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INVESTIGATION OF CHEMICAL CONSTITUENTS OF ARTEMISIA SCOPAEFORMIS

Nowadays, there is a growing interest in the scientific and practical aspects of the Artemisia (family of Asteraceae) in Kazakhstan. In this work, the quantitative and qualitative analysis of phytochemical constituents of medicinal plant Artemisia scopaeformis from Kazakhstan has been made for the first time. Biological active constituents such as alkaloids (8.55 %), saponins (7.25 %), flavonoids (6.59 %), polysaccharides (1.37 %) together with moisture content (7.03 %), ash (6.1 %), and extractives (15.06 %) of plant Artemisia scopaeformis were determined. By using the method of multi-element atomic emission spectral analysis in the ash of the plant 8 macro-micro elements were found, in which the main contents were K (84.509 mg/g), Ca (27.645 mg/g), Mg (7.0076 mg/g). Additionally, the component and quantitative compositions of the aerial parts were determined for amino and fatty acids by GLC. Twenty amino and eight fatty acids were identified from A. scopaeformis. The results showed that the major contents of amino acids were glutamate (2635 mg/100g), aspartate (1302 mg/100g) and alanine (902 mg/100g), as regards fatty acids, there were linoleic acid (67.3 %) and oleic acid (19.6 %), respectively.
Keywords: *Artemisia scopeformis, bioactive constituents, macro-micro elements, amino-, fatty acids*

Introduction.

Natural products have contributed greatly to the development of modern therapeutic drugs over the years. Plants represent various natural sources of useful compounds that might serve as lead for the development of novel drugs. Drugs of herbal origin are frequently considered less toxic and induce fewer side effects than synthetic ones. Hence, pharmacological research on phytochemicals has become mandatory to establish the claimed medicinal properties of herbs. The genus Artemisia (family of Asteraceae) includes more than 500 species. There are several reports, describing Artemisia plants as dietary foods and as traditional herbal medicines for the treatment of diseases such as malaria, hepatitis, cancer, and inflammation. A literature survey on the phytochemical properties of this genus shows that it is a source of diverse bioactive compounds such as essential oils, sesquiterpenes lactones, sesquiterpenes alkaloids, diterpenes, triterpenes, alkamides, flavonoids, coumarins, caffeoylquinic acids, sterols and acetylenes [1].

Amino acids are one of the most important classes of natural compounds. The content of amino acids in plants varies depending on the age of plants, the external conditions: from nutrition, temperature, day length, moisturizing and qualitative composition of amino acids. The number of free amino acids decreases with the age of the plant. In vegetative organs of plants, free amino acids are more than in reproductive. Valine helps stimulate muscle growth and regeneration, also involved in energy production. Phenylalanine is a precursor for the neurotransmitters tyrosine, dopamine, epinephrine and norepinephrine. It plays an integral role in the structure and function of proteins and enzymes, as well as in the production of other amino acids. Lysine plays major roles in protein synthesis, hormone and enzyme production and the absorption of calcium [2].

Fatty acids are substantial components of lipids and cell membranes in the form of phospholipids. Besides their role as a source of energy, they act as main constituents of cellular membranes. In this case, as part of the membrane phospholipids, they assure the fluidity, flexibility, permeability of the membrane and also assure the passive transport through the membrane and are interconnected with other proteins in intra and intercellular way. The fatty acids could be identified and quantified by using various analytical methods, but the most widely used technique is the gas chromatography (GC). Its main advantages are selectivity, sensibility and efficiency [3].

Materials and methods.

Plant material. The root part of plant A. scopaeformis was collected in 2018 from Almaty region. The air dried aerial parts of A. scopaeformis were cut into small pieces and stored at room temperature.

Experimental part. The quantitative and qualitative contents of biologically active constituents of underground part of the plant were determined according to methods reported in the State Pharmacopeia XI edition techniques.

In the "Center of Physico-Chemical methods and analysis", Republican State Enterprise Kazakh National Al-Farabi University, MON RK using the method of multi-element atomic emission spectral analysis in the ash of A. scopaeformis was analyzed elemental constituents. To determine the mineral composition of ashes was used Shimadzu 6200 series spectrometer.

Method for the determination of amino acids. 1 g of the analyte, hydrolyzed in 5 ml of 6N hydrochloric acid at 105 ºC for 24 hours, in ampoules sealed under a stream of argon. The resulting hydrolyzate is evaporated three times to dryness on a rotary evaporator at a temperature of 40-50 ºC and a pressure of 1 atm. The resulting precipitate is dissolved in 5 ml of sulfosalicylic acid. After centrifugation for 5 minutes, the packed liquid is passed through a column of ion exchange resin at a rate of 1 drop per second. Subsequently, the resin is washed with 1-2 ml of deionized water and 2 ml of 0.5N acetic acid; following this, the resin is washed to neutral pH with deionized water. To elute the amino acids from the column, 3 ml of a 6N NH_4OH solution is passed through it at a rate of 2 drops per second. The eluate is collected in a round bottom flask together with distilled water, which is used to wash the column to a neutral pH medium. The contents of the flask are then evaporated to dryness on a rotary evaporator at a pressure of 1 atm and a temperature of 40-50 ºC. After adding a drop of freshly prepared 1.5% $SnCl_2$ solution, 1 drop of 2,2-dimethoxypropane and 1-2 ml of propanol saturated with hydrochloric acid, it is heated to 110 ºC, keeping 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 acetyl reagent (1 volume of acetic anhydride, 2 volumes of triethylamine, 5 volumes of acetone) is introduced into the flask and heated at a temperature of 60 ºC for 1.5-2 minutes. The sample is again evaporated on a rotary evaporator to dryness and 2 ml of ethyl acetate and 1 ml of a saturated NaCl solution are added to the flask. The contents of the flask are thoroughly mixed and as the two layers of liquids are clearly formed, an upper layer (ethyl acetate) is taken for gas chromatographic analysis.

To determine the amino acids composition was made erenow of the raw material used GC/MS device. GC/MS analysis: the roots of A. scopaeformis were analyzed by Gas Chromatograph coupled to Mass Spectrometer using polar mixture of 0.31% carbowax 20 m, 0.28% silar 5 CP and 0.06% lexan in chromosorb WA-W-120-140 mesh., column (400 x 3 mm). The column temperature was programmed from 110ºC (held for 20 min), at 6ºC/min from 110ºC to 180ºC, at 32ºC/min from 185ºC to 290ºC. When it reaches to 250ºC, it should stay constant till finishing analysis of all existed amino acids. The chromatogram is counted according to an external standard.

Determination of the fatty acids composition of dried plant A. scopaeformis extracted with a chloroform - methanol mixture (2:1) for 5 minutes, the extract is filtered through a paper filter and concentrated to dryness. Then, to taked extract add 10 ml of methanol and 2-3 drops of acetyl chloride and further methylation at 60-70°C in a special system for 30 minutes. The methanol is removed by rotary evaporation and the samples are extracted with 5 ml of hexane and analyzed using a gas chromatograph.

As a result, chromatograms of methyl esters of fatty acids were obtained. By comparison with reliable samples by the time of exit from the column, eight fatty acids were identified. To determine the components, the internal normalization method was used.

Results and discussion.

Minerals have great contribution to the life of plant and human body. The content of minerals in medicinal plants is determined by ash, the amount of which is defined in the range (from 3 to 25%) depending on the type of raw material. The moisture content in plant materials is one of the numerical indicators, characterizing its good quality. For most types of medicinal plant materials, the permissible limit is up to 12-15%.

Under moisture, it is customary to take the loss in mass due to hygroscopic moisture and volatile substances, which is determined in the raw material during the dryness to constant weight.

We carried out the determination of biologically active constituents, extractives contents, moisture and total ash of the studied plant Artemisia scopaeformis. Finally, the total ash content of the plant was 6.1 %, moisture - 7.03 %, alkaloids - 8.55 %, saponins - 7.25 %, flavonoids - 6.59 %, polysaccharides - 1.37 %, coumarins - 0.29 %, organic acids - 0.21 %, extractives - 15.06 %.

Table 1 – Quantitative analysis of biologically active constituents of A. scopaeformis

Component	Content, %
Moisture	7.03
Ash	6.1
Extractives	15.06
Alkaloids	8.55
Saponins	7.25
Flavonoids	6.59
Polysaccharides	1.37
Coumarins	0.29
Organic acids	0.21

In “Center of Physico-Chemical methods of analysis”, Republican State Enterprise Kazakh National Al-Farabi University, MES RK using the method of multi-element atomic emission spectral analysis in the ash of A. scopaeformis there were determined ten macro- and microelements, shown in Table 2 and major of them were K (84.509 mg/g), Ca (27.645 mg/g), Mg (7.0076 mg/g). There were recognized 14 necessary microelements for the life: iron, cuprum, manganese, zinc, cobalt, iodine, fluorine, molybdenum, vanadium, nickel, strontium, silicon and selenium. They enhance the activity of enzymes, which catalyze biochemical processes that promote the synthesis of carbohydrates, proteins and vitamins, and are also involved in metabolism [4]. Potassium is one of the most important elements necessary for the life of plants and animals. Potassium is involved in conducting electrical impulses in nerve and muscle cells. Therefore, it is very important for normal functioning of the nervous and cardiovascular systems. In addition, potassium acts as a catalyst in carbohydrate and protein metabolism, supports the acid-base balance in the body, regulates blood pressure and helps the normal functioning of the kidneys. Calcium is very important for human health. Heart, bones, muscles, nerves need this element throughout life. It regulates metabolic processes, blood clotting, hormones and therefore it is indispensable for the female body. Calcium is the main participant in the process of blood clotting. It prevents the formation of blood clots, which in turn lead to clogging of blood vessels and death. The amount of calcium also affects the work of the adrenal glands, pancreas, thyroid gland and reproductive system. The important interaction between phosphate and magnesium ions makes magnesium essential to the basic nucleic acid chemistry of all cells of all known living organisms. More than 300 enzymes require magnesium ions for their catalytic action, including all enzymes using or synthesizing ATP and those that use other nucleotides to synthesize DNA and RNA. The ATP molecule is normally found in a chelate with a magnesium ion [5].

Table 2 – Composition of macro-micro elements in the sample of A. scopaeformis

Element	Concentration in ash, mg/g	Concentration in plant, mg/g
Ca	27.645	1.567
K	84.509	4.792
Mg	7.0076	0.3973
Na	3.0341	0.1720
Fe	2.3200	0.1315
Mn	0.3171	0.0179
Zn	0.07169	0.00407
Cu	0.04006	0.00227
Ni	0.00205	0.00012

Cd	0.00448	0.00025

In the composition of amino acids mainly were glutamate (2635 mg/100g), aspartate (1302 mg/100g) and alanine (902 mg/100g). The results shown in Table 3. Glutamate is one of the most abundant of the amino acids. In addition to its role in protein structure, it plays critical roles in nutrition, metabolism and signaling. Post-translational carboxylation of glutamyl residues increases their affinity for calcium and plays a major role in hemostasis [6]. Aspartic acid increases immunity, metabolism, deactivates ammonia, promotes the removal of chemicals, including drugs, restores working capacity. Aspartate has many other biochemical roles. It is a metabolite in the urea cycle and participates in gluconeogenesis. It carries reducing equivalents in the malate-aspartate shuttle, which utilizes the ready interconversion of aspartate and oxaloacetate, which is the oxidized (dehydrogenated) derivative of malic acid. Aspartate donates one nitrogen atom in the biosynthesis of inosine, the precursor to the purine bases. In addition, aspartic acid acts as a hydrogen acceptor in a chain of ATP synthase [7]. Alanine is one of the most widely used for protein construction and is involved in the metabolism of tryptophan and vitamin pyridoxine. Alanine is an important source of energy for muscles and central nervous system, strengthens the immune system, helps in the metabolism of sugars and organic acids, and displays a cholesterol-reducing effect in animals [8].

Quantitative composition of fatty acids in A. scopaeformis mostly contained in linoleic acid (67.3 %) and oleic acid (19.6 %), showed in Table 4. Linoleic acid is an essential fatty acid in nutrition and is used in the biosynthesis of prostaglandins and cell membranes. Oleic acid is needed by the body's cells for proper membrane fluidity — making sure the cell membrane has a thick enough layer. This is important for fighting pathogens, transporting minerals and responding to hormones. Oleic acid also serves as a major source of energy for our cells, and it's used for the production and biosynthesis of many essential metabolites [9].

Table 3 – Amino acids contents of plant A.scopaeformis

№	Amino acids	Molecular Formula	MW	Amount in plant, mg/100g
1	Alanine	$C_3H_7NO_2$	89	902
2	Glycine	$C_2H_5NO_2$	75	480
3	Leucine	$C_6H_{13}NO_2$	131	453
4	Isoleucine	$C_6H_{13}NO_2$	131	419
5	Valine	$C_5H_{11}NO_2$	117	350
6	Glutamate	$C_5H_9NO_4$	147	2635
7	Threonine	$C_4H_9NO_3$	119	346
8	Proline	$C_5H_9NO_2$	115	830
9	Methionine	$C_5H_{11}NO_2S$	149	124
10	Serine	$C_3H_7NO_3$	105	386
11	Aspartate	$C_4H_7NO_4$	133	1302
12	Cysteine	$C_3H_7NO_2S$	121	55
13	Oxyproline	$C_5H_9NO_2$	131	2
14	Phenylalanine	$C_9H_{11}NO_2$	165	338
15	Tyrosine	$C_9H_{11}NO_3$	181	400
16	Histidine	$C_6H_9N_3O_2$	155	280
17	Ornithine	$C_5H_{12}N_2O_2$	132	2
18	Arginine	$C_6H_{14}N_4O_2$	174	500
19	Lysine	$C_6H_{14}N_2O_2$	146	344
20	Tryptophan	$C_{11}H_{12}N_2O_2$	204	102

Table 4 – Fatty acid composition of plant A.scopaeformis

№	Fatty acids	Molecular Formula	MW	Amount in plant, %
1	Meristic acid $C_{14:0}$	$C_{14}H_{28}O_2$	228	0.6
2	Pentadecanoic acid $C_{15:0}$	$C_{15}H_{30}O_2$	242	1.1
3	Palmitic acid C $_{16:0}$	$C_{16}H_{32}O_2$	256	6.1
4	Palmitoleic acid $C_{16:1}$	$C_{16}H_{30}O_2$	254	0.4
5	Stearin acid $C_{18:0}$	$C_{18}H_{36}O_2$	284	4.5
6	Oleic acid $C_{18:1}$	$C_{18}H_{34}O_2$	282	19.6
7	Linoleic acid $C_{18:2}$	$C_{18}H_{32}O_2$	280	67.3
8	Linolenic acid $C_{18:3}$	$C_{18}H_{30}O_2$	278	0.4

Conclusion.

Quantitative and qualitative analysis of bioactive constituents, the moisture, total ash and extractives contents of A. scopaeformis were determined. Macro-micro elements in the ash of the medicinal plant were investigated. Moreover, total eight macro-micro elements were identified by the method of multi-element atomic emission spectral analysis. Meanwhile, twenty amino and eight fatty acids were determined from A. scopaeformis. Presence of these bioactive constituents, may indicative that the plant has substances capable to promote a better brain activity, the contractile function of the cardiac and sceletel muscles, nerve conduction.

Earlier, we carried out similar work [10, 11].

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ARTEMISIA SCOPAEFORMIS ХИМИЯЛЫҚ ҚҰРАМЫН ЗЕРТТЕУ

Түйін: Бүгінде Қазақстандағы Artemisia (Астрал) отбасының ғылыми және практикалық аспектілеріне қызығушылық артып келеді. Бұл жұмыста алғаш рет Қазақстанда Artemisia scopaeformis дәрілік өсімдігінің фитохимиялық құрамына сандық және сапалық талдау жүргізілді. A. Scopaeformis өсімдігінің алкалоидтар (8,55%), сапониндер (7,25%), флавоноидтар (6,59%), полисахаридтер (1,37%) сияқты биологиялық белсенді компоненттері мен ылғалдылық (7,03%), күлділік (6,1%) және экстрактивті заттар (15,06%) құрамы анықталды. Макро- және микроэлементтердің сандық мөлшерін анықтау мақсатында жасалған талдау нәтижесінде A. scopaeformis өсімдігінің құрамында 10 элемент бар екендігі анықталды, солардың ішінде көп мөлшерде: калий (84.509 мг / г), кальций (27.645 мг / г), магний (7.0076 мг / г) кездеседі. Минералды заттарды анықтау көпэлементті атомды-эмиссиялы спектральды талдау арқылы жүзеге асырылды. Бұдан басқа, A. scopaeformis өсімдігінің жер үсті бөлігінен амин және май қышқылдары газ-сұйықтықты хроматография арқылы анықталды. Жиырма амин қышқылдары мен сегіз май қышқылдары A. scopaeformis құрамына анықталды. Май қышқылдарының ішінде линол қышқылы (67,3 %) және олеин қышқылы (19.6%) , амин қышқылдарының ішінде глутамат (2635 мг / 100 г), аспартат (1302 мг / 100 г) және аланин (902 мг / 100 г) басым кездеседі.

Түйінді сөздер: Artemisia scopaeformis, биоактивті құрамдастар, макро-микро элементтер, амино-май қышқылдар.

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ИССЛЕДОВАНИЕ ХИМИЧЕСКОГО СОСТАВА ARTEMISIA SCOPAEFORMIS

Резюме: Сегодня растет интерес к научным и практическим аспектам семьи Artemisia (Астрал) в Казахстане. В данной работе впервые проведен количественный и качественный анализ фитохимических составляющих лекарственного растения Artemisia scopaeformis из Казахстана. Были определены биологически активные компоненты, такие как алкалоиды (8,55%), сапонины (7,25%), флавоноиды (6,59%), полисахариды (1,37%) вместе с содержанием влаги (7,03%), золы (6,1%) и экстрактивных веществ (15,06%) растения Artemisia scopaeformis. С использованием метода многоэлементного атомно-эмиссионного спектрального анализа в золе растения было обнаружено 8 макро-микроэлементов, в которых основное содержание составляли K (84.509 мг / г), Ca (27.645 мг / г), Mg (7.0076 мг / г). Кроме того, компонентный и количественные составы надземной части были определены на предмет амино- и жирных кислот методом ГЖХ. Двадцать аминокислот и восемь жирных кислот были

идентифицированы из A. scopaeformis. Результаты показали, что основными состовляющимим аминокислот являются глутамат (2635 мг / 100 г), аспартат (1302 мг / 100 г) и аланин (902 мг / 100 г), жирных кислот - линолевая кислота (67,3%) и олеиновая кислота (19,6%).
Ключевые слова: Artemisia scopaeformis, биоактивные компоненты, макро-микро элементы, амино-, жирные кислоты.