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Phytochemical and elemental composition of the *Linaria cretacea* Fisch ex Spreng *(Plantaginaceae)*

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Abstract. The article presents the results of a phytochemical study of a rare species of the Republic of Kazakhstan *Linaria cretacea* Fisch ex Spreng. Specific biologically active compounds contained in the inflorescences, roots, and leaves of plants have demonstrated their pharmacological properties and biological activity, confirming that various phytochemical groups and individual compounds have beneficial effects. Of the total number of organic compounds detected in ethanol extracts, falcarinol (with a relative content of 16,51%) and thymol (with a relative content of 0,57%) in *L. cretacea* were identified with a high degree of confidence (65-90%). These compounds are valuable biologically active substances used in medicine. Being unsaturated compounds, they can be classified as natural antioxidants. For example, falcarinol is found in the roots of American ginseng. Thymol, which is part of the structural elements of cell membranes, is also a phenolic antiseptic with pronounced antimicrobial and antifungal activity against various microorganisms. The results obtained are promising and can be further used to develop a technology for obtaining valuable biologically active substances from *Linaria cretacea* Fisch ex Spreng biomass.

Keywords: *Linaria cretacea* Fisch. ex Spreng., endemic, structural components, calcefite compound, phytochemical analysis

Introduction

Flora of the Aktobe region the large-scale, predominantly shallow-soil-low- high-altitude vegetation of Kazakhstan is characterized by a variety of successive communities, which differ both in their composition and structure. Endemic plant species are mainly found on rocks with various lithological compositions, including granites, sandstones, shales, limestones and effusions [1-3].

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The book " Plant Resources of the USSR" provides instructions for the chemical composition and use of 10 species belonging to the genus *Linaria*, including L. *vulgaris* Mill. is known in Kazakhstan as medicinal plants. *Linaria vulgaris* has a long history of use in folk medicine [4]. It is effective in the treatment of dropsy, constipation, jaundice and scrofula, and is also used as a laxative, diuretic, diaphoretic, anthelmintic and choleretic. This plant helps to improve the functioning of the stomach and especially the intestines, helps to cope with flatulence and reduces inflammatory processes, contributing to the reduction and resorption of infiltrates [5,6].

Representatives of the *Linaria* genus have a rich composition, including natural alkaloids, essential oils, phenolic and flavonoid compounds. Species containing biologically active ingredients are used to improve digestion, to relieve spasmic pain in the gastrointestinal tract, as an anti-inflammatory, soothing agent for cleaning injuries and wounds, and as an antiseptic, for sun-damaged skin, for the treatment of cystitis diseases. In Europe, medical extracts and teas are prepared from raw materials obtained from related species [7,8].

Materials and research methods

To study the composition of the extract prepared from the vegetative part of *Linaria cretacea,* samples were treated with 96% ethyl alcohol. 1 g of dry samples were analyzed using Agilent 6890n/5973n gas chromatography by gas chromato-mass spectrometer method. Analysis conditions 1 μL of the sample was divided at a temperature of 270°C 10: 1. The separation was carried out using a DB-WAX chromatographic capillary column, 30 m long, 0.25 mm in diameter and 0.25 microns thick, at a constant gas flow rate (helium) of 1 ml/min. The chromatography temperature was programmed to range from 40 °C to 300 °C for 5 minutes with a heating rate of 5 °C/min. The Agilent MSD ChemStation version 1701EA software was used to register the results and process the data obtained by gas chromatography. A mass spectrometric detector using the Wiley 7 and NIST'02 libraries was used to analyze spectral information and distinguish the obtained mass spectra results [9,10].

For sample preparation, 20 g of ground plant material was sieved through a sieve with 1 mm hole diameter and placed in a 100 ml volumetric flask. To the obtained material 50 ml of ethanol was added as extractant. The extraction was carried out using ultrasonication for 60 minutes. After completion of the process, the extract was filtered and then a 2 ml aliquot was taken for subsequent chromatographic analysis (Figure 1).

Statistical processing was performed using MS EXCEL 2010 programme and Statistica 5.0 statistical software package.

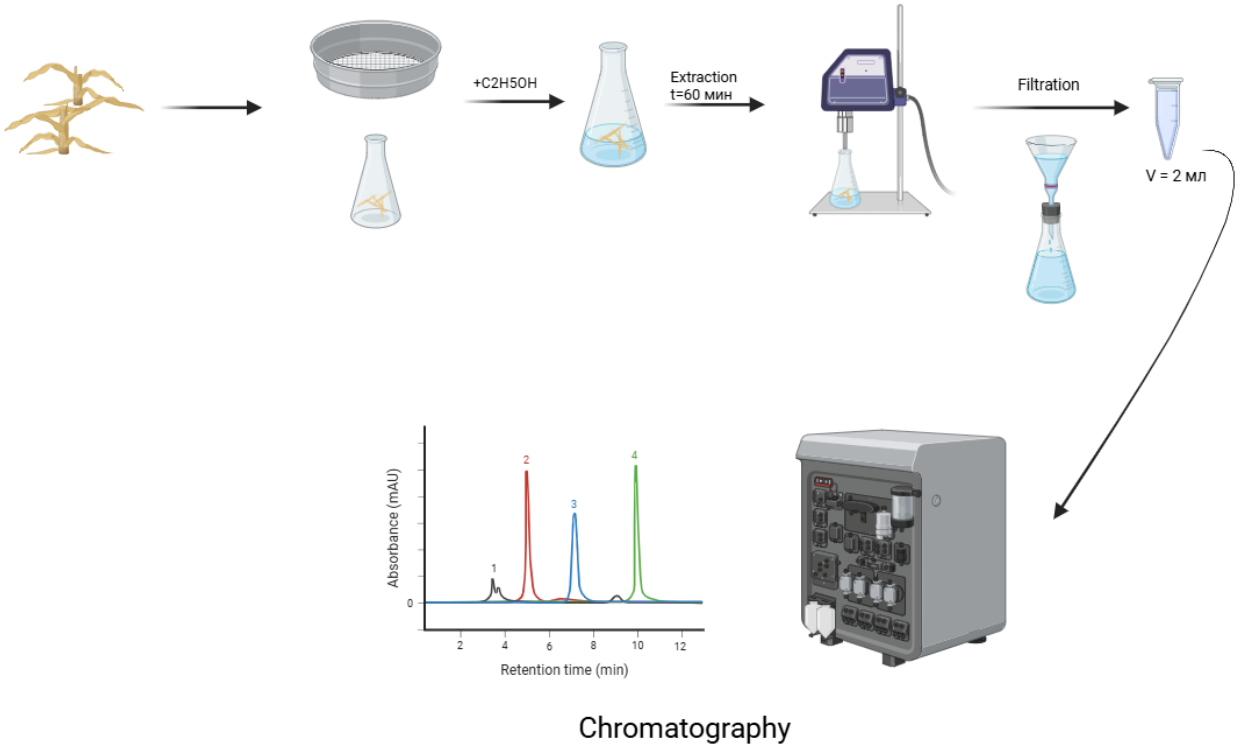

Figure 1. The analysis scheme

Results

Well, L. *cretaceae* to date, the healing properties and chemical composition of the species have not been scientifically described. Therefore, of interest is the composition of biologically active compounds in L. *cretaceae* and in which organ the substances accumulate. For use as a source of active substances, an extract from the roots, leaves and flowers of the species was prepared and the composition (Table 1) was studied in order to know exactly the amount of useful compounds in the composition and therapeutic and prophylactic properties.

Table 1

Bioactive compounds of vegetative organs of the plant extract L. *cretaceae*

№	Retention time, min	Connections	Probability of identification, %	Percentage content, %
1	10,32	Propanoic acid, 2-oxo-, methyl ester	87	1,57
2	11,54	3-Furaldehyde	88	0,38
3	12,55	2-Propanone, 1-(acetyloxy)-	69	0,16
4	12,85	Hexanoic acid	80	0,82

5	13,12	α-Phellandrene	85	1,01
6	13,74	4-Cyclopentene-1,3-dione	80	0,54
7	14,41	2-Cyclopenten-1-one, 2-hydroxy-	88	1,38
8	15,23	Phenol	82	0,49
9	15,56	Benzaldehyde	80	0,32
10	15,74	2-Furancarboxaldehyde, 5-methyl-	72	0,58
11	16,29	2(5H)-Furanone	79	0,47
12	18,14	1,3-Dioxol-2-one,4,5-dimethyl-	70	1,04
13	18,64	2-Hydroxy-gamma-butyrolactone	75	1,18
14	19,41	Phenol, 2-methoxy-	70	0,60
15	20,46	Carvenone	80	0,74
16	20,97	Ethanol, 2-(2-butoxyethoxy)-	67	0,68
17	21,46	Bicyclo[3.1.0]hexan-3-ol, 4-methylene-1-(1-methylethyl)-, (1α,3α,5α)-	80	0,84
18	21,57	Benzenemethanol, α,α,4-trimethyl-	87	0,81
19	22,08	Cyclopropyl carbinol	74	1,03
20	22,32	2-Undecanone	84	0,96
21	24,06	2,6-Octadienoic acid, 3,7-dimethyl-, methyl ester	80	0,52
22	24,68	Thymol	65	0,57
23	25,57	4-Octen-3-one, 6-ethyl-7-hydroxy-	71	2,90
24	26,19	2-Methoxy-4-vinylphenol	87	1,23
25	27,07	Hexadecane	85	0,52
26	27,38	Cetene	82	0,51
27	27,44	2-Tridecanone	83	0,89
28	28,50	3-Cyclohexene-1-methanol, α,α,4-trimethyl-, propanoate	77	0,51
29	30,15	Tetradecanal	78	0,49
30	31,42	(-)-Spathulenol	86	1,01
31	32,13	Sucrose	73	6,43
32	33,09	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	88	2,03
33	33,64	2-(3,4-Dimethoxyphenyl)-6-methyl-3,4-chromanediol	73	1,80
34	34,63	2-Pentadecanone, 6,10,14-trimethyl-	79	0,46
35	37,66	Hexadecanoic acid	86	1,38
36	39,15	4H-Indeno(2,1-d)thiazol-2-amine	67	0,56
37	39,84	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	70	0,94
38	41,07	Dibutyl phthalate	96	2,66
39	41,69	Falcarinol	90	16,51

40	44,61	2-Nonadecanone	71	6,66
41	44,80	Drometrizole	76	0,96
42	45,81	Hexanedioic acid, bis(2-ethylhexyl) ester	94	15,61
43	45,89	4,8,12,16-Tetramethylheptadecan-4-olide	68	2,36
44	46,84	2-Nonadecanone	69	1,14
45	47,95	2H-1-Benzopyran-2-one, 7-methoxy-6-(3-methyl-2-butenyl)-	87	8,67
46	51,54	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	68	0,44
47	51,83	Supraene	83	1,24
48	53,72	4-(3-Methyl-2-oxobutoxy)-7H-furo[3,2-g][1] benzopyran-7-one	72	1,30
49	55,22	7H-Furo(3,2-g)(1)benzopyran-7-one, 4-(2,3-epoxy-3-methylbutoxy)-, (S)-(-)-	78	4,11

On GC /MS analysis revealed qualitative and quantitative indicators of the main biologically active substances(surfactants) as part of the vegetative organs of the plant. As a result of phytochemical analysis of the L. *cretaceae* extract contains 49 known components. The percentage of Falcarinol is 16,51%, Hexanedioic acid, bis(2-ethylhexyl) ester 15,61% and LUP-20 (29)-En-3-ol, acetate, (3b) 2H-1-Benzopyran-2-one, 7-methoxy-6-(3-methyl-2-butenyl)-8,67% compounds (Table 1).

Most of the substances identified by the vegetative organs extract are essential oils, alcohols, and ketones. The minimum concentrations (< 10%) of many of the compounds listed in Figure 2 were determined.

The results of the study show that the vegetative organs of the L. *cretaceae* plant are rich in biologically active compounds in the form of essential oils, alcohols, alkaloids, steroids, terpenoids, flavonoids, aldehydes, ketones, carbohydrates and phenols. The percentage of chemical compounds in surface and underground organs is different (Figure 2).

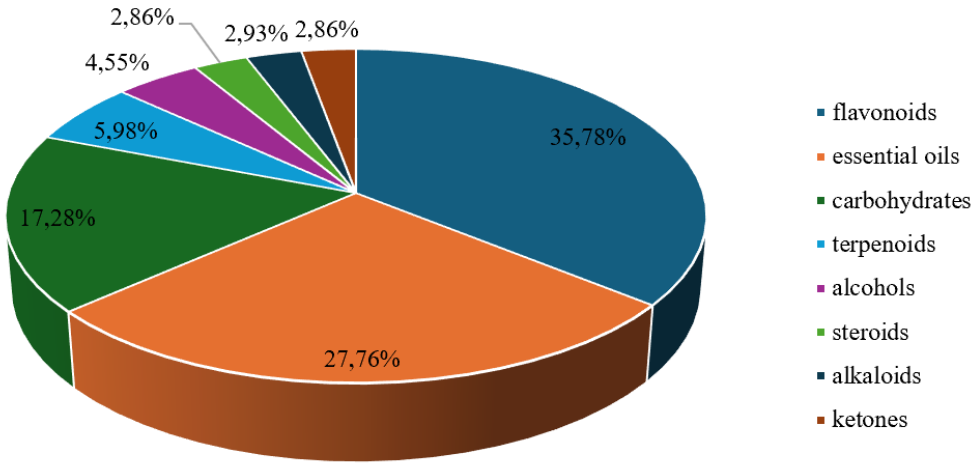

Figure 2. Biologically active compounds in L. *cretaceae* extract

While the L.cretaceae vegetative organs extract consists of 35.78% flavonoids and 27.76% essential oils of various amounts of chemical compounds. In addition to 17.28% carbohydrates and 5.98% terpenoids, alcohols are 4.55%, alkaloids are 2.93%, steroids are 2.86% and ketones are 2.86% below five percent (Figure 2,3).

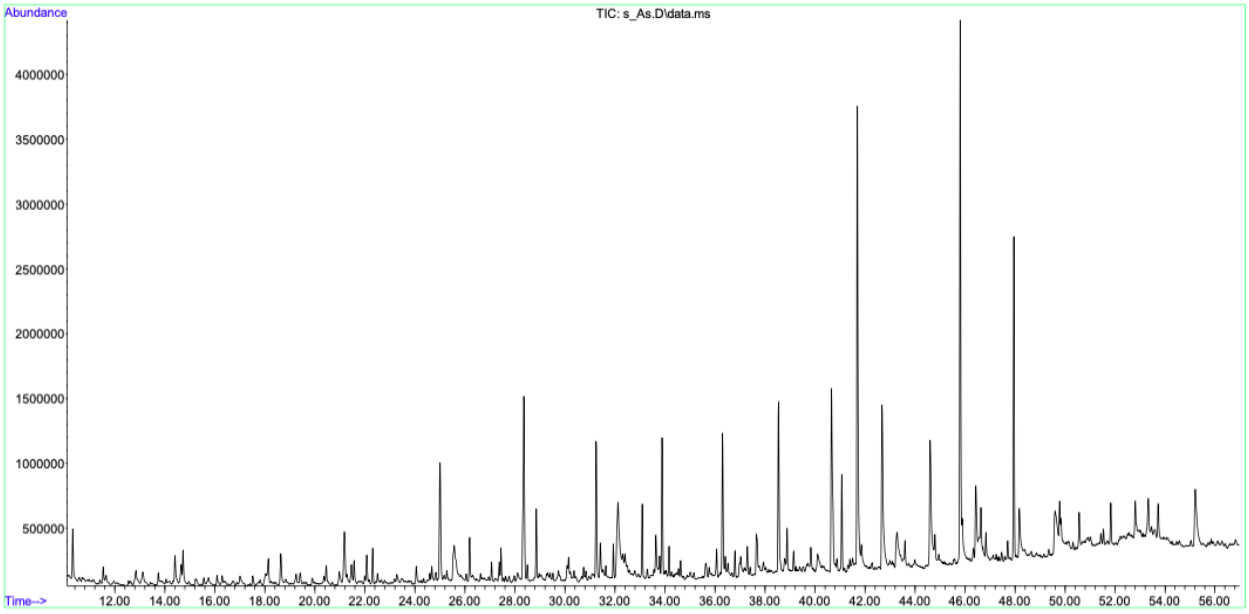

Figure 3. Chromatogram of the extract

The results show that the qualitative composition of the identified elements in the leaves, stems and roots of the plant is almost identical, while the quantitative content has minor differences depending on the population. By qualitative composition and quantitative composition of dry extracts of L. *cretaceae* leaves, stems and roots correspond to the raw material reserves.

Obtained from all three populations of 9 elements were identified in the chemical composition of the L.*cretaceae* plant (Figure 4). Various concentrations of carbon (C), oxygen (O), sodium (Na), magnesium (Mg), silicon (Si), sulfur (S), chlorine (Cl), potassium (K) and calcium (Ca) in the vegetative organs of plants perform certain physiological functions. These elements are presented in organs in varying amounts. Carbon and oxygen, which are the main macronutrients, are found in significant quantities in plant organs [11-15].

Chemical elements are characterized by a homogeneous qualitative composition, however, there is a difference in the quantitative content of individual components (Figure 4). Carbon ranks first in terms of the content of chemical elements in plant organs, its share is about 51.9-58.6% in roots, 61.0-63.9% in stems, and 55.47-78.9% in leaves. Oxygen is in second place, approximately 32.7-35.8% at the root, 28.5-35.1% at the stem and 23.6-35.8% for the sheet. Calcium is in third place, approximately 3.1-8.6% at the root, 1.4-3.8% at the stem, and 1.5-5% at the leaf. It can be seen that the elements Na, Mg, Si, S, Cl, and K are found in smaller amounts (0.1-4.2%). Nutritional elements are indicators of the soil and climatic conditions of the region. Collected from natural habitats of the Aktobe region L. *cretaceae*, the following conclusions can be drawn about the chemical composition and therapeutic properties of the plant [16-19].

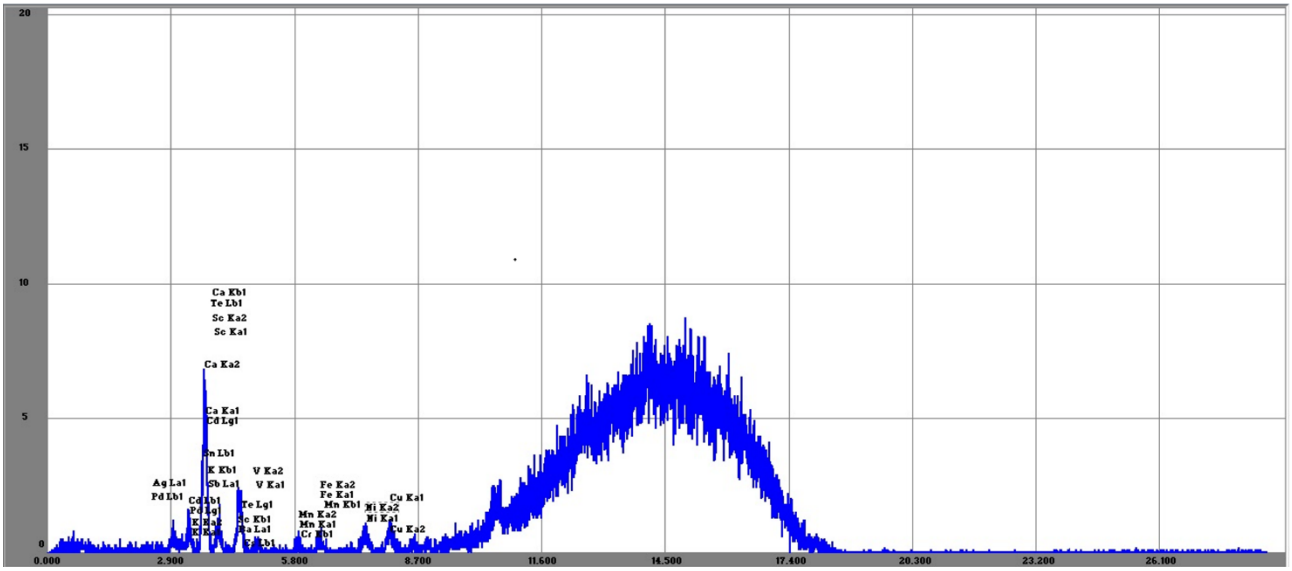

Figure 4. Element content in L. *cretaceae,* Ishkaragtau, mg/kg

Discussion

It was found that individual parts of the L. *cretaceae* species consist of different biologically active compounds in each. 49 specific biologically active compounds were found in the inflorescences, roots, and leaves of plants, which confirmed their pharmacological properties and demonstrated biological activity. These results indicate that various phytochemical groups and certain compounds have valuable beneficial properties (Table 1).

The analysis of literature information proves the medicinal properties and curative properties of bioactive compounds contained in the plant. Among the various compounds found in the inflorescence extract are anti-inflammatory, analgesic, antipyretic(1,6-Dioxaspiro[4.4]non-3-ene, 2-(2,4-hexadiynylidene, 9,12,15-Octadecatrienoic acid,2,3-dihydroxypropyl ester, (Z, Z, Z) and antimicrobial properties (Hexacosane, Lup-20 (29) - En-3-ol, acetate, (3b)) connections are present. In plant roots, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, 1, 6-Dioxaspiro [4.4]non-3-ene, 2-(2,4-hexadiynylidene), Bis (2-ethylhexyl) phthalate components exhibit antimicrobial, anti-inflammatory, and antioxidant properties, and their antibacterial properties are associated with the presence of caryophyllene oxide and squalene. Sucrose is used in the treatment of type 2 diabetes mellitus, as well as ethyl 9. cis., 11. trans. - octadecadienoate is used antitumor [20-24].

Along with antimicrobial antioxidant properties, as a local anesthetic, it has an analgesic effect, anti-inflammatory, antitumor, cytoprotective effect of gastric juice, Caryophyllene has platelet inhibition properties [25-27].

The element content reaches 3.1-8.6% at the root, 1.4-3.8% at the stem, and 1.5-5% at the leaf. It is noted that the number of other elements (Na, Mg, Si, S, Cl) and K (0.1-4.2%) is small. According to the available data, some chemical elements are absent in insignificant concentrations in most of the studied terrestrial and underground organs. For example, chlorine was not detected in the

roots of plants collected in the Akshatau region, while silicon was not detected in stems and leaves. In addition, magnesium and silicon were completely absent in the stems and leaves of plants of the planet species collected from the Ishkaragtau population. In contrast, all the elements were found in all the studied vegetative organs of the Virginian individuals collected in the Bestau area. The data presented in the tables of the study of the virgin state of plants show that the leaves, roots and stems contain the elements C, O, Na, Mg, Si, Cl, S, K, Ca.

Conclusion

Phytochemical analysis of the terrestrial and underground organs of L. *cretaceae* revealed the presence of various classes of biologically active compounds, including carbohydrates, essential oils, alkaloids, steroids, terpenoids, alcohols, flavonoids, aldehydes, phenolic compounds and ketones. Most of the identified basic and auxiliary chemical compounds determine the pharmacological action of the plant.

The reserves of 49 active substances found in the leaves of the plant compared to the roots and inflorescences confirm in medicine useful medicinal properties in the treatment of flu and cough symptoms, against expectoration, inflammatory diseases and microbes. The predominance of natural compounds of camphor, 1,8-cineol, camphene, borneol in the composition of essential oil can serve as a basis for use as a medicine. It has been shown that extracts obtained on the basis of leaves, roots, and stems of plants are a promising source of macro-and microelements. The use of only leaves without damage to roots and inflorescences in the isolation of natural compounds in the composition of plant organs for folk and medical care allows not only to rationally use an endangered species, but also to preserve a rare species. Further research is needed to fully describe the period of life and time of year when biologically active compounds and elements are preserved in the plant's composition in different parts of the species.

Among the organic compounds detected in ethanol extracts, falcarinol (with a relative content of 90%) and thymol (with a relative content of 65%) were identified with the highest reliability (up to 90%). These substances are valuable biologically active components used in medicine. As unsaturated compounds, they can be classified as natural antioxidants. For example, falcarinol is found in the roots of American ginseng. Thymol, which is part of the structural components of cell membranes, also acts as a phenolic antiseptic with pronounced antimicrobial and antifungal activity against a number of microorganisms.

In biological samples obtained from an endemic species *Linaria cretacea*, endogenous organic substances were found, among which valuable compounds are present, including falcarinol and thymol.

The obtained data represent a promising result and can be used in the future to develop technologies for obtaining valuable biologically active substances of Linaria cretacea Fisch ex Spreng biomass.

Author Contributions

M.K., A.T. – concept and supervision of the work**; M.B. –** conducting the experiments; **T.A. –** discussion of the research results; **M.B. –** writing the text; **A.B.** and **N.U. –** editing the text of the article.

Conflicts of Interest

The authors declare no conflicts of interest.

Compliance with ethical standards

This article does not contain a description of studies performed by the authors involving people or using animals as objects.

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Linaria cretacea Fisch ex Spreng. (Plantaginaceae) фитохимиялық және элементтік құрамы

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Аңдатпа. Мақалада Қазақстан Республикасының сирек кездесетін *Linaria cretacea* Fisch ex Spreng нүрін фитохимиялық зерттеу нәтижелері келтірілген. Өсімдіктердің гүлшоғырларында, тамырларында және жапырақтарында болатын ерекше биоактивті қосылыстар биоактивтілікке ие фармакологиялық қасиеттерді растады және көптеген фитохимиялық топтар мен арнайы қосылыстардың пайдалы қасиеттері бар екенін көрсетті. L. *cretacea* құрамында этанол сығындыларында кездесетін органикалық қосылыстардың жалпы санының ішінде фалкаринол (салыстырмалы құрамы 90%) және тимол (салыстырмалы құрамы 65%) ең жоғары сенімділік дәрежесімен (90% дейін) анықталды. Бұл медицинада қолданылатын құнды биологиялық белсенді заттар. Қанықпаған қосылыстар болғандықтан, оларды табиғи антиоксиданттар қатарына жатқызуға болады. Мысалы, фалькаринол американдық женьшеньдің тамырында кездеседі. Жасуша мембраналарының құрылымдық компоненттерінің құрамына кіретін тимол сонымен қатар бірқатар микроорганизмдерге қарсы айқын микробқа қарсы және саңырауқұлаққа қарсы белсенділігі бар фенолды антисептик болып табылады. Алынған нәтижелер перспективалы болып табылады және одан әрі *Linaria cretacea* Fisch ex Spreng биомассасынан құнды биологиялық белсенді заттарды алу технологиясын жасау үшін пайдаланылуы мүмкін.

Түйін сөздер: *Linaria cretacea* Fisch.ex Spreng., эндемик, құрылымдық компоненттер, кальцефиттер қауымдастығы, фитохимиялық талдау

Фитохимический и элементный состав *Linaria cretacea* Fisch ex Spreng (Plantaginaceae)

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Аннотация. В статье представлены результаты фитохимического исследования редкого для Республики Казахстан вида Linaria cretacea Fisch ex Spreng. Специфические биологически активные соединения, содержащиеся в соцветиях, корнях и листьях растений, подтвердили

фармакологические свойства, обладающие биологической активностью, и показали, что многочисленные фитохимические группы и специальные соединения обладают полезными свойствами. Из общего числа органических соединений, обнаруженных в этанольных экстрактах, фалькаринол (относительное содержание 90%) и тимол (относительное содержание 65%) были идентифицированы с наибольшей степенью достоверности (до 90%) в составе *L. cretacea.* Это ценные биологически активные вещества, которые используются в медицине. Являясь ненасыщенными соединениями, они могут быть отнесены к категории природных антиоксидантов. Например, фалькаринол содержится в корнях американского женьшеня. Тимол, входящий в состав структурных компонентов клеточных мембран, также является фенольным антисептиком с выраженной антимикробной и противогрибковой активностью в отношении ряда микроорганизмов. Полученные результаты являются многообещающими и могут быть в дальнейшем использованы для разработки технологии получения ценных биологически активных веществ из биомассы *Linaria cretacea* Fisch ex Spreng.

Ключевые слова: *Linaria cretacea* Fisch.ex Spreng., эндемик, структурные компоненты, сообщество кальцефитов, фитохимический анализ

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