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Quantitative and qualitative profile of biologically active substances extracted from purple echinacea *(Echinacea Purpurea* L.) growing in the Kemerovo region: functional foods application

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Abstract: Immunodeficiency causes a lot of modern diseases. Immunodeficiency, in its turn, is caused by such factors as polluted environment, chronic stress, sedentary lifestyle, unbalanced diet, etc. All these factors weaken respiratory organs and gastrointestinal tract, disturb hormonal regulation, and destabilize immune defence. Food industry responds to these challenges by developing functional foods and dietary supplements from medicinal plants. Dietary supplements made from natural plant extracts have more advantages than their numerous synthetic analogues. They produce a mild therapeutic effect and no pronounced side effects. Purple Echinacea (*Echinacea purpurea* L.) possesses immunomodulatory, anti-inflammatory, antiviral, and tonic properties. However, climatic and soil conditions are known to affect the qualitative and quantitative profile of biologically active substances. The present paper describes the micronutrient profile of various parts of *Echinacea purpurea* grown in the Kemerovo region. The study employed a complex of physical and chemical methods. The research featured leaves, roots, and flowers, as well as components extracted from the plant with the help of a 70% ethanol solution. The latter was chosen for its universal properties in micronutrient extraction. The methods included high performance liquid chromatography (HPLC), thin layer chromatography (TLC), and IR spectroscopy. A set of triple experiments showed that the extracts contained substances with anti-inflammatory, antioxidant, and immunomodulating properties. Thus, Echinacea extract can be recommended for functional foods and dietary supplements.

Keywords: *Echinacea purpurea*, quality extraction, extract, biologically active substances, biologically active substances, qualitative and quantitative identification, chromatography

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INTRODUCTION

One of the biggest problems the humanity is currently facing is human-environmental interactions in the aspects of human health and homeostasis. Polluted environment damages immune status irrespectively of social stratum. This problem is especially relevant for regions with bad ecology and harsh climate [5]. Functional foods and dietary supplements with immunostimulant plant agents have become a popular preventive action against immunodeficiency [20]. Functional foods with targeted properties possess a high degree of usefulness and safety, which allows them to substitute pharmaceutical products, to a certain extent [1–3].

The alarming health status of population demands that Russian scientists started using medicinal plants since they are one of the largest domestic resources in the sphere of medicine. Biologically active substances obtained from medicinal plants can be used to treat even severe diseases. Medicinal plants have become a popular source of raw materials for preventive medical treatment. All these make studies of biologically active substances, extracts, and botanical medicines extremely relevant.

Unlike their artificial analogues, botanical medicines produce a mild therapeutic effect and no pronounced

side effects [13]. Despite the growing consumers' interest, the share of botanical medicines and herbal repertories on the Russian pharmaceutical market remains small: 11–12 million US dollars, or 0.5–1.5% [16]

Botanical medicines are getting more popular in Europe as well. For instance, sales of galenicals (mostly extracts) grew by 2% (440 million Euros) in France between 2009 and 2010. And if compared to 2005, the sector of painkillers grew by 12.3%, sedatives and soporifics – by 21.5%, cough mixtures, cold remedies, and antiallergic drugs – by 0.5%, and digestants – by 7.6% [17].

Medicinal plant materials contain a variety of biologically active compounds from various chemical classes of natural substances, i.e. terpenoids, polysaccharides, phenolic compounds, alkaloids, etc. Each class and group of BAS has a specific spectrum of biological activity, which is typical of the whole group. However, this spectrum may be variable for each subgroup of biologically active compounds. In some cases, there may also be fundamental differences for each individual substance, depending on its specific chemical structure [7, 8, 34].

The list of advantageous Siberian medicinal plants includes butterfly orchid (*Platanthera bifolia* L.), yellow seet-clover (*Melilotus officinalis* L.), skullcap (*Scutellaria moniliorhiza* L.), roseroot (*Rhodiola rosea* L.), maral root (*Rhaponticum carthamoides* L.), various sorts of milk-vetch (*Astragalus* turczaninowii L., *Astragalus danicus* L., etc.), hedisarum (*Hedysarum turczaninóvii* L.), etc.

To create new efficient and safe substances and functional foods, one has to use techniques based on the most recent knowledge about life and living systems. The basic idea behind all biotechnologies is how to use bio-objects in order to produce efficient and safe functional products. The share of vegetative BAS on the market of functional foods is 60–65% [1].

To study a group of biologically active substances and predict its toxic and pharmaceutical properties, one should start with a phytochemical analysis. A study of chemical formula of vegetative organic compounds begins with a series of identification tests that make it possible to define groups of biologically active substances [19]. The thin layer chromatography (TLC) method helps to refer a micronutrient to a particular group. It is often used to identify medicinal plant materials. Hence, the method defines the further research, as well as the choice of solvent and stationary phases [11, 13]. The method of high performance liquid chromatography (HPLC) makes it possible to perform a complete chemical analysis of plant samples, including identification and potency assay [6, 18].

Purple Echinacea (*Echinacea purpurea* L.) possesses immunomodulatory, anti-inflammatory, antiviral, and tonic properties [23]. It is a valuable medicinal plant from the *Asteraceae* family. The plant originated in North America, where it grows in the wild on fields, limestone wastelands, stony hills, and dry prairies of central and southern states. Some sources define it as *Rudbeckia purpurea,* although modern botany separates these two species [21].

Echinacea purpurea is a perennial herb. It is 50–100 cm tall and has one or several cylindrical ribbed ramose caulis. For medicinal purposes, the plant is harvested during its blooming stage, while its roots are usually dug in autumn [15, 24].

One of the main groups of biologically active substances found in *Echinacea* is phenylpropanoids, namely, derivatives of cinnamic acids. Another typical component is chicory acid, which is responsible for the immunomodulating and antiviral properties of Echinacea-based pharmaceuticals. The amount of chicory acid depends on the age of the plant [12, 14]. Phenylpropanoids contained in Echinacea include caffeic and chlorogenic acids. Virus-neutralising and immunostimulating properties are due to the presence of saponins. Inulin can be found in roots and, in lesser amounts, in leaves and stems. It possesses anti-inflammatory properties. The alkamides are responsible for the analgetic effect and improve the immune system. The same is true for vitamins A, E, and C. In spite of the fact that the pharmaceutical effect of separate components is relatively low, the medicinal effect of cumulative preparations, e.g. potions, extracts, or juices, is rather high [9, 10, 12]. Hydroalcohol potions, alcoholates, extracts, and juices are used to boost immune system by improving phagocytosis, bacericyte and cytotoxic properties of macrofags, and antibody synthesis.

Chemical industry produces various artificial substitutes, e.g. flavouring agents, preparations, active components, etc. Still, natural plant extracts remain in demand in food, cosmetics, and pharmaceutical industries [22, 25]. *Echinacea purpurea* makes part of many herbal immunoamplifiers.

Chemical composition of various plant parts depends on the climate and soil of the region where the plant grows. Hence, the research objective was to study the profile of the biologically active substances found in *Echinacea purpurea* that grows in the Kemerovo region. A set of physical and chemical methods helped to substantiate their use in functional food production.

STUDY OBJECTS AND METHODS

The research featured medicinal herbs of *Echinacea purpurea*. The averaged samples originated from the village of Novostroyka near the city of Kemerovo (Kemerovo region, GPS coordinates: 55°15'14"N, 86°13'05"E). The local soil can be characterized as black, leached, and argillaceous, with enough macroelements for medicinal herbs. The amount of humus was found to be 7.7%, nitrate nitrogen ($N\text{-}NO_3$) – 45 mg/100 g; labile phosphorus (P_2O_5) – 88 mg/100 g; exchange potassium (K_2O) – 142 mg/100 g of soil. To identify soil contamination and plant mass material with heavy metals, the averaged samples were sorted according to the state standards and approved techniques [28].

The atomic absorption method was used to determine heavy metals (Zn, Pb, Co, Ni, Cd) for an averaged soil sample [26, 27]. The samples were taken from the roots at the depth of 0–20 cm, from the rhizomes, and from the herbs. Heavy metals were extracted for 24 hours using an ammonium acetate buffer with a pH of 4.8. The soil-solution ratio was 1:10. The sample prepa-

ration of rhizomes, leaves, stems, and flowers was conducted separately and took 24 hours. Dry ashing was followed by extraction with diluted nitric acid (1:1).

In the Russian Federation, there are no current standards for the toxicological assessment of medicinal raw materials for heavy metals. Hence, most researchers have to use regulations adopted for plant-based dietary supplements [30, 31].

The research featured various parts of *Echinacea*, as well as components extracted from it with the help of a 70% ethanol solution. The latter was chosen for its universal properties in extracting of a wide range of biologically active substances.

The methods included high performance liquid chromatography (HPLC), thin layer chromatography (TLC), and IR spectroscopy.

Statistical data processing was performed with the help of Microsoft Excel. The assay content of biologically active substances was defined with the help of standard curves. The concentration range was 0.5–150.0 mkg/ml.

The research involved five consecutive stages.

1. Extraction of the samples and preparation for screening and analysis of biologically active substances (secondary metabolites).

To define the sum of biologically active substances, the field test samples were air-dried (0.5 kg). An averaged sample of herbs (shoots, leaves, flowers) and foot ends (rhizomes and roots) were extracted and ground. After that, the plant material underwent a complete extraction with ethanol with the ratio of 1:10 at 10°C for 48 hours. The extract was a green-brown transparent liquid with a specific smell. The extracts were kept in the dark at 4–6°C.

2. Preparation of samples for HPLC analysis. The ground plant material (0.5 kg) underwent extraction with 70% ethanol in ratio 1:10 in a sonication bath (100 W, 35 kHz) at 40°C. The process lasted 30 min and was conducted twice. The extract was filtered through 0.2 micron membranes. Then, a vacuum rotary evaporator was used to concentrate the permeate in order to get water residue. After that, the permeate underwent a liquid-phase extraction with hexane (fraction 1) and an ethylacetat-ethanol mix (5:1). Fraction 2 was chromatographed with sorbent LH-20 with column of 10×350 mm by using a chromatograph (Bio-Rad, USA). The elution was conducted with isopropylalcohol at the gradient of 20–90%.

The extract was filtered and condensed with an 8 vacuum rotary evaporator at 72 Mbar to a thick consistency. The thickened suspension was then diluted four times with water and left for 12 hours at 40°C. The tarry residue was removed by filtration. After that, the permeate was treated with chloroform and ethyl acetate. The extract was then drained with anhydrous sodium sulphate. It was concentrated with the help of a rotary evaporator at 400°C, 240 mbar. The fractions were applied to column with an LH-20 sephadex (Pharmacia,). The fractions were mixed with a small amount of sorbent, loaded into the column, and eluted with aqueous alcohols at a ratio of 5:5; 6:4; 7:3; 8:2; and 9:1, as well as with absolute ethyl alcohol. The fractions were collected by 10–15 ml. The composition of the eluate was controlled with the help of TLC. If the fractions contained the same components, they were put together and condensed using a vacuum rotary evaporator.

After that, we defined the substances that could be classified as biologically active substances according to qualitation tests and chromatograms. Their structure was defined according to UV and IR Fourier spectra. UV-spectra were measured using СФ-2000 spectrophotometer both as pure components and with chemical reagents to specify the location of hydroxyl groups and clycosidation.

3. Preparation of samples for IR Fourier-transform spectrometry.

Two mg of a dried sample was ground in an agate mortar together with potassium bromide at a ratio of 1:100 (Fluka, Germany). A disk was formed in a press at 4,000 psi. IR spectra were measured by a single-beam interferometer with a ФСМ-1202 Fourier spectrometer (Infraspek, St-Petersburg, Russia). The spectra were registered in the range of 4.000–400 cm^1 with the resolution of 4 cm. The FSpec software 4.0.0.2 was used to process the data.

4. TLC stage. TLC analysis was performed on TLC aluminium foil analytical plates. It was followed by densitometry using a Sony densitometer (HDR-CH 405, OOO IMID, Russia). Photofixation was conducted at the waves of 254 and 365 nm and at a visual band after specific derivatization. The elution involved the following fluid systems: n-butanol – glacial acetic acid – water at the ratio of 60:15:25 and ethyl acetate – formic acid – glacial acetic acid – water at the ratio of 100:11:11:26.

In the preparative variant the chromatographic zones were cut out and subjected to further analysis.

5. HPLC conditions. The substances were separated using a Shimadzu –20 Prominence chromatograph with a photodiode array and a Shimadzu refractometric detector. The Kromasil –18 column was 250×4.6 mm, particle size – 5 µm. A mix of solvents was used as eluent components, namely methyl syanide MECN (solvent A) and 0.1% aqueous formic acid (solvent B). During separation, a gradient elution mode was used with the following isocratic components: 0 min – 20% A, 4 min – 55% A, 14 min – 55% A, 16 min – 20% A. The flow rate was 0.5 ml/min, the column temperature was 24°C, the sample volume was 20 µl, reference wave lengths were 254 and 330 nm.

Two approaches were used for identification.

1. UV spectra and retention time of peaks were compared with the reference samples. The chromatograms were developed using programme.

2. HPLC and/or TLC were used together with IR Fourier-transform sepctrometry. The column temperature was 40°C, while the volumetric flow rate of the eluent phase was 0.4 ml/min. A 0.1% water solution of formic acid (solvent A, v/v) and a 0.1% solution of formic acid in MECN (solvent B, v/v) were used as eluent. HPLC separation was conducted by gradient eluation. The eluent composition was as follows (solvent B, by volume): 0–1 min - 15%, 1–5 min – 30%, 5–15 min –

Table 1. Content of heavy metals in soil and in plant raw material of Echinacea, mg/100 g

Elements	Soil *			Plant raw material		
	MPC, TPC	Assigned value	MPC for dietary supplements [32]	Assigned value		
				roots and rhizomes	stems and leaves	flowers
Zink (Zn)	23.0	2.00 ± 0.12	–	0.93 ± 0.09	0.25 ± 0.007	0.09 ± 0.003
Lead (Pb)	3.2	0.82 ± 0.08	6	0.46 ± 0.06	0.18 ± 0.007	0.02 ± 0.002
Cobalt (Co)	5.0	1.15 ± 0.08	–	0.44 ± 0.06	0.21 ± 0.008	0.02 ± 0.002
Nickel (Ni)	4.0	1.78 ± 0.12	–	0.55 ± 0.06	0.34 ± 0.05	0.02 ± 0.002
Cadmium (Cd)	1.0	0.56 ± 0.06	1	0.22 ± 0.008	0.11 ± 0.007	–

Note: content of heavy metals measured in the active form

30 → 38%, 15–15.5 min – 48 → 45%, 15.5–23 min – 45%, 23–23.5 min – 45 → 95%. Each chromatographic fraction was analyzed after accumulation.

A quantitative assay of the secondary metabolites (flavones) was conducted with the help of standard curves at the concentration range of 1.9–235.0 mkg/ml. The equation for the standard curves was as follows:

$$y = a \times x \times X, \quad (1)$$

where X is standard concentration (mkg/ml); y is HPLC peak response (cu); and a is proportionality factor.

Formula (2) connects the peak response and the dry weight unit:

$$C = S / (a \times m \times 1000), \quad (2)$$

where C is dry flavones in the portion of dry material (mg/g); m is mass of dry weight (g); a is proportionality factor from the standard curve equation. A coefficient of 1,000 is necessary to transform C into mg/g dimension.

The concentration of chlorophylls *a* and *b* and carotinoids was measured using a UV 1800 Shimadzu spectrophotometer. The stems and leaves were used as assimilative bodies. A 0.1 g portion of plant material was ground, rubbed, put into a vile with 10 ml of 98% ethanol and was stored in a dark place. After 12 hours it was measured for pigments using spectrophotometry at the wave length of 649 nm, 665 nm, and 440.5 nm. The concentrations of chlorophylls *a* and *b* and carotinoids were measured as follows (mg/g):

$$C_{\text{chlorophyll}}a = 13.7 \cdot D_{665} - 5.76 \cdot D_{649,} \quad (3)$$

$$C_{\text{chlorophyll}}b = 25.8 \cdot D_{649} - 7.6 \cdot D_{665,} \quad (4)$$

$$C_{\text{carotinoids}} = 4.695 \cdot D_{440.5} - 0.268 \cdot (C_{\text{chlorophyll}}a + C_{\text{chlorophyll}}b), \quad (5)$$

where D_{649}, D_{665}, $D_{440,5}$ are absorbency at the wave lengths of 649 nm, 665 nm, and 440.5 nm, respectively.

RESULTS AND DISCUSSION

As a rule, pollutants enter the plant tissue from soil through the root system. They also enter the plant from the dust that settles down on the surface of its aerial organs and penetrates into the intercellular spaces through natural passages, e.g. stomata, pores, or lenticels [29, 32]. Therefore, herbal raw materials must be environmentally pollution-free. The analysis of soil samples and plant material confirmed this requirement (Table 1).

Table 1 shows that the content of heavy metals (Zn, Pb, Co, Ni, Cd) in the soil does not exceed the maximum permissible level. For food plants, including medicinal ones, the MPC content for heavy metals (HM) is stated in Sanitary Regulations and Norms 2.3.2.1078-01* for dietary supplements. However, the document features only lead and cadmium, which belong to technogenic metals and are of no biological significance for plants [31].

According to the assigned value analysis of the heavy metals, they are mostly accumulated by roots and rhizomes, and to a lesser degree – by flowers. However, the content of standardized elements (Pb, Cd) in different parts of the plant is significantly below the permissible level (Table 1). In general, the results indicate that the content of HM in the raw material corresponds to the standard indicators and can be used to obtain biologically active substances for dietary supplements and food.

Photosynthesis plays the key role in plant growth and development. Therefore, it determines the formation of the secondary metabolites, including those with biologically active properties. Chlorophylls *a* and *b,* as well as carotenoids, are involved in photochemical reactions. A high content of chlorophyll *a* and the chlorophyll *a/b* ratio may indicate a high potential photochemical activity of the leaves, and, consequently, a more active accumulation of biologically active substances [33].

Hence, it seemed important to investigate the quan in the leaves of *Echinacea purpurea* (Table 2).

The experimental data indicate that Echinacea leaves have the highest amount of chlorophyll *a* in their pigment complex. Apparently, chlorophyll *a* has the greatest stability among other pigments of photosynthesis

Table 2. Content of photosynthetic pigments in leaves of *Echinacea purpurea*

Indicator	Assigned value
Chlorophyll a, mg/g	0,700 ± 0.01
Chlorophyll b, mg/g	0,282 ± 0.01
Chlorophyll amount (a + b), mg/g	0,990 ± 0.02
Chlorophyll ratio a/ b	2.480 ± 0.01
Carotinoids, mg/g	0.190 ± 0.01
Ratio of chlorophylls and carotinoids, chlorophyll/carotinoid	5.210 ± 0.01

Note: mean values of triple consecutive tests

* Sanitary Regulations and Norms 2.3.2.1078-01. Sanitary rules and Regulations 2.3.2. 1078-01. Hygienic requirements for safety and nutritional value of food.

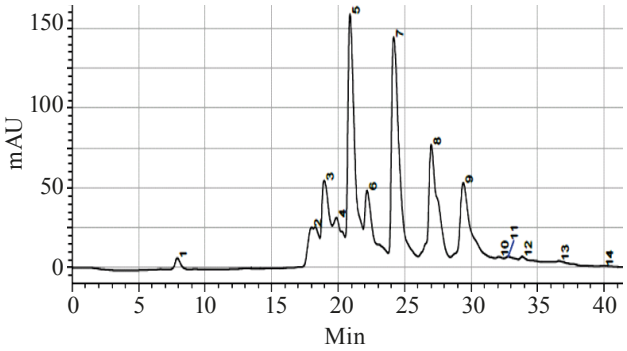

Fig. 1. HPLC chromatogram of the ethanol extract from *Echinacea purpurea* stems and leaves.

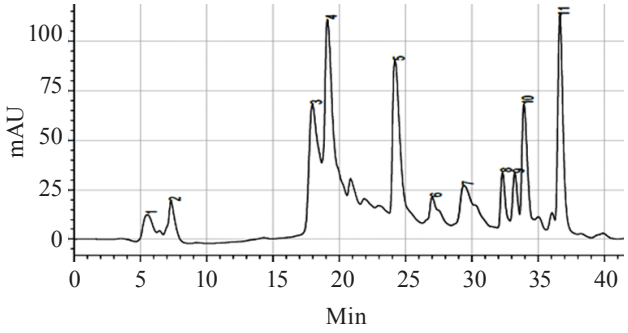

Fig. 2. HPLC chromatogram of the ethanol extract from *Echinacea purpurea* rhizomes and roots.

Table 3. Residence time Content of the main biologically active substances in the ethanol extracts from stem and leaves

Component	Concentration range mkg/ml	Correlation coefficient	Residence time t_R, min
Tetradec-8Z-en-11,13-diyn-2-one		0.9700	7.87
8-hydroxy-tetradec-9E-en-11,13-diyn-2-one		0.9600	18.07
3,4-dioxybenzoic acid		0.9800	19.01
8- hydroxy-pentadec-9E-en-11,13-diyn-2-one		0.9894	19.87
Echinacoside	0.50–50.03	0.9881	20.97
Caftaric acid	1.01–10.07	0.9602	22 22
Chlorogenic acid	0,103–50.05	0.9881	24.25
Chicoric acid	0.51–50.01	0.9891	26.98
Caffeic acid	0.501–100.02	0.9831	29.39

Table 4. Content of the main biologically active substances in the ethanol extracts of *Echinacea purpurea* stem and leaves

Component	Content, mg/g of dry weight
Tetradec-8Z-en-11,13-diyn-2-one	1,098 ± 0.01
8-hydroxy-tetradec-9E-en-11,13-diyn-2-one	0,911 ± 0.01
3,4-dioxybenzoic acid	5.84 ± 0.01
8- hydroxy-pentadec-9E-en-11,13-diyn-2-one	1.03 ± 0.01
Echinacoside	24.42 ± 0.01
Caftaric acid	5.21 ± 0.01
Chlorogenic acid	28.5 ± 0.01
Chicoric acid	17.56 ± 0.01
Caffeic acid	13.88 ± 0.01

and optimizes the photosynthetic processes. As the data show, the amount of chlorophylls (*a* + *b*) significantly exceeds the content of carotenoids – by 5.2 times. The pigment complex of Echinacea leaves has a rather high ratio of chlorophylls *a/b*. High ratios of chlorophyll *a/b* are characteristic of chloroplasts. The proportion of stromal thylakoids in chloroplasts prevails, and they have a greater light absorption and a better membrane protection of from photodamage.

Thus, a high content of chlorophyll *a* and a high chlorophyll *a/b* ratio indicate a high potential for photosynthesis of Echinacea leaves. Indirectly, it may indicate a more intensive synthesis of secondary metabolites, in particular, those with biologically active properties.

The main active biologically active substances of Echinacea plants are hydroxy acids and polysaccharides. For a more complete assessment of the Echinacea biologically active substances, we conducted a physico-chemical study of its main components, i.e. rhizomes, roots, stems, leaves, and flowers.

Table 5. Residence times of the main biologically active substances in the samples of chromatographic fractions of ethanol extracts of *Echinacea purpurea* rhizomes and roots

Component	Concentration range, mkg/ml	Correlation coefficient	Residence time t_R, min
8-hydroxy-tetradec-8E-en-11,13-diyn-2-one			5.52
Tetradec-8Z-en-11,13-diyn-2-one			7.87
8-hydroxy-tetradec-9E-en-11,13-diyn-2-one			18.07
Cynarine	1.03–150.00	0.9800	19.01
Echinacoside	0.50–5.03	0.9881	20.97
Chlorogenic acid	0,103–150.05	0.9881	24.25
Chicoric acid	0.51–50.01	0.9891	26.98
Isobutylamide dodeca-2E,4E,8Z,10Z-tetraenic acid			29.39
8- hydroxy-pentadec-9E-en-11,13-diyn-2-one			33.24
Isobutylamide undeca-2E-en-8,10-diynic acid			33.88
Methylbutylamide dodeca-2E,4Z-dien-8,10-diynic acid methylbutylamide dodeca (2E), (4Z)-dien-8,9-diynic acid			36.56

Table 6. Contents of the main biologically active substances in the ethanol extract of *Echinacea purpurea* rhizomes and roots

Component	Content, mg/g of dry weight
8-hydroxy-tetradec-8E-en-11,13-diyn-2-one	2.67 ± 0.01
Tetradec-8Z-en-11,13-diyn-2-one	3.31 ± 0.01
8-hydroxy-tetradec-9E-en-11,13-diyn-2-one	15.84 ± 0.01
Cynarine	23.68 ± 0.01
Echinacoside	16.65 ± 0.01
Chlorogenic acid	3.38 ± 0.01
Chicoric acid	3.43 ± 0.01
Isobutylamide dodeca-2E,4E,8Z,10Z-tetraenic acid	3.51 ± 0.01
8-hydroxypentadien-9E-en-11,13-diyn-2-one	3.40 ± 0.01
Isobutylamide undeca-2E-en-8,10-diynic acid	8.88 ± 0.01
Methylbutylamide dodeca-2E,4Z-dien-8,10-diynic acid	15.19 ± 0.01

For the phytochemical characteristics of the extracts, we chose those groups of compounds that were more likely to be present in the hydrophilic extracts in question.

To study the content of biologically active substances, we analyzed the extract obtained by using a 70% ethanol solution of the stem and leaves. To determine the content of the main groups of biologically active substances, we used HPLC and TLC, accompanied with an IR Fourier-transform spectrometry.

Fig. 1 and Tables 3 and 4 present a detailed analysis of the composition of the extract obtained from the stems and leaves of *Echinacea purpurea*.

The analysis showed that the leaf extract of *Echinacea purpurea* contained such biologically active substances as acetylene and alkene derivatives, as well as phenylpropanoids, which are derived from caffeic acid. The fact that the extracts from leaves and stems contain phenylpropanoids confirms the biological value of the plant. This group of organic compounds is a rich source of substances with adaptogenic, tonic, immunomodulating, hepatoprotective, and antioxidant properties.

The next stage involved an analysis of the extract obtained from the rhizomes and roots of *Echinacea purpurea*. The extraction was performed with a 70% ethanol solution.

To determine the content of the main biologically active substances, we used HPLC and TLC, accompanied with IR Fourier-transform spectrometry. Fig. 2 and Tables 5 and 6 show the results of the chemical and physical analysis of the extract from rhizomes and roots.

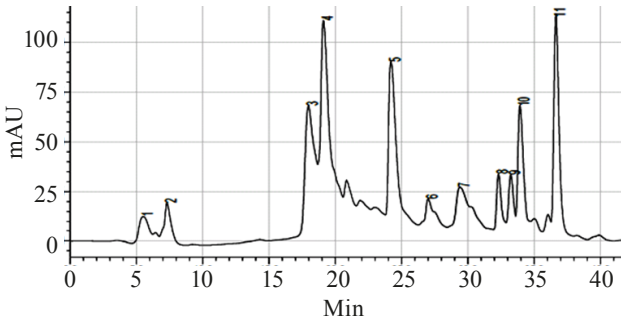

Fig. 3. HPLC chromatogram of the ethanol extract from Echinacea flowers.

Table 7. Residence times of the main biologically active substances in the samples of chromatographic fractions of ethanol extracts of *Echinacea purpurea* flowers

Component	Concentration range mkg/ml	Correlation coefficient	Residence time t_R, min
8-hydroxy-tetradec-8E-en-11,13-diyn-2-one			5.46
8- hydroxy-pentadec-9Z-en-11,13-diyn-2-one			6.03
Tetradec-8Z-en-11,13-diyn-2-one			7.87
3,4-dioxybenzoic acid	1.03–150.00	0.9800	19.01
Echinacoside	0.50–5.03	0.9881	21.78
Chlorogenic acid	0,103–150.05	0.9881	24.25
Chicoric acid	0.51–50.01	0.9891	26.98
Vanillic acid			29.70
8- hydroxy-pentadec-9E-en-11,13-diyn-2-one			33.19
Methylbutylamide dodeca-2E,4Z-dien-8,10-diynic acid			36.56

Tables 5 and 6 and Fig. 2 show that the extract obtained from Echinacea rhizome and roots contain alkylamides and phenylpropanoids.

Alkilamides demonstrate a great variety. Despite their relatively simple molecular structure, these substances have a wide spectrum of biological activity. They have an immunomodulating, antimicrobial, antiviral, insecticidal, diuretic, and antioxidant properties. In addition, they can potentiate antibiotics and inhibit prostaglandin synthesis.

The final stage of the study featured the extract obtained from Echinacea flowers. The extraction was performed with a 70% ethanol solution.

For the physical and chemical evaluation of the flower extracts, we used HPLC and TLC, accompanied

Table 8. Content of the main biologically active substances in the ethanol extract of *Echinacea purpurea* flowers

Component	Content, mg/g of dry weight
8-hydroxy-tetradec-9E-en-11,13-diyn-2-one	2.53 ± 0.01
8- hydroxy-pentadec-9Z-en-11,13-diyn-2-one	1.65 ± 0.01
Tetradec-8Z-en-11,13-diyn-2-one	1,006 ± 0.01
3,4-dioxybenzoic acid	2.45 ± 0.01
Echinacoside	10.19 ± 0.01
Chlorogenic acid	45.32 ± 0.01
Chicoric acid	4.56 ± 0.01
Vanillic acid	5.94 ± 0.01
8- hydroxy-pentadec-9E-en-11,13-diyn-2-one	3.22 ± 0.01
Methylbutylamide dodeca-2E,4Z-dien-8,10-diynic acid	19.19 ± 0.01

with an IR Fourier-transform spectrometry. Fig. 3 and Tables 7 and 8 show the results of the analysis.

The data show that the flower extract contains all the necessary biologically active substances. The most valuable substances are polar derivatives of caffeic acid and chlorogenic acids.

Chlorogenic acids possess strong antioxidant, antimicrobial, and anti-fungal properties. Therefore, they are considered valuable biological active compounds.

CONCLUSION

The experiment revealed that the soils associated with *Echinacea purpurea* in the Kemerovo region demonstrated no excess MPC of heavy metals (Zn, Pb, Co, Ni, Cd). The soils proved to be pollution-free, which makes them suitable for medicinal plants.

The content of such standardized elements as Pb and Cd in various parts of *Echinacea purpurea* is significantly below the permissible level. It makes this vegetable raw material environmentally friendly. It can be used as a source of biologically active substances to produce dietary supplements and functional foods.

The extracts obtained from Echinacea rhizomes, roots, stems, leaves, and flowers were used to study biologically active substances. A 70% ethanol solution was used as an extractant. It allowed for the maximum extraction of biologically active substances.

In order to study the quantitative and qualitative profile of Echinacea biologically active substances, a physical and chemical analysis of these extracts was performed using HPLC, TLC, and IR Fourier-transform spectrometry.

We conducted a comparative analysis of the composition of the biologically active substances in different parts of the plant. It showed that the leaf part of the plant was rich in phenylpropanoids. These compounds exhibited immunomodulatory and antioxidant properties.

The root of the plant mainly contains such significant biologically active substances as alkylamides, which possess immunomodulatory, antimicrobial, and antiviral properties.

The analysis of the ethanol extract of Echinacea flowers showed that it was rich in chlorogenic acid, which is responsible for the antioxidant property in this group of plants.

The experimental contribute to the formation of a data base on the chemical composition of medicinal raw materials that grow in various geographical zones of Russia. The research expands the existing profile of biologically active substances obtained from *Echinacea purpurea* that grows in the Kemerovo Region.

The experimentally established qualitative and quantitative profile that allows us to recommend it for the production of dietary supplements and functional foods.

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