Original Article

Trace element structure of the most widespread plants of genus *Pulmonaria**

Abstract

Aim: The aim of this work was a comparative research of trace element structure of various organs of three *Pulmonaria* species. **Materials and Methods:** The aerial parts of the most widespread plants of genus *Pulmonaria* such as *Pulmonaria officinalis* L., *Pulmonaria obscura* Dumort. and *Pulmonaria mollis* Wulf. ex Hornem., which were collected in ending of flowering and were used as the research objects. The amount of trace elements (B, K, P, V, Ca, Co, Cu, Fe, Mg, Mn, Mo, Na, Si, Zn, Ag, Al, Ba, Br, Cr, I, Ni, Se, Sr, and Ti) was determined by means of mass spectroscopy with inductively coupled plasma. **Results:** The data clustering has shown that floral shoots and rosellate leaves possess essentially various trace element status. At the same time, the trace elements' status of organs of researched plants poorly depends on a taxonomic position of the plant. Thereupon, it is obvious that pharmacological activity is defined by organs of plants from which medicines were made, but not by a species of the used plant. **Conclusions:** The significant distinction in pharmacological activity of preparations depends on the trace elements' status of used medicinal vegetative raw materials.

Key words:
Iron-deficiency anemia, mass spectroscopy with inductively coupled plasma, pulmonaria, trace element structure

Introduction

Nowadays, the majority of herbal remedies are made on the basis of biological activity compounds that are the products of a secondary metabolism of vegetative organisms.

At the same time in some cases, the substances of a primary metabolism represent definite interest for creation of effective medicines for preventive maintenance and treatment of a rather extended trace element disbalances. The iron-deficiency anemia concerns a number of similar diseases and first of all previous iron-deficiency status of organism.

Earlier, it has been shown that the extracts taken from the aerial parts of *Pulmonaria mollis* Wulf. ex Hornem. have an evident antianemic activity.[1] At the same time, the medicines from leaves of a closely related plant *Pulmonaria officinalis* growing in the Western Europe possess other properties—emollient, expectorant, astringent, and antihemorrhagic.[2] The specified action of *P. officinalis* is connected with the presence in its structure of polysaccharides and flavonoids of quercitin group.[3] Antianemic activity of extracts made from *P. mollis* is connected with the hemopoietic microelements such as Fe, Mn, Cu, and Co which were determined in its structure. Moreover, iron being the trivalent ion is founded in the polysaccharide–albuminous complex which is representing itself as matrix for trivalent iron.[4]

Talking about these plants, it is necessary to notice that on the Euroasian continent *P. officinalis* L., *Pulmonaria obscura* Dumort., and *P. mollis* Wulf. ex Hornem. are the most widespread species of the genus *Pulnomaria*. All of them are typical ephemeroids and their development cycle includes two

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*Dedicated to the late Dr. Fransworth, University of Illinois at Chicago for his pioneer work on bio-active natural products periods—the first one is a short period from the beginning of vegetation to fruitage where floral shoots are developing and the second one is a long period of rosellate leaf where leaves are growing from underground resting buds.

As a result of research of pharmacological activity of medicines which were made from different parts of the plant, it has been established that they possess various antianemic activity.[4]

The recovering of balance of iron is the most important for treatment of anemia. Therefore, the presence of the trace elements of hemopoietic complex and first of all iron are necessary to be in the medicines which are used for antianemic therapy. At the same time, it is obvious that the microelement structure of medicines (in this case extracts from an aerial part of the plant) is defined by the microelement structure of the plant.

Thereupon, the aim of this work was a comparative research of microelement structure of various organs of three *Pulmonaria* species.

Materials and Methods

The aerial parts of investigated plants, which were collected in ending of flowering, were used as the research objects [Table 1].

The collected aerial parts of plants were dried up and crushed up to the sizes of particles from 0.2 to 2 mm. Then the weighed dry plant powder was dissolved by the method of wet ashing using the mixture of acids and MWR-induced heating. After this, the amount of microelement was determined by means of inductively coupled plasma mass spectroscopy (ICP-MS). In ICP-MS, plasma is used to generate the ions. The sample of interest is introduced in the ICP-MS through a spray chamber, which is converted into an aerosol. The plasma ionizes the elements in the sample, and these ions pass through the interface and ion lens. The focused ions are sorted according to their m/z ratio (m, massa and z, charge of ion) in a quadrupole mass analyzer and are measured by the detector to produce a mass spectrum.[5] The device “ELAN DRC” of PerkinElmer Inc. was used for this measurement.

The elements for analysis have been chosen with taking their role in physiology of a vegetable and human organisms into account. The trace elements such as B, K, P, V, Ca, Co, Cu, Fe, Mg, Mn, Mo, Na, Si, and Zn are essential for both organisms so they were included in the number of researched elements. The second group of trace elements Ag, Al, Ba, Br, Cr, I, Ni, Se, Sr, and Ti have a dominant toxic influence on vegetable organism.[6] Among the trace elements of the second group, Br, I, Se, Sr, Ba, Ti, Cr, and Sr are also essential meaning for a human organism.

Table 1: Objects of research

Pulmonaria species	Collection site
Pulmonaria officinalis L.	Germany, suburb of Frankfurt am Main, hornbeam-oak forest
Pulmonaria obscura Dumort	Russia, suburb of Yaroslavl, mixed forest
Pulmonaria mollis Wulf. ex Hornem.	Russia, suburb of Novosibirsk coniferous forest

Measuring amount of trace elements was being made in five replications for the determination of reproducibility of results. Established results are given in [Table 2].

Results and Discussion

The mass spectroscopy method, which was used in this work, allowed to define all the elements, whose role in physiological processes of plants has been authentically established.[6]

At the same time, the increase in number of analyzed elements leads to the great difficulties in the generalizing and understanding of the received results. There are two methods which are used more often in the analysis of the amount of microelements. The first method is comparing the elements which are contained in objects in extreme quantities. The second method is the establishment of the pair correlations between the separate elements. Both of these methods do not appear informative enough. It becomes obvious that the analysis of the big data files demands special mathematical apparatus.

Approaching formal positions, a problem of comparison of objects which have a lot of parameters is a typical problem of one of sections of mathematical statistics—the multiple-factor analysis. In these cases, the using of the data clustering can be much effective for studying element structure.

The means of a data clustering have been applied which allow to analyze the set of all experimental data simultaneously.[7] One of the most widely used classes of methods involves hierarchical agglomerative clustering, in which two groups, chosen to optimize some criterion, are merged at each stage of the algorithm. Popular criteria include the sum of within-group sums of squares (the Ward’s method) and the shortest distance between groups, which underlies the single-link method. The Ward’s method was taken as a rule for association or communication of two groups into one cluster. The sum of root-mean-square deviations for any two (hypothetical) clusters which can be generated on each step must be minimized by this method. The distance between different clusters must be not less than a limit of variability of amount of the trace element, the value of which was taken as 10%.

The data clustering has shown that floral shoots and rosellate leaves possess essentially various trace element status [Figure 1]. At the same time, the trace elements’

Table 2: The content of trace elements (amount in ppm) in floral shoots and rosellate leaves of plants of the genus *Pulmonaria*

Elements	*P. mollis* floral shoot	*P. obscura* floral shoot	*P. officinalis* floral shoot	*P. mollis* rosellate leaf	*P. obscura* rosellate leaf	*P. officinalis* rosellate leaf
B	28,1	17	22,3	30,9	17	17,2
Na	79,5	31	66,5	152,7	151,3	57,3
Mg	1693,5	1788	2718,5	2391,3	1364,8	1348,6
Al	467	243,8	430,2	792,6	321,1	154,3
Si	2175	2865	6077,2	5177,3	5326,2	3414,1
P	2318	3508	3157,4	2093,7	2871,3	2345,4
K	60627	66736	65572,2	50457,7	44524,3	37845,5
Ca	7864	7210	8690,3	13422	6054,6	5719,2
Ti	22,1	1,89	7,3	63	48	10,2
V	0,875	0,49	0,98	1,59	1,37	0,66
Cr	5,46	6,45	8,37	4,4	2,07	1,8
Mn	84	37,6	57,9	96,7	70	58,9
Fe	405,3	234,2	345,7	200,4	281,5	138,3
Co	0,73	1,06	1,32	0,58	0,22	0,25
Ni	1,85	0,76	1,66	3,9	1,47	1,65
Cu	14,3	9,53	13,6	17,5	7,67	9,25
Zn	34,6	16,83	19,1	33,4	17,3	21,8
Se	0,24	0,314	0,86	0,85	1,1	1,03
Br	15,35	14,3	27,7	28,3	8,7	13,2
Sr	17,75	9,6	16,8	36,33	35,7	34,1
Mo	0,37	0,189	0,1	0,15	0,22	0,2
Ag	0,02	0,006	0,01	0,02	0,01	0,01
I	1,53	0,049	0,046	0,11	0,03	0,038
Ba	46,7	39	82,9	116,7	96,7	93,5

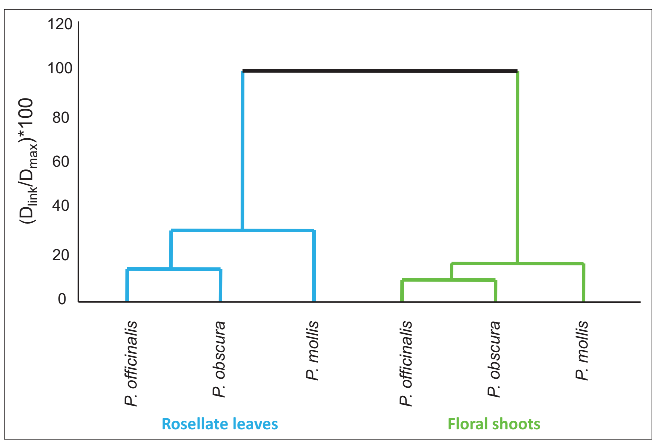

Figure 1: Hierarchical tree of investigated objects

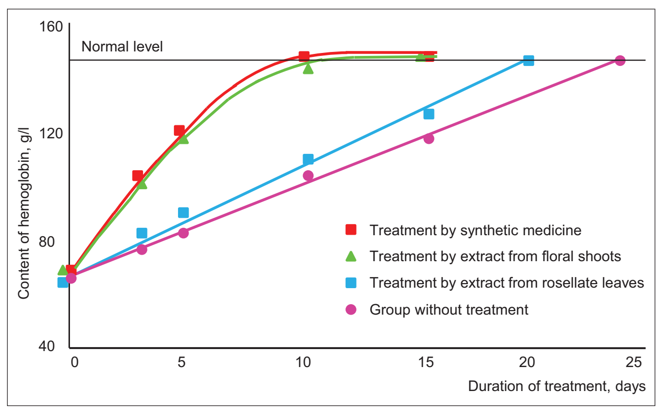

Figure 2: Restoration of hemoglobin level at rats with iron-deficiency anemia[4]

status of organs of researched plants poorly depends on a taxonomic position of the plant. Thereupon, it is obvious that pharmacological activity is defined by organs of plants from which medicines were made, but not by a species of used plant.

Earlier it has been researched that extracts made of different parts of plant have different pharmacological activity [Figure 2].[4] The extract from floral shoots possesses pathogenetic action which is equal to the action of a modern synthetic medicine on a basis of Fe^{3+}-hydroxide polymaltose complex. The treatment of experimental rats with iron-deficiency anemia by this extract has lead to restoration of level of hemoglobin for 8–9 days. It is necessary to pay attention that dependence of level of hemoglobin on duration of treatment by extract from floral shoots of plants has an identical form with similar dependence at treatment by a synthetic medicine. On treatment by extract from rosselate leaves of the plant, the dependence mentioned above has other form and the level of hemoglobin at experimental rats was restored by the 20th day (but rats without treatment have restoration of the level of hemoglobin occurring by the 25th day). Various nature of change of level of hemoglobin allows to assume various mechanisms of pharmacological effect of extracts from different parts of the plant. The extract from floral shoots of plant has pathogenetic action. At this time, the extract from rosellate leaves possesses a weak etiotropic action. This etiotropic action obviously is connected with antihemorrhagic properties most likely defined by the presence in their structure of flavanoids possessing P-vitamin activity. The pathogenetic action of extract from floral shoots is obviously caused with the microelement structure of these parts of plant.

It is also important to notice that similar dependences of pharmacological activity of extract from different parts of the plant were established for all researched plants. Comparing data received as a result of data clustering is possible to draw a conclusion that the microelement structure does not depend on a species of plant. At the same time, the different parts of plants have different microelement structure. In this case, results of the data clustering correspond to the pharmacological activity of medicines made from various parts of a plant.

Thus, it is necessary to notice that the qualitative structure of biologically active compounds is presented by flavonoids, polysaccharides, tannins, and is very close for the plants of various species and their organs.[8]

Therefore, significant distinction in pharmacological activity of medicines depends on the trace elements' status of used medicinal vegetative raw materials.

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