UDC 615.074
https://www.doi.org/10.34907/JPQAI.2020.31.52.007

DYNAMICS OF MINERAL COMPOSITION OF RAW MATERIALS OF BIRTHWORT DUTCHMAN'S-PIPE (*ARISTOLOCHIA CLEMATITIS* L.) AND SOIL FROM ITS HABITAT

I.I. Terninko, *PharmDr., Associate Professor, Head of the Testing Laboratory (Medicine Quality Control Center), Assistant Professor of the Department of Pharmaceutical Chemistry, Saint Petersburg State Chemical and Pharmaceutical University, Ministry of Health of Russia, Saint Petersburg*

I.O. Suina, *Assistant of the Department of Pharmaceutical Chemistry, Saint Petersburg State Chemical and Pharmaceutical University, Ministry of Health of Russia, Saint Petersburg, Suina.Irina@pharminnotech.com,*

Yu.E. Generalova, *Analytical Chemist in the Testing Laboratory (Medicine Quality Control Center), Saint Petersburg State Chemical and Pharmaceutical University, Ministry of Health of Russia, Saint Petersburg*

Z.R. Ditkovskaya, *Analytical Chemist in the Testing Laboratory (Medicine Quality Control Center), Saint Petersburg State Chemical and Pharmaceutical University, Ministry of Health of Russia, Saint Petersburg*

The ultimate composition of the Birthwort Dutchman's pipe (Aristolochia clematitis L.) herb and soil samples from its habitat in the dynamics of 2017–2019 was analyzed by inductively coupled plasma-atomic emission method. The majority macro- and microelements are potassium, magnesium and calcium, iron, manganese and zinc, respectively. The content of heavy metals and the coefficient of biological absorption of Birthwort Dutchman's pipe herb were determined.

Keywords: Birthwort Dutchman's pipe (*Aristolochia clematitis* L.), mineral composition, heavy metals, maximum permissible concentration

One of the important elements of a comprehensive study of the chemical composition of medicinal plant raw materials (MPRM) is the study of the element profile. Macro- and microelements have a significant impact on the biosynthesis of various groups of biologically active substances (BAS): they affect the processes of plant growth, act as components of enzymes. Thus, potassium, sodium and calcium are key elements in respiration and many other biochemical processes of plants [1], phosphorus is a part of ATP, and magnesium is a main component of chlorophyll.

In the process of obtaining the plant extracts, the mineral components, along with the rest of the BAS groups, are isolated from the MPRM and, in turn, affect the manifestation and severity of the pharmacological action of the medicine, as well as show their own activity. It was noted [2] that microelements of plant origin are better absorbed by the human body, since they are in "biological" concentrations in physiologically balanced complexes.

The chemical composition of plants is influenced by many factors: environmental, genetic, and physiological [3,4]. Phytochemical composition of MPRM depends on the growing environment, namely, the elemental composition of the soil and the ability of the plant to concentrate elements [5]. Therefore, it is advisable to study the elemental composition of medicinal plants in conjunction with soil research. The literature [6] shows that chemical elements contained in the soil significantly affect the biosynthesis of BAS in plants and are capable of selective accumulation. The identified natural concentrators of micro-elements from plants can be successfully used in practical medicine for corrective therapy. At the same time, an important issue is the environmental aspect, namely, environment pollution with heavy metals. Coming through the soil, they change the biochemical processes of plants, disrupt the normal processes of life [7] and, as a result, can be in herbal medicinal products. Therefore, it is necessary to determine and compare with the maximum permissible concentrations (MPC) the content of heavy metals in the harvested raw materials.

Plants that are considered as poisonous have always attracted the attention of researchers due to their high activity [8]. Therefore, interest in the possible pharmacotherapeutic profile of toxic compounds remains high. One of these plants is the Birthwort Dutchman's pipe (*Aristolochia clematis* L.), which contains toxic aristolochiic acids [9,10]. Previously, scientists studied the accumulation of individual elements in the A. clematitis herb growing in the Stavropol territory [11,12]. However, the geochemical aspects of vegetation have a significant impact on the elemental profile of the plant, and in addition, there is no data in the literature on the dynamics of accumulation of macro – and microelements. Therefore, the **purpose** of our work was to study the macro – and microelement composition of Birthwort Dutchman's pipe (*Aristolochia clematis* L.) herb, in comparison with soil samples from its habitat in the dynamics of the periods of harvesting the raw materials and highlight the maximum concentration.

MATERIALS AND METHODS

As the study object, we used the Birthwort Dutchman's pipe (*Aristolochia clematis* L.) herb and soil from its habitat to form a conclusion about the selective accumulative capacity of the plant. Raw materials were harvested in early July 2017, 2018, 2019 in the city of Novy Oskol, Belgorod region, according to the rules for harvesting medical herbs in the forest phytocenosis (in dry weather at noon in the afternoon from unpolluted, well-developed, healthy plants at a distance of at least 10 km from major industrial cities and no closer than 500 m from the roadside with heavy traffic). Raw material is dried using natural shade drying. The degree of grinding is 3–5 mm. The soil was prepared in early July 2017 and 2019 in the city of Novy Oskol, Belgorod region. To determine the ultimate composition of the soil, samples were taken at 3 points around the plant in a diameter of 30 cm and from a depth of 25 cm, thoroughly mixed and formed a single average sample. Then the soil was sifted and large particles were removed. For a more objective picture of changes in the concentration capacity of Birthwort Dutchman's pipe (*Aristolochia clematis* L.), the soil was collected in two years (in 2017 and 2019).

The analysis was performed by inductively coupled plasma-atomic emission method using the Optima 8000 spectrometer (Perkin Elmer, USA) in accordance with the recommendations of the RF SP XIII OFS.1.5.3.0009.15 [13] based on CCU "Analytical center".

Sample preparation: a sample weight of about 0.4 g (exact weight) was placed in a Teflon vessel ("bomb"). 5 ml of concentrated nitric acid for inductively coupled plasma-atomic emission (65%, Sigma Aldrich), 3 ml of hydrogen peroxide were added to the weighted sample, carefully mixed, and left for 10 minutes to remove vapors. Then the vessel was placed in the BERGHOF SpeedWave Entry Two microwave system, and the temperature conditions were set for mineralization of plant objects. After cooling, the mineralizate was transferred to polymer measuring flasks with capacity of 50 ml, brought to the mark with purified water of type 1 (solution No.1). 250 ml of solution No.1 was placed in a polymer measuring flask with a capacity of 25 ml with a mechanical dispenser and brought to the mark with solvent (15.4 ml of concentrated nitric acid and 30 ml of hydrogen peroxide were brought in a polymer measuring flask with purified water of type 1 to 500 ml) – solution No.2. The resulting solution No. 2 was used for quantitative determination of macro-elements in the solution

As standard samples, we used Multi-Element Calibration Standard-3 with certified element values of 10 mg /l and Pure Plus Mercury 10 mg /l (Perkin Elmer, USA).

Test conditions: plasma feed rate – 10 l/min, auxiliary – 0.2 l/min, spray – 0.7 l/min. Power 1300 W, the viewing position is axial. The mud flow rate (peristaltic pump) – 1.5 ml/min, for washing the rapid feed of solvent – 2.5 ml/min, the delay time – 25 sec., read time – 0.1–1.0 sec., repetitions (replicas) – 3.

The choice of analytical waves is based on the minimum value of sensitivity and the maximum intensity. Several analytical wavelengths (2–3 lengths) are pre-viewed, the wavelengths, where the overlapping spectra of other elements (for example, iron), are noticeable, are removed, the position of the peak and the baseline is adjusted, and the calculation is provided relative to the solvent by the peak area. The monochromatic spectrometer registers the signal intensity and gives the value of the calibration concentration according to the calibration curve. The reliability of the results is evaluated by RSD (the acceptance criterion of RSD is 2%, it is acceptable for micro-concentrations of RSD up to 30%). The content of elements (X, mg/kg) in the sample was calculated using the formula:

$$\mathbf{X, mg/kg} = \frac{C_x \times V_1 \times V_2}{m \times V_3} \times 1000 \times 1000,$$

where C_x – element concentration according to the calibration curve, mg/l; V_1 – solution volume, l; V_2 – dilution volume, ml; V_3 – solution volume assumed for dilution, ml; m_1 – sample weight, mg; 1000 – conversion of mg into g; 1000 – conversion of g into kg.

RESULTS AND DISCUSSION

According to the results of the study of macro- and microelements in the Birthwort Dutchman's pipe (*Aristolochia clematis* L.) herb and soil from its habitat, the content of 17 elements was found in 2017, 11 elements in 2018 and 2019 (see Table 1).

Comparing the results of the ultimate composition in dynamics over the years of harvesting, we can say that the majority macro-and microelements in the Birthwort Dutchman's pipe (*Aristolochia clematis* L.) herb do not change, they are potassium, magnesium and calcium, iron, manganese and zinc, which is a normal physiological element profile of plants. The content of magnesium in the raw materials harvested in 2017 and 2019 significantly exceeds its content in the soil (7361.3 mg /kg – 42.04 mg/kg; 2188.5 mg/kg – 979.2 mg/kg, respectively). Considering that magnesium is a pigment-forming element, its quantitative variability over the years in a significant range is normal. The increase in the content of calcium in herb in 2019 (12629.5 mg/kg) by 25% compared to the soil can be explainedby unevenness of the harvested herb elements (the predominance of stems compared to leaves) and accumulation of physiological inclusions of calcium oxalate.

The data from the analysis of birthwort Dutchman's-pipe herb harvested in different years correlate by the majority components, but differ in the content of individual elements, especially, normalized heavy metals. Thus, it is possible to trace a gradual decrease in the

Table 1

RESULTS OF THE STUDY OF MACRO-AND MICROELEMENT COMPOSITION OF THE BIRTHWORT DUTCHMAN'S PIPE (*ARISTOLOCHIA CLEMATIS* L.) HERB AND SOIL FROM ITS HABITAT

Element	Content of elements, mg/kg (n=5)				
	Raw material, 2017	Soil, 2017	Raw material, 2018	Raw material, 2019	Soil, 2019
Al	230.12	19903.23	83.65	63.4	3838.5
As (MPC 0,5 mg/kg)	**–**	**–**	**0.07**	**–**	**–**
Ba	83.29	107.31	not determined	*20.4*	17.0
Ca	7112.76	13838.71	not determined	*12629.5*	10051.0
Cd (MPC 1,0 mg/kg)	**0.58**	**0.86**	**0.34**	**–**	**–**
Co	–	–	–	–	–
Cr	2.39	42.58	0.75	–	–
Cu	*49.14*	16.45	*27.95*	*6.6*	2.6
Fe	374.81	5707.53	119.2	82.0	3085.0
K	*16016.46*	13591.40	not determined	*25730.0*	1546.5
Li	–	200.00	not determined	–	–
Mg	*7361.32*	42.04	not determined	*2188.5*	979.2
Mn	110.86	142.90	40.70	35.4	82.8
Na	*91.19*	17.44	not determined	*137.3*	69.7
Ni	3.13	18.71	1.48	–	–
Pb (MPC 6,0 mg/kg)	***2.39***	**–**	***1.34***	**–**	**–**
Se	*6.42*	–	not determined	–	–
Sr	–	–	–	27.7	20.8
Zn	*109.05*	0.91	*54.21*	*22.3*	15.2
Hg (MPC 0,1 mg/kg)	***4.61***	**0.22**	**0.03**	**–**	**–**
Bi	*0.082*	–	not determined	–	–

Note: bold font – elements whose content is normalized by the RF SP; italics with underscores – elements whose content in raw materials exceeds the same indicator in soil

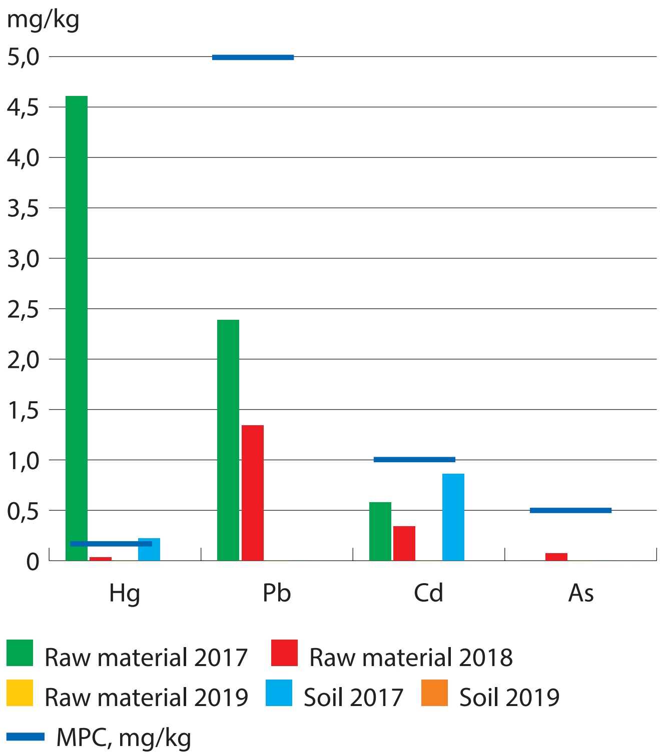

FIG. *Dynamics of the content of heavy metals and arsenic (mg /kg) in the Birthwort Dutchman's pipe (Aristolochia clematis L.) herb and soil from the Belgorod region in different years*

content of mercury, lead and cadmium in the birthwort Dutchman's-pipe herb 3 years until the disappearance. The content of mercury in the raw material harvested in 2017 is 4.61 mg/kg and exceeds the MPC by 40 times. The content of this heavy metal in the soil is 0.22 mg/kg, which allows us to conclude that the raw material of birthwort Dutchman's-pipe is able to concentrate mercury. The mercury content in the herb harvested in 2018 is much lower (0.03 mg/kg) and is within normal limits, and in raw materialand soil in 2019 the mercury was not identified. The content of other heavy metals is normal and does not exceed the MPC. However, lead and arsenic were identified in raw materials (2.39/1.34 mg/kg (2017 and 2018) and 0.07 mg/kg (2018), respectively) and were not identified in the soil. This proves that the birthwort Dutchman's-pipe herb concentrates heavy metals of any origin. In comparison with 2017, in 2019 the content of all identified heavy metals does not exceed the MPC. Thus, we can assume that the plant is the most representative indicator of the degree of environmental pollution in a particular region, since it is able to accumulate certain toxic elements. Based on the research data, we can assume that the environmental situation in the Belgorod region deteriorated in 2016–2017. The results of the content of heavy metals and arsenic in the birthwort Dutchman's-pipe herb and soil from the habitat and MPC according to the RF SP OFS.1.5.3.0009.15 "Determination of the content of heavy metals and arsenic in medicinal plant raw materials and medicinal plant medicines" are shown in the figure [13].

The coefficients of biological absorption (CBA – the ratio of the element content in the aboveground part to its content in the soil) were

Table 2

CBA FOR THE BIRTHWORT DUTCHMAN'S PIPE (*ARISTOLOCHIA CLEMATIS* L.) HERB IN DYNAMICS OF 2017 AND 2019

CBA	2017	2019
Elements of energetic and strong accumulation (CBA>1)	**Cu, K, Mg, Na, Zn, Hg**	**Cu, K, Mg, Na, Sr, Zn, Ba, Ca**
Elements of weak accumulation and average capture (1>CBA>0,1)	**Ba, Ca, Cd, Mn, Ni**	**Mn**
Elements of weak capture (CBA<0,1)	**Al, Cr, Fe**	**Al, Fe**

calculated by the N.F. Ganzhar scale [14] for the Birthwort Dutchman's pipe (*Aristolochia clematis* L.) herb harvested in different years (Table 2).

It is established that regardless of the year of harvesting, the Birthwort Dutchman's pipe (*Aristolochia clematis* L.) herb is Cu, K, Mg, Na, and Zn accumulator. These elements are distributed in descending order as follows: raw materials harvested in 2017 – Mg, Zn, Na, Cu, K; raw materials harvested in 2019 – K, Cu, Mg, Na, Zn. This allows us to consider the accumulation of these elements as an additional chemical marker for the Birthwort Dutchman's pipe (Aristolochia clematis L.) herb.

CONCLUSION

The macro-and microelement composition of the Birthwort Dutchman's pipe (*Aristolochia clematis* L.) herb and the soil on which it grows in the dynamics of 2017–2019 was determined. The content of 17 elements was determined in 2017 and 11 elements in 2018 and 2019, respectively. Moreover, the data from the analysis of the Birthwort Dutchman's pipe (*Aristolochia clematis* L.) herb harvested in different years correlate by the majority components (potassium, magnesium, calcium, iron, manganese and zinc) and accumulation elements (copper, potassium, magnesium, sodium and zinc), but differ in content. It was found that the Birthwort Dutchman's pipe (*Aristolochia clematis* L.) herb is able to concentrate heavy metals (mercury and lead), the content of which in the raw material harvested in 2017 is significantly higher than in the soil. This fact makes it possible to consider the raw material of the Birthwort Dutchman's pipe (*Aristolochia clematis* L.) as a biological marker of mercury contamination of the environment and to assume the detoxification properties of the raw material.

REFERENCES

1. *Stal'naya M.I. Study of the elemental composition of plants // New technology. – 2007. – №3. URL: https://cyberleninka.ru/article/n/issledovanie-elementnogo-sostava-rasteniy (Access date: 05.09.2019).*
2. *Skalny A.V. Bioelements in medicine / A.V. Skalny, I.A. Rudakov. – M.: Publishing house "ONYX 21 century": Mir, 2004. – p. 272*
3. *Kovalsky A.L. Plant biogeochemistry / A.L. Kovalsky. – Novosibirsk: Nauka. Sib. Department, 1991. – p. 288*
4. *Lovkova M.Ya. Characteristics of chemical properties of medicinal plants (review) / M.Ya. Lovkova, G.N. Buzuk, S.M. Sokolova et al. // Applied Biochemistry and Microbiology. – 2001. – №3. – Vol. 37. – pp. 261–273.*
5. *Buzuk G.N. Determination of correlation dependencies between the content of alkaloids and chemical elements using mathematical modeling / G.N. Buzuk, M.Ya. Lovkova, S.M. Sokolova et al. // Reports of the Academy of Sciences. – 2001. – №5. – Vol. 376. – pp 690–693.*
6. *Avtsin A.P., Zhavoronkov A.A., Rish M.A. – M.: Medicine, 1991. – p. 496.*
7. *Rozhdestvenskaya T.A., Yelchininova O.A., Puzanov A.V. Elemental chemical composition of plants of the Altai Mountains and factors that define it // Materials of the International Conf. "Biodiversity, environmental problems of the Altai Mountains and neighboring territories: present, past and future". – Gorno-Altaisk. – 2008. – pp. 110–114.*
8. *Lisyany N.I. Immunomodulating and antitumor effect of celandine medications on brain tumors / N.I. Lisyany, L.N. Belskaya, A.I. Klyuchnikova / Ukrainian Neurosurgical Journal. – 2011. – №1. – pp. 23–29.*
9. *Heinrich Michael. Local uses of Aristolochia species and content of nephrotoxic aristolochic acid 1 and 2 – A global assessment based on bibliographic sources / Michael Heinrich, Jennifer Chan, Stefan Wanke, Christoph*

Neinhuis // Journal of Ethnopharmacology. – 2009. – №125. – P. 108–144.

10. *Margaret L. Aristolochic acid in the etiology of renal cell carcinoma / L. Margaret et al. // Cancer Epidemiol Biomarkers Prev. – 2016. – №12. – P. 1600–1608.*
11. *Samsonova O.E., Belous V.N., Dudar Yu.A. Pharmacognostic study of the Birthwort Dutchman's pipe (Aristolochia clematis L.) of the Stavropol Territory flora// Chemical and pharmaceutical journal. – 2006. – Vol. 40. – №4. – pp 23–24.*
12. *Samsonova O.E. Bioelements Mn, Cu, Zn in some useful and poisonous plants of Stavropol territory // Bulletin of Orenburg State University. – 2006. – №12–2.*
13. *State Pharmacopoeia of the Russian Federation, XIII Edition, 2015. Vol. 1. p. 1470; Vol. 2. p. 1004. – URL: http://femb.ru/feml. (Date of access: 01.09.2019).*
14. *Ganzhara N.F., Baibekov R.F., Boyko O.S., Koltykhov D.S., Areshin A.V. Geology and landscape studies. – Moscow: Association of scientific publications of CMC. – 2007. – p. 380 + p. 56*