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BIOLOGICALLY ACTIVE SUBSTANCES OF COMPOSITIONS BASED ON PLANTS OF THE GENUS HAPLOPHYLLUM

Abstract. The article presents the results of research of biologically active substances of compositions based on the plant of the genus *Haplophyllum* (*Tuberculatum*), which grows in the Bakanas district of Almaty region, Bidens (*Frondosa*), collected in the Enbekshikazakh district and Polygonum (*Aviculare*) from the territory of Tashtykara district of Almaty region. The mineral composition was studied, and the article also provides data on vitamin, amino and fatty acid compositions.

Medicinal raw materials contain many compounds that are potentially harmful to the human body, these can be both mineral components, for example, salts of heavy metals, and various organic substances, both being natural metabolites of the plant and trapped in raw materials from the environment (pesticides used in agriculture or industrial emissions).

The study of mineral composition was carried out using atomic absorption analysis on the basis of the center of physico-chemical methods of research and analysis (cfhma). As a result, information was obtained about the micro- and macronutrient composition of compositions based on a plant of the genus *Haplophyllum* (*Tuberculatum*).

The content of fatty acids was determined by the GVC method and using the following chromatography conditions: carrier gas-helium, flame ionization detector, carrier gas speed 30ml / min, detector temperature 188°C, furnace temperature 230°C, analysis time 1 hour, steel column 0.4×3mm filled with polyethylene glycoladipinate (20%) on cellulite-545.

Studies of amino acids were carried out on the amino acid analyzer "Carlo Erba", using the gzhx method, chromatography conditions: carrier gas-helium, flame ionization detector 300°C, evaporator temperature 250°C, on the WAW chromosome.

The content of vitamins a (retinol) and (tocopherol) was determined by the fluorimetric method on the spectrofluorimeter device. Vitamin C in biological samples was determined by titrimetric method.

Keywords: Haplophyllum (Tuberculatum), Bidens (Frondosa),Polygonum (aviculare), mineral composition, amino acids, fatty acids.

Introduction. Minerals are of great importance for the normal existence of organisms. They are classified as macro-and microelements. Macronutrients include potassium, sodium, calcium, magnesium, phosphorus, chlorine, and sulfur. Comparatively, they come in large quantities.

Trace elements are a group of chemical elements that are contained in the human body and animals in very small amounts, within the range of 10-3-10-12 mg %, these include Nickel, cadmium, lead, chromium, mercury and other d - elements [1].

In ecologically unfavorable areas, a very large number of heavy metals, such as lead, Nickel, chromium, mercury, and any changes in the optimal ratios of trace elements in their composition can lead to unforeseen consequences [2].

In an acidic environment, the movement of molybdenum decreases, but the movement of copper, manganese, zinc, and cobalt increases. Trace elements such as chalk, fluorine, and iodine also move in acidic and alkaline environments. Some trace elements, for example, form compounds that are soluble with organic matter, while others (iodine and copper) are fixed and become inaccessible to plants. The

lack or excess of trace elements in the soil leads to their deficiency or excess in the plant and animal body. At the same time, changes in the nature of accumulation (deposition), weakening or strengthening the synthesis of biologically active substances, repeated violation of the processes of interline exchange, creating a new adaptation or violations that lead to endemic diseases of humans and animals develop [3].

Thus, the mineral composition of plants is directly affected by natural and anthropogenic factors. All this should be taken into account when preparing vegetable raw materials.

Currently, there are 14 trace elements necessary for life: iron, copper, manganese, zinc, cobalt, iodine, fluorine, molybdenum, vanadium, Nickel, strontium, silicon and selenium. They increase the activity of enzymes, catalyze biochemical procedures, and promote the synthesis of carbohydrates, proteins, and vitamins. It also participates in metabolism. Trace elements are part of plant preparations and influence their activity[4].

Most studies of *Haplophyllum tuberculatum* evaluated medicinal and phytochemical properties, analysis of some of which produced two alkaloids called alkaloids, lignans, glycosides and flavonoids, etc. in the phytochemical study of *Haplophyllum tuberculatum*, alkaloids such as acutifolium, halilofitin were obtained [5]. The chemical composition of *Polygonum aviculare* is rich and varied. It is a natural storehouse of vegetable protein (17 %), biologically active substances (44%), vegetable fiber (27%), ash compounds (8.9%), simple and complex sugars (2.5%), resin, wax, and tannins. Useful trace elements such as calcium, phosphorus, zinc, and silicon were also found in it [6]. *Bidens frondosa* contains organic acids, essential oil, tannins, polysaccharides, coumarins (umbelliferon, skoletin), triterpenoids, vitamin C (up to 0.9%), carotenoids (0.05 %), carotenes, a large amount of vitamin C (up to 1000 mg%), manganese salts, polyacetylenes, and aromatic acids containing derivatives and thiophenes, flavonoids - (glycosides luteolin, Butin, sulfuretin, Auron) [7-12].

Fatty acids are the basis of metabolic processes in the body, an integral part of the biological synthesis of the body's processes along with the components of lipids. Several fatty acids that have essential properties have vitamin-like effectiveness: olein, arachidone, linol, linolene. Essential fatty acids have cardioprotective properties, improving blood circulation and the cardiovascular system.

Amino acids are organic compounds whose molecules simultaneously contain carboxyl and amine groups. These are substances of primary synthesis, they are present in all organs of all plants. Amino acids are divided into α, β, γ, σ, and others. amino acids, depending on the location of the amino and carboxyl groups. Of these, the most common are α, β and γ. α - l-configuration amino acids are the most important components of peptides and proteins. Also, plants may contain monobasic diamino-and dibasic monoaminoacids.

Vitamin A and E occur in pairs. They improve the condition of the skin, vision, and bones, helping to assimilate each other, increase resistance to infections, and protect the mucous membrane. Retinol is one of the vital vitamins necessary and vital for our body, which belong to the fat-soluble group. The usefulness of vitamin A for health is invaluable: it participates in redox processes, affects protein synthesis, and cell membranes. Retinol interacts well with another fat-soluble vitamin-tocopherol (vitamin E). if the body lacks vitamin E, the absorption of retinol is absorbed, so it is optimal to take these vitamins together.

Ascorbic acid is an organic compound similar to glucose, the sour taste is found in the form of a white crystalline powder. It performs the biological functions of a coenzyme and a reducing agent of certain metabolic processes, and is an antioxidant. Vitamin C strengthens the immune system and protects it from viruses and bacteria, accelerates the healing process, promotes the synthesis of several hormones, regulates the processes of the circulatory system and normalizes capillary permeability, participates in the synthesis of collagen protein. This is necessary for the growth of tissue cells, bones and cartilage of the body, regulates metabolism, improves bile secretion, and restores the external function of the pancreas and thyroid gland.

Materials and methods. *Determination of mineral composition.* The objects of the study were plants of the genus *Haplophyllum (Tuberculatum)* growing in the Bakanas district of Almaty region, Bidens (Frondosa) collected in the Enbekshikazakh district and *Polygonum (aviculare)* from the territory of Tashtykara district of Almaty region, harvesting was carried out in compliance with sanitary requirements and the requirements Of the state Pharmacopoeia of the Republic of Kazakhstan. The quantitative content of micro-and macronutrients was determined from ash residues obtained by the following method.

About 1 g of the drug or 3-5 g of crushed medicinal plant raw materials (exact weight) is placed in a pre-calcined and precisely weighted porcelain, quartz or platinum crucible, evenly distributing the substance along the bottom of the crucible.

Then the crucible is carefully heated, allowing the substance to burn or evaporate at the lowest possible temperature. The burning of the remaining coal particles must also be carried out at a lower temperature as possible; after the coal has burned almost completely, the flame is increased. In case of incomplete combustion of coal particles, the residue is cooled, moistened with water or a saturated solution of ammonium nitrate, evaporated in a water bath and the residue is calcined. If necessary, repeat this operation several times.

Calcination is carried out at low red heat (about 500°C) to a constant mass, avoiding fusing the ash and sintering with the walls of the crucible. At the end of calcination, the crucible is cooled in a desiccator and then the resulting ash is burned again at 600°C until a uniform gray color is obtained.

If the result is not achieved, the remainder is dissolved in concentrated nitric acid, after which it is heated on a tile removing the nitric acid and then in a muffle at 400°C for 30 minutes.

Finally, the precipitate is dissolved in 5 ml of HNO_3 (1:1) when heated. The resulting solution must be heated on the tile to wet salts. The result is dissolved in 10-15 ml of 1H HCl or 1H HNO_3 (the Second option is preferable) and transferred to a 25ml volumetric flask, bringing the volume to the label.

In parallel, a single experiment is carried out, which consists in preparing a solution of the same concentration from the same acid using the same utensils.

Then the finished samples were transferred to the center for physical and chemical methods of research and analysis to determine the mineral composition by atomic adsorption spectroscopy on the ASSIN device of the Karl Zeiss company.

The results obtained are presented below [13,14].

It was revealed that the amount of heavy metals does not exceed the permissible norms of their presence in medicinal raw materials.

Study of the fatty acid composition of the obtained extracts by the GLC method. The content of fatty acids was determined using the GLC method using the following chromatography conditions: carrier gas-helium; flame ionization detector; carrier gas speed 30ml / min; detector temperature 188ºC; furnace temperature 230ºC; analysis time 1H; steel column 0.4 m × 3mm filled with polyethylene glycoladipinate (20%) on cellulite-545.

Sample preparation: 10ml of methanol, 2-3 drops of acetyl chloride are added To the chloroform extracts of the test samples, and then methylation is performed at 60-70^{0}C in a special system for 30 minutes. Methanol is removed using a rotary evaporator, and samples are extracted with 5ml of hexane and analyzed in a Carlo-Erbo-4200 gas chromatograph[15].

Study of the amino acid composition of the obtained extracts by the GLC method. 1 g of the analyte is hydrolyzed in 5ml of 6h hydrochloric acid at 105ºC for 24 hours, in ampoules sealed under a jet of argon. The resulting hydrolysate is evaporated three times to dry on a rotary evaporator at a temperature of 40-50ºC. The resulting precipitate is dissolved in 5ml of sulfosalicylic acid. After centrifugation for 5 minutes, the supernatant is passed through a column with an ion-exchange resin Daux 50, with a speed of 1 drop per second. After that, the resin is washed to a neutral pH.

For elution of amino acids from the column, 3ml of 6h NH_4OH co solution is passed through it at a rate of 2 drops per second. The eluate is collected in a round-bottomed flask with distilled water, which is used to wash the column to a neutral pH. Then the contents of the flask are evaporated dry on a rotary evaporator at a pressure of 1 atm. and a temperature of 40-50ºC.

After adding to this flask, 1 drop of freshly prepared 1.5 % $SnCl_2$ solution, 1 drop of 2.2-dimethoxypropane and 1-2ml of hydrochloric acid-saturated propanol, ee is heated to 110 ºC, maintaining this temperature, for 20 minutes, and then the contents are again evaporated from the flask on a rotary evaporator.

In the next step, 1 ml of freshly prepared acelizing reagent (1 volume of acetic anhydride, 2 volumes of triethylamine, 5 volumes of acetone) is injected into the flask and heated at a temperature of 60 ^{0}C for 1.5-2min. Then the sample is again evaporated on a rotary evaporator to dry and 2ml of ethyl acetate and 1ml of saturated NaCl solution is added to the flask. The contents of the flask are thoroughly mixed and, as 2 layers of liquids are clearly formed, the upper one (etiacetate) is taken for gas chromatographic analysis, which was performed on the gas-liquid chromatograph "Carlo-Erba-4200" (Italy-USA) [16].

Study of vitamins A, E, and C. 0.2 ml of the sample, 1ml of alcohol and 1ml of distilled water are placed in centrifuge tubes, tightly closed with lids and mixed with careful shaking. Add 5ml of hexane and shake again. The contents are centrifuged for 10 minutes at 1500rpm. the Separated hexane layer (3 ml) is used for measurements at wavelengths of 292-310 nm (for tocopherol) and 335-430 nm (for retinol), respectively [17-22].

The content of vitamin C in biological samples was determined by titrimetric method. A sample of at least 0.3 ml is taken in a centrifuge tube, the walls of which are covered with sodium citrate powder. After centrifuging the sample for 30 minutes at 3000 rpm, it is transferred to another test tube and an equal amount of bidistilled water and double the amount of freshly prepared 5% metaphosphoric acid solution is added. The protein precipitate is stirred with a stick and centrifuged for 10 minutes at 3000 rpm. The nadosadochnuyu liquid in an amount (0.1-0.5 ml) is introduced into porcelain titration cuvettes (2 parallel samples) and titrated 0.001 n-0.0005 n with a solution of the sodium salt of 2.6 dichlorophenolindophenol from a special micropipette with a capacity of 0.1 ml.

Results and discussion. To create complexes, first of all, the composite correspondence of plant raw materials to each other was studied and the ratio was chosen. Depending on which ones show up, they were obtained in three types. The percentage of components in the complexes is theoretically justified by taking into account the contribution of each component to the last therapeutic effect and evaluating the activity of plant components. This takes into account the phenomenon of interaction of plant components (interference), since some types of plant raw materials show the greatest activity in an individual or a mixture, and when it changes, there is a decrease in the influence of the participating components. The plant complex was obtained in different ratios. The results are presented in table 1.

Table 1 - plant ratio

№	complex
1	1:1:3
2	2:1:1
3	2:3:1

Based on the data from table 1, the mineral composition of plant complexes was studied in 3 different ratios.

Data on the mineral composition were obtained by atomic absorption analysis conducted at the material and technical base of the center for physical and chemical analysis methods. The results are presented in tables 2 and 3.

Table 2 - Quantitative content of trace elements

Element	Zn	Cu	Pb	Cd	Fe	Ni	Mn
1:1:3							
Content in the sample, %	0.0004892	0.0002358	0.00009821	0.00001683	0.01304	0.00006447	0.001113
2:1:1							
Content in the sample, %	0.0006301	0.0002323	0.00006677	0.00001795	0.01558	0.00006005	0.001059
2:3:1							
Content in the sample, %	0.0007055	0.0004068	0.0001103	0.00002102	0.01162	0.00008746	0.001415

Table 3 - Quantitative content of macronutrients

Element	K	Na	Ca	Mg
1:1:3				
Content in the sample, %	0.6040	0.01851	0.4576	0.07897
2:1:1				
Content in the sample, %	0.5823	0.02051	0.3448	0.08191
2:3:1				
Content in the sample, %	0.6166	0.02578	0.3786	0.06799

Comparing the data given in table 2, we can conclude that the quantitative content of iron dominates all samples of plant complexes in 3 different ratios. Iron is a part of herbal preparations and affects their activity. It is also known that iron increases the activity of enzymes and catalyzes biochemical processes. Promotes the synthesis of carbohydrates, proteins and vitamins.

Among the macro elements presented in table 3 and figure 2, the largest number of elements are K, Ca. They are part of the cell nucleus. Calcium plays an important role in the growth and action of cells. It has a significant impact on the exchange process and contributes to the full digestion of food substances. By strengthening the body's protective function, it increases resistance to external adverse conditions, especially infections.

The content of fatty acids was determined by the GZHC method. The results are presented in table 4.

Table 4 - Quantitative analysis of fatty acids

№	Fatty acids		%
1	$C_{14:0}$	Miristin ($C_{14}H_{28}O_2$)	0,8
2	$C_{15:0}$	Pentadecan ($C_{16}H_{30}O_2$)	1,2
3	$C_{16:0}$	Palmitin ($C_{16}H_{32}O_2$)	4,6
4	$C_{16:1}$	Palmitalein ($C_{16}H_{30}O_2$)	0,4
5	$C_{18:0}$	Stearin ($C_{18}H_{36}O_2$)	2,3
6	$C_{18:1}$	Olein ($C_{18}H_{34}O_2$)	36,5
7	$C_{18:2}$	Linol ($C_{18}H_{32}O_2$)	53,9
8	$C_{18:3}$	Linolene ($C_{18}H_{30}O_2$)	0,3

From the data obtained, it follows that 8 fatty acids were detected. In terms of quantitative content, the dominant fatty acids were oleic and linoleic acids, relatively palmitic and stearic acids. Linolenic acid, which showed the lowest index, plays a large role in the body. Fatty acid, most necessary for the functioning of cell and subcellular membranes.

Amino acids were identified from the plant complex. Studies of amino acids were carried out on the amino acid analyzer "Carlo Erba", using the gzhx method. The data is presented in table 5.

Table 5 - Quantitative analysis of the amino acid composition of aqueous extracts of compositions

	Name of amino acids		Amino acids, mg / 100 g
1	Ala	Alanine	612
2	Gly	Glycine	234
3	Val	Valine.	195
4	Leu	Leucine.	370
5	Ile	Isoleucine	334
6	Thr	Threonine	177
7	Ser	Serine	317
8	Pro	Proline	415
9	Met	Methionine	51
10	Asp	Asparate	1360
11	Cys	Cystine	22
12	O-Prp	Oxyproline	1
13	Phe	Phenylalanine	264
14	Glu	Glutamate	2546
15	Orn	Ornithine	1
16	Tyr	Tyrosine	288
17	His	Arginine	370
18	Arg	Lysine	318
19	Lys	Histidine	189
20	Trp	Tryptophan	72

The analysis revealed their qualitative identity and insignificant quantitative relationships, and also found that the dominant amino acids are asparate, glutamate, alanine: 1360 mg / 100g, 2546 mg/100g, 612 mg / 100g.

The content of vitamins a (retinol) and (tocopherol) was determined by the fluorimetric method on the spectrofluorimeter device . Vitamin C in biological samples was determined by titrimetric method. The data is presented in table 6.

Table 6 - Quantitative content of vitamins A, E and C

Vitamins	mg/100g
A	0,09
E	2,8
C	22

Comparing the data shown in table 6 and figure 2, we can conclude that the quantitative content of vitamin C dominates.

Conclusion

1. For the first time, biologically active substances were studied, including mineral composition, vitamins, amino and fatty acids of compositions based on a plant of the genus Haplophyllum (Tuberculatum) growing in the Bakanas district of Almaty region, Bidens (Frondosa) collected in the Enbekshikazakh district and Polygonum (aviculare) from the territory of the Tashtykara district of Almaty region.

2. It was found that the level of heavy metal content in the studied samples does not exceed the maximum permissible norm.

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HAPLOPHYLLUM ТЕКТЕС ӨСІМДІКТЕР НЕГІЗІНДЕГІ КОМПОЗИЦИЯЛАРДЫҢ БИОЛОГИЯЛЫҚ БЕЛСЕНДІ ЗАТТАРЫ

Аннотация. Мақалада Алматы облысының Бақанас ауданында өсетін Haplophyllum (Tuberculatum) туысының өсімдігі және Еңбекшіқазақ ауданында жиналған Bidens (Frondosa) және Алматы облысының Таштықара ауданының аумағынан Polygonum (aviculare) өсімдітер негізінде композициялардың биологиялық белсенді заттарын зерттеу нәтижелері келтіріледі. Минералды құрам зерттелді, мақалада витаминдік, амин және майлы қышқылдық құрам туралы мәліметтер келтірілген.

Дәрілік шикізат құрамында адам ағзасына зиян келтіретін көптеген қосылыстар бар, олар минералды компоненттер болуы мүмкін, мысалы, ауыр металдардың тұздары, сондай-ақ әр түрлі органикалық заттар, олар өсімдіктердің табиги метаболиттері болып табылады және қоршаған ортадан шикізатқа түседі (қолданылатын пестицидтер) ауыл шаруашылығында немесе өнеркәсіптік шығарындыларда).

Минералдық құрамды зерттеу физика - химиялық зерттеу және талдау әдістері орталығы (ЦФХМА) базасында атомдық - абсорбциялық талдау әдісімен жүргізілді. Нәтижесінде haplophyllum (Tuberculatum) тұқымының негізіндегі композициялардың микро - және макроэлементтік құрамы туралы мәліметтер алынды.

Май қышқылдарының құрамын ГЖХ әдісімен және хроматографиялаудың келесі шарттарын қолдану арқылы анықтадық: газ-тасығыш – гелий, жалынды-иондаушы детектор, газ тасығыштың жылдамдыгы 30мл/мин, детектордың температурасы 188°С, пештің температурасы 230°С, талдау уақыты 1с, Болат баганасы 0.4×3мм, целлит-545 полиэтиленгликольадипинатпен толтырылган (20%).

Аминқышқылдарын зерттеу "Карло Эрба" аминқышқылды талдағышта ГЖХ әдісімен жүргізілді, хроматографиялау шарттары: газ-тасығыш – гелий, 3000с жалынды-иондау детекторы, булау температурасы 250°С, WAW хромосорбасында жүргізілді.

А (ретинол) және Е (токоферол) витаминдері спектрофлуориметр аспабында флуориметрлік әдіспен анықталған. С витамині биологиялық үлгілерде титриметриялық әдіспен анықталды.

Түйін сөздер: Haplophyllum (Tuberculatum), Bidens (Frondosa), Polygonum (aviculare), минералды құрамы, амин қышқылдары, майлы қышқылдар.

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БИОЛОГИЧЕСКИ АКТИВНЫЕ ВЕЩЕСТВА КОМПОЗИЦИЙ НА ОСНОВЕ РАСТЕНИЯ РОДА HAPLOPHYLLUM

Аннотация. В статье приводятся результаты исследования биологически активных веществ композиций на основе растения рода Haplophyllum (Tuberculatum), произрастающего в Баканасском районе Алматинской области, Bidens (Frondosa), собранный в Енбекшиказахском районе и Polygonum (aviculare) из территории Таштыкараского района Алматинской области. Были исследованы минеральный состав, в статье также приведены данные по витаминному, амино- и жирнокислотному составам.

В лекарственном сырье содержится множество соединений, потенциально способных нанести вред организму человека, это могут быть как минеральные компоненты, например, соли тяжёлых металлов, так и различные органические вещества, как являющиеся естественными метаболитами растения, так и попавшие в сырьё из окружающей среды (пестициды, применяемые в сельском хозяйстве, или выбросы промышленных предприятий).

Исследования минерального состава проводились методом атомно- абсорбционного анализа на базе центра физико- химических методов исследования и анализа (ЦФХМА). В результате получены сведения о микро- и макроэлементном составе композиций на основе растения рода Haplophyllum (Tuberculatum).

Содержание жирных кислот определяли методом ГЖХ и использованием следующих условий хроматографирования: газ-носитель – гелий, пламенно-ионизационный детектор, скорость газо-носителя 30мл/мин, температура детектора 188^{0}С, температура печи 230^{0}С, время анализа 1ч, стальная колонка 0.4×3мм, заполненная полиэтиленгликольадипинатом (20%) на целлите-545.

Исследования аминокислот проводили на аминокислотном анализаторе «Карло Эрба» методом ГЖХ, условия хроматографирования: газ-носитель – гелий, пламенно-ионизационный детектор 300^{0}С, температура испарителя 250^{0}С, на хромосорбе WAW.

Содержание витаминов А (ретинол) и (токоферол) определили флуориметрическим методом на приборе спектрофлуориметр. Витамин С в биологических образцах определили титрометрическим методом.

Ключевые слова: *Haplophyllum (Tuberculatum), Bidens (Frondosa), Polygonum (aviculare),*минеральный состав, аминокислоты, жирные кислоты.

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