19	12.86 ± 1.15	7.8	2.28	1.31	57.5
23	17.00 – 17.93	17.62 ± 0.53	8.5	1.98	1.13	57.1
24	17.46 – 18.00	17.64 ± 0.31	6.6	2.48	1.20	48.4
25	13.33 – 14.59	14.08 ± 0.66	7.0	1.93	0.82	42.5
26	14.80 – 15.93	15.55 ± 0.65	5.3	2.77	1.27	45.8
27	19.46 – 20.59	20.26 ± 0.69	8.6	2.24	0.94	41.9
28	17.53 – 19.86	19.00 ± 1.27	8.7	2.19	0.85	38.8
29	12.86 – 16.13	14.17 ± 1.72	5.9	2.23	0.64	28.7
30	22.33 – 24.13	23.37 ± 0.93	6.2	3.77	1.09	28.9
31	20.39 – 21.79	21.26 ± 0.75	4.8	4.36	1.10	25,2
32	18.79 – 21.46	19.75 ± 1.48	7.6	4.10	3.57	87.1
33	18.99 – 24.46	20.84 ± 3.13	7.6	4.50	2.31	51.3
34	7.53 – 10.00	8.73 ± 1.23	2.1	2.78	1.31	47.1
35	4.86 – 7.19	6.24 ± 1.22	3.4	2.99	0.84	28.1
36	4.53 – 5.53	4.97 ± 0.50	1.9	3.68	0.83	22.6
37	n.d.	n.d.	n.d.	2.82	1.14	40.4
38	4.26 – 9.19	5.90 ± 2.84	2.0	3.53	2.34	66.3

Table 2. cont.

Sample No.	Phytate P (μg/mL)			Total P	$P\text{-}PO_4$	$P\text{-}PO_4$/ total P (%)
	Range	Mean ± SD	Phytate P/ total P (%)	mean (mg/g)		
Fruits, seeds (*Fructus, Semen)*						
39	17.79 – 17.93	17.86 ± 0.07	8.3	3.11	0.41	13.2
40	8.79 – 10.79	9.90 ± 1.01	2.9	3.21	0.33	10.3
41	2.26 – 4.60	3.71 ± 1.26	10.0	1.16	0.56	48.3
42	19.06 – 20.33	19.48 ± 0.73	2.5	1.37	0.82	59.9
43	7.33 – 8.33	7.97 ± 0.55	10.5	1.27	0.49	38.6
44	1.53 – 3.53	2.44 ± 1.01	5.2	1.19	0.79	66.4
45	18.59 – 19.93	19.22 ± 0.67	0.4	3.52	0.50	14.2
46	10.79 – 13.06	11.66 ± 1.22	4.5	4.01	0.65	16.2
47	15.72 – 16.53	16.06 ± 0.43	2.6	3.83	1.05	27.4
48	21.46 – 23.73	22.39 ± 1.18	10.0	1.55	0.54	34.8
49	17.00 – 18.93	18.11 ± 0.99	13.4	1.66	0.74	44.6
50	9.06 – 11.66	10.24 ± 1.31	7.7	1.40	0.16	11.4
51	30.26 – 31.32	30.90 ± 0.56	7.5	1.37	0.43	31.4
52	2.67 – 3.67	3.27 ± 0.52	26.9	1.02	0.11	10.8
53	10.00 – 12.33	11.51 ± 1.30	1.0	2.70	0.65	24.1
54	21.86 – 23.46	22.73 ± 0.80	10.2	1.04	n.d.	n.d.
55	n.d.	n.d.	n.d.	3.65	0.50	13.7
56	17.00 – 17.10	17.11 ± 0.11	41.7	0.82	0.25	30.5
57	5.10 – 5.30	5.22 ± 0.10	0.5	3.03	0.24	7.9
58	13.26 – 13.73	13.48 ± 0.25	4.4	3.06	1.24	40.5
59	8.46 – 10.86	9.84 ± 1.23	5.3	4.00	n.d.	n.d.

n.d. – not detected (below detection limit)

metric flasks and diluted up to 50 mL with redistilled water (Heraeus Quarzglas, Switzerland).

Extraction/tea brewing procedure

The dry sample of a medicinal herb (a bag of 1-2 g) was placed in a 250 mL beaker, and 100 mL of boiling redistilled water was added. Then, the sample was brewed during 15 min under glass cover and the bag was removed from the extract, which was transferred into a volumetric flask and diluted up to 100 mL with redistilled water.

Determination of phytate phosphorus

Phosphate P was determined spectrophotometrically with the Wade reagent (9). To 2 mL of the aqueous extract (tea) of a medicinal herb, 10 mL of the Wade reagent consisting of a 0.03% $FeCl_3$ solution and a 0.25% 5-sulfosalicylic acid solution, 3 + 1 (v/v) was added. Then, the sample was diluted up to 25 mL with redistilled water and the absorbance was measured at 500 nm using a UV/Vis spectrophotometer (Metertek SP-870, South Korea).

The calibration curve was characterized by regression equation: y = 0.6879 – 0.015x for the range of 5.33-40 μg/mL (r = 0.9899). The method was validated (17) by determining limits of detection (1.6 μg/mL) and quantification (5.33 μg/mL). The accuracy (recovery) of the method was evaluated by the standard addition method as 80%.

Determination of total and inorganic phosphorus

Total P in the digested herbal samples and inorganic P in the aqueous extracts of medicinal herbs were determined based on phospho-molybdenum blue reaction (4). The absorbance was measured at 650 nm. The accuracy and precision of the method were checked using the Certified Reference Material: Mixed Polish Herb (INCT-MPH-2). These values are shown in Table 1.

Table 3. Results of determination of trace and major elements in the studied medicinal plant samples.

Trace elements (mg/kg)			
Element	Range	Mean ± SD	Median
Fe total	27.49 – 408.64	131.00 ± 90.60	105.91
Fe extr.	1.78 – 31.39	6.85 ± 5.04	5.48
Zn total	4.56 – 85.36	39.86 ± 18.36	40.43
Zn extr.	0.35 – 49.35	12.21 ± 10.22	8.91
Mn total	7.41 – 243.01	75.53 ± 63.5	45.96
Mn extr.	0.34 – 92.05	24.15 ± 24.81	16.10
Cu total	3.48 – 56.85	10.43 ± 7.61	8.92
Cu extr.	0.25 – 23.93	4.06 ± 4.32	3.44
Major elements (mg/g)			
Mg total	0.79 – 11.42	3.84 ± 2.89	2.85
Mg extr.	0.09 – 10.85	1.44 ± 1.52	1.12
Ca total	1.39 – 133.89	31.48 ± 29.30	25.09
Ca extr.	0.09 – 76.01	8.92 ± 13.72	3.89
Na total	0.24 – 6.84	2.16 ± 1.88	1.19
Na extr.	0.02 – 0.62	0.10 ± 0.11	0.07
K total	0.27 – 1.70	0.73 ± 0.25	0.70
K extr.	0.04 – 0.57	0.26 ± 0.13	0.22

extr. – water extractable form of the element

Table 4. Statistically significant correlations indicating high correlation ($r > 0.5$; $\alpha < 0.05$) between elements in the studied medicinal plant samples.

Pair of elements	r	p
P-PO_4 – K extr.	0.52	0.000044
P-PO_4 – Zn total	0.50	0.000062
P-PO_4 – Zn extr.	0.60	0.000001
Ca total – Ca extr.	0.72	0.000000
Na total – Na extr.	0.67	0.000000
K total – K extr.	0.76	0.000000
Zn total – Zn extr.	0.51	0.000058
Mn total – Mn extr.	0.80	0.000000
Cu total – Cu extr.	0.81	0.000000

extr. – water extractable forms, p – significance level calculated by *Statistica* program

Determination of metallic elements

Total concentrations and the water-extractable forms of Fe, Zn, Mn, Cu, Mg, Ca, Na and K were determined by AAS in the digested medicinal plants, and in herbal teas. The 250 Plus Atomic Absorption Spectrometer (Varian, Australia) was used. The standard analytical parameters were as follows: air/acetylene flame, the analytical wavelengths (in nm): Fe (248.3), Zn (213.9), Mn (279.5), Cu (324.8), Mg (285.2), Ca (422.7), Na (589.0), and K (766.5). The accuracy and precision of the method were checked using the Certified Reference Material: Mixed Polish Herb (INCT-MPH-2), and these values are listed in Table 1.

Statistical evaluation of the results

The program Statistica 7.1 (Statsoft, Poland) was applied for all statistical calculations. The start-

ing database for PCA had the dimensions of 19 × 59, i.e., the concentrations of phytate P, inorganic P, total P, as well as total and water-extractable forms of Mg, Ca, Na, K, Fe, Zn, Mn, and Cu (19 parameters), determined in 59 analyzed samples.

RESULTS AND DISCUSSION

Phytate phosphorus

Phytate P in herbal teas fell in the range of 2.44-36.90 μg/mL, as shown in Table 2. The average level of extraction yield obtained for the aqueous extracts from different plant parts, ranged from 5.9% of total P in flowers to 16.7% of total P in leaves (Fig. 2). As only two samples of the seeds were analyzed, the fruits and seeds were treated as one group in statistical evaluation. The differences in the extraction yield of phytate P in the herbal teas are statistically significant only for the leaves, as confirmed by the Student's *t*-test ($t = 2.18$, $p = 0.04$, $\alpha < 0.05$).

As compared to the literature data, the phytate P level in the studied medicinal plants is much lower than that in the bean, lentil and legumes samples (7-9). It appears, according to our findings, that medicinal plants, in spite of containing quite high levels of total and inorganic P, in the order of mg/g d.w. (4, 5), don't have comparable levels of phytate P. However, when comparing our results of phytate P determination with the data obtained for African medicinal plants (16), the level of phytate P appears in the similar range of concentrations.

Relating the concentration of phytate P recalculated in mg/kg d.w., to total P level in the same samples, as shown in Table 2, it is on average about 8% of total P. This is in contrast to the level of inorganic P, which constitutes much higher part of total P, on average 44.6%.

Inter-elemental relations

The results of quantification of metallic elements in the plant samples are shown in Table 3. The average total level of trace elements follows the order: Fe > Mn > Zn > Cu, while for the major elements: Ca > Mg > Na > K. When considering the median values this order remains the same. These results are compatible with the literature data (4, 18).

A correlation analysis enabled to recognize relations among phytate P, total and inorganic P, as well as among the trace and major elements in the studied medicinal herbs and teas obtained from them. From 210 calculated correlation coefficients, only 51 were statistically significant ($\alpha < 0.05$). The values of $r > 0.5$ were considered as a high correlation, and they are presented in Table 4. Surprisingly, the relations of phytate P to other P forms were below the significance level, similarly as the relations of phytate P to metallic elements. However, there are several characteristic relations of inorganic

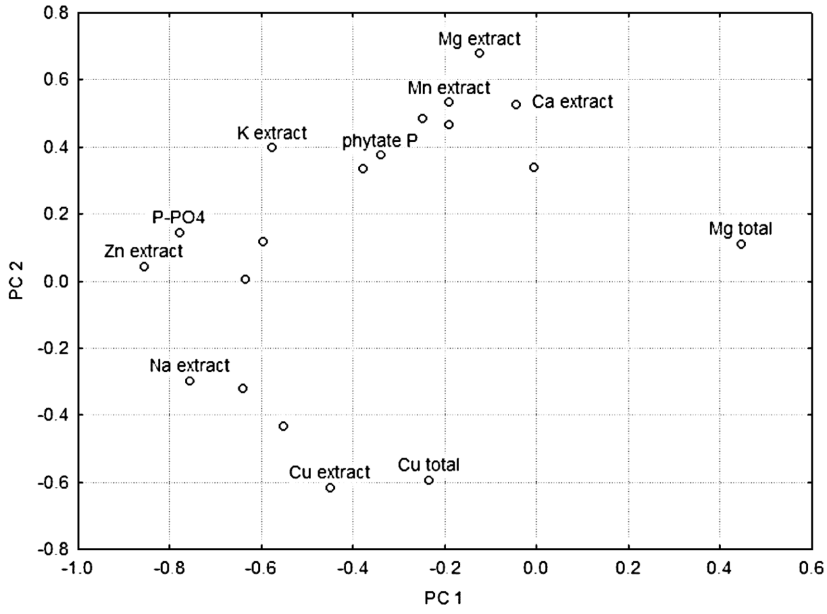

Figure 4. The two-dimensional loading plot of PC1 *vs*. PC2 obtained for medicinal herbal materials. Term "total" denotes the total content of essential elements in herbal materials and term "extract" refers to the content of water extractable forms

P to water extractable K, Zn, and total Zn, which can reveal a synergistic interaction between them. Also positive relations between total and water extractable amounts of the metallic elements were found.

Principal Component Analysis

One of the pattern recognition methods widely used for interpretation of experimental results is Principal Component Analysis, PCA (19). This statistical method is particularly useful in situations, when an experimental database comprises multivariate data describing a number of analyzed samples. The most important advantage of PCA is reducing the multidimensionality and the possibility to extract those factors, which influence the variability of the studied samples. These arguments have led us to application of PCA to the experimental results.

The results of PCA calculations revealed that first three principal components (PCs) explain together 54% of the variability among the studied medicinal plant samples, and first two PCs 40.8%. Therefore, a two-dimensional plot of first two PCs was used. A graphical interpretation of the PCA results (Fig. 3) shows that there are several characteristic groups of the studied plant samples. For example, in the left area of the plot there is a group of three samples of *Calendulae Flos* (*32-34*, sample numbers given in parenthesis). They all have a similar PC2 value, but the PC1 value differentiates one of them (*34*) from the remaining two representing the same plant species. Two samples of *Euphrasiae Herba* (*1* and *2*) are grouped in the central zone of the plot. In the right area there are also two characteristic clusters. Underneath there is one group of *Anisi Fructus* (*40* and *41*), and below a single sample of *Foeniculi Fructus* (*46*). In the upper right area of the plot, there is one cluster grouping all four samples of *Salviae Folium* (*13-16*), as well as one sample of *Hyperici Herba* (*7*) and one of *Urticae Herba* (*21*). Such a location of the samples given above as examples is caused by similar concentration of several elements in them or water extractable forms of the elements.

The loading plot of first two PCs, presented in Figure 4, shows which elements or their water extractable species have the highest impact on the distribution of the analyzed plant samples in a two-dimensional space. Total Mg concentration is positively correlated to PC1, but a stronger negative correlation of water-extractable Zn, inorganic P and of water extractable Na, can be seen. Regarding the correlation of PC2 with original parameters, one can notice that it is positively correlated to water extractable Mg, as well as to water extractable species of Mn and Ca. On the other hand, PC2 is negatively correlated to total and water extractable levels of Cu. After PCA calculation, it was also found that PC3 was negatively related to water-extractable form of Mn, but this could not be shown on the 2D plot.

CONCLUSIONS

It was found that herbal teas contained phytate P at a relatively low level, of the order of μg/mL. The extraction yield for phytate P obtained from the leaves of medicinal plants was the highest in comparison with the other analyzed plant organs. The flowers represented the lowest extraction yield for phytate P among all the studied plant organs. It was also found that no statistically significant relations occurred between phytate P and the analyzed trace and major elements. However, inorganic P concentration in herbal teas was positively correlated to water extractable K, Zn, and total Zn, which reveals a synergistic interaction between these essential elements.

PCA was a helpful multivariate statistical method, which enabled the analyzed samples to be grouped based on similarity of their elemental contents, as well as to state that inorganic P, water extractable Zn and Na, had a crucial impact on differentiation of the studied plant materials.

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