Foods and Raw Materials. 2025;13(2)

ISSN 2308-4057 (Print)
ISSN 2310-9599 (Online)

Research Article
Open Access

Available online at http://jfrm.ru/en
https://doi.org/10.21603/2308-4057-2025-2-649
https://elibrary.ru/UTMTYG

The phytochemical composition of Kuzbass medicinal plants studied by spectrophotometry and chromatography

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Received 27.04.2024; Revised 02.07.2024; Accepted 06.08.2024; Published online 16.10.2024

Abstract:
Flavonoids are plant polyphenols that exhibit biological activity with antibacterial, antiviral, antioxidant, anti-inflammatory, antimutagenic, and anticarcinogenic effects. The medicinal plants of Kuzbass have high contents of flavonoids and other polyphenolic compounds. Therefore, they can be used in medicinal preparations to prevent or treat serious diseases.
We studied the following plants collected in Kuzbass: common thyme (*Thymus vulgaris* Linn., leaves and stems), woolly burdock (*Arctium tomentosum* Mill., roots), alfalfa (*Medicago sativa* L., leaves and stems), common lungwort (*Pulmonaria officinalis* L., leaves and stems), common yarrow (*Achillea millefolium* L., leaves and stems), red clover (*Trifolium pratense* L., leaves and stems), common ginseng (Panax ginseng, roots), sweetvetch (*Hedysarum neglectum* Ledeb., roots), and cow parsnip (*Heracleum sibiricum* L., inflorescences, leaves, and stems). To extract flavonoids, we used ethanol at concentrations of 40, 55, 60, 70, and 75%. Spectrophotometry was used to determine total flavonoids, while high-performance liquid chromatography was employed to study the qualitative and quantitative composition of the extracts.
The highest yield of flavonoids was found in *H. sibiricum* leaves (at all concentrations except 70%), followed by the 55% and 70% ethanol extracts of *T. vulgaris* leaves and stems, as well as the 75% ethanol extract of *A. millefolium* leaves and stems. Thus, these plants have the greatest potential in being used in medicines. High-performance liquid chromatography showed the highest contents of polyphenolic compounds in the samples of *P. officinalis*, *A. millefolium*, *T. vulgaris*, and *T. pratense*.
Our results can be used in further research to produce new medicinal preparations based on the medicinal plants of Kuzbass.

Keywords: Flavonoids, medicinal plants, spectrophotometry, chromatography, HPLC, extraction, plant extracts

Funding: Our study was financially supported by the Russian Science Foundation (RSF) (grant No. 23-16-00113).

Please cite this article in press as: Velichkovich NS, Dunchenko NI., Stepanova AA, Kozlova OV, Faskhutdinova ER, Yustratov VP., *et al.* The phytochemical composition of Kuzbass medicinal plants studied by spectrophotometry and chromatography. Foods and Raw Materials. 2025;13(2):219–232. https://doi.org/10.21603/2308-4057-2025-2-649

INTRODUCTION

Flavonoids are low-molecular-weight polyphenolic phytochemicals secreted by plants as secondary metabolites in response to stress. Secondary metabolites have a wide range of therapeutic effects that are beneficial to humans. Flavonoids are widely distributed in the plant kingdom and have long been used in various herbal medicines due to their antibacterial, antiviral, antioxidant, anti-inflammatory, antimutagenic, and anticarcinogenic properties [1–3].

There have been many studies on medicinal plants to explore their therapeutic potential in treating disease. Herbal treatments have minimal or no side effects and are therefore safer than synthetic drugs. Medicinal plants have shown efficiency in treating a number of difficult-to-cure diseases [4, 5]. Ayurveda has successfully used medicinal herbs for many years to prevent and treat serious diseases [6].

Flavonoids protect plants from biotic and abiotic stress. They function as signaling molecules, detoxifiers, UV filters, allopathic compounds, and phytoalexins, as well as play an important role in drought and frost resistance [7].

Acting as antioxidants, flavonoids protect plants, animals, and humans from the effects of reactive oxygen species. They suppress the formation of reactive oxygen

species by either inhibiting the enzyme or chelating trace elements involved in the formation of free radicals, i.e., by removing reactive oxygen species or by enhancing antioxidant protection [8].

Plant flavonoids exhibit antiviral activity helping inhibit various enzymes involved in the viral life cycle [9]. A wide variety of medicinal plants produce antimicrobial effects due to large numbers of flavonoids that are released in response to bacterial infection [10]. Many studies have shown the effectiveness of flavonoids in cardioprotection and prevention of hypertension and atherosclerosis. Flavonoids reduce atrial pressure, enhance the vasorelaxant process, and prevent endothelial dysfunction [11]. Also, flavonoids have antidiabetic properties that regulate carbohydrate digestion, insulin secretion, insulin signaling, glucose uptake, and fat deposition [12]. Finally, flavonoids exhibit anticancerous activity and inhibit the proliferation of tumor cells by suppressing the formation of reactive oxygen species. They also inhibit the enzymes xanthine oxidase, cyclooxygenase-2, and 5-lipoxygenase, which play an important role in tumor development [13].

Plants accumulate different types and amounts of bioactive substances depending on the region of growth, time of collection, plant organs, and other factors. Kemerovo Oblast – Kuzbass is a region that has a variety of soils and climates, with large areas covered by medicinal herbs. In particular, Kuzbass is home to common thyme (*Thymus vulgaris* Linn.), woolly burdock (*Arctium tomentosum* Mill.), alfalfa (*Medicago sativa* L.), common lungwort (*Pulmonaria officinalis* L.), common yarrow (*Achillea millefolium* L.), red clover (*Trifolium pratense* L.), common ginseng (*Panax ginseng*), sweet-vetch (*Hedysarum neglectum* Ledeb.), and cow parsnip (*Heracleum sibiricum* L.) [14, 15]. These plants have great medicinal potential and can be used in medicines against various diseases.

T. vulgaris is a perennial flowering plant belonging to the *Lamiaceae* family, native to southern Europe [16]. *T. vulgaris* is most widely used as a flavoring agent in the food, perfume, and cosmetic industries, as well as a preservative for chicken, meat, and fish. Due to its healing and antiseptic properties, it was used in traditional medicine to treat wounds and skin diseases [17]. It has also been used as essential oil to treat food poisoning due to a high content of polyphenols such as carvacrol and thymol [18]. Modern studies have revealed the effectiveness of *T. vulgaris* in improving oxidative stress and cell-mediated immune response [19, 20]. Studies also confirm the plant's antiparasitic and antihelminthic activity due its content of monoterpenes [21].

A. tomentosum belongs to the *Asteraceae* family and grows in Europe, Asia, and North America [22]. Due to its polyphenolic compounds, the plant is widely applied in traditional medicine. Its root and leaf extracts have a diuretic effect and improve metabolism. They are also used to treat gastrointestinal diseases and diabetes mellitus in the early stages [23, 24]. The leaves and roots of *A. tomentosum* are applied externally to relieve skin inflammation. Burdock contains compounds of the lignan group, such as lappaol A, lappaol C, lappaol F, matairesinol, arctiin, arctigenin, and arctigenic acid [22]. Its leaves are rich in flavonoids (luteolin, quercetin, quercitrin, and rutin) and phenolic acids, while its root extracts are rich in dicafoylquinic acid isomers and their derivatives, polysaccharides, and derivatives of polyunsaturated fatty acids [22, 25].

M. sativa is one of the most important forage species that has recently been used for human consumption, mainly due to its content of bioactive phenolic compounds [26]. According to numerous studies, *M. sativa* contains bioactive phytochemicals, such as alkaloids, saponins, phenols, tannins, polysaccharides, and phytoestrogens, which have antioxidant, anti-inflammatory, and anticarcinogenic properties [27–29]. Several classes of alfalfa's phenolic compounds have been described in literature, especially phenolic acids and flavonoids.

P. officinalis is a long-lived, shade-tolerant perennial plant belonging to the *Boraginaceae* family [30]. Although lungwort is native to Central and Eastern Europe, its scattered populations are also found in the UK and Denmark. The plant has emollient, antitussive, expectorant, antimicrobial, diuretic, cleansing, antilithiatic, and anti-inflammatory effects and is used to treat diseases of the respiratory tract and urinary system [31]. The biological properties of *P. officinalis* can be attributed to a diverse set of phytochemicals such as anthocyanins, alkaloids, flavanones, flavonols, flavones, hydroxycinnamic acid, lignans, polyphenols, polyphenolic acids, and other phytochemicals present in various parts of the plant [31]. The extract of *P. officinalis* leaves contains such compounds as naringin, hesperidin, naringeninm, apigenin-7-glucoside, rutin, chlorogenic acid, myricetin, hyperoside, acacetin, and gallic acid [32].

A. millefolium is a herbaceous flowering plant that grows wild in Asia, Africa, Europe, and America [33]. In traditional medicine, its extract is widely used to treat inflammatory, hepatobiliary, cardiovascular, and respiratory diseases, as well as diabetes and diabetes-related diseases [34, 35]. The main phytochemical compounds isolated from *A. millefolium* are essential oil and flavonoid derivatives including apigenin, rutin, lutein, and kaempferol [36].

T. pratense is a valuable forage plant growing in temperate and humid regions [37]. It is used to treat diabetes, as well as cardiovascular, neurodegenerative, and other diseases [38–41]. Isoflavones and flavonoids have been found in *T. pratense*, such as formononetin, biochanin A, genistein, and daidzein.

P. ginseng has been used in traditional medicine since ancient times. It has a positive effect on cardiovascular and neurodegenerative diseases, cancer, and diabetes mellitus [42–46]. Extensive research has associated the biological activities of Korean *P. ginseng* and its products with various functional components, including ginsenosides, polyacetylenes, phenolic compounds, alkaloids, polysaccharides, oligopeptides, and essential oils [47].

H. neglectum is a plant of the legume family [48]. Known in traditional medicine as a "red root," *H. neglectum* is used as an anti-inflammatory agent, as well as to treat gastrointestinal, cardiovascular, and other diseases. The plant contains polysaccharides, flavonoids, catechins, tannins, alkaloids, and other bioactive substances [49, 50].

H. sibiricum is a perennial taproot plant widespread in the central part of Russia, Central Europe, Ciscaucasia, and Western Siberia [51]. *H. sibiricum* is actively used as a forage plant for cattle, pigs, rabbits, and birds [52]. In medicine, its roots and leaves are used to treat diseases of the nervous and digestive systems, convulsions, and skin diseases. Preparations based on this plant have antimicrobial and anti-inflammatory effects. The leaves are harvested during the flowering period, while the roots are collected and dried in the autumn. Preparations made from the roots of *H. sibiricum* are considered more effective than those based on its leaves [53]. *H. sibiricum* contains essential oils (up to 3%), coumarins (up to 2.5%), tannins, flavonoids, resins, phenols, and other compounds.

Thus, using the medicinal plants of Kuzbass in drugs and preparations can be effective in treating many diseases, including oncological, gastrointestinal, cardiovascular, and neurodegenerative diseases, diabetes, etc. For this, polyphenols in plant materials need to be identified and quantified.

In this study, we aimed to identify phytochemicals in Kuzbass medicinal plants isolated from different plant organs by extractants at various concentrations in order to select the plants rich in bioactive substances to be further extracted and studied by spectrophotometry and high-performance liquid chromatography.

The novelty of our study lies in its focus on medicinal plants that had to adapt to the specific climatic conditions of Kuzbass and therefore developed unique chemical profiles and biological activity. Our data can be further used to develop effective medicines and dietary supplements.

STUDY OBJECTS AND METHODS

Spectrophotometry and high-performance liquid chromatography (HPLC) were employed to analyze medicinal plant materials collected in Kemerovo Oblast – Kuzbass from May to August 2023, namely:

– *Thymus vulgaris* Linn. (leaves and stems) collected in Zhuravlevo village;
– *Arctium tomentosum* Mill. (roots) collected in the Topkinsky Municipal District, Sukhaya Rechka village, and Mamaevsky settlement;
– *Medicago sativa* L. (leaves and stems) collected in Metallploshchadka settlement, Topkinsky Municipal District, Pugachi village, Zhuravlevo village, and Belovsky District;
– *Pulmonaria officinalis* L. (leaves and stems) collected in the Topkinsky Municipal District, Yaya District, Zhuravlevo village;
– *Achillea millefolium* L. (leaves and stems) collected in the Yaya District and Belovsky District;
– *Trifolium pratense* L. (leaves and stems) collected in Kemerovo city, Pugachi village, Mamaevsky settlement, and Zhuravlevo village;
– *Panax ginseng* (roots) collected in Sheregesh village;
– *Hedysarum neglectum* Ledeb. (roots) collected in Sheregesh village; and
– *Heracleum sibiricum* L. (inflorescences, leaves, and stems) collected in the Topkinsky Municipal District, Pugachi village, and Zhuravlevo village.

Morphological identification was carried out by the working group of the grant team.

The plant materials were collected in 2023 and dried according to the State Pharmacopoeia XIII [54]. In particular, the collected materials were washed, crushed, and dried (first, at room temperature in a well-ventilated room and then at 50°C until the samples had a recommended moisture, averaging 6–13%). The samples were stored in bags in a dry, cool place at the Laboratory for Biotesting Natural Nutraceuticals, Kemerovo State University.

Extracts were prepared to isolate flavonoids from the plant materials. For this, we took samples of medicinal plants (1 g each), weighed them in a conical flask, and added 30 mL of ethanol.

Solvents such as mixtures of water with alcohol (methanol or ethanol) are the most suitable systems for polyphenol extraction. Water acts as a swelling agent for the plant material, increasing the contact surface. Pure alcohols dehydrate and disrupt the plant cells, causing the breakdown of the dissolved cell wall bond. Therefore, alcohol is mixed with water for a synergistic effect, and the optimal concentration of alcohol can increase the extraction of flavonoids [55]. We used different concentrations of ethanol (40, 55, 60, 70, and 75%) for all the samples to determine the best yield of flavonoids. For some samples, we used a two-phase extraction system (70% ethanol + oil). Then, the flask was placed in a reflux condenser and heated in a boiling water bath for 30 min, with periodical shaking. The resulting supernatant was filtered into a 100-mL measuring flask, preventing the plant particles from getting on the filter.

Flavonoids were extracted in triplicate for each plant sample with different concentrations of ethanol (30 mL) added to the filtrate. Then the filtered materials were combined and cooled to 20°C. In the volumetric flask, the filtrate was brought to the mark with ethanol.

Spectrophotometry and HPLC were used to determine the mass fraction of flavonoids in the plant extracts, as well as to examine their qualitative and quantitative composition.

Spectrophotometric analysis involved measuring the optical density of the solutions [56]. For this, 5 mL of the extracts was poured into two 25-mL flasks. Then, 4 mL of a 5% solution of aluminum chloride was added to one of the flasks (test solution). Both flasks were brought to the mark with ethanol and thoroughly mixed. To prepare the aluminum chloride solution, 5 g of aluminum chloride was weighed in a 250-mL conical flask and dissolved in 50 g of ethanol; then, the solution was brought

to 100 g with the same alcohol and thoroughly mixed. After 30 min, the optical density of the test solution was determined against the reference solution (without aluminum chloride) at 410 nm (maximum absorption) in cuvettes with a 1-cm optical layer on a UV 1800 spectrophotometer (Shimadzu, Japan).

Then, we determined the amount of rutin (mg) in 25 mL of the solution of the state standard reference rutin sample (Sigma-Aldrich) using a calibration graph. To prepare the solution, 0.050 g of rutin was weighed in a 50-mL volumetric flask, mixed with 40 mL of ethanol, and heated to 50–60°C until rutin completely dissolved. Then the solution was cooled to room temperature, brought to the mark with ethanol (at the same concentration as was used to prepare the solutions of medicinal plants), and stirred. To plot the calibration graph, two 25-mL volumetric flasks were filled with 0.2 mL of the prepared rutin solution at a concentration of 1 mg/mL. Then, 4 mL of aluminum chloride solution was added to one of the flasks (test solution), and both flasks were brought to the mark with ethanol and mixed. New solutions were similarly prepared with 0.4, 0.6, 0.8, 1.0, and 1.2 mL of the rutin solution as described above and kept for 30 min. The optical density of the test solutions was measured against the reference solution (without aluminum chloride) at 390–420 nm (maximum absorption) in cuvettes with a 1-cm optical layer.

The mass fraction of flavonoids in terms of rutin (X, %) was determined according to the following Eq. (1):

$$X = C \times 100 \times 100 \times m \times 5 \quad (1)$$

where C is the amount of rutin in 25 mL according to the calibration graph, mg; 100 is the volume of the extracts, mL; 100 is the volume of the extracts, %; m is the mass of the medicinal plant samples, g; 5 is the volume of the medicinal plant extracts, mL.

Flavonoid compounds were also determined qualitatively and quantitatively by HPLC on an LC-20 Prominence Shimadzu chromatograph with a Shimadzu SPD-20MA diode-matrix detector (Shimadzu, Japan) and a Kromosil C18 chromatographic column (5 µm, 110 A, 250×4.6 mm). The conditions included an injection volume of 20 µL, a column temperature of 400, and a reference wavelength of 254 nm.

Microsoft® Excel was used for statistical data processing. The absolute calibration method with the rutin standard was employed to quantify the flavonoids.

RESULTS AND DISCUSSION

Total flavonoids were spectrophotometrically determined in the following medicinal plant materials: *Thymus vulgaris* Linn. (leaves and stems), *Arctium tomentosum* Mill. (roots), *Medicago sativa* L. (leaves and stems), *Pulmonaria officinalis* L. (leaves and stems), *Achillea millefolium* L. (leaves and stems), *Trifolium pratense* L. (leaves and stems), *Panax ginseng* (roots), *Hedysarum neglectum* Ledeb. (roots), and *Heracleum sibiricum* L. (inflorescences, leaves, and stems). Ethanol extractant was used at different concentrations to identify the highest yield of flavonoid compounds (Figs. 1–4).

The extract of *T. vulgaris* leaves and stems with 55% ethanol showed the highest yield of flavonoids (1.124%). In the study by Malankina *et al.*, this indicator was significantly higher, varying from 1.64 to 2.83% [57]. The authors found that the flavonoid contents tended to increase in the years with lower average daily temperatures during the harvesting period, which might explain the differences. The extracts of *M. sativa* and *A. millefolium* leaves and stems with 75% ethanol had the maximum yields of flavonoids (0.939% each).

Compared to the flavonoid content in the *A. millefolium* extract in our study, Tikhonov *et al.* reported a flavonoid content of 1.28% (0.34% higher) extracted with 40 and 70% ethanol for 24 h [58]. Longer extraction

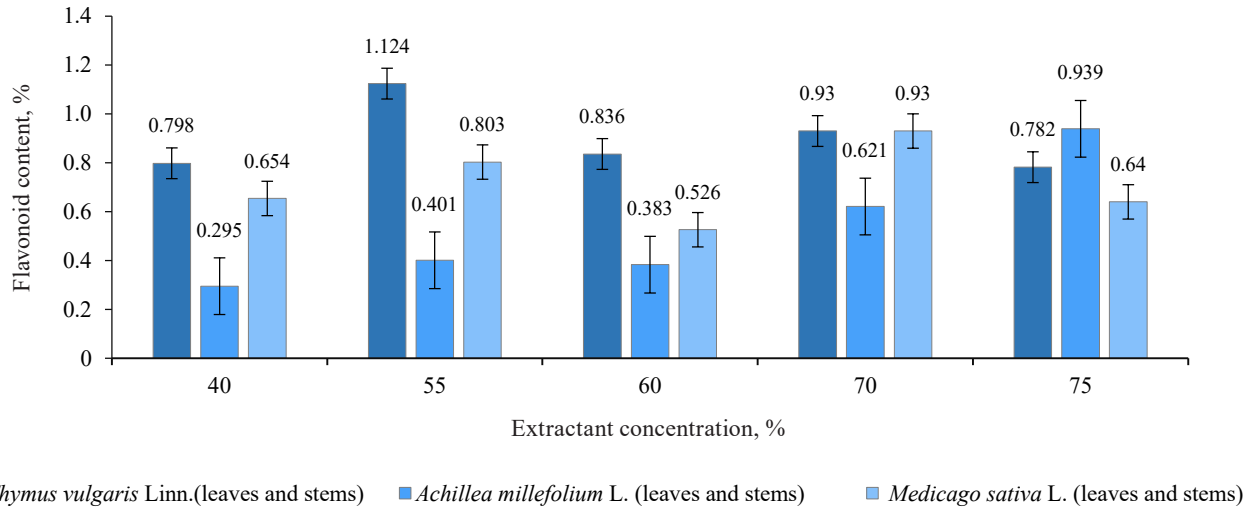

Figure 1 Total flavonoids in the extracts of *Thymus vulgaris* Linn., *Achillea millefolium* L., and *Medicago sativa* L. (leaves and stems)

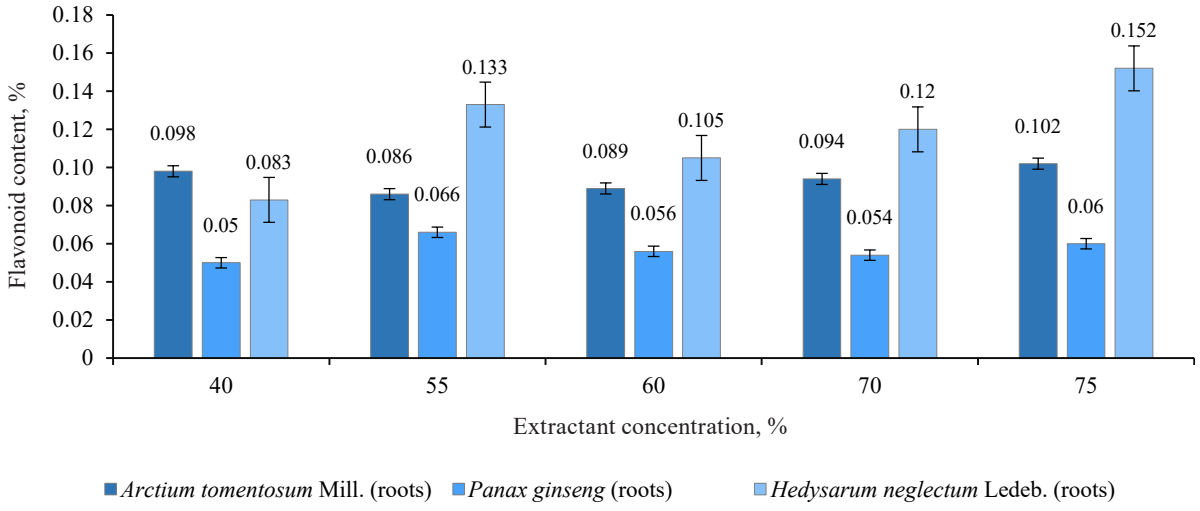

Figure 2 Total flavonoids in the extracts of *Arctium tomentosum* Mill., *Panax ginseng*, and *Hedysarum neglectum* Ledeb. (roots)

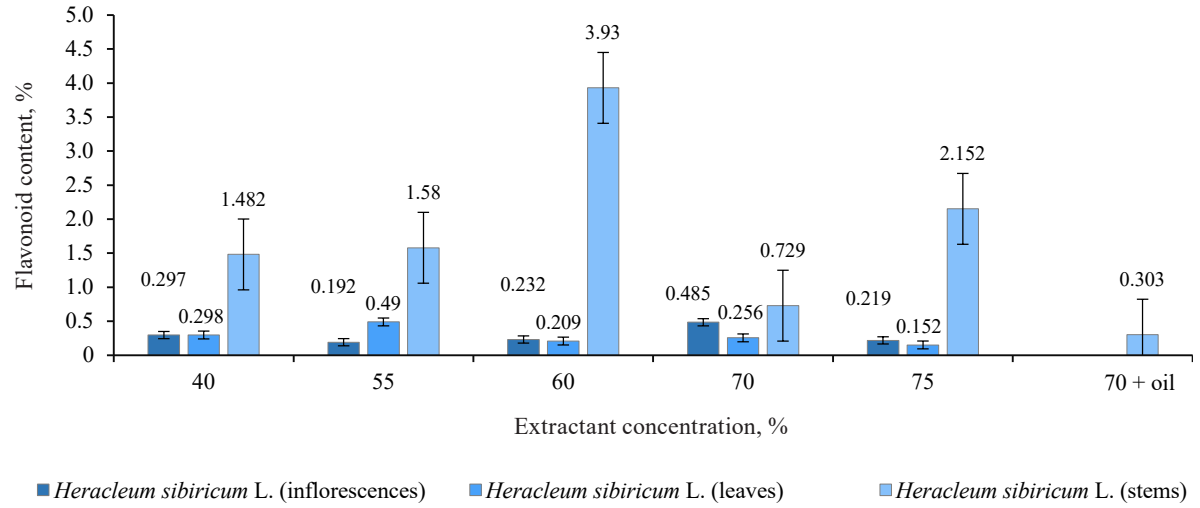

Figure 3 Total flavonoids in the extracts of *Heracleum sibiricum* L. (inflorescences, leaves, stems)

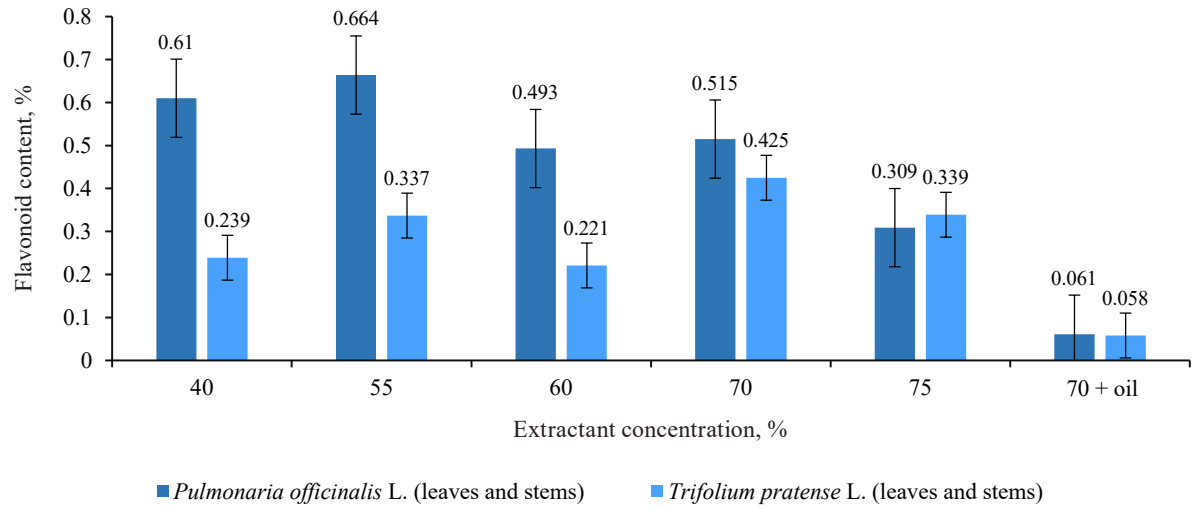

Figure 4 Total flavonoids in the extracts of *Pulmonaria officinalis* L. and *Trifolium pratense* L. (leaves and stems)

can contribute to higher yields of target substances, but it is not always rational for the production process due to high electricity costs. Nevertheless, we are planning to optimize the extraction process in terms of its duration and yield of flavonoids in further research. Another interesting fact is that in the study by Tikhonov *et al.*, the contents of flavonoids were quantitatively the same in the extracts based on 40 and 70% ethanol, while our study showed a 0.33% difference. This can be explained by a number of factors, mainly the difference in the extraction time. It might be that the 24-h extraction in the study by Tikhonov *et al.* ensured the maximum yield of flavonoids regardless of the ethanol concentration. Another possible explanation is the variability in the composition of flavonoids caused by differences in the place of plant growth.

The extracts of *A. tomentosum*, *P. ginseng*, and *H. neglectum* roots extracted with 75, 55, and 75% ethanol had the highest contents of flavonoids, namely 0.102, 0.066, and 0.152%, respectively. In comparison, Shkolnikova *et al.* reported a 0.06% lower flavonoid content (0.09%, in terms of rutin) in the *H. neglectum* roots collected in the Republic of Altai [59]. This is likely due to the difference in the region of growth, including climatic conditions, soil composition, and other environmental factors. Our results indicate great potential of *H. neglectum* grown in Kemerovo Oblast as a source of flavonoids.

The *H. sibiricum* inflorescence, stem, and leaf extracts had the maximum flavonoid contents (0.485, 0.490, and 3.93%, respectively) with 70, 55, and 60% ethanol, respectively. Modern literature lacks information on total flavonoids in different parts of *H. sibiricum*, so our results contribute significantly to understanding the plant's phytochemical characteristics.

The leaf and stem extracts of *P. officinalis* and *T. pratense* showed the highest yields of flavonoids (0.664 and 0.425%, respectively) with 55 and 70% ethanol, respectively. According to modern literature, the content of flavonoids in *T. pratense* varies greatly depending on the variety and region of cultivation. For example, Konovalenko *et al.* reported 0.58% of flavonoids, while Kasatkina and Nelyubina found 1.3–2.4% of flavonoids in terms of rutin [60, 61]. Our values were significantly lower, which may be due to differences in the methods of extraction and quantification. We used spectrophotometry, while the above studies [60, 61] used thin-layer chromatography, which might affect the accuracy of the results.

According to our spectrophotometric analysis, the highest yield of flavonoids was observed in the *H. sibiricum* leaf extract, followed by the stem and leaf extracts of *T. vulgaris* and *M. sativa*. Therefore, these plants are the most promising for being used in medicinal preparations. We found no correlation between the concentration of the extractant and the yield of flavonoids. However, 55% ethanol produced the maximum yield of flavonoids more often than the other concentrations.

Then, we identified and quantified bioactive substances in the *M. sativa* extract by HPLC (Fig. 5 and Table 1).

As can be seen, the *M. sativa* extract contained significant amounts of tricine and quercetin-3-O-glycoside. Also, we detected 3,3',4,5-tetrahydroxyflavone, formononetin, and naringenin. Karimi *et al.*, who studied the methanol extract of *M. sativa* leaves in Iran, reported

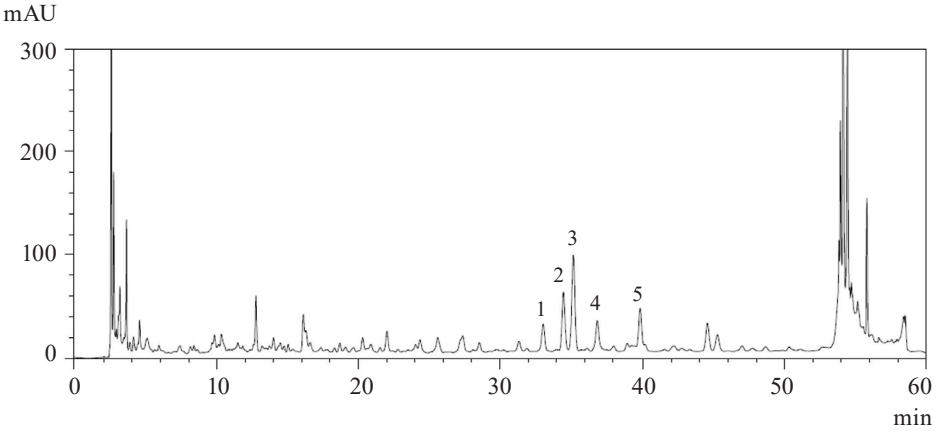

Figure 5 The chromatogram of bioactive substances in the *Medicago sativa* L. extract: 1 – 3,3',4,5-tetrahydroxyflavone, 2 – quercetin-3-O-glycoside, 3 – Tricine, 4 – Formononetin, 5 – Naringenin

Table 1 The contents of bioactive substances in the *Medicago sativa* L. extract

Peak No.	Bioactive substances	Retention time, min	Content, mg/g
1	3,3',4,5-tetrahydroxyflavone	33.0100	0.6400 ± 0.0011
2	Quercetin-3-O-glycoside	34.2100	1.3000 ± 0.0027
3	Tricine	35.1400	2.0900 ± 0.0300
4	Formononetin	36.8900	0.7300 ± 0.0021
5	Naringenin	39.7800	0.9800 ± 0.0026

the phenolic compounds of apigenin (0.214 mg/g), myricetin (0.456 mg/g), naringin (0.738 mg/g), quercetin (0.574 mg/g), and daidzein (0.335 mg/g) [62]. By comparison, the content of naringin in our study was 0.24% higher. The content of tricine, however, has not been previously reported for *M. sativa* extracts. Thus, the flavonoid composition of *M. sativa* differs depending on the region of growth. Further research is needed to identify factors that affect flavonoid contents in the plant and to assess their biological activity.

The results of HPLC analysis of the *P. officinalis* extract are shown in Fig. 6 and Table 2.

As can be seen, the *P. officinalis* extract contained gallic acid, triterpene saponin, ferulic acid, and rosmarinic acid. These results are consistent with those reported by Dushlyuk *et al.* [63]. The authors detected ferulic, gallic, caffeic, rosmarinic, and chlorogenic acids, as well as triterpene saponins, rutin, isorhamnetin, and quercetin in the 70% extract of the *P. officinalis* callus culture. It is worth noting that the contents of caffeic, rosmarinic, and chlorogenic acids were higher in the callus culture than in the above-ground parts of the plant.

The results of HPLC analysis of the *P. ginseng* extract are shown in Fig. 7 and Table 3.

According to HPLC analysis, the *P. ginseng* extract contained syringic acid, ginsenoside LC_1, panaxen, ginsenoside RB_1, panaxoside, and gomisin A.

Ginsenoside RB_1, which showed the highest yield among all the compounds, is one of the most important components of ginseng that contributes to its therapeutic effect [64]. This compound has numerous beneficial effects on human health, including the cardiovascular and central nervous systems, as well as antidiabetic and antitumorous activity. Notably, we detected significant amounts of syringic acid, which had not been previously reported for *P. ginseng* extracts.

The results of HPLC analysis of the *T. vulgaris* extract are shown in Fig. 8 and Table 4.

The HPLC analysis of the *T. vulgaris* extract determined significant contents of apigenin, thymol, quercetin, and hesperidin, as well as lower contents of gallic and caffeic acids.

Thymol is considered the main component of *T. vulgaris*, which was confirmed by our study. However, apigenin was present in higher contents than thymol. Apigenin has anticarcinogenic, anti-inflammatory, antiviral, and antioxidant properties [65]. Mărculescu *et al.* reported that the *T. vulgaris* extract contained caffeic (436.4 mg/100 g), chlorogenic (25.5 mg/100 g), p-coumaric (19.1 mg/100 g), and ferulic (41.6 mg/100 g) acids, as well as luteolin (658.8 mg/100 g) and apigenin (57.4 mg/100 g) [66].

The results of HPLC analysis of the *A. millefolium* extract are shown in Fig. 9 and Table 5.

As can be seen, the *A. millefolium* extract contained chlorogenic and caffeic acids, apigenin, vicenin-2, luteolin, rutin, hesperidin. Asyakina *et al.*, who studied the chemical composition and biological activity of *A. millefolium* cell cultures, detected caffeic (22.21 mg/mL) and 4,5-dicaffeoylquinic (12.70 mg/mL) acids, as well as coumarosides (14.55 mg/mL), luteolin (9.27 mg/mL), vicenin-2 (4.18 mg/mL), rutin (2.96 mg/mL), and other compounds [67].

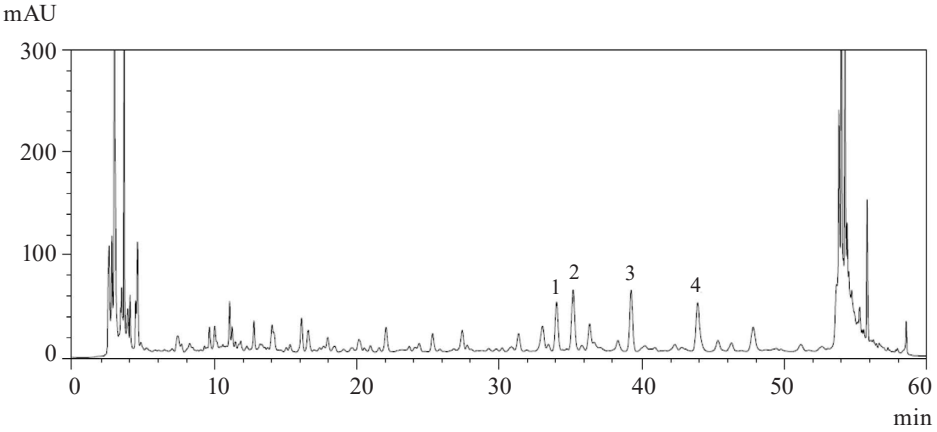

Figure 6 The chromatogram of bioactive substances in the *Pulmonaria officinalis* L. extract: 1 – Gallic acid, 2 – Triterpene saponin, 3 – Ferulic acid, 4 – Rosmarinic acid

Table 2 The contents of bioactive substances in the *Pulmonaria officinalis* L. extract

Peak No.	Bioactive substances	Retention time, min	Content, mg/g
1	Gallic acid	34.0500	3.2900 ± 0.0019
2	Triterpene saponin	35.2200	4.3200 ± 0.0031
3	Ferulic acid	39.1000	4.6800 ± 0.0039
4	Rosmarinic acid	43.9500	4.0100 ± 0.0045

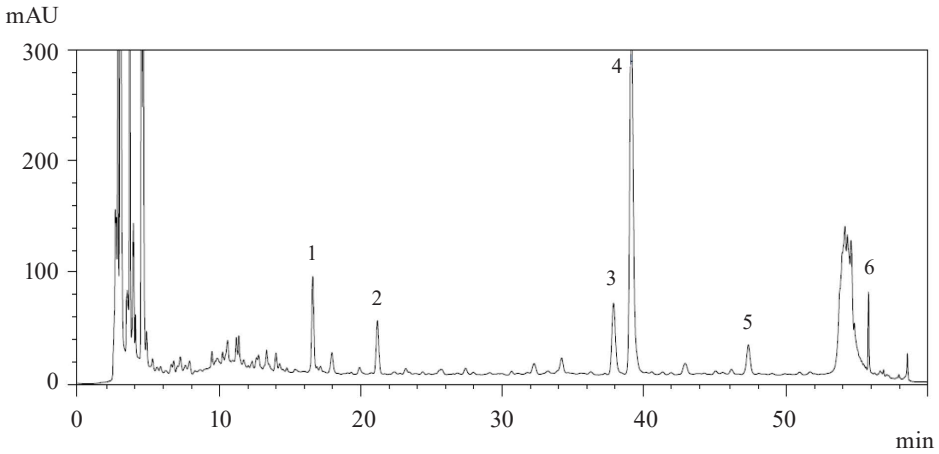

Figure 7 The chromatogram of bioactive substances in the *Panax ginseng* extract: 1 – Syringic acid, 2 – Ginsenoside LC_1, 3 – Panaxen, 4 – Ginsenoside RB_1, 5 – Panaxoside, 6 – Gomisin A

Table 3 The contents of bioactive substances in the *Panax ginseng* extract

Peak No.	Bioactive substances	Retention time, min	Content, mg/g
1	Syringic acid	16.3400	0.9500 ± 0.0020
2	Ginsenoside LC_1	21.1800	0.5400 ± 0.0012
3	Panaxen	37.9500	0.7100 ± 0.0017
4	Ginsenoside RB_1	39.1300	0.7100 ± 0.0017
5	Panaxoside	47.1800	0.3400 ± 0.0014
6	Gomisin A	55.9700	0.8300 ± 0.0019

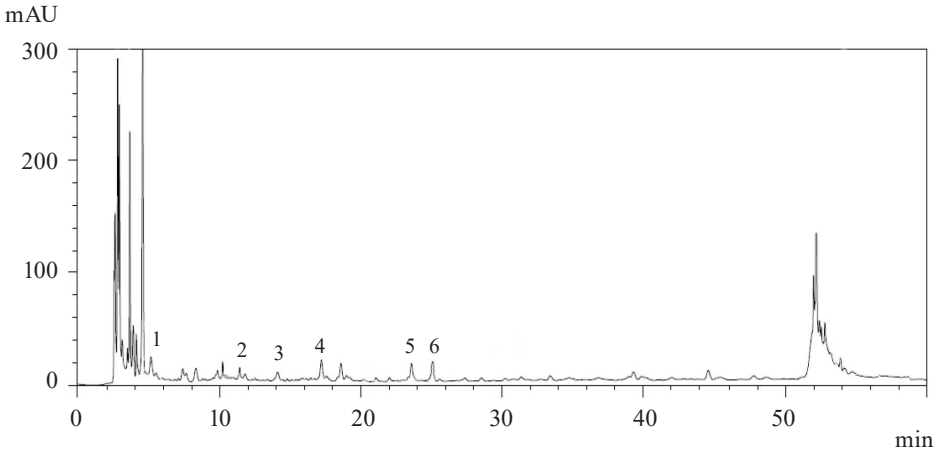

Figure 8 The chromatogram of bioactive substances in the *Thymus vulgaris* Linn. extract: 1 – Quercetin, 2 – Gallic acid, 3 – Caffeic acid, 4 – Apigenin, 5 – Hesperidin, 6 – Thymol

Table 4 The contents of bioactive substances in the *Thymus vulgaris* Linn. extract

Peak No.	Bioactive substances	Retention time, min	Content, mg/g
1	Quercetin	5.1800	1.6000 ± 0.0025
2	Gallic acid	11.3400	1.0900 ± 0.0021
3	Caffeic acid	14.0700	0.3600 ± 0.0013
4	Apigenin	17.0100	2.0700 ± 0.0042
5	Hesperidin	23.6500	1.5600 ± 0.0027
6	Thymol	25.1300	1.8300 ± 0.0034

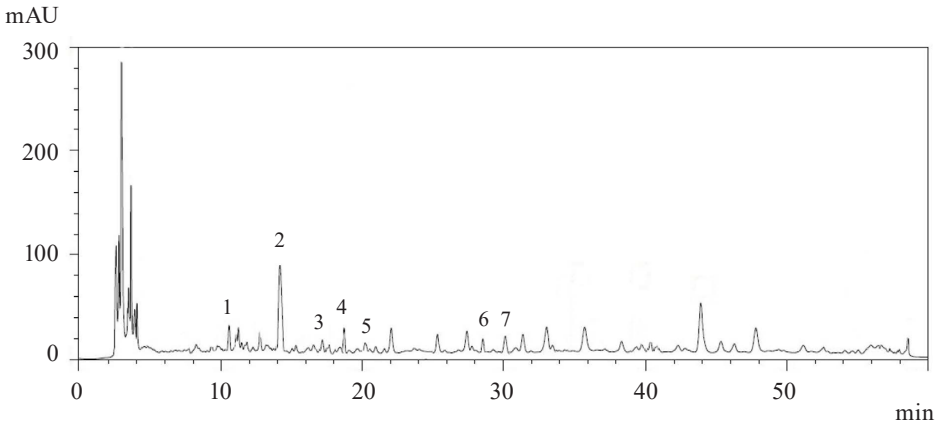

Figure 9 The chromatogram of bioactive substances in the *Achillea millefolium* L. extract: 1 – Chlorogenic acid, 2 – Caffeic acid, 3 – Apigenin, 4 – Vicenin-2, 5 – Luteolin, 6 – Rutin, 7 – Hesperidin

Table 5 The contents of bioactive substances in the *Achillea millefolium* L. extract

Peak No.	Bioactive substances	Retention time, min	Content, mg/g
1	Chlorogenic acid	10.2100	1.0300 ± 0.0027
2	Caffeic acid	14.0700	5.2900 ± 0.0059
3	Apigenin	17.0400	0.3900 ± 0.0016
4	Vicenin-2	18.6900	1.0200 ± 0.0023
5	Luteolin	20.3400	0.2400 ± 0.0008
6	Rutin	28.6100	0.4600 ± 0.0010
7	Hesperidin	30.0900	0.7000 ± 0.0022

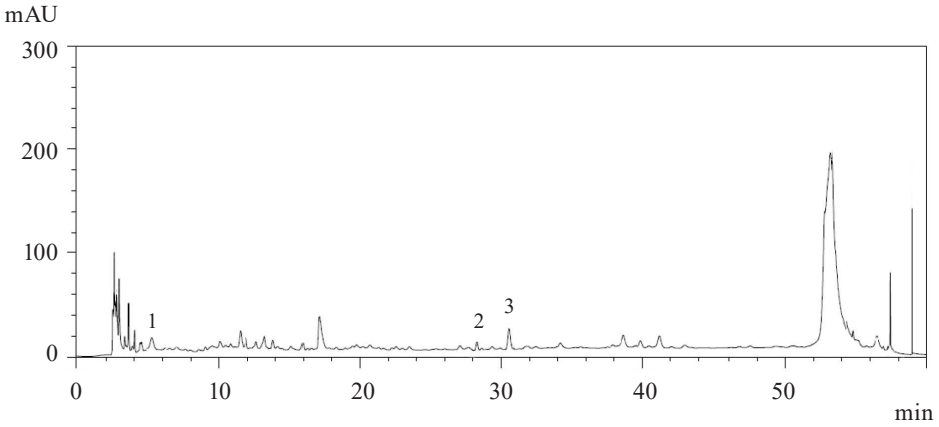

Figure 10 The chromatogram of bioactive substances in the *Hedysarum neglectum* Ledeb. extract: 1 – Quercetin, 2 – Rutin, 3 – Mangiferin

Table 6 The contents of bioactive substances in the *Hedysarum neglectum* Ledeb. extract

Peak No.	Bioactive substances	Retention time, min	Content, mg/g
1	Quercetin	4.9900	0.3800 ± 0.0016
2	Rutin	28.3000	0.1300 ± 0.0007
3	Mangiferin	30.6100	0.7200 ± 0.0021

The results of HPLC analysis of the *H. neglectum* extract are shown in Fig. 10 and Table 6.

According to Table 6, the *H. neglectum* extract contained quercetin, rutin, and mangiferin. Babich *et al.* reported the presence of coumaric acid (0.460 mg/kg), hyperoside/rutin (11.628 mg/kg), and quercetin-3-glycoside (10.410 mg/kg) in the *H. neglectum* extract [68].

The results of HPLC analysis of the *T. pratense* extract are shown in Fig. 11 and Table 7.

According to HPLC results, the *T. pratense* extract contained significant amounts of quercetin, biochanin A, and daidzein. Smaller amounts were detected of genistein, apigenin, rutin, luteolin, formononetin, and naringin. Tundis *et al.* found luteolin (16.7 mg/g),

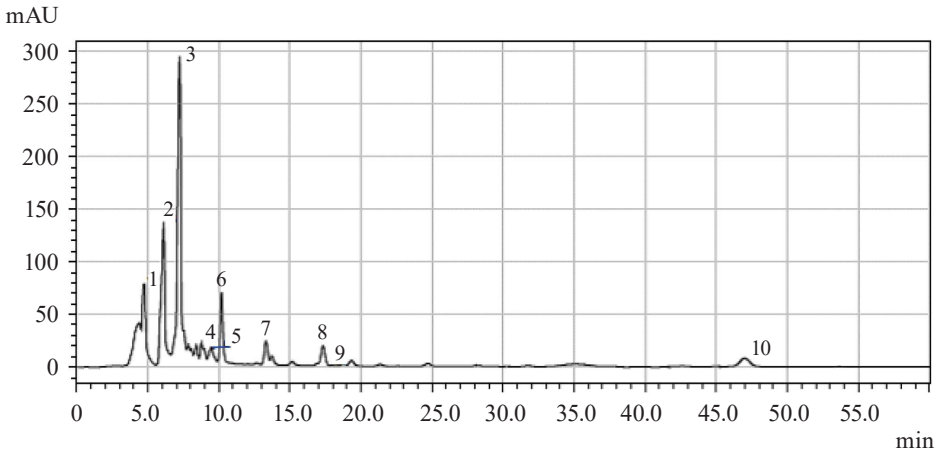

Figure 11 The chromatogram of bioactive substances in the *Trifolium pratense* L. extract: 1 – Guercetin, 2 – Biochanin A, 3 – Daidzein, 4 – Genistein, 5 – Apigenin, 6 – Luteolin, 7 – Formononetin, 8 – Naringin, 9 – Rutin

Table 7 The contents of bioactive substances in the *Trifolium pratense* L. extract

Peak No.	Bioactive substances	Retention time, min	Content, mg/g
1	Quercetin	4.9800	2.8400 ± 0.0048
2	Biokhanin A	6.7300	3.1900 ± 0.0053
3	Daijouin	10.0900	6.3400 ± 0.0076
4	Genistein	15.0000	1.2800 ± 0.0031
5	Apigenin	16.9200	0.4300 ± 0.0012
6	Rutin	28.2900	0.6900 ± 0.0015

kaempferol (0.8 mg/g), and myricetin (0.5 mg/g) in the extract of *T. pratense* flowers [69].

As shown by the tables above, the HPLC analysis revealed the highest contents of polyphenolic compounds in the samples of *P. officinalis*, *A. millefolii*, *T. vulgaris*, and *T. pratense*. Therefore, these plants can be considered the most promising for developing medicinal preparations against various diseases.

CONCLUSION

Flavonoids play a valuable role in human health, which explains their increasing consumption worldwide. The medicinal plants of Kemerovo Oblast – Kuzbass are a rich source of these natural antioxidants. Flavonoids require new methods and technologies to be developed for their study and use in production.

According to the spectrophotometric analysis, the highest yield of flavonoids was determined in the extract of *Heracleum sibiricum* L. leaves with 60% ethanol. Quite high contents were also detected in the 75% ethanol extract of *H. sibiricum* leaves, the 55 and 70% ethanol extracts of *Thymus vulgaris* Linn. leaves and stems, and in the 75% ethanol extract of *Achillea millefolium* L. leaves and stems.

High-performance liquid chromatography showed the highest contents of polyphenolic compounds in the samples of *Pulmonaria officinalis* L., *A. millefolium*, *T. vulgaris*, and *Trifolium pratense* L.

Thus, our results for the qualitative and quantitative composition of medicinal plants grown in Kemerovo Oblast – Kuzbass can be further used to study the biological activity of the extracts in order to create new medicinal preparations to maintain health and reduce the risk of life-threatening diseases such as diabetes, cancer, stroke, and cardiovascular diseases.

CONTRIBUTION

The authors were equally involved in writing the manuscript and are equally responsible for plagiarism.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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