

DETERMINATION OF THE ELEMENTAL AND AMINO ACID COMPOSITION OF THE DIURETIC DRUG “EKUSTIM”

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ABSTRACT

The purpose of these studies is to determine the elemental and amino acid composition of the diuretic drug “Ekustim” dry extract obtained from a mixture of 8 medicinal plants: false amber grass, woolly willow grass, horsetail grass, creeping anchor grass, yarrow flowers, cucumber seeds, corn stigmas and licorice roots. Quantitative determination of macro - and microelements was carried out by inductively coupled plasma mass spectrometry (ICP-MS), and determination of amino acid composition by high-performance liquid chromatography (HPLC).

The data obtained indicate that of the detected trace elements, K, Mg, Ca, Fe, Na, P and AI are contained in the largest amount, which is essential when creating a diuretic drug, and the concentrations of heavy metals and arsenic in the analyzed preparation did not exceed the regulated limit according to the requirements of WHO and GF XIV.

KEYWORDS: *Dry Extract, Biologically Active Substances, Amino Acid Composition, Elemental Composition, HPLC, ICP-MS, Macro- And Microelements.*

INTRODUCTION

Currently, herbal medicines occupy an important place in medical practice, since they have a wide range of biological effects, which allows them to be used for the prevention and treatment of many diseases. The use of medicinal plants, especially in collections, simultaneously with treatment gives the body additionally various natural substances (vitamins, chemical elements, etc.). Medicinal plants can be used prophylactically for a long time without fear of any complications, which cannot be said about synthetic drugs.

As can be seen from observations, the diuretic properties of plants eliminate stagnation of urine, enhance urination without irritation of the renal epithelium. In addition, it was found that at the same time the work of the gastrointestinal tract and sweat glands improves, the metabolism, both mineral and acid-base, is normalized, salts and small concretions from the kidneys and bladder are removed.

Medicinal plants of diuretic action have various biologically active substances, affect various systems and by various mechanisms. To ensure a more complete and rapid therapeutic effect, it

is advisable to use several herbal components at the same time. This problem is successfully solved with the help of such dosage forms as herbal preparations.

The review considers aspects of active phytopreparations, their mechanisms and concomitant pharmacological properties of medicinal plants and preparations based on them. Along with the positive effects of herbal remedies, there are some residual effects on kidney function of some phyto preparations [1].

Today, diuretics are mostly synthetic and have a number of side effects. Therefore, it is extremely important to develop drugs with a diuretic effect from local medicinal plants. Amino acids and chemical elements play an important role for all physiological processes occurring in the human body [2-3]. The constancy of their composition is one of the most important and mandatory conditions for them [4]. In this regard, it is of interest, used in folk medicine for a wide range of diseases [5-6] of normal functioning and development of the body. Amino acids and individual chemical elements not only have a certain pharmacological effect themselves, but can also show synergism with respect to a number of substances, therefore, combined drugs with multifunctional properties can be obtained from plants. A deviation in the composition of amino acids and elements in the body leads to a deterioration in the state of health [7]. One of the possibilities of timely correction of violations of elemental and amino acid homeostasis in the human body is the use of medicinal plants.

Currently, numerous medicinal collections are known that have already found application in official medicine, such as: Brusniver, Bekvorin, Herbafol, diuretic collection No. 2, urological or diuretic collection, tutukon (Great Britain), phytolysin, canephron (CanephronumN) - a combined drug.

Taking into account the rich resources of medicinal plants growing in the Republic of Uzbekistan and have long been used in folk medicine as diuretics, the development and introduction into medical practice of new effective import-substituting diuretic drugs based on raw materials of local medicinal plants seems very relevant.

On the basis of the conducted research, a combined diuretic drug was created in UzKFITI, consisting of a mixture of dry extracts of the following 8 medicinal plants: false amber grass, woolly willow grass, horsetail grass, creeping anchor grass, yarrow grass, cucumber seeds, columns with corn stigmas and licorice roots. This composition was given the conditional name "Ekustim". The drug "Ekustim" is protected by the patent of the Republic of Uzbekistan IAP [8-16].

The purpose of this work is to study the amino acid and mineral composition of the drug "Ekustim".

The Experimental Part

To obtain a dry extract of the drug "Ekustim", vegetable raw materials were crushed and sieved through a sieve for leaves and herbs up to a size of 7 mm, for tubers, roots - up to a size of 5 mm, for fruits up to a size of 0.5 mm.

Dry extract of the drug "Ekustim" was obtained by triple extraction of a mixture of crushed raw materials with hot water ($t = 70-80\text{ }^{\circ}\text{C}$) in a ratio of 1:30 (first extraction), 1:15 (second extraction), 1:7 (third extraction). The duration of extraction is 2.5 hours. The obtained aqueous extracts were combined and concentrated by distilling water at a temperature of $70\text{ }^{\circ}\text{C}$ under

vacuum. The resulting thick extract was dried in a vacuum drying cabinet at a temperature of 60 °C to a constant weight.

In order to study the chemical composition, a study was conducted to study the elemental composition of the dry extract “Ekustim”. The study was performed on an inductively coupled plasma mass spectrometer (ICP-MS). In the analysis, an argon inductively coupled plasma was used as an ion source.

To determine the elemental composition, a quadrupole mass spectrometer with inductively coupled plasma manufactured by Agilent Technologies 7700 was used. A system for decomposing samples consisting of 36 or 54 cells of the type Hot Block (Environmental Express) or Digi Black (Lab Tech). Electric cookers with a closed spiral and adjustable power according to GOST 14919-83 or other heating means providing a heating temperature of at least 200 °C. Teflon cups with lids with a capacity of 40 cm³. Polyethylene bags with a capacity of 22 cm³. Polyethylene tubes with a capacity of 15 cm³ and 50 cm³. Single-channel mechanical dispensers for 100 µL; from 200 µL to 1000 µL; from 1 mL to 5 mL manufactured by “FinBio” Finland. Standard samples of the composition (CO) corresponding to UZDST 8.004:2004 with certified component contents established with a certification error insignificant in comparison with the error of the methodology.

Nitric acid manufactured by “Merck”, Germany, cat. No. 1.00452.2500 (Nitric Acid 65% GR, ISO); Hydrochloric acid manufactured by Merck, Germany, cat. No. 1.00317.1000 (Hydrochloric Acid fuming 37 %); Chloric acid manufactured by “Merck”, Germany, cat. No. 1.00518.1000 (Perchloric Acid GR for analysis); hydrofluoric acid manufactured by “Merck”, Germany, cat. No. 1.00338.500 (Hydrofluoric Acid 40%, GR for analysis); Paper filters, disinfected “Blue ribbon” with a diameter of 9 cm; High purity argon gas according to GOST 10157-79.

Mass spectrometric determination of the content of elements in the samples is carried out with the following parameters of the mass spectrometer: Generator output power (W) 1500; Type of sprayer angle. Plasma-forming flow rate Ar (L/min) 13; Auxiliary flow rate Ar (L/min) 0.7; Flow rate Ar in the atomizer (L/min) 0.5; Mass registration area (AU) from 5 to 242; Number of scans 100. Permissible relative standard deviations of measurement results calculated with a confidence probability of 0.95.

Isolation of free amino acids. Precipitation of proteins and peptides of aqueous extract in centrifuge cups. To do this, 1 ml (exact volume) of 20% trichloroacetic acid was added to 1 ml of the test sample. After 10 min, the precipitate was separated by centrifugation at 8000 rpm for 15 min. After separating 0.1 ml of the infusion fluid, it was dried lyophilically.

Analysis of HPLC of FTC-derived amino acids. Synthesis of FTC (phenylthiocarbomoyl) derivatives of free amino acids was carried out by the method of Steven A., Cohen Daviel.

Identification of FTC amino acids is carried out on an Agilent Technologies 1200 chromatograph on a column 75x4.6 mm Discovery HS C18. Solution A: 0,14M CH₃COONa + 0,05 % TЭA pH 6,4, B:CH₃CN. Flow rate 1.2 ml/min, absorption of 269 nm. Gradient % Evergreen / min: 1-6 % / 0-2. 5 min; 6-30 % / 2.51-40 min; 30-60 %/40.1-45 min; 60-60 %/45.1-50 min; 60-0 % /50.1-55 min.

Discussion of the results

Table 1 shows the data of the elemental analysis of the dry extract “Ekustim”.

The analysis showed that the identified elements by the degree of reduction of their quantitative content can be represented as the following series: Ca = K = Na = Mg = Fe > Al = P > S = Mn > Se > Zn = Cu = Cr = Ni > B > Co = Mo = Pb = Rb = Ga = As = Sn = V > Ag = Cd = Li = Be = Au The table shows that the composition of the dry extract “Ekustim” contains macronutrients Na, K, Mg, Ca, Fe, the amount of which is up to 50 mg / kg, which is important to the human body. The analyses showed the presence of 20 mg/kg of phosphorus in the extract, a high content of such important elements as calcium, potassium, sodium, magnesium, iron, phosphorus, etc., which is a direct proof of the high pharmacological activity of the analyzed drug.

As you know, potassium and magnesium together with other elements such as calcium, sodium, phosphorus, fluorine, sulfur, oxygen, make up more than 90% of the ionic composition of the human body. The lack of potassium and magnesium leads to disruption of the cardiovascular and central nervous systems [17, 18, 19]. The AI content reaches up to 20 micrograms/kg. The substance also contains trace elements: the presence of Mn (10 mcg / kg) enhances water, gas and mineral metabolism, the presence of Se (3 mcg / kg) increases the immune properties of the body and protects vitamins and lipids of biological membranes from destruction. Copper (1 mcg/kg) participates in metabolic processes and chromium (1 mcg/kg) helps glucose assimilation. At the same time, the substance contains heavy metals in the amount of up to 0.1 micrograms / kg in acceptable doses.

TABLE 1. THE ELEMENTAL COMPOSITION OF THE DRY EXTRACT “EKUSTIM”

| № | Element | The value of indicators, kg/kg | № | Element | The value of indicators, mcg/ kg |
|-----|---------|--------------------------------|----|---------|----------------------------------|
| 1. | Na | 50 | 14 | Zn | 1,0 |
| 2. | K | 50 | 15 | B | 0,2 |
| 3. | Mg | 50 | 16 | Mo | 0,1 |
| 4 | Ca | 50 | 17 | Sn | 0,1 |
| 5. | Fe | 50 | 18 | Rb | 0,1 |
| 6. | P | 20 | 19 | Sr | 0,1 |
| 7. | Al | 20 | 20 | Y | 0,1 |
| 8. | Mn | 10 | 21 | Pb | 0,1 |
| 9. | S | 10 | 22 | Sb | 0,1 |
| 10. | Se | 3,0 | 23 | Be | 0,05 |
| 11. | Cr | 1.0 | 24 | Li | 0,05 |
| 12. | Cu | 1.0 | 25 | Au | 0,05 |
| 13. | Ni | 1.0 | 26 | Ag | 0,05 |

Chromatographic analysis of amino acids (Table 2) shows that the drug “Ekustim” contains essential amino acids, such as valine, threonine, isoleucine, leucine, phenylalanine, histidine, lysine and argenine, which determines the usefulness of the protein and its nutritional significance, as well as medicinal properties in the medical aspect. The record holder in the composition of the dry extract “Ekustim” is tryptophan 18.74% (5,907,104 mg/g). Since the body does not produce tryptophan, the presence of this amino acid is necessary for the supply in the body.

TABLE 2. AMINO ACID COMPOSITION OF DRY EXTRACT “EKUSTIM”

| Name of amino acids | Extract, mg/g | Amino acid content, % |
|---------------------|-----------------|-----------------------|
| Aspartic acid | 1,163366 | 3,69 |
| Tryptophan | 5,907104 | 18,74 |
| Glutamic acid | 0,669704 | 2,12 |
| Serin | 0,477143 | 1,51 |
| Glycine | 1,252706 | 3,97 |
| Asparagine | 1,266225 | 4.01 |
| Glutamine | 0,313966 | 0,99 |
| Cysteine | 0,671733 | 2,13 |
| Threonine | 0,594292 | 1,88 |
| Arginine | 0,582381 | 1,84 |
| Alanin | 4,098862 | 13,00 |
| Proline | 4,875216 | 15,47 |
| Tyrosine | 3,831587 | 12,15 |
| Valin | 0,888256 | 2,81 |
| Methionine | 1,901173 | 6,03 |
| Isoleucine | 0,729351 | 2,31 |
| Leucine | 0,757149 | 2,40 |
| Histidine | 0,442055 | 1,40 |
| Phenylalanine | 0,419260 | 1,33 |
| Lysine HCl | 0,670048 | 2,12 |
| Total: | 31,51158 | 100,00 |

It is interesting to note that 15.47% (4.875216 mg/g) of proline is observed in the composition of the drug, which is very important for improving the immunity of the body, for improving the general condition of the kidneys and liver. In addition, proline promotes the restoration of damaged tissues and promotes wound healing. The preparation contains alanine 13.00% (4.098863 mg/g), which is necessary to improve the overall condition of the body.

The presence of tyrosine in the substance in an amount of 12.15% (3.831587 mg/g) helps the body to adapt and improves the condition of the blood. The composition also contains 6.03% methionine (1.901173 mg/g), which plays an important role in liver infiltration. The remaining amino acids in the composition of the drug are found to be up to 2%, which also play an important role for the body.

CONCLUSIONS

Thus, based on the conducted studies, it was found that 20 amino acids and 12 elements were found in the composition of the drug “Ekustim” and it can be considered as a means of correcting the imbalance of amino acids and elements in the body.

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