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Д.В.Сокольский атындағы «Жанармай,
катализ және электрохимия институты» АҚ

Х А Б А Р Л А Р Ы

ИЗВЕСТИЯ

НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК
РЕСПУБЛИКИ КАЗАХСТАН
АО «Институт топлива, катализа и
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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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CHEMICAL CONSTITUENTS OF THE ROOTS OF *FRITILLARIA PALLIDIFLORA*

Abstract. The roots of *F.pallidiflora* collected in Kazakhstan were investigated for chemical constituent. The quantitative and qualitative analysis of the medicinal plant have been made. Biological active constituents such as organic acid (1.08%), flavonoids (0.17%) together with moisture content (7.13 %), ash (20.796%), and extractives (12.85%) of plant *F.pallidiflora* were determined. By using the method of multi-element atomic emission spectral analysis in theash of the plantwas found 8 macro-micro elements, in which the main contents were Potassium (440.420 mcg/ml) and Sodium (33.7475 mcg/ml). In addition, twenty amino and eight fatty acids were identified from *F.pallidiflora*. The results showed that the major contents of amino acids were glutamate (2650 mg/100g), aspartate (1185 mg/100g) and alanine (802 mg/100g), and fatty acids were linoleic (24.2%) and oleic acids (48.4%), respectively.

Key words: *Fritillaria pallidiflora,* bioactive constituents, macro-micro elements, amino-, fatty acids.

Introduction

Fritillaria - is a genus of about 130−165 species within the monocot family Liliaceae, and is native to temperate regions of the Northern Hemisphere. *Fritillaria* is a botanical source for various pharmaceutically active components which have been used in traditional Chinese medicine for thousands of years. Many species (such as *F. cirrhosa*, *F.thunbergii* and *F. verticillata*) are used in traditional Chinese cough remedies. Increasing interest in *Fritillaria* medicinal resources has led to additional discoveries of steroidal alkaloids, saponins, terpenoids, glycosides and many other compounds in various *Fritillaria* species, and to investigations on their chemotaxonomy, molecular phylogeny and pharmacology [1]. *Fritillaria pallidiflora Schrenk* belongs to the *Fritillaria* genus of *Liliaceae* family widely distributed in Xinjiang province of China and finds widespread applications as antitussive, antiasthmatic and expectorant medicine [2]. The chemical constituents of *F. pallidiflora* have been studied, and steroidal saponins and alkaloids are regarded as their main ingredients [3]. In recent years, steroidal saponins have attracted more attention for their significant bioactivities, including their anti-tumor [4], anti-thrombotic [5], anti-inflammatory [6], anti-fungal [7] activities.

In the steppe zones, meadow places, on the slopes of the alpine and subalpine belt of Kazakhstan, there are 5 species of growing [8]. In addition, the bulbs contain organic acids, terpenoids, phytosterols and some vitamins. In small doses, the alkaloids contained in the bulbs have a therapeutic effect. Thus, in Chinese medicine, on the basis of the alkaloids contained in the bulbs, expectorants and soothing agents are made. In large doses of hazelnut bulbs are dangerous to health [9].

Proteins are large, complex molecules that are critical for the normal functioning of the human body. They are essential for the structure, function, and regulation of the body's tissues and organs. Proteins are made up of hundreds of smaller units called **amino acids** that are attached to one another by peptide bonds, forming a long chain. First point of investigation was on amino acids, which is organic (carboxylic) acids, the molecules of them contain one or more amino groups (NH_2-groups), the basic structural elements of protein molecules [10].

It is known that fatty acids - components of lipids exist in plants, animals, and microorganisms. Lipids are necessary for our body, because without them, metabolism cannot be carried out normally, and toxins and toxins accumulate in cells and tissues, as purification processes are slowed down. Many of fatty acids

cannot be synthesize in human organism. Those fatty acids are required, however, for cellular processes and the production of other necessary omega-3 and omega-6 fatty acids. In addition fatty acids have a wide range of commercial applications, for example, they are used not only in the production of numerous food products but also in soaps, detergents, and cosmetics. Soaps are the sodium and potassium salts of fatty acids [11].

In the present study, the quantitative and qualitative analysis of bioactive components such as moisture, total ash, and extractives contents of *F.pallidiflora* have been carried out, as well as amino-fatty acid contents were determined. Determination of main bioactive constituents, macro, micro elemts and amin-, fatty acid contents of the root of *F.pallidiflora* which growing in Almaty region of Kazakhstan were reported for the first time.

Materials and Methods

The root part of plant *F.pallidiflora* was collected in May 2017 from the piedmont steppe of the Toraigir Mountains of Almaty region and identified by Dr. AlibekYdyrys. Plant was 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 *F.pallidiflora* were cut into small pieces and stored at room temperature.

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

In the "Center of Physico-Chemical methods of research 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 *F.pallidiflora* 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°C for 24 hours, in ampoules sealed under a stream of argon. The resulting hydrolysate is evaporated three times to dryness on a rotary evaporator at a temperature of 40-50°C 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 thenevaporated to dryness on a rotary evaporator at a pressure of 1 atm and a temperature of 40-50°C. After adding a drop of freshly prepared 1.5% SnCl2 solution, 1 drop of 2,2-dimethoxypropane and 1-2 ml of propanol saturated with hydrochloric acid, it is heated to 110°C, 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 triethyl amine, 5 volumes of acetone) is introduced into the flask and heated at a temperature of 60°C 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 by using GC/MS device. GC/MS analysis: amino acid content of the roots part of *F.pallidiflora* 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 110°C (held for 20 min), at 6°C/min from 110°C to 180°C, at 32°C/min from 185°C to 290°C. When it reaches to 250°C, it should stay constant till fishing of exit of all amino acids. The chromatogram is counted according to an external standard.

Results and discussion

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

The ash of plant raw materials is the balance of inorganic substances obtained after burning the raw material and then calcining the residue to a constant mass. The ash of plants consists of a mixture of various inorganic substances characteristic of the plant, and mineral impurities that can get into the raw material during collection and drying. The moisture of plant materials is the loss in the mass due to hygroscopic moisture and volatile substances detecting when plant material is dried till constant weight. The moisture content in medicinal plant raw materials serves as one of the numerical indicators characterizing its quality. Medicinal plant raw materials should not contain moisture above the permissible standards, because with high humidity, during storage conditions are created that contribute to a decrease in its quality. For most types of medicinal plant raw materials, the permissible moisture limit is usually 12-15%.

Table-1 - Quantitative analysis of bioactive constituents of the root *F.pallidiflora*

Content, %				
Moisturecontent	**Ash**	**Extractives**	**Organicacids**	**Flavonoids**
7.13	20.7	12.8	1.08	0.17

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 *F.pallidiflora* there were determined eight macro- and micro elements, shown in Table 2 and major of them are Potassium (440.420 mcg/ml) and Sodium (33.7475 mcg/ml). One of the main factors of nutrition, affecting health, working capacity and active longevity, are micronutrients - macro- and micro elements. The body does not produce microcircuits and should receive them in ready form, for example, with food. The ability to store these substances in the body is absent. Sodium is the maintains the osmotic pressure and pH of the medium, participates in the formation of gastric juice, activates the enzymes of saliva and pancreatic juice, together with potassium, forms the electrical potential of cell membranes, through which a signal is transmitted in nerve cells, muscle cells, etc. Potassium is the most important component of intracellular fluid, controlling acid-base balance, muscular activity, and synthesis of proteins and glycogen [12].

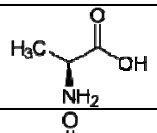
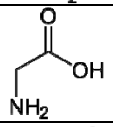
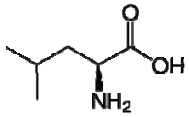
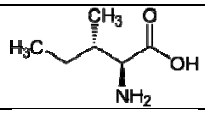
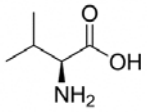
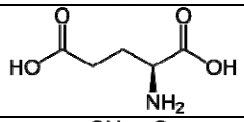
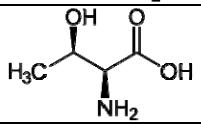
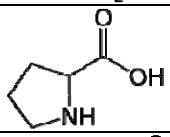
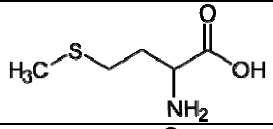
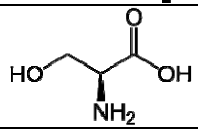
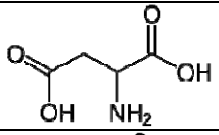
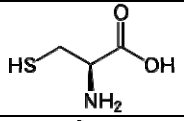
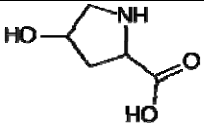
Table 2 - Composition of macro-micro elements in the ash of *F.pallidiflora ash*

Element	**K**	**Mg**	**Ca**	**Mn**	**Fe**	**Zn**	**Cu**	**Na**
mcg/ml	440.4200	25.6850	28.0300	0.4254	3.4152	5.8052	0.1731	33.7475

Determination of fatty acid composition of raw material, and dried plant *F.pallidiflora* 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 "CARLO-ERBA-420" allocated the Kazakh Academy of Nutrition for 1 hour. 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.

Quantitative composition of fatty acids in *F.pallidiflora* mostly were linoleic acid (24.2%) and oleic acid (48.4%). Linoleic acid has received much attention in recent years because of its interesting biological benefits. The main health effects described for linoleic acid include reduction of carcinogenesis, atherosclerosis, inflammation, obesity, diabetes, as well as growth promoting and bone formation-promoting properties [13]. 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 [14].

Table 3 - Amino acids from the root of *F.pallidiflora*

№	Amino acids	Molecular formula	Structure	MW	Amount in plant, mg/100g
1	2	3	4	5	6
1	Alanine	$C_3H_7NO_2$		89	802
2	Glycine	$C_2H_5NO_2$		75	326
3	Leucine	$C_6H_{13}NO_2$		131	415
4	Isoleucine	$C_6H_{13}NO_2$		131	402
5	Valine	$C_5H_{11}NO_2$		117	328
6	Glutamate	$C_5H_9NO_4$		147	2650
7	Threonine	$C_4H_9NO_3$		119	290
8	Proline	$C_5H_9NO_2$		115	652
9	Methionine	$C_5H_{11}NO_2S$		149	75
10	Serine	$C_3H_7NO_3$		105	480
11	Aspartate	$C_4H_7NO_4$		133	1185
12	Cysteine	$C_3H_7NO_2S$		121	50
13	Oxyproline	$C_5H_9NO_3$		131	1

Окончание таблицы

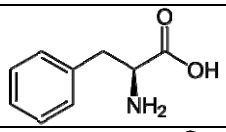
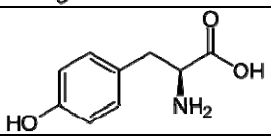
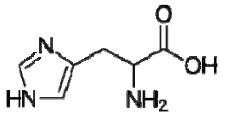
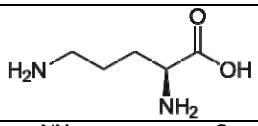
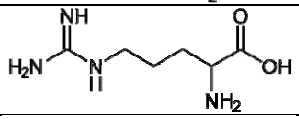
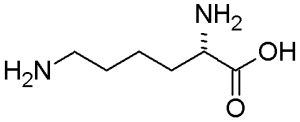
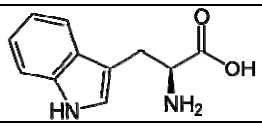
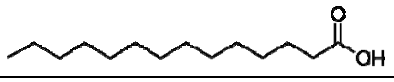
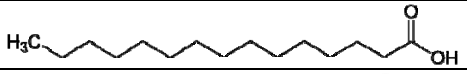
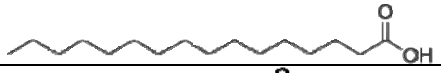
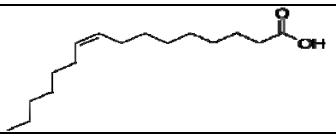
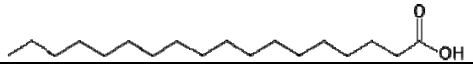
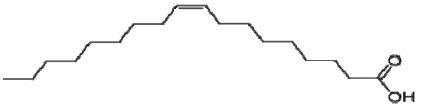
1	2	3	4	5	6
14	Phenylalanine	$C_9H_{11}NO_2$		165	303
15	Tyrosine	$C_9H_{11}NO_3$		181	348
16	Histidine	$C_6H_9N_3O_2$		155	200
17	Ornithine	$C_5H_{12}N_2O_2$		132	2
18	Arginine	$C_6H_{14}N_4O_2$		174	498
19	Lysine	$C_6H_{14}N_2O_2$		146	160
20	Tryptophan	$C_{11}H_{12}N_2O_2$		204	90

Table 4 - Fatty acids from the root of *F. pallidiflora*

№	**Fatty acids**	**Molecular formula**	**Structure**	**MW**	**Amount in plant, %**
1	Meristic acid $C_{14:0}$	$C_{14}H_{28}O_2$		228	2.1
2	Pentadecanoic acid $C_{15:0}$	$C_{15}H_{30}O_2$		242	1.2
3	Palmitic acid C $_{16:0}$	$C_{16}H_{32}O_2$		256	18.3
4	Palmitoleic acid $C_{16:1}$	$C_{16}H_{30}O_2$		254	0.9
5	Stearin acid $C_{18:0}$	$C_{18}H_{36}O_2$		284	3.8
6	Oleic acid $C_{18:1}$	$C_{18}H_{34}O_2$		282	48.4
7	Linoleic acid $C_{18:2}$	$C_{18}H_{32}O_2$		280	24.2
8	Linolenic acid $C_{18:3}$	$C_{18}H_{30}O_2$		278	1.1

In the composition of amino acids mainly were glutamate (2650 mg/100g), aspartate (1185 mg/100g) and alanine (802 mg/100g). Glutamate is replaceable amino acid, which plays the role of a neurotransmitter with high metabolic activity in the brain, stimulates redox processes in the brain, the exchange of proteins. Normalizes the metabolism, changing the functional state of the nervous and endocrine systems [15]. 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 [16]. Alanine plays a significant role in metabolic processes, as well as to regulate the level of sugar in the bloodstream. This amino acid protects against the development of cancer of the pancreas and prostate gland, it is an important part of sports nutrition, increases physical endurance and allows to build muscle mass [17].

Conclusion

Quantitative and qualitative analysis of bioactive constituents and the moisture, total ash and extractives contents of roots *F.pallidiflora* were determined. Besides, macro-micro elementsin the ash of the medicinal plant were investigated, and 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 *F.pallidiflora*. The results showed that the major contents of amino acids were glutamate (2650 mg/100g), aspartate (1185 mg/100g) and alanine (802 mg/100g), and fatty acids were linoleic (24.2%) and oleic acid acids (48.4%), respectively.

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АҚШЫЛ СЕПКІЛГҮЛ ТАМЫРЫНЫҢ ХИМИЯЛЫҚ ҚҰРАМЫН ЗЕРТТЕУ

Аннотация. Қазақстанда жиналған *F. pallidiflora* тамырының химиялық құрамы зерттелді. Дәрілік өсімдіктің сандық және сапалық талдауы жүргізілді. Өсімдіктің ылғалдылығы (7,13%), күлділігі (20,796%) және экстрактивтілігі (12,85%),сонымен бірге органикалық қышқыл (1,08%), флавоноидтар (0,17%) сияқты биологиялық активті компоненттер құрамы анықталды. Атомдық эмиссия спектральды талдау әдісін қолдана отырып, өсімдіктің күліндегі 8 микро және макро элементері анықталдыжәне оның негізгі құрамы калий (440.420 мкг / мл) және натрий (33.7475 мкг / мл) элементтерінен тұратыны анықталды. Бұдан басқа, *F.pallidiflora*-дан жиырма амин және сегіз майлы қышқыл анықталды. Алынған нәтижелер бойынша аминқышқылдардың негізгі құрамы глутамат (2650 мг / 100 г), аспартат (1185 мг / 100 г) және аланин (802 мг / 100 г) және май құрамында линол (24,2 %) және олеин қышқылдары (48,4 %).

Түйін сөздер: *Fritillaria pallidiflora*, биоактивті құрамдастар, макро-микро элементтер, амино-майлы қышқылдар.

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ИССЛЕДОВАНИЕ ХИМИЧЕСКОГО СОСТАВА РЯБЧИКА БЛЕДНОЦВЕТКОГО

Аннотация. Был исследован химический состав корней *F. pallidiflora*, собранного в Казахстане. Сделан количественный и качественный анализ лекарственного растения. Определены биологически активные компоненты, такие как органические кислоты (1,08%), флавоноиды (0,17%), а также содержание влаги (7,13%), зольность (20,796%) и экстрагенты (12,85%) растения *F.pallidiflora*. При использованием метода многоэлементного атомно-эмиссионного спектрального анализа в золе растения было найдено 8 макро-микро элементов, основными веществами из которых были калий (440.420 мкг / мл) и натрий (33.7475 мкг / мл). Кроме того, из корней *F.pallidiflora* были идентифицированы двадцать аминокислот и восемь жирных кислот. Результаты показали, что основным содержанием аминокислот являются глутамат (2650 мг / 100 г),

аспартат (1185 мг / 100 г) и аланин (802 мг / 100 г), а среди жирных кислот - линолевые (24,2%) и олеиновые кислоты (48,4%) соответственно.

Ключевые слова: *Fritillaria pallidiflora*, биоактивные компоненты, макро-микро элементы, амино-, жирные кислоты.

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