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Short communication

Trace elements analysis of *Echinacea purpurea*—herbal medicinal

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Abstract

Elemental composition of *Echinacea purpurea* (Asteracae), grown in Serbia under strongly controlled conditions, has been studied. To distinguish elemental patterns of different parts of the plant, the content of Zn, Fe, Cu, Mn, Ca, Mg, Sr, Ni, and Li in root versus upper plant parts were determined, by flame atomic absorption and flame atomic emission spectrometry. Analyses of the mentioned elements in soil and in an ethanolic extract of *E. purpurea* were made, too. The trace element data were evaluated by multivariate methods, i.e. principal component analysis and hierarchical cluster analysis. This revealed two groups of elements (I: Fe, Cu, Mn, Li; II: Ca, Mg, Zn, Ni), while trace element profiles of root, stem, leaves, and flowers of this plant differed significantly. However, no significant difference in the trace element patterns between the summer and the autumn harvest samples was found.

Keywords: *Echinacea purpurea*; Trace elements; Atomic absorption spectrometry; Atomic emission spectrometry; Chemometrics

1. Introduction

Echinacea purpurea (Asteraceae), also known as the purple coneflower, is an herbal medicine with positive effects on various immune parameters [1]. It is usually used in supportive therapy of colds and chronic infections of the respiratory and the lower urinary tract. Although many of the active compounds of *E. purpurea* have been identified, the mechanism of its action remained unknown [2]. Specifically, there are some recent literature data on the determination of numerous molecular constituents of this plant [3–11] and also on the Echinacea products activity [12]. But, as far as trace and minor elements determination in this medicinal plant is concerned, the data are still missing. These elements are essential for normal growth of plants, their protection against plant viruses, immunity and the completion of the life cycle [13,14].

The objective of this study was to quantify the content of various elements that might be respon-

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sible for some properties of Echinacea, such as its immunostimulatory and anti-inflammatory activities. Investigated elements were chosen (Zn, Fe, Cu, Mn, Ca, Mg, Sr, Ni and Li) according to their role and importance in many biological mechanisms.

Quantitative determination of the mentioned elements in different parts of *E. purpurea*, grown in the eastern part of Serbia (samples from the summer and autumn harvests of 2001), as well as within the soil itself, was performed. In addition, an evaluation of their distribution in plant tissues was discussed.

In order to provide a better insight into the elemental pattern, a common chemometrics approach to data analysis was used. Several multivariate methods have been used to analyze the data sets obtained by chemical measurement of trace elements in various plants [15,16]. However, except one article [6] dealing with multivariate analysis of organic compounds data set, there are no data on trace elements in *E. purpurea* analyzed by means of chemometrics. Principal component analysis and cluster analysis were used in this work to study the association of nine trace elements in *E. purpurea* and to evaluate their distribution between different parts of this plant.

2. Experimental

2.1. Solutions and reagents

Standard solutions were prepared by dilution of single-element standards (1000 μg/ml) obtained from Merck (Darmstadt, Germany) with 1 M HCl. Concentrations of these working standard solutions (five standards for each element) were in the linear calibration range of each element. All acids (HCl, HNO_3, $HClO_4$ and HF) used in this work were purchased from Merck and were of reagent grade quality. All aqueous solutions and dilutions were prepared with bidistilled water.

2.2. Sample preparation

E. purpurea cultivated in eastern Serbia, under strongly controlled conditions, was collected in July (summer harvest) and in autumn (the end of September and the beginning of October) in 2001. The roots (root samples were collected in autumn only) were slightly washed in field and again in laboratory with bidistilled water in order to remove soil; each plant sample was cut into five parts, stored in brown paper bags, and dried at 105 °C until constant weight was obtained. All samples were then ground through a stainless steel mill. An ethanolic extract of *E. purpurea* commercially available at the Serbian market was used, too. The plant samples were prepared according to AOAC 975.03 and 985.01 methods [17]. Accurately weighted samples of roots, steams, leaves, flowers and herbs (~ 1 g each) and 10 ml of the extract were slightly heated on a hot plate at 100–120 °C, for 15 min, then placed into a furnace and further heated at 500 °C for 2 h. After cooling, ten drops of bidistilled water and then 4.0 ml of 8 M HNO_3 were added into each sample, slightly heated on the hot plate to dryness then placed in the furnace at 500 °C, for 1 h. After cooling, 10 ml of 6M HCl was added in each sample and the contents were quantitatively transferred into 50 ml volumetric flasks.

Soil samples (~ 0.2 g) were totally decomposed in a mixture of concentrated acid (5 ml of $HClO_4$ and 10 ml of HF) by gentle heating for 20 min at 100–120 °C. Ten milliliters of HF was added into the samples twice into the samples during digestion for additional 40 min. Beside 'total' decomposition, the soil samples were leached with 0.1 M HCl (30 min at 100 °C), and the contents were quantitatively transferred to 25 ml volumetric flasks. This second procedure was employed in order to 'simulate' acid soil.

2.3. Instrumentation

Samples were analysed for Zn, Fe, Cu, Mn, Ca, Mg, Sr and Ni by flame atomic absorption spectrometry (AAS) and for Li by flame atomic emission spectrometry (AES), using a Perkin–Elmer Model 403 atomic spectrometer (Perkin–Elmer, Norwalk, USA). The operating parameters and the analyte characteristics are given in Table 1. The signals were measured with the background correction (deuterium lamp) at optimal flame

Table 1
Operating conditions and analyte characteristics

Element	λ[b] (nm)	Slit (nm)	Flame type	CC[c] (μg/ml)	Range[d] (μg/ml)	DL[e] (μg/ml)
Cu	324.8	0.7	A–Ac	0.077	5.0	0.012
Fe	248.3	0.2	A–Ac	0.110	6.0	0.03
Mn	279.5	0.2	A–Ac	0.052	2.0	0.01
Zn	231.9	0.7	A–Ac	0.018	1.0	0.01
Ni	232.0	0.2	A–Ac	0.140	2.0	0.04
Li[a]	670.8	0.7/1.4	A–Ac	0.035	3.0	0.01
Sr	460.7	0.2/0.4	N–Ac	0.110	5.0	0.05
Mg	285.2	0.2	N–Ac	0.036	0.50	0.01
Ca	422.7	0.2	N–Ac	0.048	5.0	0.012

A–Ac: air–acetylene; N–Ac: nitrous oxide-acetylene.
[a] Emission intensity measured by AES
[b] Wavelength.
[c] Characteristic concentration (sensitivity).
[d] Linear range.
[e] Detection limits obtained under experimental conditions.

heights. Ionization was controlled by adding 5 ml (10 g/l CsCl+100 g/l La) buffer solution (Merck, p.a.) to all samples and standards. The concentrations of different elements in these samples were determined by the external standard method using the corresponding calibration curves. The employed AAS and AES methods are not only sufficiently sensitive but enable cost-benefit analysis to be made [18].

2.4. Data analysis

The statistical data processing was performed by using the SPSS software package, using the logarithmically transformed trace element concentration data set. The principal component analysis and the hierarchical cluster analysis were applied to analyze trace element patterns in different parts of the Echinacea plant and its ethanolic extract, and to relate them with the soil composition.

3. Results and discussion

The biological effects of estimated elements (Zn, Fe, Cu, Mn, Ca, Mg, Sr, Ni and Li) in living systems strongly depend on their concentration [14,19] and thus should be carefully controlled [20], especially when herbs and their products are used in human medicine as it is the case with Echinacea. Trace element concentrations in plants vary widely with the soil type, pH, fertilizer and organic content, climate, species, etc. To distinguish the elemental pattern of different parts of this plant, grown in Serbia, under strongly controlled conditions, the content of Zn, Fe, Cu, Mn, Ca, Mg, Sr, Ni and Li in root versus upper plant parts were determined. In addition, the soil samples and the ethanolic extract of Echinacea were analyzed, too.

The concentrations of nine trace elements in *E. purpurea*, expressed on a dry weight basis, are listed in Table 2. Each result is the mean value of five sample measurements. The relative standard deviations were in the range from 0.2 to 2.5% confirming good reproducibility of the applied method. The results obtained after 'total' decomposition of the soil samples are too high (Cu—50.1 μg/g, Fe—29.8 mg/g, Li—51.0 μg/g, Mg—6.85 mg/g, Mn—945 μg/g, Ni—22.8 μg/g, Sr—87.5 μg/g, Zn—50.8 μg/g and Ca—9.55 mg/g) to be taken into account in data evaluation. This large difference between the soil and plant concentrations could be explained by the fact that the root absorption mechanism is not able to absorb all the elements in their total amount.

The concentration of trace elements decreases as follow: Ca > Mg > Fe > Mn > Cu > Zn > Sr >

Table 2
Concentrations of elements in *E. purpurea*

Sample	Cu (μg/g)	Fe (μg/g)	Mn (μg/g)	Zn (μg/g)	Ni (μg/g)	Li (μg/g)	Sr (μg/g)	Mg (mg/g)	Ca (mg/g)
$Soil_{ex}$	15.1	4800	410	15.8	2.90	7.1	5.9	1.45	5.45
Root[a]	11.5	170	13.1	4.50	0.50	2.8	7.6	3.24	4.97
Stem[a]	9.40	101	18.9	6.40	3.70	0.9	4.4	3.39	8.98
Leaf[a]	11.8	292	67.6	12.7	5.10	3.2	10.7	8.38	29.3
Flower[a]	15.9	184	29.9	18.6	9.30	2.3	4.4	3.49	14.6
Herbs[a]	10.2	220	42.0	12.6	4.30	2.8	6.8	6.3	23.5
Stem[b]	4.4	32.2	8.80	3.9	3.90	0.5	9.4	3.21	5.14
Leaf[b]	8.9	189	50.6	11.7	7.70	3.3	27.3	10.5	41.4
Flower[b]	14.7	62.4	14.8	14.8	9.30	2.9	10.9	4.01	11.5
Herbs[b]	9.1	65.7	15.5	10.9	3.40	1.3	10.2	5.01	10.8
$Extract_{EtOH}$	7.0	4.00	23.0	66.0	18.0	2.0	5.0	17.8	31.3

[a] Autumn harvest.
[b] Summer harvest.

Ni > Li. Manganese, Cu, Zn, Sr, Ni and Li were found in traces while the concentration of Ca, Mg and Fe was somewhat higher. The differences in concentrations of various elements are attributed to the differences in botanical structure of the particular part of the plant, as well as to the mineral composition of the soil in which plants were cultivated.

Both Zn and Ni are essential elements for plant growth and are mobile within the plant [13], which probably explains their highest concentration in flowers. Magnesium is not only essential, but it is also a constitutive element of chlorophyll, so that its highest concentration was found in leaves. The distribution of Ca is similar to that of Mg.

Two sample series, from two harvests, were analyzed in order to investigate the seasonality. The higher content of Fe (1.5–3.3 times), Mn (1.3–2.7 times) and Zn (1.1–1.5 times) was found in the autumn samples, and of Sr (2.1–2.5 times) in the summer samples, while the content of Ni and Li is practically the same. The content of Cu, Mg and Ca depends on both the season and the plant tissue.

The ethanolic extract of *E. purpurea*, phytopharmaceutical formulation used as a therapeutic immunostimulant (commercially available at the Serbian market), was also analyzed and the results are presented in Table 2. As expected, much lower concentrations of elements than those in the dried plant material were obtained. Zinc (beside Fe, Mn and Ca) is one of the elements present in considerably significant concentration and this may be correlated with immunostimulant abillity of the extract.

To study trace element patterns as well as different plant parts grouping, a chemometrics approach was also used. In the first step of the statistic evaluation, using Shapiro–Wilk's test [21] (significance level α was 0.05), it was found that the data set (Table 2) deviates from the normal distribution. In contrast, the log-transformed data that are normally distributed. The data matrix was also tested by applying Grubb's test [22] detecting no outliers.

Table 3 presents the correlation matrix of nine elements. Over 50% of the correlation coefficients in the matrix are over 0.3. The data set of concentration measurements was subjected to principal component and hierarchical cluster analysis in order to highlight the relations between the elements. Principal component analysis (PCA) removes the highly inter-correlated nature of variations in trace element concentrations. The initial statistics of eigen analysis is given in Table 4. It can be seen that three principal components (PCs) appeared to account for 86% of the variance in the data. According to the Kaiser criterion [23], only the first three PCs were retained because subsequent eigenvalues are all less than one. Hence, reduced dimensionality of the descriptor space is three.

Table 3
The correlation matrix of trace element data (Pearson correlation)

	Cu	Fe	Mn	Zn	Ni	Li	Sr	Mg
Fe	0.61							
Mn	0.49	0.77						
Zn	0.22	−0.25	0.34					
Ni	−0.07	−0.42	0.06	0.74				
Li	0.75	0.62	0.77	0.42	−0.02			
Sr	−0.17	0.05	−0.02	−0.22	0.02	0.12		
Mg	−0.33	−0.63	−0.20	0.54	0.55	−0.04	0.31	
Ca	0.01	−0.26	0.15	0.61	0.66	0.24	0.34	0.87

Table 4
Eigen analysis of the correlation matrix

Variable	Eigenvalue	Cumulative
Cu	3.4018	0.378
Fe	3.0425	0.716
Mn	1.3117	0.862
Zn	0.5458	0.922
Ni	0.4066	0.968
Li	0.2544	0.996
Sr	0.0267	0.999
Mg	0.0099	1.000
Ca	0.0005	1.000

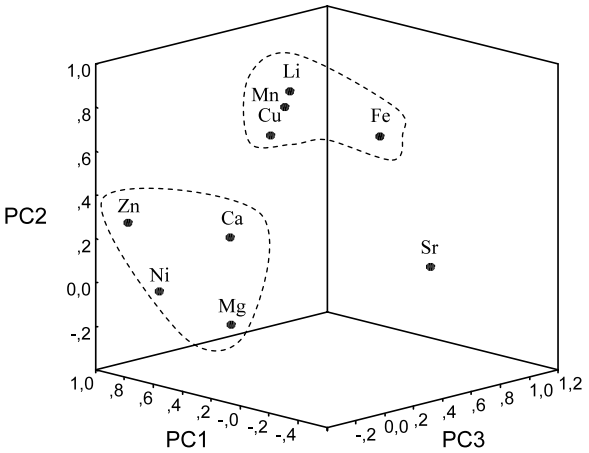

Fig. 1. Principal component plot in rotated space.

One of the main objectives of PCA is to identify factors that are substantively meaningful. In this case, the first principal component shows a high positive correlation with variables Ni, Zn, and the second principal component a high correlation with variables Li, Mn, Cu and Fe. The third principal component comprises Li, Mn, Fe and Sr. Fig. 1 illustrates principal component plot in rotated space in which the elements can be clustered.

A graphical depiction of different plant parts groupings was obtained by means of hierarchical cluster analysis (HCA) of standardized concentrations using Ward's method [24] as an amalgamation rule and the squared Euclidean distance as a measure of the proximity between samples. A dendrogram is shown in Fig. 2. As a result of applying HCA to the principal component score matrix, the parts split to two main groups. Nevertheless, the soil and Echinacea extract are well separated from other plant parts, while the extract is more similar to one of the groups.

Generally, both principal component analysis and cluster analysis certified that there is a correlation between trace elements in *E. purpurea*. However, no clear difference between the summer and the autumn harvest was observed.

E. purpurea was analyzed in order to get some useful information to be used in the preparation of drugs from this plant material. As there are no reports in literature on the trace element content in Echinacea, this paper should be considered as a contribution to that course. Being far from knowing exactly the mechanisms of action and the formation of active constituents of this medicinal plant, a direct link between the elemental content and its curative effect remains to be established.

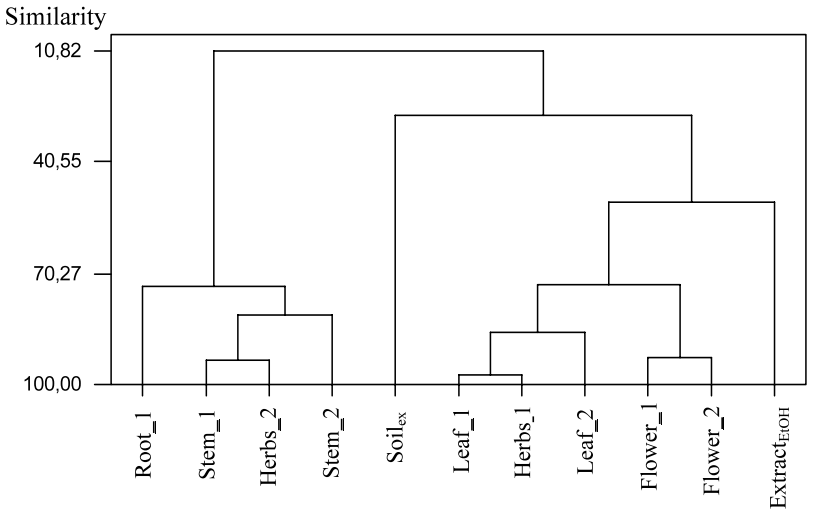

Fig. 2. Dendrogram of cluster analysis of *E. purpurea*.

4. Conclusion

E. purpurea from eastern Serbia exhibits two different elemental distribution patterns within the plant. Flowers contain the highest concentration of Cu, Zn and Ni, while the concentration of Mg, Ca, Fe, Mn, Li and Sr is the highest in leaves. In order to develop a stronger basis to estimated the curative effects of *E. purpurea*, the extract has to be investigated both in terms of elemental composition and biochemical make-up.

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