Research Article

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Investigations of metallic elements and phenolics in Chinese medicinal plants

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Abstract: Traditional Chinese Medicines (TCM) can be contaminated with heavy metals, and therefore, the aim of this study is to analyze the Fe, Mn, Zn, Cu, Cd, Pb, Cr, and phenolic compounds contents in TCM plants used against civilization diseases. Metals were determined by flame atomic absorption spectroscopy (FAAS) for Fe, Mn, Zn, and Cu and inductively coupled plasma-optical emission spectroscopy (ICP-OES) for Pb, Cd, and Cr. The total phenolic, flavonoid, and phenolic acid contents were determined by HPLC and UV/vis spectrometry. The contents of the studied elements were highest in *Radix Rehmanniae*, whereas lowest in *Fructus Lycii* and *Fructus Crataegi*. The studied metals were assayed in the decreasing order: Fe, Zn, Mn, Cu, Cr, Pb, and Cd. *Radix Rehmanniae Glutinosae Preparata* showed the lowest phenolic composition, while *Fructus Lycii* showed the richest content. Principal component analysis (PCA) revealed that the contents of ferulic acid, caffeic acid, rutin, and Cu, Cr, and Cd were among the most important factors responsible for the differentiation between the investigated medicinal plants. Cluster analysis (CA) showed that the TCM samples originating from the same botanical plant species were often found in the same cluster, which confirms the similar level of studied elements determined within the samples.

Keywords: TCM, civilization diseases, metallic elements, phenolics, statistical methods

1 Introduction

Natural drugs prepared from medicinal plants have been known in human populations for centuries. There is written evidence that in China, several plants have been used to treat various diseases, dating back to 5,000 BC [1]. Traditional Chinese medicine (TCM) comprises the use of not only herbs, but also animal parts and minerals, in addition to acupuncture, physical exercise, and special diets. Therefore, the need to standardize medicinal plant materials used in TCM is growing [2].

Currently, in modern society, there is an increasing tendency to fight against a growing number of civilization diseases. A literature report stated that “civilization diseases kill and invalidate more people than all wars and ethnic conflicts” [3]. As consequences of civilization development, many diseases have appeared in higher numbers, such as hypertension, insomnia, stress, alcoholism, obesity, allergies, mental disorders including depression, and diabetes [4]. The use of TCM may help to act against civilization stress and/or to prevent humans from numerous diseases mentioned above.

Among TCMs used against civilization diseases are goji berries and hawthorn fruits. Goji berries are recommended for weakness in organisms because they increase *yin* according to TCM principles, have a positive impact on the eyes, and contain significant amounts of microelements, such as Mn [1]. Hawthorn fruits support blood circulation, release phlegm, and are used as agents to provide sweet and sour tastes to food [1]. Fleece flower roots have been applied in TCM to nourish blood and *yin*, stimulate the intestines, and eliminate itching of the skin and they are believed to rejuvenate humans and help with sexual problems, especially erection [1]. *Rehmanniae* roots nourish *yin* and stimulate the secretion of body fluids, improve blood parameters, and decrease side effects during

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chemotherapy, and they are applied for exasperation and insomnia. These roots also decrease fever and are used to reduce flushing of the face caused by malaria [1]. Numerous studies have examined the total levels of essential and toxic elements in Chinese medicinal plants and tea samples, including several speciation analyses, performed mainly by applications of analytical techniques, such as atomic absorption or emission spectroscopy, and by the use of inductively coupled plasma as the excitement source [5–12].

A problem of the application of TCMs has been associated with their contamination with heavy metals and organic compounds. This can pose a severe risk to human health. Recent studies investigated TCMs in terms of their contamination with metallic elements [13] and pesticides [14,15].

Phenolic compounds are phytochemicals with both well-known functional and health-promoting properties [16,17]. They have been mainly considered as secondary plant metabolites and are among the largest classes of bioactive compounds containing one or more aromatic rings along with one or more hydroxyl groups in their backbone structure [18–20]. They are classified into various groups of phenolic acids, flavonoids, tannins, and their hydrolyzed products, as well as derivatives. Phenolic compounds are acknowledged as strong natural antioxidants having key role in wide range of biological and pharmacological properties such as anti-inflammatory, anticancer, antimicrobial, antiallergic, antiviral, antithrombotic, hepatoprotective, food additive, signaling molecules, and many more [21,22].

Taking this into consideration, the aim of the study was to quantitatively analyze the concentrations of Fe, Mn, Zn, Cu, Cd, Pb, and Cr as well as phenolic compounds in medicinal plant materials originating from China that are used against civilization diseases. Fe, Mn, Zn, and Cu are indispensable elements for human organism, and Cd, Pb, and Cr are the most common toxic elements; therefore, these elements were determined. The study was also undertaken to find TCMs rich in the analyzed elements and to monitor the levels of toxic elements contained within them.

2 Materials and methods

2.1 Preparation of samples prior to analysis

TCM products were purchased from importing companies in dry form, as shown in Table 1, ground using a sample mill (Foss Tecator, Sweden), and kept in glass jars in dark until analysis. Next, the TCM materials were digested with a microwave digestion unit (Jupiter B, Sineo, China)

Table 1: The list of TCM materials under analysis

Sample no.	Chinese name (pin yin)	Latin name	English name	Origin	Importer
1	Gou Qi Zi	<i>Fructus Lycii</i>	Goji berry (wolfberry)	China	CMC Polska, Brzeg, Poland
2	Shan Zha (Sheng)	<i>Fructus Crataegi</i>	Hawthorn fruit	China, Shandong	CMC Polska, Brzeg, Poland
3	He Shou Wu (Sheng)	<i>Radix Polygoni Multiflori</i>	Fleeceflower root	China, Sichuan	Medboom, Green Nature, Amsterdam, The Netherlands
4	He Shou Wu (Sheng)	<i>Radix Polygoni Multiflori</i>	Fleeceflower root (prepared)	China, Sichuan	Medboom, Green Nature, Amsterdam, The Netherlands
5	He Shou Wu (Zhi)	<i>Radix Polygoni Multiflori preparata</i>	Fleeceflower root (prepared)	China, Sichuan	Medboom, Green Nature, Amsterdam, The Netherlands
6	He Shou Wu (Zhi)	<i>Radix Polygoni Multiflori preparata</i>	Rehmannia root	China	CMC Polska, Brzeg, Poland
7	Di Huang (Sheng)	<i>Radix Rehmanniae Glutinosae</i>	Rehmannia root	China	CMC Polska, Brzeg, Poland
8	Di Huang (Shu)	<i>Radix Rehmanniae Glutinosae preparata</i>	Rehmannia root (prepared)	China	CMC Polska, Brzeg, Poland

and the following digestion program: step I – 150°C, 10 min; step II – 160°C, 5 min; step III – 180°C, 5 min; and step IV – 190°C, 15 min. The digestion mixture consisted of 11 mL of a concentrated HNO₃ solution + 1 mL of a 30% H₂O₂ solution for 0.5 g of plant sample. Each TCM sample was digested and later analyzed in triplicate (three samples were obtained from the same plant: therefore, $n = 3$).

2.2 Determination of the elements

The contents of four essential elements, namely, Fe, Mn, Zn, and Cu, were determined in the obtained digests by the flame atomic absorption technique (SpectrAA 250 Plus, Varian, Australia) using standard analytical procedures and an external calibration method. An air–acetylene mixture was used during the measurements along with the following analytical wavelengths (nm) for the particular metallic elements: 248 (Fe), 280 (Mn), 214 (Zn), and 325 (Cu).

The other analyzed elements (Pb, Cd, and Cr) were assayed by the inductively coupled plasma–optical emission spectroscopy (ICP–OES) technique using a PerkinElmer Optima 7000DV and inductively coupled plasma–optical emission spectrometer.

2.3 HPLC analysis

The chromatographic separation and quantitation of the phenolic compounds were performed on a Hypersil Gold C18 column (250 × 4.6 mm, 5 µm particles) (Thermo Scientific, Runcorn, UK), maintained at 25°C, using acetonitrile–0.1% acetic acid solution (solvent A) and a water–0.1% acetic acid solution (solvent B) as the mobile phase [23]. The separation was performed at a constant flow rate (1 mL/min) with the following conditions: linear gradient from 5 to 25% of A in 30 min, from 25 to 40% in 10 min, from 40 to 63% in 10 min, and from 65 to 5% in 10 min. The absorbance was monitored at 280 nm for gallic acid; 320 nm for caffeic, chlorogenic, and ferulic acids; and 370 nm for quercetin and rutin. Chromatographic separation of selected phenolic compounds is shown in Figure 1.

Identification of the analytes was based on comparison of the retention times to those of their standard compounds. Additionally, a selected sample was spiked with the standard compounds and analyzed again. Calibration

Figure 1: HPLC profile of the extract prepared from *Fructus Crataegi* (samples no. 2) recorded with UV-Vis detector: for gallic acid (5.12 min, GA), chlorogenic acid (7.54 min, CGA), caffeic acid (8.85 min, CA), ferulic acid (25.76 min, FA), rutin (27.98 min, RUT), and quercetin (30.89 min, Q).

curves of known concentrations (10–100 µg/mL) for each phenolic standard were used to estimate the phenolic compound contents.

2.4 Total phenolic content

The Total phenolic content (TPC) of the white mulberry extracts was determined using the Folin–Ciocalteu method as previously described by Singleton [24] with some modifications. Briefly, an appropriate amount of the extract was mixed with 0.4 mL of Folin–Ciocalteu reagent. The mixture was left to settle for 3 min, and then 2 mL of 7% (w/v) Na₂CO₃ solution was added followed by incubation in the dark at room temperature for 1 h. The absorbance of the mixture was measured at 760 nm using an SP-870 Metertek UV-Vis spectrophotometer (South Korea). The gallic acid calibration curve (0.1–0.154 mg/mL) was used to express the results as milligrams of gallic acid equivalents (GAE) per gram of dry weight (mg GAE/g DW).

2.5 Total flavonoid content

The total flavonoid content (TFC) of the TCMs extracts was determined according to the method described in the European Pharmacopeia [25] with some modifications. An appropriate amount of the extract was mixed with 0.1 mL of 5% (w/v) AlCl₃ solution. The mixture was incubated for 30 min in the dark at room temperature and the absorbance was then measured at 430 nm using an SP-870 Metertek UV-Vis spectrophotometer (South Korea). The

Table 2: Statistical evaluation of results of metals determination using flame atomic absorption spectroscopy (FAAS) (a) and ICP-OES (b). Concentrations are shown as mg/kg DW ($n = 3$)

Element	Range	Arithmetic mean	Median	SD	RSD [%]
Fe ^a	66.39–778.44	216.10	135.31	236.72	109.5
Zn ^a	16.50–31.33	23.60	22.87	5.23	22.2
Mn ^a	2.12–17.27	8.05	6.70	6.27	77.9
Cu ^a	1.38–5.27	2.58	1.75	1.56	60.5
Pb ^b	0.67–2.91	1.22	1.05	0.72	59.0
Cd ^b	0.09–0.25	0.16	0.15	0.06	37.5
Cr ^b	0.61–9.36	2.54	1.61	2.83	111.4

TFC is expressed in milligrams of quercetin equivalents (QE) per gram of dry weight (mg QE/g DW) using a calibration curve constructed from quercetin standard solutions (5–50 µg/mL).

2.6 Total phenolic acid content

The procedure described in the Polish Pharmacopeia VI [26] was used for Total phenolic acid content (TPAC) determination with Arnov's reagent. An appropriate amount of the extract was mixed with 0.2 mL of 0.5 M HCl, 0.2 mL of Arnov's reagent, and 0.2 mL of 1 M NaOH. The absorbance was measured at 490 nm using an SP-870 Metertek UV-Vis spectrophotometer (South Korea). The results are expressed in milligrams of caffeic acid equivalents (CAE) per gram of dry weight (mg CAE/g DW) based on a calibration curve constructed for caffeic acid (5–40 µg/mL).

For statistical evaluation of experimental data, the Statistica 7.1 program (Tulsa, USA) was used.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 Metallic element determination

The metallic element determination results are presented in Tables 2 and 3. The first table shows the range of concentrations of the elements in all 8 TCM materials together with their basic evaluation, including values such as the arithmetic mean, median, standard deviation (SD), and relative standard deviation (RSD). Based on these data, the order in which the studied metals were assayed was as follows: Fe, Zn, Mn, Cu, Cr, Pb, and Cd,

taking into consideration both the mean and median values. The SD calculated for the determination of Fe together with the RSD value for this element showed large difference between the Fe levels in TCM analyzed samples. This fact is supported by Figure 2, which presents the average concentration of iron in all samples. The Fe content was the highest among all samples in *Radix Rehmanniae*, especially *Rehmanniae preparata*, where its content was determined to be approximately 780 mg/kg of dry weight (DW). The levels of the other metallic elements did not show as wide a spread among the studied samples; therefore, the results were presented graphically only for that microelement.

The results for the other TCM materials presented in Table 3 show that Fe levels above 100 mg/kg DW were found in the samples of *Radix Polygoni multiflori preparata* (samples 5 and 6) and in one sample of *Radix Polygonii multiflori* (sample 4). The lowest Fe concentration was determined in both fruits: 80.05 mg/kg DW in *Fructus Lycii* and 66.39 mg/kg DW in *Fructus Crataegi*. The highest Mn levels were found in *Radix Rehmanniae preparata* in an amount above 17 mg/kg DW and in *Fructus Lycii* at 16.47 mg/kg DW.

Zinc was found in all TCM samples at a more balanced level, from 16.50 mg/kg DW in *Radix Polygoni multiflori preparata* (sample 5) to 31.33 mg/kg DW in *Radix Rehmanniae preparata* (sample 8), as shown in Table 3. Taking the Cu level in all studied medicinal plant samples into consideration, the highest concentration of this microelement was found in TCM fruits: 5.27 mg/kg DW in *Fructus Lycii* and 4.82 mg/kg DW in *Fructus Crataegi*.

Table 3 shows that based on the concentrations of toxic elements (Pb, Cd, and Cr), the TCM material with the highest Pb level and the lowest Cr concentration was *Fructus Lycii*. The highest Cr level was found in *Radix Rehmanniae preparata* (sample 8), while the highest Cd concentration was determined to be in one sample of *Radix Polygonii multiflori* (sample 3). From Table 2, the difference between the mean and median values for Pb

Table 3: Results of elements determination. The arithmetic mean [mg/kg DW] $\pm$ SD is given ($n = 3$)

Sample no.	Latin name	Fe ^a	Mn ^a	Zn ^a	Cu ^a	Pb ^b	Cd ^b	Cr ^b
1	<i>Fructus Lycii</i>	80.05 $\pm$ 2.75	16.47 $\pm$ 2.46	22.07 $\pm$ 0.66	5.27 $\pm$ 0.69	0.93 $\pm$ 0.05	0.19 $\pm$ 0.01	0.61 $\pm$ 0.05
2	<i>Fructus Crataegi</i>	66.39 $\pm$ 8.96	2.12 $\pm$ 0.36	23.67 $\pm$ 1.32	4.82 $\pm$ 0.22	2.91 $\pm$ 0.05	0.09 $\pm$ 0.02	2.82 $\pm$ 0.05
3	<i>Radix Polygoni Multiflori</i>	98.32 $\pm$ 5.09	9.77 $\pm$ 0.62	28.63 $\pm$ 2.33	1.76 $\pm$ 0.18	0.67 $\pm$ 0.06	0.25 $\pm$ 0.01	1.06 $\pm$ 0.06
4	<i>Radix Polygoni Multiflori</i>	155.65 $\pm$ 2.00	10.29 $\pm$ 1.92	27.70 $\pm$ 2.45	1.74 $\pm$ 0.17	1.13 $\pm$ 0.08	0.13 $\pm$ 0.02	1.56 $\pm$ 0.06
5	<i>Radix Polygoni Multiflori preparata</i>	119.19 $\pm$ 2.43	6.97 $\pm$ 0.45	16.50 $\pm$ 0.97	1.66 $\pm$ 0.26	1.32 $\pm$ 0.05	0.21 $\pm$ 0.01	1.65 $\pm$ 0.06
6	<i>Radix Polygoni Multiflori preparata</i>	151.43 $\pm$ 8.23	6.41 $\pm$ 1.22	18.31 $\pm$ 1.59	1.38 $\pm$ 0.26	1.19 $\pm$ 0.06	0.18 $\pm$ 0.01	1.22 $\pm$ 0.05
7	<i>Radix Rehmanniae Glutinosae</i>	279.36 $\pm$ 12.43	4.86 $\pm$ 0.23	20.59 $\pm$ 2.75	1.59 $\pm$ 0.06	0.97 $\pm$ 0.08	0.11 $\pm$ 0.01	2.06 $\pm$ 0.05
8	<i>Radix Rehmanniae Glutinosae preparata</i>	778.44 $\pm$ 17.39	17.27 $\pm$ 2.08	31.33 $\pm$ 4.75	2.41 $\pm$ 0.06	0.67 $\pm$ 0.07	0.10 $\pm$ 0.01	9.36 $\pm$ 0.05

Determined by using FAAS (a) and ICP-OES (b).

was not very large, and for Cd, these values were practically the same. However, in the case of Cr, the difference between the mean and median values was large, which points to a diversification in the level of this metal in the studied TCM materials.

Comparing the results of the metallic elements obtained in this study with other research, it can be stated that, for example, the Pb level determined in the TCMs in our study was approximately 2 times higher than that reported in Chinese medicinal plants, such as *Angelica sinensis*, *Bacopa monnieri*, *Bupleurum sinensis*, and others [27]. Previously reported cadmium concentration results, on the other hand, were found to be very similar to those found in our research, in amounts from less than 0.1 to above 0.2 mg/kg DW [27]. Another study on seabuckthorn leaves, a plant material used in TCM, showed that the Pb level was 1.8 mg/kg DW and the Cd level was 0.007 mg/kg DW, which is higher than the Pb concentration found in our study, but much lower than our results for Cd [28]. Further comparison of the results obtained for other metallic elements (Fe, Zn, Mn, and Cu) in our study with the levels of these elements determined by other researchers was carried out. For example, the Fe level in medicinal plants growing in Serbia ranged from less than 100 mg/kg DW to amounts above 300 mg/kg DW [28], which is quite similar to our study, with the exception of *Radix Rehmanniae*, where the Fe level was much higher. Next, the Zn, Mn, and Cu levels in Serbian medicinal plants [29] represent similar range of concentrations, as those determined in the TCM materials in our study, and the accumulation of metals depended on the analyzed plant species among other factors. These factors comprise climatic conditions, rainfall, contamination of soils with heavy metals, and other impacts of industrial or agricultural activity in the area of plant growth.

The correlation analysis revealed that the element that was most frequently correlated with other metals appeared to be iron. This element was highly correlated with the Cr and Pb levels in the studied TCM materials. The weaker correlation, with the correlation coefficients of approximately 0.5, was found for relations Fe–Zn and Fe–Mn. Analysis of other correlations revealed that Cu was negatively correlated with Pb and Cd levels. Zn was positively correlated with the Cr concentration, and Mn was negatively correlated with Cd. It must be stressed that in earlier research, several significant correlations were also found between the levels of essential elements, including Mn–Zn and other pairs of metallic elements crucial for biochemical transitions in living organisms [30,31]. These correlations point to the cooperation of

Figure 2: Fe content in the studied TCM materials [mg/kg DW].

metal ions in the physiological processes that occur in plants [32].

3.2 Phenolic compound analysis

Since polyphenols are considered among the most biologically active constituents that contribute greatly to antioxidant activity, the TPC, TFC, and TPAC in the studied Chinese medicinal plants were determined. The results shown in Table 4 reveal that the TPCs ranged from 0.58

(sample 8) to 3.91 mg GAE/g DW (sample 1), while the TFCs and TPACs were 0.66 mg QE/g DW and 1.31 mg CAE/g DW, respectively. In all cases, sample 8 (*Radix Rehmanniae Glutinosae Preparata*) displayed the lowest TPC, while sample 1 (*Fructus Lycii*) had the highest TPC. The TPCs, TFCs, and TPACs obtained in this study differ from those in the literature. Lin et al. [33] found higher TPCs in the extracts of *Crataegi Fructus*, *Polygoni Multiflori Radix*, and *Lycii Fructus* at 11.43, 4,580, and 6,172 μmol GAE/100 g DW, respectively. Kostic et al. [34] detected TPCs and TFCs in the extracts of *Crataegus*

Table 4: Results of analysis of phenolic compounds

Sample	GA	RUT	FA	CA	CGA	Q	TPC	TFC	TPAC
	$\mu\text{g/g}$						mg GAE/g	mg QE/g	mg CAE/g
1	664.14	88.98	6.68	34.92	46.40	320.87	3.91	1.64	5.55
2	874.49	86.87	8.45	56.94	56.97	538.11	2.81	0.98	1.28
3	96.15	77.69	3.57	20.26	6.19	124.53	2.55	0.65	1.25
4	230.68	80.46	3.74	21.47	4.14	113.90	2.62	0.64	0.70
5	289.53	80.69	3.57	21.60	5.09	200.72	1.38	0.42	0.87
6	368.01	80.55	3.63	21.13	6.64	241.61	1.03	0.41	0.43
7	519.38	81.76	4.16	21.87	43.68	211.34	0.77	0.38	0.37
8	474.81	81.94	4.19	22.18	43.68	301.37	0.58	0.12	0.20

GA – gallic acid; RUT – rutin; FA – ferulic acid; CA – caffeic acid, CGA – chlorogenic acid; Q – quercetin; TPC – total phenolic contents; TFC – total flavonoid contents; TPAC – total phenolic acid contents.

oxycantha L. in the range of 2.12 to 30.63 mg GAE/g fresh sample and 0.556 to 0.990 mg QE/g fresh sample, respectively. These results are in agreement with those obtained in this study.

Based on the data summarized in Table 4, the concentrations of phenolic acids and flavonoids in the Chinese medicinal plants can be represented in the following order: gallic acid > quercetin > rutin > caffeic acid > chlorogenic acid > ferulic acid. Among the analyzed samples, sample 1 was the richest in determined phenolic constituents. Magiera and Zareba [35] found lower levels of rutin, ferulic acid, and caffeic acid in *Lycium barbarum* L. fruits, ranging from 2.24 to 11.5, from 10.2 to 27.0, and from 5.45 to 12.3 $\mu\text{g/g}$ DW, respectively.

3.3 Statistical evaluation of the results

To achieve a more complex interpretation of the obtained TCM metallic element concentration results, cluster analysis (CA) and principal component analysis (PCA) were applied. As indicated by earlier studies, these statistical methods can help to answer many questions that arise when a relatively large dataset must be interpreted [30,31]. Moreover, these methods can be useful for the

classification of the studied TCM materials and in searching for the elements that are mostly responsible for the differentiation of the studied medicinal plant material. The CA results shown in Figure 3 clearly present the linkage of the studied plant samples based on their elemental content similarity. On the left portion of the dendrogram presented in Figure 3, it can be noted that both *Radix Rehmanniae glutinosae* samples, especially sample 8 (*Radix Rehmanniae preparata*), are well-separated from the other. This is due to the high Fe level in this TCM. On the other hand, in the right portion of the dendrogram, two samples (1 and 2) representing the analyzed fruits – *Fructus Lycii* and *Fructus Crataegi*, are grouped into one cluster, which illustrates their similar elemental contents.

PCA has confirmed the tendencies noticed in the CA results of the studied TCMs. The distribution of samples shown in Figure 4 indicates characteristic TCM materials. For example, in the left area of the two-dimensional plots, PC1 and PC2, there are two characteristic plant materials, *Fructus Lycii* and *Fructus Crataegi*. All four samples of *Radix Polygoni multiflori* are located in the right lower corner of the plot. In the right upper portion of the plot, there is *Radix Rehmanniae preparata* sample, which is very much well-separated from the other TCMs, and below there is *Radix Rehmanniae* sample. These

Figure 3: CA dendrogram for the studied TCM materials obtained based on their metallic elements content.

Figure 4: PCA score plot obtained for the studied TCM materials in two-dimensional plain PC1 and PC2.

Figure 5: PCA loading plot obtained for the studied TCM materials in two-dimensional plain W1 and W2.

observations are similar to those observed in the CA plot. PCA also revealed that the contents of ferulic acid, caffeic acid, rutin, Cu, and Cd, associated with PC1, and Cr and Fe,

associated with PC2, were among the most important factors responsible for the differentiation among the investigated medicinal plants. These results are shown in Figure 5.

4 Conclusions

This study on selected TCMs used against civilization diseases enabled the selection of medicinal products rich in the studied elements – *Radix Rehmanniae* and *Radix Rehmanniae preparata*, and one product containing high amounts of phenolics – *Fructus Lycii*. PCA also revealed that the contents of ferulic acid, caffeic acid, rutin, Cu, Cr, and Cd were among the most important factors responsible for the differentiation of the investigated medicinal plant materials.

Conflict of interest: The authors declare no conflicts of interest.

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