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DETERMINATION OF MACRO AND MICROELEMENTS CONTENTS IN *GENTIANA MACROPHYLLA* PALL. HERB

V. A. Tsukurova [*1], O. V. Evdokimova [2] and V. I. Prokofieva [3]

Department of Pharmaceutical and Toxicological Chemistry [3], Institute for Translational Medicine and Biotechnology [1], Sechenov First Moscow State Medical University, 8-2 Trubetskayast, Moscow, Russian Federation, 119991.

FSBI [2] (Scientific Centre for Expert Evaluation of Medicinal Products) of the Ministry of Health of the Russian Federation, Moscow, Russian Federation.

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Correspondence to Author:
V. A. Tsukurova

Ph.D. Student,
Institute for Translational Medicine and Biotechnology, Sechenov First Moscow State Medical University, 8- 2 Trubetskayast, Moscow, Russian Federation, 119991.

E-mail: vasa1805@gmail.com

ABSTRACT: Macro and microelements are integrally linked to hormones, vitamins, amino acids, and enzymes in the human body. They affect the normal course of physiological processes. Some medicinal plants contain substantial contents of micro-and macro-elements, which makes it possible to use them purposefully for the prevention and treatment of various diseases. The target of the research was samples of *G. macrophylla* herb harvested and procured in the Republic of Tyva. The aim of this research provided a quantitative determination of the elemental composition in the herb of *G. macrophylla* Pall. as a possible source of macro and microelements. The determination was carried out with the atomic absorption spectrophotometry technique. As was discovered, Fe, Mn, Zn, Cu, Cr, Se, Mo, B, and Ag are present in the *G. macrophylla* herb. The maximum admissible contents of heavy metals in the specimen under study did not exceed the limits recommended for the quality assessment of herbal raw materials.

INTRODUCTION: Macro and microelements play a critical part in human life and activities; they affect enzyme activity, are part of vitamins and hormones, perform the regulatory function, and maintain homeostasis in the body [1]. The following groups are distinguished among macro-and micro-elements: essential (Ca, K, P, Cl, Na, Mg, Zn, Cu, Cr, Fe, I, Mn, *etc*.), conventionally essential (Ag, Al, Au, B, Li, Si, etc.), conventionally toxic (Al, Bi, Ti, Rb, Sr, Be, *etc*.) and toxic elements standardized by the State Pharmacopoeia of the Russian Federation (Pb, As, Hg, Cd) [2, 3].

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Lack of a certain element in the body may cause various diseases. For example, iron is part of various proteins varied by their function, including enzymes. It takes part in the transport of electrons, oxygen, ensures the course of oxidation-reduction reactions and activation of peroxidation. Under consumption results in hypochromic anemia, myoglobin deficient atony of skeletal muscles, undue fatigability, myocardiopathy, and atrophic gastritis [4, 5].

Manganese participates in the formation of osteal and connective tissue is part of the enzymes participating in the metabolism of amino acids, carbohydrates, and catecholamine is necessary for the synthesis of cholesterol and nucleo-tides. Deficiency of manganese in the body may lead to reproductive system diseases, diabetes, arthronosos, dysimmunity, allergic responses, and arrested development in children [6, 7].

Zinc is part of over 300 enzymes, participates in the processes of synthesis and degradation of carbohydrates, lipos, nucleic acids, and in the regulation of expression of several genes. Under consumption leads to anemia, secondary immunodeficiency, hepatic cirrhosis, sexual dys-function, presence of congenital fetus anomaly [8, 9].

There exist the norms of daily demand and toxicity threshold for essential macro-and microelements for the human as established by the Federal Service for a Supervision of Consumer Rights Protection and Human Welfare (Rospotrebnadzor), which are represented in **Table 1**.

TABLE 1: NORMS OF DAILY DEMAND IN MACRO AND MICROELEMENTS FOR THE HUMAN[2]

Element	Dailydem and mg	Toxicity Threshold, mg	Blotting Capacity, %
Calcium (Ca)	500-1200	2500	30
Phosphorus (P)	550-1400	-	80
Magnesium (Mg)	200-500	-	30
Potassium (K)	1000-4000	-	100
Sodium (Na)	1300-1600	-	100
Chlorides (Cl)	2000-2500	-	
Iron (Fe)	8-10 (for men) 15-20 (for women)	-	10
Zinc (Zn)	9.5-15	25	50
Iodine (I)	0.13-0.2	0.6	100
Copper (Cu)	0.9-3.0	5	50
Manganese (Mn)	2-5	5	10
Selenium (Se)	0.03-0.08	0.3	50
Chromium (Cr)	0.03-0.1	-	25
Molybdenum (Mo)	0.05-0.1	0.6	80
Fluorine (F)	1.5-4.0	10	100

The following maximum allowable norms of contents (mg/kg) for heavy metals and arsenic in herbal raw material are established: for lead (Pb) – 6.0; arsenic (As) – 0.5; mercury (Hg) – 0.1; cadmium (Cd) – 1.03.

In Tibetan orthodox medicine, the *G. macrophylla* herb is part of prescriptions to cure the diseases of the respiratory tract, gastrointestinal tract, hepatitis, neurasthenia, influenza, as well as hemostatic teas. In the Chinese orthodox medicine, this herbal raw material is used as a bile-expelling, analgetic, vulnerary, anti-inflammatory, hepato-protective agent for treatment of rheumatism and influenza. In Mongolia, it is used on the fever of epidemic nature as an antifebrile and analgetic agent, in the Russian Federation on freezing injuries and burns, as a blood stopper, vulnerary, immunomodulating agent, and as a digestion amendment agent [10-12].

The *G. macrophylla* herb containing flavonoids, iridoids, xanthones, tanning substances, vitamins, organic acids, and other substances are actively used in the orthodox medicine of China, Mongolia, Tibet for a wide spectrum of diseases [12-14]. Data on macro and microelements in the raw material is missing. Previously, a study was conducted on the accumulation of macro and microelements in the roots and flowers of *G. macrophylla*, collected in different phases of vegetation of the plant [15]. However, the study of the mineral composition of the herb of *G. macrophylla* was not carried out.
In addition, the content of toxic elements (Pb, As, Hg, Cd) was not determined in this morphological group of raw materials. The purpose of this paper is to determine the elemental composition of *G. macrophylla* herb as a possible source of macro-, microelements, and the content of toxic elements.

MATERIALS AND METHODS: The target of the research was samples of *G. macrophylla* herb harvested and procured in the Republic of Tyva in August 2016, in the blooming period. The study of the contents of macro-and microelements of the target object was carried out with the atomic absorption spectrophotometry technique. Deter-mination of micro-and macroelements was carried out on the atomic absorption spectrophotometer AAS KVANT-Z (Russia) equipped with dedicated lamps having a hollow cathode as the radiation source, at a specific wavelength for each element. The Quant Zeeman for Windows software was used to process data. For the purpose of analysis, an assay of the *G. macrophylla* herb was comminuted to the size of particles that penetrated through a sieve with holes of 1 mm in diameter. About 1.0 g (precisely weighed quantity) of comminuted grass was placed into a porcelain crucible which was then moved to an electric hot plate. It was dried up by gradually increasing the heat and exposed on the plate till the herb started coaling. The crucibles with dried assays were placed into a cold electric furnace. By gradually raising the temperature to 450 °C, mineralization was affected till gray ash has been formed. After liming, the crucibles were cooled to the room temperature, the ash was wetted with water and nitric acid concentrated in (1:1) ratio, evaporated to dryness on the electric hot plate with weak heating, and again the assays were placed into the electric furnace, thus gradually bringing the temperature to

300 °C and и exposing within 30 min. The mineralization was regarded as complete if the ash turned white or slightly stained color without coaled particles.

If any coaled particles remained, the ash was repeatedly processed with water and nitric acid concentrated in (1:1) ratio and then subjected to extra ashing. The produced ash was dissolved while heating in water, and nitric acid concentrated in (1:1) ratio in the amount of 5 ml of acid per weighed quantity. The solution was evaporated to dryness.

The dry residue was dissolved in 20 ml of 1% nitric or hydrochloric acid. On incomplete ash dissolution, the produced nitric acid solution with its residue was evaporated to dryness, and then it was dissolved in 10 ml of hydrochloric acid of 6 M solution and boiled out to wet salts.

The produced salts were dissolved in 20 ml of 1% hydrochloric acid. The solution was filtered through a filter rinsed with a solvent into a 25 ml volumetric flask; the residue on the filter was washed, bringing to the mark using the same solvent and was stirred.

TABLE 2: PARAMETERS FOR DETERMINATION OF MACRO AND MICROELEMENTS WITH THE ATOMIC ABSORPTION SPECTROPHOTOMETRY TECHNIQUE[16]

Element	Wavelength, nm	Determination Range, mkg/ml
Potassium (K)	404.4	2-8
Iron (Fe)	372.0	10-30
Manganese (Mn)	279.5	1000-4000
Copper (Cu)	324.8	20-60
Molybdenum (Mo)	313.3	2-12
Zinc (Zn)	213.9 and 307.6	20-80
Selenium (Se)	196.0	2-20
Chromium (Cr)	357.9	20-60
Boron (B)	249.8	10-30
Silver (Ag)	328.1	0.5-5.0
Aluminum (Al)	309.3	10-100
Bismuth (Bi)	223.1	5-50
Cadmium (Cd)	228.8	0.5-2.0
Mercury (Hg)	253.7	10-30
Lead (Pb)	283.3	10-30
Arsenic (As)	193.7	10-30

In parallel, 2 references (idle) tests were prepared to determine the purity of chemicals and the glassware by adding all the chemicals into the crucible and precisely reproducing all the conditions (quantity of chemicals, temperature, heating time), in which the assay mineralization was effected, but without the weighed quantity of assay [16]. In parallel, the signal of solutions for standard samples of respective elements of a known concentration was measured **Table 2**.

The content (X) of an element in the raw materials in mkg/g was calculated with the formula:

$$X = (Cn - Cx)\ V.\ A / a$$

Where: Cn- Concentration of element in the solution under study calculated with the software program, with the concentration of the standard solution, mkg/ml, taken into account; Cx - concentration of an element in the solution of an "idle" assay calculated using the software program, with the concentration of the standard solution, mkg/ml, taken into account; a - weighed quantity of raw material, g; V - the volume of the solution of assay produced after sample processing, ml; A - dilution factor.

RESULTS: The results of the determination of the mineral composition of *G. macrophylla* herb are represented in **Table 3**.

TABLE 3: CONTENTS OF MINERAL SUBSTANCES IN *G. MACROPHYLLA* HERB

Element	Contents, mkg/g
Macroelements	
Potassium (K)[a]	9650.0±1930.0
Microelements	
Iron (Fe)[a]	223.04±44.61
Manganese (Mn)[a]	81.0±16.0
Copper (Cu)[a]	8.49±1.70
Molybdenum (Mo)[a]	0.53±0.11
Zinc (Zn)[a]	27.8±5.6
Selenium (Se)[a]	0.81±0.16
Chromium (Cr)[a]	1.84±0.37
Boron (B)[b]	759.0±152.0
Silver (Ag)[b]	0.52±0.10
Aluminum (Al)[c]	44.54±8.91
Bismuth (Bi)[c]	0.77±0.15
Cadmium (Cd)[d]	0.024±0.005
Mercury (Hg)[d]	0.025±0.005
Lead (Pb)[d]	0.21±0.04
Arsenic (As)[d]	0.087±0.017

[a] - essential elements; [b] - conventionally essential elements; [c] - conventionally toxic elements; [d]- standardized toxic elements of Russia State Pharmacopoeia

It was shown that *G. macrophylla* herb contains potassium (K). As was established, the following essential microelements are present in *G. macrophylla* herb: Fe, Cu, Se, Mn, Mo, (Zn), and Cr, whose concentrations decrease in the following order: Fe>Mn>Zn>Cu>Cr>Se>Mo. Detected was also (B) and silver (Ag) which are related to conventionally essential microelements. The obtained results are indicative of the fact that the contents of cadmium (Cd), mercury (Hg), lead (Pb) and arsenic (As) in *G. macrophylla* herb does not exceed the allowable levels of values.

CONCLUSION: A research into the determination of the elemental composition of *G. macrophylla* herb was conducted. As was shown, Fe, Mn, Zn, Cu, Cr, Se, Mo, as well as B and Ag, are present in *G. macrophylla* herb. This herbal raw material can be a source of the following essential elements: Cr, Mn, Fe, Se. The maximum admissible contents of heavy metals and arsenic in the specimen under study do not exceed the limits recommended for the quality assessment of herbal raw materials.

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