Химия растительного сырья. 2024. №1. С. 148–155.
Khimiya rastitel'nogo syr'ya, 2024, no. 1, pp. 148–155.
DOI: 10.14258/jcprm.20240112476

UDC 581.192:582.998.2:543.421/424

CHROMATOGRAPHIC AND SPECTRAL STUDY OF *ARCTIUM LAPPA* AND *ARCTIUM TOMENTOSUM* FRUCTUS CULTIVATED IN ALTAI*

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Burdock is an ingredient of official and traditional medicines in different countries, its roots are used for food, biologically active additives, functional nutrition products, fertilizers, etc. A great demand led to the situation when many countries including Russia began the plant's cultivation. Despite that, the aboveground parts of the plant including its fructus still remain expendable. At the moment, burdock fructus are officially used only in China and Japan. In Russia they are not used and studies of their chemical composition and pharmacological properties are scarce.

The research showed that fructus of *Arctium lappa* and *A. tomentosum* cultivated in Altai using by traditional way of growing, and new of agrotechnics of cultivation contain lignans, hydroxycinamic acid, fatty acids and their derivatives, polysaccharides and anthocyanin's. The use of a new cultivation technology increased the content of lignans and other BAS by 10–25%. Hydroxycinamic acid is one of the dominant substances in fructus by content, therefore it is possible to standardize raw material according to this parameter. The presence of methyl cis-9, cis-15-octadecadienoate and 11-Eicosenoic acid methyl ester in fructushas been discovered for the first time. Ethyl linoleate and methylfolate or methyl cis-9-octadecenoate are found to be dominant components of fatty acids and their derivatives.

Additional studies of chemical composition, pharmacological properties, clinical approbation are needed to obtain evidence of the efficiency of burdock fructus utilization for clinical purposes and prevention.

Keywords: cultivate, burdock fructus, methyl-cis-9,cis-15-octadecadienoate, 11-eicosenoic acid methyl ester, hydroxycinnamic acid, anthocyanin's.

For citing: Kolomiets N.E., Boev R.S., Zhalnina L.V., Ali Abdujalil Kaid Hasan. *Khimiya Rastitel'nogo Syr'ya*, 2024, no. 1, pp. 148–155. (in Russ.). DOI: 10.14258/jcprm.20240112476.

Introduction

Burdock (Arctium) is an easily recognizable genus of plants of the Asteraceae family by its leaves which are awl-shaped and hooked on the edges. These plants grow in different climatic conditions, some develop significant thickets. According to the updated data, the Arctium genus includes 19 species that are found in Russia, Asia, Europe, the USA, Uruguay, Argentina, the Himalayas, China, and Japan [1, 2].

Some types of burdock are used for food, as ingredients for bread making, or added when cooking side dishes, soups, and in production of functional nutrition products, food additives for humans and animals, fertilizers, plant protection products or used as a natural remedy for prevention and treatment of various ailments. Therefore, common burdock is cultivated in such places as North America, Europe, Australia, China, the Philippines, and Vietnam. Some authors emphasize the great expediency of cultivating burdock due to difficulties related to harvesting roots of its wild species and the effectiveness of applied agrotechnical measures which make it possible to grow significant biomass in a much shorter time than in nature [2–5].

Altai Krai is the largest agricultural region of Russia located in the south-east of Western Siberia. Chernozem-rich soils are concentrated here; there is a sufficient amount of precipitation and a temperate climate which allow developing ecological types of production (beekeeping, animal husbandry, horticulture including medical plants).

* This article has electronic supplementary material (appendix), which is available to readers on the journal's website. DOI: 10.14258/jcprm.20240112476s.

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There are several manufacturing companies in Altai Krai that grow roots of *Arctium lappa, Arctium tomentosum* for the needs of pharmaceutical and para-pharmaceutical industries. During production processes, a large amount of aboveground phytomass is wasted as it is currently not utilized in Russia [2, 4].

As of now, the biological activity of the Arctium genus species has been studied to different degrees. Pharmacological properties of *Arctium lappa* have been the most studied so far with only a few separate publications on *Arctium nemorosum*, *Arctium palladinii*, *Arctium tomentosum* and *Arctium minus*, while the remaining species of the genus have not been studied at all.

There is evidence of antioxidant, anti-inflammatory, estrogenic properties, anti-allergic, schistomicidal, antiherpetic effects, strengthening of the intestinal barrier function of *A. lappa* fructus arctigenin. Cytotoxic properties of arctiin, arctigenin and other lignans are shown *in vitro* and in vivo on cell cultures and grafted tumours. Arctiin is an effective treatment of glomerulonephritis and has radio-protective properties. The mucus of fructus has a therapeutic effect on dryness, itching and skin burns, improves trophism, and prevents the formation of wrinkles [2, 5–7, 9–11].

Infusion of *A. tomentosum* seeds in all models of ulceration shows a pronounced gastro-protective effect and seeds' extract leads to significant changes in the functional activity of the stomach and an increase in protective properties of the supra-epithelial mucous layer of the small bowel [12, 13].

The chemical composition of *Arctium lappa* has been the most studied so far, while *Arctium leiospermun*, *Arctium minus* and *Arctium tomentosum* have been studied only partly. The chemical composition of the other species has not been studied yet [2, 7, 14–22].

Fatty oils represented by hydroxy acids; epoxy acids (9.10-epoxy-cis-octadeca-12-enoic acid; myristic, palmitic, stearic, oleic, linoleic acids; triacylglycerols of coronary, vernolic, cis-9, 10-epoxyoctadecanoic and trans-9.10-epoxyoctadecanoic acids; higher aliphatic triterpene alcohols, steroids, acylglycerols were found in fructus of *Arctium* tomentosum Mill. [2, 17, 22, 23]. Fatty oil is partially hydrolyzed and contains four free fatty acids one of which can be 3 (t), 9 (c), 12 (c)–octadecatrienoic acid [23]. Flavonoids (luteolin, quercetin rhamnoside, quercitrin, quercetin), caffeic acid, chlorogenic acid, gallic acid, caffeylquinic acid, cinnamates, 14 lignans, organic acids (malic, citric), bitter alkaloid lappin, saponins, coumarins, isochlorogenic acid, sesquiterpene lactones were also found. In 2016 arctieseskineolignan B and arctiphenol glycoside A were extracted from the fructus extract [2, 7, 8, 24–28].

In Russia, studies of pharmacological properties and chemical composition of the fructus of common burdock and woolly burdock are sporadic [2, 8, 12]. Therefore, studying them is still essential, together with the study of pharmacological properties, pre-clinical and clinical research of fructus-based drugs in order to introduce them into medical practice.

Materials and methods

Harvesting, identification, and preparation of plant materials. The object of the research was the fructus harvested in the autumn during fruiting season of 2020–2021 from cultivated *Arctium lappa* L. and *Arctium tomentosum* Mill. Cultivated *Arctium lappa* and *Arctium tomentosum* were harvested at the production site of LLC "Wisterra" (Altai Krai, Russia).

During plant raw materials' harvesting, areas with similar conditions of shading/illumination and moisture were selected. Harvesting was carried out during the same part of the day. The authenticity of samples was proven by the Pharmacy Department of Kemerovo State Medical University, Russia.

The new agrotechnical measures applied at the experimental sites consisted in pre-sowing seed treatment, care of crops and seedlings, and the introduction of nutrient solutions into the soil. Currently, the methodology used by us is undergoing the patenting procedure.

Extraction of fructus samples for examination by TLC, HPLC, GS-MS methods. About 4100 g of dried crushed fructus of each species were extracted in a conical flask with a reverse refrigerator for1 hour three times 12 liters of 1 : 1 chloroform-methanol mixture. The extracts were concentrated on a rotary evaporator under reduced pressure at 35–40 °C.

Extraction of fructus samples for examination by UV spectroscopy. To quantify the content of hydroxycyanocinnamic acids, about 0.5 g of crushed seeds were placed in a conical flask with a capacity of 100 ml with 25 ml of 70% ethyl alcohol inside. The flask was attached to a reverse refrigerator and boiled in a water bath for 30 min. Extraction was performed twice. The extract was filtered through a paper filter into a 50 ml volumetric flask. An aliquot of 1 ml was taken from the resulting solution and placed in a 25 ml volumetric flask [29].

To assess the possible content of anthocyanins in seeds, about 0.5 g of crushed raw material were extracted twice in a conical flask with a capacity of 250 ml with 50 ml of 95% ethyl alcohol containing 1% hydrochloric acid (pH 2). The flask was attached to a reverse refrigerator and heated in a boiling water bath for 30 minutes. Then the contents of the flask were cooled to room temperature and the resulting extraction was filtered through a paper filter into a 100 ml volumetric flask.

To quantify polysaccharides, fructosides and fructosans a technique for burdock roots of State Pharmacopoeia XIV of Russia and Inulahelenium by Belyakov, adapted for seeds was used [30, 31].

Extraction of arctigenin. The extraction was carried out by the column chromatography method using hexane fraction. Activated neutral aluminum oxide according to Brockman I (Sigma Aldrich) was used as a sorbent (for stationary layer). Elution was carried out by ethyl alcohol acidified with acetic acid (0.05 ml per 50 ml). The resulting fraction was evaporated until dry to obtain colorless crystals.

TLC. TLC analysis of hexane fraction was carried out on 10×10 cm Sorbfil TLC plates (produced by CJSC Sorbpolymer, Russia) covered with a layer of aluminum oxide. The mobile phase of diethylamine : ethylacetate : isopropanol (1 : 15 : 5) was used. The plates were developed with the grade reagent. At the same time chromatograms were covered with standard substances – arctigenin (PHL80354, PhytoLab) and arctiin (PHL89531, PhytoLab). The substances separation quality control on chromatogram was carried out by HPLC method. Each adsorption region was separated, placed in glass tubes and extracted from the sorbent by 95% ethyl alcohol (MRAB, Russia). Alcohol was removed by nitrogen current up to 1 ml. Then a sample was taken and examined by the HPLC method.

HPLC. The HPLC analysis of adsorption regions and hexane fractions was carried out on Milichrome A-02 chromatograph (SDB Nauchpribor, Russia) using the "Multichrome" data processing program. Analysis conditions: the volume of injected sample is 2 µl; the elution rate is 100 µl/min; the composition of eluents: buffer A – acetic acid 50 µl in 50 ml (pH=3), buffer B – acetonitrile 100%; gradient profile: time (min.) 0, 5, 25; amount B, % 20, 60, 100; regeneration – 6 minutes with a starting buffer (20% B); Prontosil 120-5-C18 AQ column diameter 2 mm, length 75 mm, particle size 5 µm; T=35 °C (thermostat); detection at UV detector wavelengths: 210 nm, 240 nm, 280 nm.

GC-MS. GC-MS was performed on Agilent gas chromatograph (USA) model 6890N with a mass-selective detector (model 5973N) (USA). Sensitivity under the following conditions: capillary column – DB-5DB ((5% Phenil)-methylpolysiloxane); column length 30 m; column thickness 0.32 mm; fixed phase film thickness 0.25 µm; injector temperature – 280 °C; initial temperature of column thermostat – 50 °C; final temperature of column thermostat – 290 °C; column temperature increased at a rate of 10 °C/min; exposure at final temperature – 10 min; detector's interface temperature – 280 °C; carrier gas – helium; the volume of injected sample – 1 µl. The samples were injected into the chromatograph in the non-flow divider mode. The analysis was carried out under conditions of constant gas flow rate. The mass-selective detector operated in the electronic shock mode (70 eV). Chromato-mass spectrograms were recorded using total ion current.

Identification of Components. Identification of components was carried out by comparing the obtained peaks of the mass spectra on the chromato-mass spectrogram with the data of the mass spectra of the libraries (NIST-MS Library 05; WILEY GC-MS Library 2007) as well as comparison with the mass spectra of standard substances.

UV-spectroscopy. Spectral analysis was carried out by measuring the optical density of extracts on SF-2000 spectrophotometer (OKB Spektr, Russia) in a 10 mm cell in 210–700 nm wavelength range. Extraction solvents used in extracts' preparation were used as compensation liquids. All used physical and chemical methods were tested for linearity, precision, and accuracy. Validation was carried out in accordance with the requirements of the Russian Pharmacopoeia (14th edition), the analysis met its requirements [30].

Discussion of the results

Extraction of fructus samples for analysis using TLC, HPLC, GC-MS methods. The mass of the obtained fructus extract of *Arctium lappa* was 535 g (574 ml), *Arctium tomentosum* – 562 g (608 ml). Both extracts were a viscous oily liquid of dark green color with characteristic smell. The extracts were treated with a mixture of hexane : 95% ethyl alcohol : water (10 : 1 : 9) and separated into hexane and aqueous layers. The hexane layer was evaporated and hexane fraction was obtained. The aqueous layer was treated with chloroform and separated into aqueous layer and chloroform layer which was later evaporated and chloroform fraction was obtained. Fractions were used for the extraction of substances and their analysis by various methods.

Obtaining fatty acids extract for analysis. Chloroform fractions and ethanol extract were placed in a 0.5 ml tube after which 5 ml of hexane were added. Into the resulting solution 0.5 ml of methanol solution of potassium hydroxide were added and then it was intensively shaken for 2 minutes. The resulting solution was settled for 5 minutes after which the top layer was decanted and examined by GC-MS method.

Extraction of arctigenin. The colorless crystals extracted from hexane fraction (the yield from *Arctium lappa* – 0.39%; from *Arctium tomentosum* – 0.51%) did not dissolve in non-polar solvents but slightly dissolved in water. For further investigation, the crystals were dissolved in methanol (Sigma Aldrich) and analyzed by GC-MS method.

TLC. After the analysis of chromatograms of hexane fractions and standard substances at 365 nm wavelength, several adsorption regions were detected (at least 4 regions on *Arctium lappa* chromatogram; at least 6 on *Arctium tomenosum* chromatogram). The adsorption regions with R_f 0.65 cm and R_f 0.16 on chromatograms of both extracts coincided with similar regions of standard substances of arctigenin and arctiin. After grade reagent's treatment, the adsorption regions corresponding to arctigenin were purple in colour and the regions corresponding to arctiin were bright blue.

Further, the adsorption regions on chromatograms of hexane fractions corresponding to arctigenin and arctiiinwere studied by HPLC and compared to standard substances.

HPLC. Similar *Arctium lappa* and *Arctium tomentosum* chromatograms of hexane fractions were obtained by HPLC. In them, substances eluted from chromatograms appeared between 6^{th}–9^{th} minute identically to standard substances arctiin and arctigenin.

The concentration of substances was calculated by the method of absolute calibration using solutions of arctiin and arctigenin with precisely known concentration and detector signal values at 210 nm wavelength to build a calibration graph (Table 1).

Due to the fact that some types of pharmacological activity of burdock are associated with arctigenin and arctiin, in particular antitumor activity [32], we evaluated the content of these lignans in wild populations, samples cultivated in the traditional way and using a new cultivation technology. As follows from the data presented in Table 1, the use of a new cultivation technology increased the content of lignans in these samples by 15–25%.

GS-MS. GC-MS method confirmed the structure of arctigenin extracted from *A. lappa* and *A. tomentosum* fructus. The UV spectrum of the extracted substance has characteristic absorption maxima at 230 and 280 nm coinciding with similar maxima of the standard substance and similar research data (Fig. 1a of the electronic application).

The analysis of the HPLC chromatogram and mass spectra of the extracted substances and standard substance arctigenin showed identical values of molecular and fragmentary ions with m/z 372.2 and m/z 137.0 (Fig. 1b, 2–4 of the electronic application).

The analysis of chloroform fractions of *A. lappa* and *A. tomentosum* showed the presence of at least 19 fatty acids and their derivatives (Table 2, Fig. 5 of the electronic application). The dominant ones include methyl linoleate and methyl oleate or methyl cis-9-octadecenoate. Two substances – methyl cis-9,cis-15-octadecadienoate and 11-Eicosenoic acid, methyl ester – were discovered for the first time.

The analysis of ethanol extract of *A. lappa* and *A. tomentosum* showed the presence of at least 12 fatty acids and their derivatives (Table 3, Fig. 6 of the electronic application). The dominant substances as well as in chloroform fraction include methyl linoleate and methyl oleate or methyl cis-9-octadecenoate.

UV spectrum. The analysis of UV spectra of solutions obtained for the evaluation of GCC, polysaccharides, anthocyanins showed the presence of absorption maxima peculiar for these groups of substances. The content of hydroxycinnamic acids sum as a percentage (X) in terms of chlorogenic acid and the oven-dry mass of raw material is calculated by the following formula (1):

$$X = \frac{A \cdot V_1 \cdot V_2 \cdot 100}{a \cdot 507 \cdot (100 - W) \cdot V_3 \cdot 100},$$

where A – optical density of solution B; a – mass of raw material in grams; V_1 – volume of solution A (100), ml; V_2 – volume of solution B (25), ml; V_3 – aliquot volume of solution A (2), ml; W – weight loss during raw material's drying in %; 507 – specific absorption rate $E^{1\%}_{1cm}$ of chlorogenic acid at 327±3 nm.

Table 1. Contents of arctiin, artigenin, mgr/ml

Substance name / species name	*Arctium lappa*			*Arctium tomentosum*		
	wild	Traditionl way of growing	New cultivation technique	*wild*	Traditionalway of growing	New cultivation technique
Arctiin	0.40±0.02	0.41±0.02	0.52±0.03	0.50±0.02	0.49±0.02	0.61±0.03
Arctigenin	0.07±0.004	0.08±0.005	0.09±0.006	0.09±0.006	0.08±0.005	0.12±0.01

Table 2. Composition of the chloroform fraction of *Arctium lappa* and *Arctium tomentosum*

No	t_R	Amount,%		Substance type
		A. lappa	*A. tomentosum*	
1	13.156	0.06674	0.03521	methyltetradeconate
2	14.232	0.00962	0.00633	methylpentadecanoate
3	15.020	0.018074	0.007531	7-hexadecanoicacid
4	15.065	0.256192	0.19467	9-hexadecanoicacid
5	15.265	9.59446	7.31737	methylpalmitate
6	16.239	0.016104	0.011834	heptadecanoicacid, methylester
7	16.971	64.78151	56.38552	methyllinoleate
8	17.015	19.30404	14.33711	methyl oleateили methyl cis-9-octadecenoate
9	17.186	4.040754	2.83921	methylstearate
10	18.554	0.388816	0.27842	methyl cis-9,cis-15-octadecadienoate
11	18.679	0.050262	0.037925	methylcis 9,10-epoxystearate
12	18.737	0.22081	0.13712	11-Eicosenoic acid, methylester
13	18.938	0.755012	0.52731	eicosanoicacid, methylester
16	20.562	0.262702	0.18932	docosanoicacid, methylester
17	21.328	0.010866	0.00998	tricosanoic,methylester
18	21.910	0.012462	0.00933	15-tetracosanoic,methyl ester
19	22.071	0.11764	0.09368	tetracosanoicacid

Table 3. Composition of the ethanol extract of *Arctium lappa* and *Arctium tomentosum*

No	t_R	Amount, %		Substance
		A. lappa	*A. tomentosum*	
1	13.156	0.029977	0.021032	methyl tetradeconate
2	15.064	0.158947	0.095521	9-hexadecanoicacid
3	15.259	7.578475	6.378823	Methylpalmitate
4	16.941	74.29212	68.15321	Methyllinoleate
5	16.972	14.88342	13.62113	methyl oleate or methyl cis-9-octadecenoate
6	17.178	2.158944	2.001512	Methylstearate
7	17.520	0.123017	0.095121	ethyl linoleate or (Z,Z)-9,12-octadecadienoic acid ethyl ester; mandenol $C_{20}H_{36}O_2$
8	18.552	0.335687	0.286844	methyl cis-9,cis-15-octadecadienoate
9	18.679	0.04883	0.038286	methylcis 9,10-epoxystearate
10	18.735	0.048358	0.037673	11-eicosenoicacid, methylester
11	18.937	0.211624	0.185338	eicosanoicacid, methylester
12	20.561	0.040347	0.028957	docosanoicacid, methylester

The content of anthocyanins sum as a percentage (X) in terms of cyanidin-3-glucoside and the oven-dry mass of raw material is calculated by the following formula (2):

$$X = \frac{A \cdot V_1 \cdot V_2 \cdot 100}{E^{1\%}_{1cm} \cdot a \cdot V_3 \cdot (100 - W)},$$

where A–optical density of the test solution;a–mass of raw material in grams;V_1 – volume of solution A (100), ml; V_2 – volume of solution B (25), ml; V_3 – aliquot volume of solution A (1), $E^{1\%}_{1cm}$ – specific absorption index of cyanidin-3,5-diglycoside at 510 nm wavelength, equal to 453; W – raw material moisture content, %.

The content of polysaccharides sum in terms of fructose and oven-dry raw material as a percentage (X) is calculated by the following formula (3):

$$X = \frac{A \cdot V_1 \cdot V_2 \cdot V_3 \cdot 100}{E^{1\%}_{1cm} \cdot a \cdot V_4 \cdot V_5 \cdot (100 - W)},$$

where A – optical density of solution C; $E^{1\%}_{1cm}$ – specific absorption index of reaction products of fructose with resorcinol in acidic medium at 482 nm wavelength, equal to 298; a – mass of raw material in grams, g;V_1 – solution volume A (200), ml; V_2 – volume of solution B (100), ml; V_3 – volume of solution C (25); V_4, V_5 – aliquot volume (5); W – moisture content of raw material, %.

The content of fructosides and fructosans sum in terms of fructose and oven-dry raw materials as a percentage (X) is calculated by the following formula (4):

$$X = \frac{A \cdot 100000}{E^{1\%}_{1cm} \cdot a \cdot (100 - W)},$$

where A – optical density of solution; $E^{1\%}_{1cm}$ – specific absorption index of reaction products of fructose with resorcinol interaction in acidic medium at 520 nm wavelength, equal to 95; a – mass of raw material in grams, g; W – moisture content of raw material, %.

As the results presented in Table 4 prove, *Arctium lappa* fructus differ from the fructus of *Arctium tomentosum*. The content of hydroxycoric acids in *Arctium lappa* exceeds that of *Arctium tomentosum* by 1.7 times; fructosides, fructosans by 1.2 times; anthocyanins by 1.4 times. However, *Arctium tomentosum* fructus contain a greater amount of polysaccharides which exceeds that of *Arctium lappa* by 1.4 times. The content of the studied groups of biologically active substances in burdock samples grown using the new cultivation technology is 10–20% higher.

In the given research, groups/substances characteristic of *Arctium lappa* and *Arctium tomentosum* fructus cultivated in Altai Kraiwere discovered. Quantities of hydroxycinnamic acids, polysaccharides, anthocyanins, fatty acids in fructus of burdock species cultivated in Altai Kraiwere established for the first time. We believe that hydroxycinnamic acids are a group of substances by which standardization of burdock raw material can be carried out.

The presence of arctigenin and arctiinwas confirmed in the fructus of *Arctium lappa* and *Arctium tomentosum* cultivated in Altai Krai by TLC and HPLC methods. The quantitative content of these substances in fructus differs from quantitative contents obtained by other authors who found arctiin to be the dominant component the content of which exceeds the content of arctigenin by 18 times (for example, in *Arctium lappa*) [11].

Previously, other authors reported the results according to which the fructus of *Arctium tomentosum* contain fatty oil the dominant component of which is 9,10-epoxy-cis-octadeca-12-enoic acid [9]. Oleic glycerides (19.1%) and linoleic glycerides (58.5%) dominate in the composition of the fatty oil of *Arctium lappa* fructus [32]. In the given research, other dominant components of the fatty oil of the fructus of these two types of burdock were identified – methyl linoleate and methyl oleate or methyl cis-9-octadecenoate.

Table 4. Content of biologically active substances in fructus, %

Name of substance group, method / species	*Arctium lappa*		*Arctium tomentosum*	
	traditional way of growing	new cultivation technique	traditional way of growing	new cultivation technique
Hydroxycinnamicacids, spectrophotometric	0.59±0.04	0.64±0.06	0.99±0.07	1.2±0.1
Anthocyanins, spectrophotometric	0.013±0.001	0.015±0.001	0.0096±0.0008	0.01±0.001
Polysaccharides:				
amount of polysaccharides in terms of fructose	1.68±0.2	1.93±0.2	1.21±0.1	1.45±0.1
Fructosides, fructosans	4.60±0.3	5.45±0.5	3.73±0.2	4.4±0.4

Conclusion

The given research is the first research studying fructus of *Arctium lappa* and A*rctium tomentosum* cultivated in Altai Krai, Russia. The results presented in this research can be an additional valuable source of information about the composition of metabolites of cultivated *Arctium lappa* and *Arctium tomentosum* fructus. The obtained data can be used in the future for efficient identification of raw materials, development of suitable standardization methods, explanation of pharmacological action mechanisms in current and future studies. Burdock fructus have great potential for further research of their chemical composition, pharmacological and therapeutic applications. It is shown that the use of a new cultivation technology allows to increase the content of lignans – substances that are important for the manifestation of the pharmacological properties of these species.

Supplementary Information
The electronic supplement to the article (DOI: http://www.doi.org/10.14258/jcprm.20240112476s) provides additional experimental material that reveals the main points set out in the article.

Funding
This work was supported funding Siberian State Medical University, Kemerovo State Medical University and Visterra. No additional grants to carry out or direct this particular research were obtained.

Conflict of Interest
The authors of this work declare that they have no conflicts of interest.

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Received April 3, 2023

Revised July 7, 2023

Accepted August 27, 2023

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