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PHYTOCHEMICAL PROFILE OF UNDERGROUND PART OF *CICHORIUM INTUBYS* L.

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Abstract. *Cichorium intybus* L. (chicory) is one of the medicinal plants widely used in pharmaceutical and traditional medicine due to its high content of bioactive compounds. One of its key components is inulin, a prebiotic polysaccharide present in significant amounts, which has a beneficial effect on gut microbiota. This study focuses on the quantitative and qualitative analysis of bioactive compounds and mineral elements in the underground parts of *C. intybus* L. Moisture, ash, and extractive content were evaluated to determine the plant material's chemical profile. The main identified bioactive compounds included organic acids, flavonoids, polysaccharides, coumarins, tannins, and vitamin C. The total content of bioactive substances, which high concentrated, was determined as follows: coumarins – 1.77 %, polysaccharides – 1.72 %, tannins – 1.79 %. The mineral composition of the plant material was analyzed using spectrophotometric methods, confirming the presence of essential elements such as calcium (4.47788 mg/g), magnesium (1.17590 mg/g), iron (0.16184 mg/g), and

potassium (13.56730 mg/g), which contribute to its nutritional and therapeutic value. According to the data, by Gas Chromatography-Mass Spectrometry (GC-MS) analysis 49 compounds have been identified in the petroleum ether fraction. GC-MS analysis determined the dominant fatty acids, including 9,12-octadecadienoic acid, hexadecanoic acid, and their derivatives, which are known for their antioxidant, anti-inflammatory, and hepatoprotective properties. The obtained results confirm the high medicinal potential of *C. intybus* L., highlighting its promising application in the development of pharmaceuticals, nutraceuticals, and functional food products.

Keywords: *Cichorium intybus* L., phytochemical constituents, bioactive compounds, , quantative and qualatative analysis, macro-microelements, GC-MS.

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CICHORIUM INTYBUS L. ӨСІМДІГІНІҢ ЖЕР АСТЫ БӨЛІГІНІҢ ФИТОХИМИЯЛЫҚ ПРОФИЛІ

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Аннотация. *Cichorium intybus* L. (цикорий) – құрамында биоактивті қосылыстардың көп болуына байланысты фармацевтикада және дәстүрлі

медицинада кеңінен қолданылатын дәрілік өсімдіктердің бірі. Оның негізгі компоненті – инулин, едәуір мөлшерде болатын пребиотикалық полисахарид, ол ішек микробиотасына пайдалы әсер етеді. Бұл зерттеу *C. intybus* L. өсімдігінің жерасты бөлігіндегі биоактивті қосылыстар мен минералды элементтердің сандық және сапалық талдауына бағытталған. Өсімдік материалының химиялық профилін анықтау үшін ылғалдылық, күлділік және экстрактивті заттар мөлшері анықталды. Негізгі анықталған биоактивті қосылыстарға органикалық қышқылдар, флавоноидтар, полисахаридтер, кумариндер, тері илегіш заттар және С дәрумені кіреді. Жоғары концентрациядағы биоактивті заттардың жалпы мөлшері келесідей анықталды: кумариндер – 1.77 %, полисахаридтер – 1.72 %, тері илегіш заттар – 1.79 %. Өсімдіктің тамыр бөлігінің минералдық құрамы спектрофотометриялық әдістерді қолдану арқылы талданды. Бұл кальций (4.47788 мг/г), магний (1.17590 мг/г), темір (0.16184 мг/г) және калий (13.56730 мг/г) сияқты маңызды элементтердің болуын растайды, олар оның нутритикалық құндылығына және оның пайдалы қасиеттеріне ықпал етеді. Деректерге сәйкес, газ хроматографиясы-масс-спектрометрия (ГХ-МС) талдауы арқылы мұнай эфир бөлігінде барлығы 49 қосылыс анықталды. ГХ-МС талдауы антиоксиданттық, қабынуға қарсы және гепатопротекторлық қасиеттерімен танымал 9.12-октадекадиен қышқылы, гексадекан қышқылы және олардың туындылары сияқты май қышқылдарының концентрациясы жоғары болды. Алынған нәтижелер *C. intybus* L.-ның жоғары емдік қасиетін растайды және оның фармацевтикалық, нутрацевтикалық және функционалды тамақ өнімдерін дамытудағы перспективалы қолданылуын көрсетеді.

Түйін сөздер: *Cichorium intybus* L., фитохимиялық құрамдас бөліктер, биоактивті қосылыстар, сандық және сапалық талдау, макро-микроэлементтер, ГХ-МС.

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ФИТОХИМИЧЕСКИЙ ПРОФИЛЬ ПОДЗЕМНОЙ ЧАСТИ *CICHORIUM INTYBUS* L.

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Аннотация. *Cichorium intybus* L. (цикорий) – одно из лекарственных растений, широко применяемых в фармацевтике и народной медицине благодаря высокому содержанию биологически активных соединений. Его основным компонентом является инулин - пребиотический полисахарид, присутствующий в значительных количествах и оказывающий благотворное влияние на микробиоту кишечника. Данное исследование было сосредоточено на количественном и качественном анализе биологически активных соединений и минеральных элементов в подземной части растения *C. intybus* L. Для определения химического профиля растительного материала определяли содержание влажности, зольности и экстрактивных веществ. Основные выявленные биоактивные соединения включают органические кислоты, флавоноиды, полисахариды, кумарины, дубильные вещества и витамин С. Общее количество биологически активных веществ в высоких концентрациях составило: кумарины – 1.77 %, полисахариды – 1.72 %, дубильные вещества – 1.79 %. Минеральный состав корня растения анализировали спектрофотометрическими методами. Это подтверждает наличие важных элементов, таких как кальций (4.47788 мг/г), магний (1.17590 мг/г), железо (0.16184 мг/г) и калий (13.56730 мг/г), которые обуславливают его пищевую ценность и полезные свойства. Согласно полученным данным, методом газовой хроматографии-масс-спектрометрии (ГХ-МС) во фракции петролейного эфира было идентифицировано 49 соединений. Анализ ГХ-МС выявил высокие концентрации жирных кислот, таких как 9,12-октадекадиеновая кислота, гексадекановая кислота и их производные, которые известны своими антиоксидантными, противовоспалительными и гепатопротекторными свойствами. Полученные результаты подтверждают высокий терапевтический потенциал *C. intybus* L. и указывают на перспективность его применения при разработке фармацевтических, нутрицевтических и функциональных пищевых продуктов.

Ключевые слова: *Cichorium intybus* L., фитохимические компоненты, биологически активные соединения, количественный и качественный анализ, макро-микроэлементы, ГХ-МС.

Introduction.

In modern pharmaceutical science, creating medicinal products from medicinal plant materials is considered one of the promising trends. Such medicinal products are known

for their safety and pharmacological effectiveness, comparable to synthetic drugs. One widely used medicinal form today is extracts from medicinal plant materials. They are widely used in both the pharmaceutical industry and cosmetic activities. According to the literature, one such plant is *Cichorium intybus* L. The relevance and role of extracts from these plants are evidenced by the list of 40 registered extracts from plant materials in the State Register of the Republic of Kazakhstan (Tynybekov, 2011).

Cichorium intybus L. is a plant widely distributed throughout the world. From ancient times to the present, chicory has been considered a valuable medicinal plant. Its raw materials are used to produce various medicines. The roots of *Cichorium intybus* L. are used in medicine, less often the aboveground part and inflorescences of the plant.

In domestic medicine, antioxidant, anti-inflammatory, neuroprotective, and antitumor properties of chicory have been identified. *Cichorium intybus* L. is recommended for the treatment and prevention of atherosclerosis, disorders of mineral metabolism, osteochondrosis, gout, pancreatic diseases, joint diseases, and the cardiovascular system. Also, the raw material *Cichorium intybus* L. shows a pronounced therapeutic effect in the prevention of diabetes mellitus and its treatment: it reduces blood glucose levels and normalizes the metabolic process in the body. The properties of chicory raw materials to help with the above diseases are due to the biologically active substances (BAS) of the plant (Singh et al, 2019; Balzarini et al, 2018).

Cichorium intybus L. is often a perennial or biennial plant, less commonly an annual herb, with short-stalked, toothed, entire or arcuate-pinnately-lobed leaves that are usually basal and form a rosette. The aboveground and underground parts of this plant are often used in both folk and scientific medicine as a means with hepatoprotective, antioxidant, and hypoglycemic functions (Ahmed et al, 2011; Mehmood et al, 2012).

Chicory is a plant of the *Asteraceae* family. It has a woody character, reaching a height of 20 to 1.5 meters, with about 1 meter attributed to its branched herbaceous part. The root system consists of a taproot that deeply penetrates the soil. The stem is erect, with few branches and a thickened apex. The leaves gradually change their structure as they approach the root: the upper leaves are small and entire, while the lower leaves resemble basal leaves. The leaf shape varies from lanceolate to lanceolate-ovate. The plant is covered in glandular hairs.

Figure 1 – *Cichorium intybus* L. underground and aboveground parts

Figure 1 represents the plant's flowers, which are bright blue and occasionally white and measure 15 to 25 mm in length. They are bisexual, toothed, and have short stalks. The flower heads are grouped together or occur singly on lateral branches or the apex. Sometimes they can also be found in the axils of upper and middle stem leaves. Inflorescences formed in the axils of upper leaves, numbering from 1 to 2, are located on short peduncles and have a diameter of 2.5-4.5 cm. The fruit of the plant is a small seed with a slightly ribbed, inversely ovate structure. The seeds are yellowish-brown and measure 2-3 mm in length, with a truncate upper part and a tuft of hairs measuring 0.2-0.3 mm.

The main habitat conditions for chicory are river floodplains, meadows, forest clearings, as well as rocky and grassy slopes up to 2000 meters in altitude. It can also be found as a weed in gardens, cultivated fields, and meadows. The plant usually blooms from June to October, and fruiting occurs from July to October (Street et al, 2013; Aisa et al, 2020; Saybel et al, 2020; Janda et al, 2021).

Relevance of the research topic. Currently, one of the urgent tasks of pharmaceutical science in the Republic of Kazakhstan is the creation and introduction of import-substituting medicines, including medicines from plant raw materials. Creation and introduction of competitive import-substituting medicines, on the one hand, will contribute to the successful implementation of the State Program on Forced Industrial and Innovative Development of the Republic of Kazakhstan from 25.12.2014 № 984 (with amendments and additions), and on the other hand, will ensure the security of the country. Moreover, in the first quarter of 2024, 74% of sold packages and 87% of the value of pharmacy sales were imported drugs. Accordingly, the share of domestic drugs was 26% in physical terms and only 13% in monetary terms. It is important to note that this ratio has not changed significantly in recent years. The relevance of such research is also due to the fact that according to the WHO forecast, in 15-20 years the share of phytopreparations in the total assortment of medicines may increase to 60%. This forecast is explained by the WHO strategy in the field of traditional medicine.

In this research, medicinal herb *Cichorium intybys* L. was determined authencity of plant by moisture, total ash and extractive content. Futhermore, it was studied for its quantative composition of biologically active constituents, specially organic acids, flavonoids, ploysaccharides, coumarins, tannins and vitamin C. During this work, the content of macro-microelements in total plant was used spectrophotometer technique.

Materials and methods.

Plant material. Chicory (*Cichorium intybus*) roots were purchased from Jiangsu Yabang Chinese Medicine Co., Ltd., Jiangsu, China. The voucher specimen of the plant material (RINPT - 202403) was deposited at the Research Institute for Natural Products & Technology, Almaty, Kazakhstan.

The quantitative and qualitative analysis. The quantitative and qualitative analysis of main bioactive compounds of underground part of *Cichorium intybus* L. was accorded to methods reported in the monograph (Muzychkina R.A., 2004). The extractive content of root of *C. intybus* L. was identified by using 96% ethanol solution in accordance with methods reported in the State Pharmacopeia [State Pharmacopoeia of the RK, 2014].

Mineral composition analysis. The mineral composition was determined using a Shimadzu 6200 series spectrophotometer. First, 3 g of raw material was precisely weighed and placed in a pre-calcined ceramic crucible. The material was gently heated, allowing it to burn at the lowest possible temperature before gradually increasing the flame. A temperature of 500°C was used to achieve a consistent mass during calcination. The crucible was cooled in a desiccator and the resulting ash was further burned at 600°C until it turned a gray color. The plant ash was then dissolved in 10.0 mL of 40% nitric acid by heating and the solution was boiled to get wet salts. Then, it was dissolved in 15.0 mL of 1 N nitric acid and moved into a 25.0 mL analytical volumetric flask.

Gas Chromatography-Mass Spectrometry (GC-MS) determination. To the crude ethanol extract was added water and dissolved at room temperature. The solution then was transferred to a separation funnel, where petroleum ether was added in a 1:1 ration three times. After mechanically shaking the mixture and allowing it to stand a certain amount of time, the petroleum ether part was separated from the aqueous layer and dried using an EYELA N-1300 rotary evaporator at 35 ºC to obtain the desired concentrate. Then, same method was repeated with dichloromethane.

The samples were analyzed using gass chromatography-mass spectrometry (7890A/5975C). The gas chromatography system was controlled and the data was recorded and processed using Agilent MSD ChemStation software (version 1701EA). The data processing involved determining retention times, peak areas and analyzing the spectral information from the spectrometric detector. The Wiley 7th edition and NIST'02 libraries, containing over 550000 spectra, were used to interpret the obtained mass spectra.

Results and discussion. The data in the table 1 and 2 present a detailed quantitative and qualitative analysis of the biologically active constituents and mineral composition of *Cichorium intybus* L.

Table 1 – Quantitative and qualitative analysis of main biologically active constituents of *C.intybus* L. (%)

Plant	Moisture	Ash	Extractives (50%)	Organic acids	Flavonoids	Polysaccharides	Coumarins	Tannins	Vitamin C
***Cichorium intybus* L.**	7.10	7.50	48.20	0.07	0.01	1.72	1.77	1.79	0.57

The moisture content of the plant is 7.10%, while the ash content is 7.50%. A significant amount of extractive substances, measured at 48.20, was obtained using a 50% ethanol mixture, indicating a high solubility of plant compounds in these conditions.

Organic acids were present at 0.007%, while flavonoids, a class of compounds known for their antioxidant properties, were found in small amounts – 0.01% (Cook et al, 1996). Polysaccharides, known for their roles in energy storage and cell structure, contributed 1.72% to the total plant composition. The vitamin C content is 0.57%.

Other notable bioactive compounds include coumarins (1.77%) and tannins (1.79%), both recognized for their therapeutic applications. Coumarins are a significant class of low-molecular-weight phenolic compounds. These natural substances are found in various plant sources and have been widely applied in the prevention and treatment

of numerous diseases. They exhibit a range of biological activities, including anti-inflammatory, antioxidant, anticancer, antiviral and anticoagulant properties, making them highly promising in pharmaceutical applications (Kostova et al, 2011; Matos et al, 2015; Khai, 2024). While, tannins are a group of naturally occurring polyphenolic compounds found in a variety of plants. They are known for their ability to bind and precipitate proteins, which contributes to the astringent taste of foods and drinks such as wine, tea, and some fruits. They also exhibit a variety of biological activities, including antibacterial property and have been studied for their potential health benefits, particularly in the prevention of cardiovascular disease and cancer (Farha et al, 2020; Das et al, 2020).

Table 2 – Composition of macro-microelements in the plant (mg/g)

Elements	Concentration in plant, mg/g
Cd	0.00052
Ni	0.00343
Pb	0.00204
Cu	0.00477
Mn	0.02669
Zn	0.01453
Fe	0.16184
Ca	4.47788
Mg	1.17590
Na	0,40810
K	13.56730

Table 2 outlines the concentrations of macro-microelements found in chicory. Among the trace elements, cadmium (Cd), nickel (Ni) and lead (Pb) were found in very low quantities, at concentrations of 0.00052 mg/g, 0.00343 mg/g and 0.00204 mg/g, respectively. Copper (Cu), manganese (Mn) and zinc (Zn), which are important micronutrients for plant health, were present at concentrations of 0.00477 mg/g, 0.02669 mg/g and 0.01453 mg/g.

Iron (Fe), a crucial element for photosynthesis and respiration, was present at 0.16184 mg/g. Among the macronutrients, calcium (Ca) and magnesium (Mg) were found in significant amounts with concentrations of 4.47788 mg/g and 1.17590 mg/g in the plant. Sodium (Na) was measured at 0.40810 mg/g and potassium (K), a vital element for plant cellular processes, was the most abundant with a concentration of 13.56730 mg/g.

The mineral composition analysis provided further insight into the plant's therapeutic potential. The presence of essential macronutrients such as calcium (4.47 mg/g), magnesium (1.17 mg/g), and potassium (13.56 mg/g) highlights *C. intybus* L.'s contribution to bone health, metabolic functions, and cellular processes. The presence of micronutrients such as iron (0.16 mg/g), zinc (0.014 mg/g), and copper (0.004 mg/g) underscores the plant's potential role in supporting immune function, promoting healthy skin, and contributing to enzymatic processes within the body.

Table 3 – The most concentrated compounds of *C. intybus* L.'s petroleum ether part

№	Retention time, min	Compounds	Content, %
1	24,74	1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)-	1,02
2	25,83	Hexadecanoic acid, ethyl ester	7,76
3	29,29	Ethyl Oleate	4,56
4	30,13	9,12-Octadecadienoic acid, ethyl ester	10,48
5	30,91	9,12,15-Octadecatrienoic acid, ethyl ester	2,70
6	31,99	Dibutyl phthalate	1,71
7	32,54	cis-11-Eicosenoic acid, methyl ester	1,77
8	35,43	Hexadecanoic acid	15,42
9	35,64	Docosanoic acid, ethyl ester	2,65
10	39,24	9,12-Octadecadienoic acid	17,97
11	39,56	Gamolenic Acid	2,03
12	39,92	9,12,15-Octadecatrienoic acid	8,26
13	40,74	Eicosanoic acid	1,41
14	41,77	Arborescin	2,77
15	43,26	Docosanoic acid	2,59
16	43,52	Erucic acid	1,37
17	45,09	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	4,33
18	47,92	γ-Sitosterol	1,65

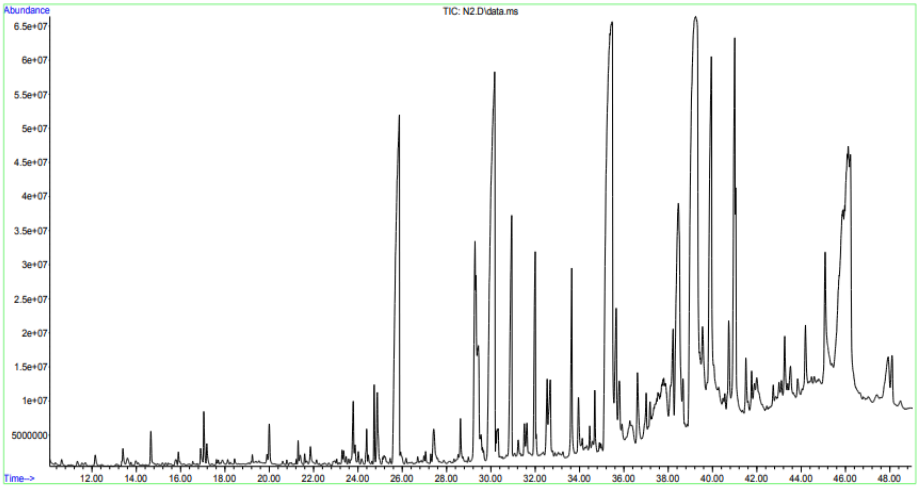

Figure 2 – Chromatogram of petroleum ether part of *C. intybus* L

Additionally, during the study, petroleum ether (PE) fraction of underground part of this medicinal plant were extracted using ultrasonic extraction and subsequently analyzed via gas chromatography with mass scpectrometry. The chromatogram for PE

part is presented in Figure 2. The main compounds were identified and categorized among all the detected liposoluble components as shown in Table 3.

Generally, according to the data, there are 49 compounds in PE part. According to the information in Table 3, there are three components with higher concentration, which are 9,12-Octadecadienoic acid, ethyl ester, hexadecanoic acid and 9,12-Octadecadienoic acid and they all categorized as fatty acids.

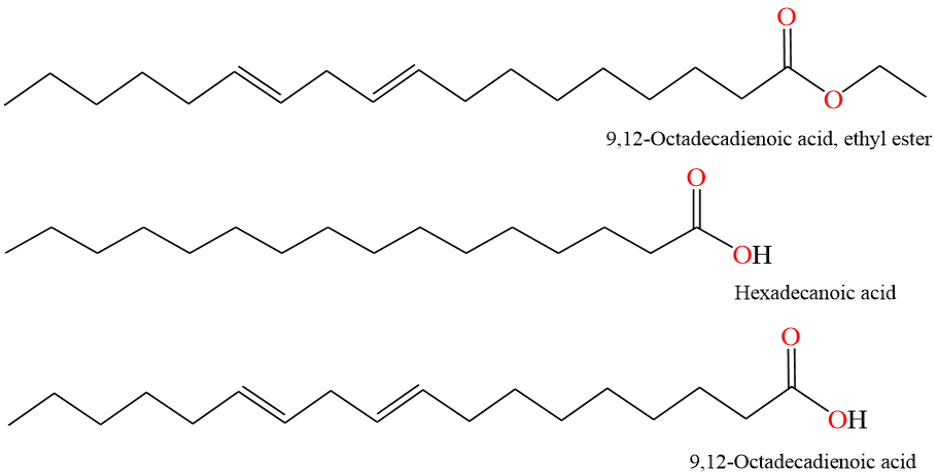

Figure 3 – Chemical structure of three fatty acids in PE part of *Cichorium intybus* L.

9,12-Octadecadienoic acid, ethyl ester is polyenoic fatty acid and noted for its cholesterol-lowering properties. Furthermore, it has protective effect against liver damage (Garuba et al, 2020).

Hexadecanoic acid, also known as Palmitic acid, is the common saturated fatty acid in human body. This compound attracts with antimicrobial, antioxidant and 5-alpha-reductase inhibitory properties (Olivia et al, 2021).

9,12-Octadecadienoic acid or Linoleic acid – is the most popular polysaturated fatty acid, which is necessary for human organism and shows several activities. For example, the latest studies outlined their anti-inflammatory and neuroprotective actives in Parkinson's disease models (Castañeda-Corral et al, 2024)

Conclusion

The present study thoroughly investigated *Cichorium intybus* L., focusing on its bioactive constituents and elemental composition to evaluate its medicinal potential. Chicory has been recognized since ancient times for its therapeutic uses, and its wide distribution makes it a valuable resource in both traditional and modern medicine. The results obtained from the quantitative and qualitative analysis confirm that *Cichorium intybus* L. contains numerous biologically active compounds, including organic acids, flavonoids, polysaccharides, coumarins, tannins, and vitamin C, which significantly contribute to its medicinal value. The moisture content of the plant was found to

be relatively low, indicating good storage stability, while the ash content suggests a substantial presence of mineral constituents. The most notable among the bioactive compounds were coumarins and tannins, present in concentrations of 1.77% and 1.79%, respectively. The mineral composition analysis provided further insight into the plant's therapeutic potential. The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of *C. intybus* L.'s petroleum ether extract further enhanced the understanding of its medicinal potential. Among the identified compounds, 9,12-Octadecadienoic acid, Hexadecanoic acid, and their derivatives were the most concentrated. Therefore, *Cichorium intybus* L. has the potential to serve as a natural ingredient for the creation of multifunctional pharmaceuticals and bioactive supplements.

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