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To a phytochemical study of Kazakhstani species *Fritillaria karelinii* (Fisch. ex D. Don) Baker

Present article provides the results of a primary phytochemical analysis of *Fritillaria karelinii* (Fisch. ex D. Don) Baker (syn. *Rhinopetalum karelinii* Fisch. ex D. Don) from family *Liliaceae* Juss., collected from different fields of populations in the Almaty region. A comparative analysis of water-alcohol isolations allowed identifying three substances belonging to phenolic nature, and the quantitative contents of flavonoids (in the range of 0.7–1.03 % in terms of air dry matter) and some other biological active compounds (alkaloids, amino acids, organic acids). There is a correlation in the quantitative content of arginine (max) and methionine (min) out of 13 amino acids that were identified in bulbs of three studied samples. By accumulation of amino acids, the Ili plant sample shows quantitatively different from the other two, for example arginine, proline (0.475), leucine (0.372), lysine, valine, phenyl, threonine, and alanine (0.51–0.31) prevail, and methionine and histidine were less than 0.1 %. Valine, threonine and proline are contained in the range of 0.298–0.193 %, while lysine, leucine, phenylalanine, and serine are in the range of 0.175–0.161 % for a sample collected near Nurly village. The content of other identified amino acids was below 0.15 %.

Keywords: *Fritillaria karelinii* bulbs, phytochemical analysis, amino acids, fat acids, quantitative content.

Introduction

Developed worldwide research in the field of chemistry of natural compounds makes it possible to isolate and obtain total extracts from wild plants, and further determine their active components, and access biological activity. Thereby it contributes to the replenishment of the range of medicinal plants used in official medicine. In this regard, the search for new sources of medicinal plant materials is an actual task.

Early spring bulbous wild plants adapting to changes in external factors and too harsh growing conditions (temperature and water conditions, salinization, soil erosion, etc.) have great potential as a source of new biologically active substances. The defense mechanism of such plants is due to the accumulation and compatibility of osmolytes which affect the biosynthesis of macromolecules [1].

The prerequisite for this study was the analysis results of the medicinal flora in Kazakhstan [2] which revealed poor knowledge of the local flora species.

The object of our research is a perennial plant – Karelin's hazel grouse, *Fritillaria karelinii* (Fisch. ex D. Don) Baker (syn. *Rhinopetalum karelinii* Fisch. ex D. Don) from the family *Liliaceae* Juss. According to Plants of the World Online, the genus *Fritillaria* Tourn. ex L. has 152 species, 6 of which described in the flora of Kazakhstan, and 2 species include inside genus *Rhinopetalum* Fisch [2, 3].

In traditional Chinese and other Asian countries medicine, some species of *Fritillaria* are used as a medicinal raw material for the production of effective antitussive and expectorant drugs [4–7]. Tubers of whorled hazel grouse *F. verticillata* are used for complicated diseases of the digestive system and also “cure the heat of blood vessels the heart” [8].

Some species of the genera *Veratrum* L. and *Fritillaria* Tourn. (*Liliaceae*) contain C₂₇-alkaloids of the C-nor-D-homosteroid series with a wide spectrum of biological activities [9–10], in particular, having inotropic effect on the heart [11].

In literatures data on isolation and identification of steroid alkaloids from *F. verticillata* and *F. puqiensis* were found [12, 13], and in both the aboveground and in the bulbs veratrum (jervanine and cevanine subgroups) alkaloids were found.

The phytochemical composition of Kazakh hazel grouse species is practically not studied. There is information about the isolation of the alkaloid alginine (C₂₃H₂₉NO₃) from *F. sewerzowii* collected in the western part of Tien Shan (Kyrgyzstan), the structure of which has not been determined in detail [14].

Researches from the plant resources laboratory has identified the distribution features and anatomical and morphological characteristics of *F. karelinii* species collected in southeast Kazakhstan [15–16].

The purpose of our research is a phytochemical study (qualitative and quantitative determination of the main groups of biological active compounds) of *F. karelinii* collected from various natural populations in the Almaty region (2020–2021).

Experimental

The research materials (bulbs) of *F. karelinii* were collected in March 2020–2021 from natural populations in southeast Kazakhstan (within the Enbekshi kazakh, Balkhash, and Ili administrative districts of the Almaty region).

During the harvesting of plant raw materials, plants were dug up, removed from the soil, and dried under the air shadow after cutting the bulbs into several parts, and the final dry plant raw material was milled.

Phytochemical studies to identify extractives, determine the quality of plant raw materials, quantitative content of some groups of BAS were performed according to the methods described in the SP RK and others [17, 18].

The quantitative determination of vitamins, amino- and fatty acids was carried out in the accredited laboratory “Food Safety” at Almaty Technological University.

Aminoacids were analyzed on a Capel 105 M capillary electrophoresis system (RF) after preliminary hydrolysis of plant materials with hydrochloric acid (1:1) at 105 °C for 14–16 hours [19]. The acid hydrolysate evaporated up to a minimum volume and after converted into phenylisothiocarbonyl derivatives. Detection was carried out in the UV region at 254 nm.

The content of fatty acids was done on a Kristal-4000 gas-liquid chromatograph (RF) after preliminary extraction of lipids with hexane in a Soxhlet apparatus for 5 hours. The extract was evaporated at a temperature of 30–40°C.

Ethylating was in a mixture of hexane (97 %), 25 % sodium ethoxide in absolute ethanol and acetic acid. The resulting mixture was stirred, settled, and filtered through a paper filter; the final solution was analyzed on a Kristal-4000 GLC with a flame ionization detector and NetChrom software [20]. Chromatographic separation was carried out under the following conditions: injector temperature, 188°C; detector temperature, 230°C; thermostat temperature, 188°C; column content: polyethylene glycol adipate (20 %) on celite — 545.

The determination of vitamins was carried out according to the standard methods described in the manual M-04-41-2005 and GOST 32043-2012 [21, 22].

Results and Discussion

During the determination of the quality of plant raw materials there the moisture content varies from 6.32 to 8.74 % was revealed and the quantitative content of extractives in a 70 % water-alcohol solution was 20–25 %. Substances of a phenolic nature (flavonoids, phenolic acids), alkaloids, steroids, and sterols have been qualitatively detected. The results of the quantitative content of some groups of biological active compounds are shown in Table 1.

Table 1

Indicators of plant raw materials and the content of some biologically active substances and vitamins in studied *Fritillaria karelinii* plant samples

Plant raw material indicators	Kapshagai population		Bogeti population		Ili population	
	2020	2021	2020	2021	2020	2021
Losson drying	6.42	8.74	7.01	6.75	7.94	6.32
Determination of extractive substances (water solution)	25.24	23.42	25.03	17.04	24.84	22.88
Determination of extractive substances (70 % alcohol solution)	23.98	24.03	20.71	24.29	25.09	23.69
Quantitative content						
Vitamin C mg/100 g	-	3.42	-	4.28	-	5.63
Vitamin E mg/100 g	-	0.24	-	0.23	-	0.28
Amino acids	1.24	1.28	0.94	1.45	1.64	1.72
Organic acids	3.03	3.28	2.98	2.68	2.83	3.21
Flavonoids	1.08	1.18	1.32	1.28	1.32	2.01
Alkaloids	0.73	0.98	0.78	1.03	0.83	1.28

According to the literature data the chemical composition of the aboveground and underground parts of *F. karelinii* has not been studied, we have begun a preliminary study of the flavonoid composition. A comparative analysis of water-alcohol extracts allowed establishing the qualitative composition of phenolic substances in all studied samples and it is almost the same. By two-dimensional paper chromatography and using various solvent systems revealed 3 substances of phenolic nature. According to the location of the spots on the chromatogram the substances were tentatively classified as biglycosides of flavonoids. The content of flavonoids was determined in the range of 0.7–1.03 % on air dried weight.

The content of alkaloids varied within 0.73–1.23 % on air dried weight, while according to some literary sources, the content of the total alkaloids for species of this genus varies from 0.15 to 1.77 % [23].

Determination of the amino and fatty acids content in the studied samples growing in the territory of southeast Kazakhstan is being investigated for the first time. As a result of the study 13 amino acids and 9 substances of the lipid form (fatty acids and their ethyl esters) were identified (Tables 2, 3).

Table 2

The content of amino acids in the studied samples of *Fritillaria karelinii*

Amino acids	Kapshagai population	Bogeti population	Ili population
Arginine	0.990	0.632	1.859
Lysine	0.134	0.175	0.351
Tyrosine	0.076	0.126	0.268
Phenylalanine	0.169	0.165	0.330
Histidine	0.078	0.116	0.087
Leucine + Isoleucine	0.118	0.174	0.372
Methionine	0.060	0.047	0.058
Valine	0.120	0.298	0.351
Proline	0.124	0.193	0.475
Threonine	0.144	0.228	0.330
Serene	0.134	0.161	0.289
Alanine	0.099	0.140	0.310
Glycine	0.132	0.116	0.268

Table 3

Content of fatty acids in *Fritillaria karelinii* bulbs (% of air dried weight)

Fatty acids	Kapshagai population	Bogeti population	Ili population
Caproic acid (C _{6:0})	0.2	0.39	0.25
Palmitic acid (C _{16:0})	4.7	5.65	4.59
Stearic acid (C _{18:0})	0.8	0.67	0.42
Oleic acid (C _{18:1})	1.4	1.79	1.19
Linoleic acid (C _{18:2})	3.5	5.99	5.12
Ethyl ester of palmitic acid	10.4	5.49	4.80
Ethyl ester of stearic acid	2.6	1.25	0.99
Ethyl ester of linoleic acid	10.6	5.74	3.92
Docosanoic acid ethyl ester	4.1	2.39	2.04

There is a correlation in the quantitative content of arginine (maximum) and methionine (minimum) out of the 13 amino acids found in the bulbs of the three samples studied. The Ili samples quantitatively differ from the other two populations by accumulation of the identified amino acids. For example, arginine, proline (0.475), leucine (0.372), lysine, valine, phenyl, threonine, and alanine (0.351–0.31), methionine and histidine predominate — less than 0.1 %.

The samples from Bogeti population contained amino acids: valine, threonine, and proline, the content of which varies from 0.298–0.193 %, and the content of lysine, leucine, phenylalanine, and serine vary from 0.175–0.161 %. The rest of the identified amino acids have content below 0.15 % (Table 2).

Three saturated and two unsaturated fatty acids as well as their ethyl derivatives were found in the studied plant raw material (Table 3). The content of saturated fatty acids (caproic and stearic) is more than 0.2 %, while palmitic in three studied plant samples significantly exceeds (4.6–5.65 %).

Linoleic (3.5–5.99 %) and oleic (1.19–1.79 %) unsaturated fatty acids were identified. It should be noted that the content of ethyl derivatives of the above-mentioned acids correlates with the data of their acids themselves notably the values of palmitic acid ethyl ester (4.8–10.4 %) and linoleic acid ethyl ester (3.92–10.6 %).

Conclusion

Thus, the primary phytochemical screening of *F. karelinii* bulbs made it possible to identify and quantify the content of amino and fatty acids, alkaloids, and phenolic substances (flavonoids). The peculiarities of the accumulation of amino acids and fatty acids depending on the place of growth have been established. Differences have been established in the accumulation of individual substances between populations.

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***Fritillaria karelinii* (Fisch. ex D. Don) Baker қазақстандық түрінің фитохимиялық зерттеуі**

Мақалада Алматы облысының әртүрлі популяцияларынан жиналған *Liliaceae* Juss. тұқымның *Fritillaria karelinii* (Fisch. ex D. Don) Baker (син. *Rhinopetalum karelinii* Fisch. ex D. Don) пиязшығының бастапқы фитохимиялық талдауы берілген. Су-спирт сығындыларының салыстырмалы талдауы фенолдық сипаттағы үш затты анықтауға, флавоноидтардың сандық құрамын (а.қ.ш. бойынша 0,7–1,03 % аралығында) және кейбір басқа биологиялық белсенді заттарды (алкалоидтар, амин қышқылдары, органикалық қышқылдар) анықтауға мүмкіндік берді. Қазақстанның оңтүстік-шығысында өсетін, зерттелген үлгілердегі амин және май қышқылдарының құрамы алғаш рет зерттеліп отыр. Зерттеу нәтижесінде 13 амин және 9 май қышқылдары анықталды. Үш зерттелген үлгінің пиязшықтарында анықталған 13 амин қышқылының аргинин (максимум) мен метиониннің (минимум) сандық құрамында корреляция бар. Іле үлгілері басқа екеуінен анықталған аминқышқылдарының жинақталу тенденциясы бойынша сандық жағынан ерекшеленеді, мысалы, аргинин, пролин (0,475), лейцин (0,372), лизин, валин, фенил, треонин және аланин (0,351–0,31), метионин мен гистидин 0,1 %-дан аз. Бөгеті популяция үлгілерінде келесі аминқышқылдар табылды: валин, треонин және пролин, олардың мөлшері 0,298–0,193 % аралығында, ал лизин, лейцин, фенилаланин және серин мөлшері 0,175–0,161 % аралығында кездеседі. Қалған аминқышқылдарының құрамы 0,15 %-дан төмен.

Кілт сөздер: *Fritillaria karelinii* пиязшығы, фитохимиялық талдау, амин және май қышқылдары, сандық құрамы.

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К фитохимическому изучению казахстанского вида *Fritillaria karelinii* (Fisch. ex D. Don) Baker

В статье приведены результаты первичного фитохимического анализа луковиц *Fritillaria karelinii* (Fisch. ex D. Don) Baker (син. *Rhinopetalum karelinii* Fisch. ex D. Don) из сем. *Liliaceae* Juss., собранных из разных популяций Алматинской области. Сравнительный анализ водно-спиртовых извлечений позволил выявить три группы веществ фенольной природы, определить количественное содержание флавоноидов (в пределах 0,7–1,03 % в пересчете на воздушно-сухой вес) и некоторых других биологически активных веществ (алкалоидов, аминокислот, органических кислот). Содержание amino- и жирных кислот в исследуемых образцах, произрастающих на территории юго-востока Казахстана, исследованы впервые. В результате выявлены и идентифицированы 13 amino- и 9 жирных кислот. Из 13 аминокислот, найденных в луковичах, выявлена корреляция по количественному содержанию аргинина (максимум) и метионина (минимум) в трех исследуемых образцах. Илийские образцы количественно отличаются от двух других, характеризуются тенденцией накопления выявленных аминокислот, так, например, после аргинина превалирует пролин (0,475 %), лейцин (0,372), лизин, валин, фенил, треонин и аланин (0,351–0,31), метионин и гистидин — менее 0,1 %. У образцов Богетинской популяции обнаружены аминокислоты: валин, треонин и пролин, содержание которых варьирует в пределах 0,298–0,193 %, а содержание лизина, лейцина, фенилаланина и серина — в пределах 0,175–0,161 %. У остальных идентифицированных аминокислот содержание ниже 0,15 %.

Ключевые слова: луковицы *Fritillaria karelinii*, фитохимический анализ, amino- и жирные кислоты, количественное содержание.

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