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КАТАЛИЗ ЖӘНЕ ЭЛЕКТРОХИМИЯ ИНСТИТУТЫ» АҚ

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ИЗВЕСТИЯ

НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК
РЕСПУБЛИКИ КАЗАХСТАН

АО «ИНСТИТУТ ТОПЛИВА, КАТАЛИЗА И
ЭЛЕКТРОХИМИИ ИМ. Д.В. СОКОЛЬСКОГО»

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JSC «D.V. SOKOLSKY INSTITUTE OF FUEL,
CATALYSIS AND ELECTROCHEMISTRY»

ХИМИЯ ЖӘНЕ ТЕХНОЛОГИЯ СЕРИЯСЫ

♦

СЕРИЯ ХИМИИ И ТЕХНОЛОГИИ

♦

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АЛМАТЫ, ҚР ҰҒА АЛМАТЫ, НАН РК ALMATY, NAS RK

NAS RK is pleased to announce that News of NAS RK. Series of chemistry and technologies scientific journal has been accepted for indexing in the Emerging Sources Citation Index, a new edition of Web of Science. Content in this index is under consideration by Clarivate Analytics to be accepted in the Science Citation Index Expanded, the Social Sciences Citation Index, and the Arts & Humanities Citation Index. The quality and depth of content Web of Science offers to researchers, authors, publishers, and institutions sets it apart from other research databases. The inclusion of News of NAS RK. Series of chemistry and technologies in the Emerging Sources Citation Index demonstrates our dedication to providing the most relevant and influential content of chemical sciences to our community.

Қазақстан Республикасы Ұлттық ғылым академиясы "ҚР ҰҒА Хабарлары. Химия және технология сериясы" ғылыми журналының Web of Science-тің жаңаланған нұсқасы Emerging Sources Citation Index-те индекстелуге қабылданғанын хабарлайды. Бұл индекстелу барысында Clarivate Analytics компаниясы журналды одан әрі the Science Citation Index Expanded, the Social Sciences Citation Index және the Arts & Humanities Citation Index-ке қабылдау мәселесін қарастыруда. Webof Science зерттеушілер, авторлар, баспашылар мен мекемелерге контент тереңдігі мен сапасын ұсынады. ҚР ҰҒА Хабарлары. Химия және технология сериясы Emerging Sources Citation Index-ке енуі біздің қоғамдастық үшін ең өзекті және беделді химиялық ғылымдар бойынша контентке адалдығымызды білдіреді.

НАН РК сообщает, что научный журнал «Известия НАН РК. Серия химии и технологий» был принят для индексирования в Emerging Sources Citation Index, обновленной версии Web of Science. Содержание в этом индексировании находится в стадии рассмотрения компанией Clarivate Analytics для дальнейшего принятия журнала в the Science Citation Index Expanded, the Social Sciences Citation Index и the Arts & Humanities Citation Index. Web of Science предлагает качество и глубину контента для исследователей, авторов, издателей и учреждений. Включение Известия НАН РК в Emerging Sources Citation Index демонстрирует нашу приверженность к наиболее актуальному и влиятельному контенту по химическим наукам для нашего сообщества.

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INVESTIGATION OF CHEMICAL CONSTITUENTS OF *LIGULARIA NARYNENSIS*

Abstract. In this work, the quantitative and qualitative analysis of phytochemical constituents of medicinal plant *Ligularia narynensis* from Kazakhstan has been made for the first time. Total bioactive components of *L. narynensis* such as organic acids (0.64 %), flavonoids (0.52 %) and together with moisture content (5.14 %), total ash (13.24 %), and extractives content (27.7 %) were determined. Eleven macro-micro elements from the ash of plant were identified, main contents of them were K (2214.13 μg/ml), Ca (391.31 μg/ml), and Fe (311.73 μg/ml) by using the method of multi-element atomic emission spectral analysis. In addition, twenty amino and eight fatty acids were analyzed from the plant. The results showed that major contents of amino acids were glutamate (2452 mg/100g), aspartate (1238 mg/100g) and alanine (748 mg/100g), as well as in fatty acids were oleic (33.5 %) and linoleic (41.2 %) acids, respectively.

Key words: *Ligularia narynensis,* bioactive constituents, macro-micro elements, amino-, fatty acids.

Introduction

Ligularia is the genus of perennial herbs of the family Compositae, containing about 180 Eurasian species, 17 species growing in mountains of Kazakhstan [1]. Some species in this genus have been used for a long time as folk remedies for their antibiotic, antiphlogistic, and antitumor activities [2-5]. More than 27 *Ligularia* species have been used as traditional Kazakh and Chinese medicinal herbs for the treatment of fever, pain, inflammation, and intoxication, and to invigorate blood circulation [6-9]. Previous studies confirmed the presence of sesquiterpenes, triterpenes, sinapyl alcohol derivatives, lignans, alkaloids, and steroids in *Ligularia* [10]. Eremophilane sesquiterpenes are considered as the major secondary metabolites and taxonomic markers of *Ligularia* genus. More than 500 eremophilane sesquiterpenes have been reported from this genus [11, 12]. Additionally, oplopane sesquiterpenes have been reported from *L. narynensis* [13].

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. An increase in the total amount of free amino acids is observed with a reduced nutrition of plants with potassium, phosphorus, sulfur, calcium and magnesium. The same action occurs when a number of microelements are lacking: zinc, copper, manganese, iron. This is due to the weakening of the synthesis of proteins from amino acids under these conditions. An increase in the amino acids content is also observed with an improvement in nitrogen nutrition [14].

Fatty acids are structural components of lipoproteins of cell membranes and participate in the implementation of a number of important biochemical processes in the cell. The greatest biological

activity is observed in fatty acids with two or more double bonds. It is to such unsaturated fatty acids are linoleic, linolenic, arachidonic acids. Unsaturated fatty acids prevent the development of atherosclerosis, reduce blood clotting and reduce the possibility of thrombosis. They increase the protective properties of the organism and its resistance to infections, relevant to the development of many skin diseases. There are data on the ability of such acids to prevent the action of substances that cause the development of tumors [15].

This study has made the investigation of the chemical constituents from Kazakh medicinal plant of *L. narynensis* grown in Almaty region of Kazakhstan for the first time.

Materials and methods

Plant material. The root part of plant *L. narynensis* was collected in September 2017 from Butakovskoe gorge of the Zailiysky Alatau Mountains of Almaty region and identified by Dr. Alibek Ydyrys. Specimens (1217-БН-17) were deposited in the Herbarium of Laboratory Plant Biomorphology, Faculty of Biology and Biotechnology, Al-Farabi Kazakh National University, Almaty, Kazakhstan. The air dried roots of *L. narynensis* 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 *L. narynensis* 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. After this, the resin is washed with 1-2 ml of deionized water and 2 ml of 0.5N acetic acid; then 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 [16] of the raw material used GC/MS device. GC/MS analysis: the roots of *L. narynensis* 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 *L. narynensis* 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 was used the internal normalization method.

Results and discussion

The quantitative and qualitative analysis of biologically active constituents together with moisture content, total ash, and extractives contents were determined from roots of *L. narynensis*. The results are shown in Table 1.

Table 1 – Quantitative analysis of bioactive constituents of *L. narynensis*

Content, %				
Moisture content	**Ash**	**Extractives**	**Organic acids**	**Flavonoids**
5.14	13.24	27.7	0.64	0.52

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 *L. narynensis* there were determined eleven macro- and microelements, shown in Table 2 and major of them was K (2214.13 μg/ml), Ca (391.31 μg/ml), Fe (311.73 μg/ml). Potassium is involved in the process of carrying out nerve impulses and transferring them to innervated organs, promotes better brain activity, is also necessary for the implementation of contractions of skeletal muscles. Calcium plays a very important role in many intra- and extracellular processes, including the contractile function of the cardiac and skeletal muscles, nerve conduction, regulation of enzyme activity, and the action of many hormones. It is also a cofactor of the activation of many enzymes or the formation of a number of enzyme complexes in complex, multistage processes of blood coagulation. Iron is a part of the hemoglobin of erythrocytes, myoglobin and many enzymes, participates in hematopoiesis [17].

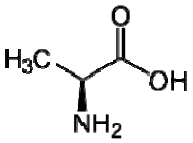
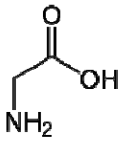
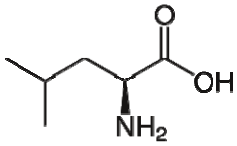
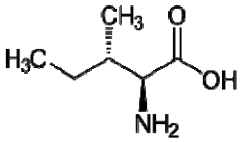
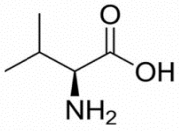
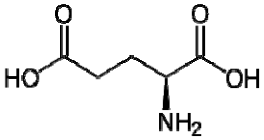
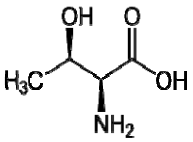
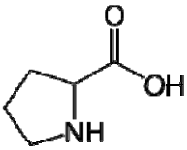
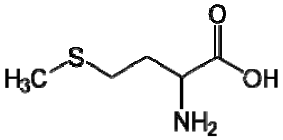
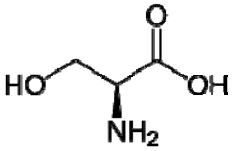
Table 2 – Composition of macro-micro elements in the ash of plant *L. narynensis*

Element	**Cu**	**Zn**	**Cd**	**Pb**	**Fe**	**Ni**	**Mn**	**K**	**Na**	**Mg**	**Ca**
μg /ml	1.57	2.58	0.05	0.66	311.73	0.36	11.73	2214.13	31.74	288.08	391.31

In the composition of amino acids mainly were glutamate (2452 mg/100g), aspartate (1238 mg/100g) and alanine (748 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 [18]. Aspartic acid increases immunity, metabolism, deactivates ammonia, participates in the formation of ribonucleic acids, promotes the removal of chemicals, including drugs, restores working capacity. Studies conducted by scientists have proved the effectiveness of taking asparaginic acid preparations for increasing testosterone levels. Aspartic acid is taken as an additive by bodybuilding athletes to improve strength, increase libido and testosterone in the blood [19]. Alanine also increases immunity and provides energy for brain and central nervous system, the muscle tissue. This amino acid protects against the development of cancer of the pancreas and prostate gland [20].

Quantitative composition of fatty acids in *L. narynensis* mostly contained in linoleic acid (41.2 %) and oleic acid (33.5 %), showed in Table 4. Linoleic acid is an essential fatty acid in nutrition and is used in the biosynthesis of prostaglandins and cell membranes [21]. Oleic acid can inhibit the progression of diseases affecting the brain and adrenal glands, as well as improve memory and reduce blood pressure, but there is evidence that the substance can provoke cancer, in particular breast cancer [22].

Table 3 – Amino acids contents of *L. narynensis*

№	Amino acids	Molecular formula	Structure	MW	Amount in plant, mg/100g
1	2	3	4	5	6
1	Alanine	$C_3H_7NO_2$		89	748
2	Glycine	$C_2H_5NO_2$		75	296
3	Leucine	$C_6H_{13}NO_2$		131	329
4	Isoleucine	$C_6H_{13}NO_2$		131	290
5	Valine	$C_5H_{11}NO_2$		117	278
6	Glutamate	$C_5H_9NO_4$		147	2452
7	Threonine	$C_4H_9NO_3$		119	275
8	Proline	$C_5H_9NO_2$		115	528
9	Methionine	$C_5H_{11}NO_2S$		149	80
10	Serine	$C_3H_7NO_3$		105	356

Окончание таблицы 3

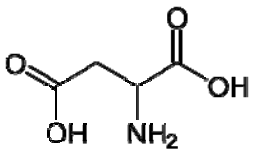
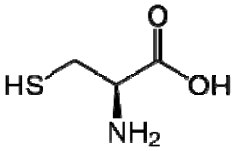
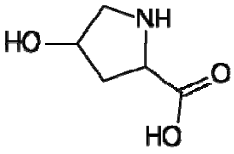
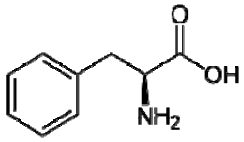
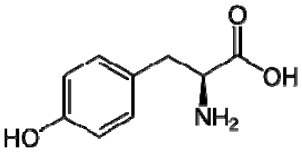
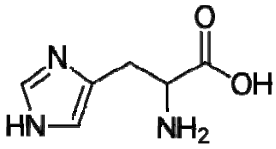
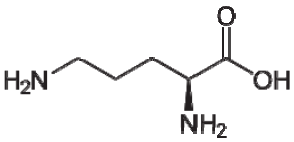
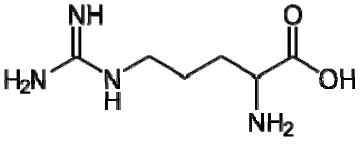
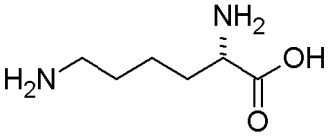
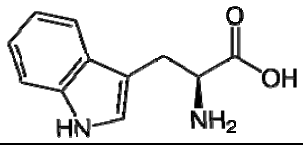
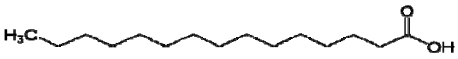
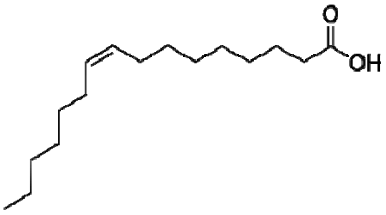
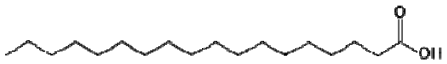
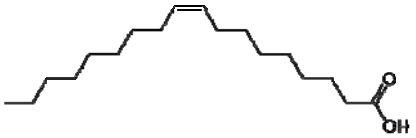
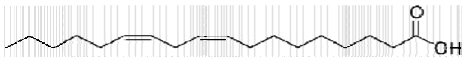
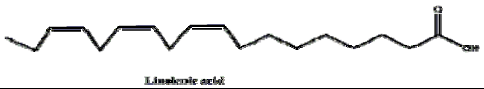
1	2	3	4	5	6
11	Aspartate	$C_4H_7NO_4$		133	1238
12	Cysteine	$C_3H_7NO_2S$		121	34
13	Oxyproline	$C_5H_9NO_3$		131	2
14	Phenylalanine	$C_9H_{11}NO_2$		165	290
15	Tyrosine	$C_9H_{11}NO_3$		181	345
16	Histidine	$C_6H_9N_3O_2$		155	218
17	Ornithine	$C_5H_{12}N_2O_2$		132	2
18	Arginine	$C_6H_{14}N_4O_2$		174	510
19	Lysine	$C_6H_{14}N_2O_2$		146	296
20	Tryptophan	$C_{11}H_{12}N_2O_2$		204	120

Table 4 – Fatty acids contents of *L. narynensis*

№	Fatty acids	Molecular formula	Structure	MW	Amount in plant, %
1	Meristic acid $C_{14:0}$	$C_{14}H_{28}O_2$		228	2.5
2	Pentadecanoic acid $C_{15:0}$	$C_{15}H_{30}O_2$		242	1.4
3	Palmitic acid C $_{16:0}$	$C_{16}H_{32}O_2$		256	14.3
4	Palmitoleic acid $C_{16:1}$	$C_{16}H_{30}O_2$		254	1.1
5	Stearin acid $C_{18:0}$	$C_{18}H_{36}O_2$		284	5.2
6	Oleic acid $C_{18:1}$	$C_{18}H_{34}O_2$		282	33.5
7	Linoleic acid $C_{18:2}$	$C_{18}H_{32}O_2$		280	41.2
8	Linolenic acid $C_{18:3}$	$C_{18}H_{30}O_2$		278	0.8

Conclusion

In summary, the quantitative and qualitative analysis of phytochemical constituents from root of medicinal plant *L. narynensis* of Kazakhstan have been made for the first time. As the results of this study, total bioactive components of *L. narynensis* were determined, eleven macro-micro elements from the ash of plant were identified together with twenty amino and eight fatty acids were quantified from medicinal plant. Presence of these bioactive constituents, may indicative that the plant has substances capable of promote a better brain activity, the contractile function of the cardiac and skeletal muscles, nerve conduction, and the action of many hormones, which play major roles in nutrition, in protein structure, metabolism, signaling, in hemostasis, increase immunity, protect against the development of cancer of the pancreas and prostate gland. The plant *L. narynensis* has high research potential and demands multidimensional study.

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LIGULARIA NARYNENSIS ХИМИЯЛЫҚ ҚҰРАМЫН ЗЕРТТЕУ

Аннотация. Бұл жұмыста Қазақстанда өсетін дәрілік өсімдіктің *Ligularia narynensis* фитохимиялық құрамының сандық және сапалық талдауы бірінші рет жүргізілді. Өсімдіктің ылғалдылығы (5.14 %), күлділігі (13.24 %) және экстрактивтілігі (27.7 %), сонымен бірге органикалық қышқыл (0.64 %), флавоноидтар (0.52 %) сияқты биологиялық активті компоненттер құрамы анықталды. Атомдық эмиссия спектральды талдау әдісін қолдана отырып, өсімдіктің күліндегі он бір макро- және микроэлементтері

зерттелді және оның негізгі құрамы K (2214.13 мкг/мл), Ca (391.31 мкг/мл), Fe (311.73 мкг/мл). Бұдан басқа, жиырма амин және сегіз майлы қышқыл анықталды. Алынған нәтижелер бойынша аминқышқылдардың негізгі құрамы глутамат (2452 мг/100г), аспартат (1238 мг/100г) және аланин (748 мг/100г), май құрамында – олеин (33.5 %) және линол (41.2 %) қышқылдары.

Түйін сөздер: *Ligularia narynensis*, биоактивті құрамдастар, макро-, микроэлементтер, амино-, майлы қышқылдар.

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ИССЛЕДОВАНИЕ ХИМИЧЕСКОГО СОСТАВА *LIGULARIA NARYNENSIS*

Аннотация. В данной работе впервые был сделан количественный и качественный анализ фитохимических составляющих лекарственного растения Казахстана *Ligularia narynensis*. Определены биологически активные компоненты *L. narynensis,* такие как органические кислоты (0,64 %), флавоноиды (0,52 %) вместе с содержанием влаги (5,14 %), общей золы (13,24 %) и экстрактивных веществ (27,7 %). При использовании метода многоэлементного спектрального анализа атомной эмиссии в золе растения были идентифицированы одиннадцать макро-, микроэлементов, основными из которых являются K (2214,13 мкг/мл), Ca (391,31 мкг/мл), Fe (311,73 мкг/мл). Кроме того, были проанализированы двадцать аминокислот и восемь жирных кислот, содержащихся в растении. Результаты показали, что основным составляющим аминокислот являются глутамат (2452 мг/100г), аспартат (1238 мг/100г) и аланин (748 мг/100г); жирных кислот – олеиновая (33,5 %) и линолевая (41,2 %) кислоты.

Ключевые слова: *Ligularia narynensis*, биоактивные компоненты, макро-, микроэлементы, аминокислоты, жирные кислоты.

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