

## STUDY OF THE CHEMICAL COMPOSITION OF THE *RUBUS VULGARIS* PLANT

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**Abstract.** *Introduction:* *Rubus vulgaris*, a member of the *Rosaceae* family within the genus *Rubus*, encompasses a diverse array of woody prickly plants characterized by their herbaceous multicolored or often vertical growth habits, featuring climbing or elongated branches adorned with simple or complex leaves. Among these species, *Rubus vulgaris* stands out as a shrub renowned for its prized fruits, commonly referred to as blackberries. These fruits hold significant importance due to their multifaceted utility in medicinal, cosmetic, and nutritional domains. Recognized as a concentrated source of valuable nutrients and biologically active compounds with therapeutic potential, *Rubus vulgaris* earns recognition as a functional food. The fruits and leaves of *Rubus vulgaris* harbor a rich reservoir of phenolic acids, including ellagic, gallic, caffeic, and p-coumaric acids, alongside flavonoids such as quercetin, hyperoside, kaempferol, myricitin, (+)-catechin, (–)-epicatechin, epicatechin gallate, procyanidin B<sub>2</sub>, and quercetin-3-D-glucoside. This intricate chemical composition underscores the plant's significance in various industries. Further analysis of *Rubus vulgaris* composition reveals its moisture content (6.71 %), ash content (3.48 %), extractive substances (16.48 %), organic acids (1.43 %), flavonoids (0.23 %), tannins (1.56 %), alkaloids (3.98 %), coumarin (2.18 %), saponins (3.80 %), and carbohydrates (2.12 %). Additionally, employing multi-element atomic emission spectral analysis unveiled the presence of eight elements in the plant ash, prominently featuring potassium (162 mg/g), calcium (29 mg/g), phosphorus (22 mg/g), and magnesium (20 mg/g). Such comprehensive elucidation of *Rubus vulgaris*'s chemical composition not only underscores its nutritional significance but also paves the way for its utilization in diverse fields ranging from pharmaceuticals to food science and beyond.

**Key words.** *Rubus vulgaris*, extraction, biologically active substances, chromatography, minerals, etc.

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## 1. Introduction

The flora of Kazakhstan is characterized by a variety of medicinal plant raw materials, many types of which can be used on an industrial scale. One of the most common plants in Kazakhstan is the common blackberry (lat. *Rubus vulgaris*). *Rubus vulgaris* is a spiny shrub with tapering thorns that curve downward to the base of the stem. The leaves are palmately compound with five leaflets. The flowers are pale lavender in color. Berries are black. The species is sometimes considered synonymous with *R. commutatus*. *Rubus vulgaris* plant blooms in May - September, bears fruit in June - November, before the first frosts. It grows in forests, shrubs, on the banks of rivers and streams, in meadows, gardens, roads and hedges. It is found in all regions of Kazakhstan and quite high in the mountains. Blackberry *vulgaris* is a fruit plant of the genus *Rubus* of the pink family, a semi-shrub, reaches a height of 50-150 cm to 3 m [1].

Phenolic acids such as ellagic, gallic, caffeic and p-cumaric acids and flavonoids such as quercetin, hyperoside, kaempferol, myricetin, (+)-catechin, (-)-epicatechin, epicatechin gallate, procyanidin B2 and quercetin-3-D were found in the fruits and leaves of *Rubus vulgaris*- glucoside.

The object under study is rich in chemical composition: blackberries contain a large number of vitamins, including vitamins of group C, organic acids, sugars, flavonoids, amino acids, micro – and macroelements - components of the quality of the raw materials under study. Active components are sources of therapeutic activity: they have a beneficial effect on the body, especially on the work of the gastrointestinal tract, and have a pronounced immunostimulating effect. Blackberry has a neuroprotective effect and enhances brain activity, reduces the likelihood of neurodegenerative diseases (Alzheimer's disease, Parkinson's disease) [2-3].

Humanity recognizes the wide therapeutic effect of blackberries. According to sources, the medicinal plant is used in the treatment of catarrhal and other intestinal diseases of the intestine, in particular, in the fight against spotting, accompanied by the excretion of metabolic products from the body - in metabolism. In addition, blackberries have antiseptic and blood-purifying effects, improve intestinal motility, and are useful for cystitis and kidney diseases. Fresh berries of the medicinal plant are able to quench thirst, as they consist mainly of water. It has been established that they have an antipyretic effect, as a result of which they are often recommended for acute respiratory diseases: obstructive bronchitis, pneumonia. The effectiveness of the studied raw materials as a folk treatment was recorded [4-6].

*Object of research* to study of the chemical composition of the *Rubus vulgaris* plant, widely distributed in the mountainous regions of the Almaty region.

*Relevance of the work* - one of the main requirements for vegetable raw materials currently used for medical preparations is the presence of phenolic compounds in the composition, as well as low toxicity, absence of side effects and

allergic reactions. In recent years, the way of obtaining various medicines from Qarabulag plant raw materials has been of interest.

*Material and methods.* Humidity and ash content of medicinal plant raw materials were determined in accordance with the requirements of the State Pharmacopoeia [7].

The Center of Physico-Chemical methods and Analysis of the NAO "Al-Farabi Kazakh National University" analyzed the elemental components in the ash of *Centaurea diffusa* lam plants using the method of atomic emission spectral analysis. A Shimadzu 6200 series spectrometer was used to determine the mineral composition of the ash. To do this, the raw materials were placed in a preheated and precisely measured porcelain crucible. The crucible was then carefully heated, first allowing the substance to burn at the lowest temperature. The temperature is gradually increasing. Burning was carried out at a temperature of 500 °C until a constant mass was obtained. At the end of calcination, the crucible was cooled in a desiccator. *Rubus vulgaris* ash (0.1254 g) dissolves in 10 ml of 40 % hydrochloric acid when heated. After that, the resulting solution is heated to obtain salts. Next, 15 ml was dissolved in 1 normal hydrochloric acid solution and transferred to a 25 ml container for analysis [8].

The quantitative content of biologically active substances in the plant (organic acids, saponin, coumarin, flavonoids, tannins, alkaloids) has been established.

## 2. Experimental part

The analysis was carried out according to generally accepted methods in accordance with the methods of the State Pharmacopoeia of the Republic of Kazakhstan and the Pharmacopoeia of the USSR.

The extract was obtained from the *Rubus vulgaris* plant. To obtain the extract, the plant material was first soaked in 96 % ethanol (raw material:extractant-1:8) at room temperature for 3 weeks, filtered and distilled under low pressure using a rotary evaporator. The resulting thick extract was dried. From 500 g of plant material, 45.6 g of dry extract was obtained. Then it is divided into fractions with petroleum ether, hexane, ethyl acetate, butanol and the corresponding extracts are obtained.

HPLC - one of the methods for conducting qualitative and quantitative analysis. The analysis was performed using (Perkin Elmer Series 200). A Zorbax Bonus RP column (4.6×150 mm, 5 μM, Agilent, USA) is used as a chromatographic column. Absorbance is measured at 254 nm. Contains water/0.1 % acetic acid (solvent A) and acetonitrile (solvent B), 10 % B (0-5 min), 20 % B (5-12 min), 30 % B (12-20 min), and linear, mobile phase gradient profile with 95% B (20-30 min) was maintained at room temperature on a column with a flow rate of 1.0 ml/min and was carried out by injecting 10 μl of sample into the HPC system.

*Determination of the moisture content of medicinal plant materials:* prepare a ratio of 3-5 g of pre-dried plant complex, crushed to a particle size of 10 mm,

and place it in a weighed container. It is calculated by the time it rises to 100-105 °C, including raw materials. We expect the first measurement to take place in 2 hours. We dry the raw materials to a constant weight, perform the work by drying for 30 minutes and cooling for 30 minutes. The difference in each measurement, not exceeding 0.1 grams, indicates that a constant weight of the raw material has been achieved. We measure the weight of an object using the Bux and determine the differences from each other by putting the formula.

*Method for determining the amount of ash:* to do this, take 5 grams of the above-ground part of the blackberry plant, place it in an iron crucible and gradually turn it into ash on an electric stove. After the raw material has completely turned into ash, we place it in a muffle furnace and burn it to constant weight at a temperature of 550-650°C. After firing, place a small, chilled crucible in a desiccator, allow it to cool completely, and measure the contents of the crucible to determine the percentage.

*Quantitative method of flavonoids:* 2 g (exact size) of crushed raw material is placed in a flask with a capacity of 150 ml with a suspension, 30 ml of a solution consisting of 90% ethyl alcohol and 1% concentrated hydrochloric acid or 10% sulfuric acid (for hydrolysis of glycosides), the flask is returned in the refrigerator, combine, heat in a water bath for 1 hour, cool to room temperature, filter through filter paper into a 100 ml volumetric flask. The extraction process is repeated 2 times according to the above method, the filter is washed with 90% ethyl alcohol and the flask is adjusted to the specified size with alcohol (solution A). Take 2 ml of solution A into a 25 ml volumetric flask, add 1 ml of a 1% solution of aluminum chloride in 95% alcohol and fill the flask to the mark with 95% alcohol. After 20 minutes, measure the optical density of the solution (10 mm thick) in a spectrophotometer at a wavelength of 430 nm. For comparison, the measurement is carried out several times using the initially prepared solution A.

The atomic emission spectrum method is used to determine macro- and microelements in the composition of the plant complex. This method is based on absorption, scattering, dispersion and refraction of the spectrum.

*Determination of the fatty acid content of the plant (*R. vulgaris*):* the isolated extract of a chloroform-methanol mixture (2:1) is filtered through a paper filter for 5 minutes and concentrated to dry mass. Then 10 ml of methanol and 2-3 drops of acetyl chloride are added to the extracted extract and methylated at 60-70°C for 30 minutes. Methanol is pumped through a rotary evaporator, samples are extracted with 5 ml of hexane and analyzed on a CARLO-ERBA-420 gas chromatograph for 1 hour at the Kazakh Academy of Nutrition. As a result, chromatograms of fatty acid methyl esters were obtained.

### 3. Research results

Quantitative analysis of plants includes determination of raw material moisture, ash content, as well as quantitative indicators of the content of extractive substances. The results of this analysis are presented in Table 1.

The humidity of the raw material is a colloidal liquid that remains in the tissues and cells of the raw material when it is dried to a constant mass.

Ash of medicinal raw materials is the residue of inorganic substances remaining after burning the raw materials and drying them to a constant mass.

Plant ash consists of a mixture of various inorganic substances present in the plant itself. The amount of ash in plant raw materials depends on the raw material itself, growing conditions, soil composition, its collection and drying.

The humidity of medicinal raw materials is one of the quantitative indicators characterizing its weight. Medicinal plant materials should not contain excess moisture, since during storage it reduces its quality. The humidity of most medicinal plant materials should not exceed 12-15%.

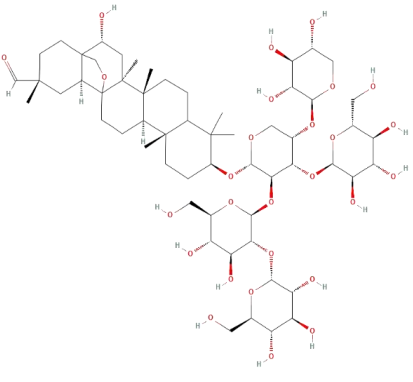
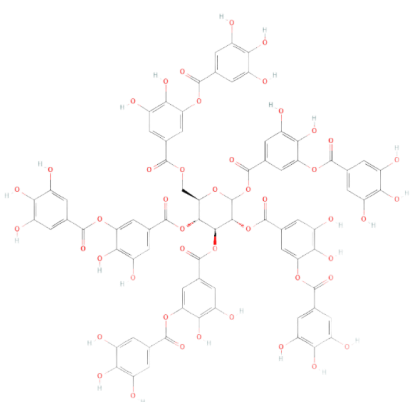
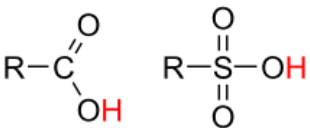
**Table 1** - *Rubus vulgaris* authenticity indicators.

<i>Plant name</i>	<i>% Share in terms of absolute dry raw materials</i>	
	Humidity	General ashiness
<i>Rubus vulgaris</i>	6.71 %	3.48 %

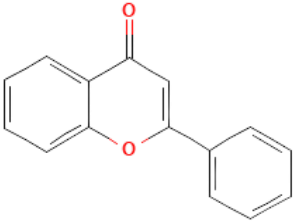
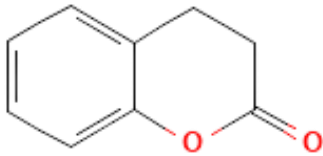
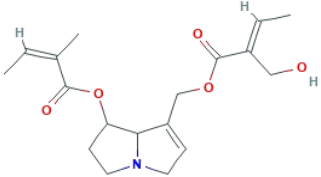
The results of the study showed that the creeping Mustard plant has high humidity and low ash content.

When using 70 % ethyl alcohol for the determination of extractive substances, the content of biologically active substances that entered the alcohol was high. It was effective for the isolation of biologically active substances from plant raw materials. Therefore, 70 % alcohol was used for the research. As a result of the study, the surfactant size was 16.48 %.

Table 2 - The main biologically active substances of the *Rubus vulgaris* residue.

Biologically active substances contained in raw materials:	Molecular formula	Definition	Structure	Percentage % content in raw materials:
Saponins	$C_{58}H_{94}O_2$ 7	A type of glycoside widely distributed in plants. Each consists of a sapogenin as the aglycone moiety, and a sugar. The sapogenin may be a steroid or a triterpene and the sugar may be glucose, galactose, a pentose, or a methylpentose.		3.80
Tannins	$C_{76}H_{52}O_4$ 6	Tannins are polyphenolic biomolecules with carbohydrate backbones that are found in a wide range of plants. Tannic acid is a specific tannin that formally contains 10 galloyl (3,4,5-trihydroxyphenyl) units surrounding a glucose center.		1.56
Organic acids		Carboxylic acid, with the general formula of R-COOH, is the most common organic acid that we are familiar with.		1.43

Continuation of Table-2.

Flavonoids	$C_{15}H_{10}O_3$	Flavonoids, a group of natural substances with variable phenolic structures, are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine.		0.23
Coumarins	$C_9H_6O_2$	A toxic white crystalline lactone with an odor of new-mown hay found in plants or made synthetically and used especially in perfumery and as a parent compound in anticoagulant agents		2.18
Alkaloids	$C_{18}H_{25}NO_5$	Alkaloids are found primarily in plants and are especially common in certain families of flowering plants.		3.98

Determination of mineral substances from the composition of the *Rubus vulgaris* plant was carried out by the Shimadzu 6200 series atomic absorption spectrometer. They:

- increase the activity of enzymes;
- catalyze biochemical processes;
- promote the synthesis of carbohydrates, proteins and vitamins;
- participate in metabolism.

The composition of ash often includes the following elements: K, Na, Mg, Ca, C, Si, P and, to a lesser extent, Cu, Al, Mn and other elements. These elements are present in ash in the form of oxides or salts of phosphorus, sulfur and other acids.

In addition to organic compounds, minerals are often found in medicinal plants. Depending on the size of the plant body, mineral elements are divided into the following groups:

1) Macronutrients contain elements from 0.01 to 10 %. In addition to organogens, this group includes: Si, Ca, K, Na, P, S, Al;

2) trace elements in quantities from 0.01 to 0.00001 %: Mn, B, Cu, Zn, Li, Ti, Mo, Co, etc. Includes;

3) ultramicroelements with smaller quantities: Cs, Se, Cd, Hg, Ag, Au, Ra, etc. belong.

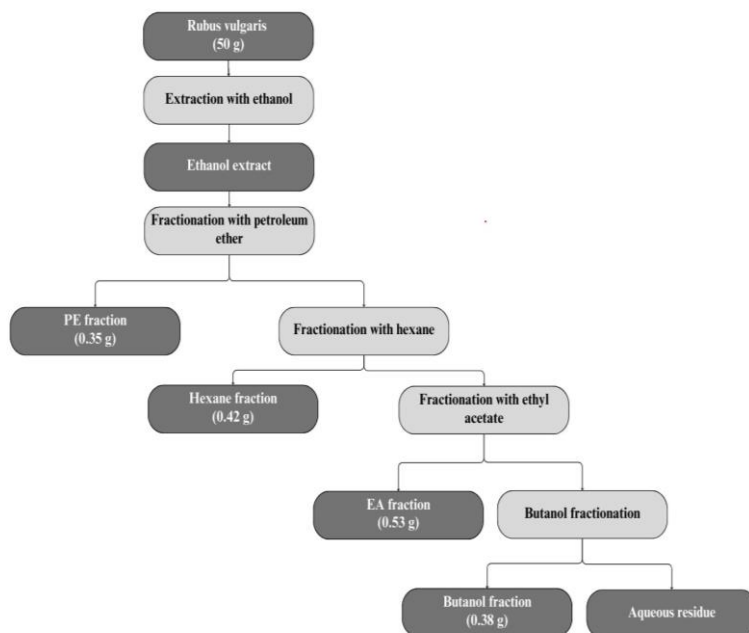
According to the results presented in Table 3, it is determined that macroelements such as K, Ca, P dominate in the *Rubus vulgaris* plant.

In addition, it is known that trace elements, which are part of herbal preparations, affect their activity. The results of the study are presented in Table 3.

**Table 3** - Determination of minerals from the composition of the *Rubus vulgaris* plant.

Elements	K	Ca	Na	Cu	Zn	Mg	Mn	P
Concentration in ash.	162 mcg/ml	29 mcg/ml	1.065 mcg/ml	0.256 mcg/ml	0.516 mcg/ml	20.312 mcg/ml	0.625 mcg/ml	22.21 mcg/ml

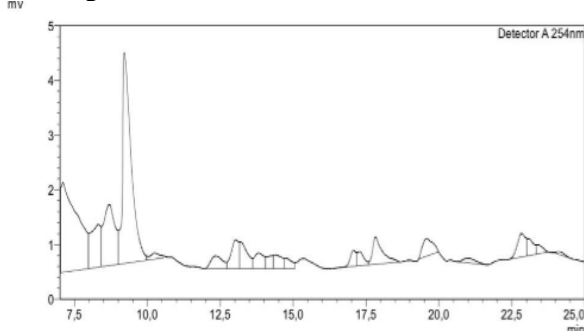
The extract was obtained from the *Rubus vulgaris* plant. To obtain the extract, the plant material was first soaked in 96 % ethanol ([raw material:extractant] – [1:8]) at room temperature for 3 weeks, filtered and distilled under low pressure using a rotary evaporator. The resulting thick extract was dried. From 50 g of plant material, 2.37 g of dry extract was obtained. Then it was divided into fractions with petroleum ether, hexane, ethyl acetate, butanol, and the corresponding extracts were obtained (Figure 1).



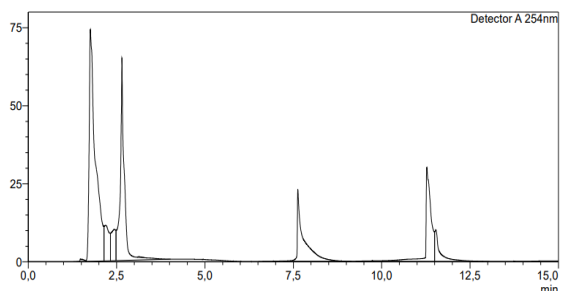
**Figure 1** - Flow diagram for the isolation of biologically active complexes from the plant *Rubus vulgaris*.



High-performance liquid chromatography is one of the methods for qualitative and quantitative analysis. The ethyl acetate extract was analyzed by HPLC (Perkin Elmer Series 200). Gallic acid was used as reference. Zorbax Bonus RP (4.6×150 mm, 5 μM, Agilent, USA) column is used as a chromatographic column. Absorbance is measured at 254 nm. Contains water/0.1 % acetic acid (solvent A) and acetonitrile (solvent B), 10 % B (0-5 min), 20 % B (5-12 min), 30 % B (12-20 min), A linear profile of the mobile phase gradient with 95 % B (20-30 min) was maintained at room temperature on the column at a flow rate of 1.0 mL/min and was performed by injecting 10 μl of the sample into the HPLC system (Figure 2, 3).



**Figure 2** - HPLC spectrum of ethyl acetate extract of *Rubus vulgaris*.



**Figure 3** - HPLC spectrum of the butanol fraction of *Rubus vulgaris*.

#### 4. Conclusion

Due to the presence of mineral compounds such as magnesium, manganese, potassium, it is widely used as a raw material in the preparation of prophylactic drugs used to prevent osteoporosis, strengthen bones, blood clotting, and diabetes. *Rubus vulgaris* contains vitamins, steroids, lipids and minerals, flavonoids, glycosides, terpenes, acids, and tannins in the aerial parts with various pharmacological properties such as antioxidant, anticarcinogenic, anti-inflammatory, antimicrobial, antidiabetic, antidiarrheal and antiviral.

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**Authors' contributions**

Concept development – N. Bolatkyzy, A.S. Nurmakhanova, G.E. Berganaeva, M.A. Dyusebaeva

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**RUBUS VULGARIS ӨСІМДІГІНІҢ ХИМИЯЛЫҚ ҚҰРАМЫН ЗЕРТТЕУ**

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**Түйіндемe.** *Kipicne:* *Rubus vulgaris* - раушан гүлділер тұқымдасына, *Rubus* туысына жататын өсімдік түрі. *Rubus* - шөптесін көп түсті немесе жиі тік, өрмелейтін немесе ұзартылған бұтақтары және қарапайым немесе күрделі жапырақтары бар ағаш тәрізді тікенді өсімдіктер. Осы түрлердің ішінде *Rubus vulgaris* әдетте *қаражидек* деп аталатын бағалы жемістерімен танымал бұта ретінде ерекшеленеді. Бұл жемістердің медицинадағы, косметикадағы және тамақтанудағы көп қырлы артықшылықтарына байланысты үлкен маңызға ие. *Rubus vulgaris* емдік потенциалы бар құнды қоректік заттар мен биоактивті қосылыстардың шоғырланған көзі ретінде танылады және функционалдық тағам ретінде танылады. *Rubus vulgaris* жемістері мен жапырақтарында фенол қышқылдарының бай қоры бар, оның ішінде *эллаг, галль, кофеин және p-кумар қышқылдары*, сонымен қатар *кверцетин, гиперозид, кемпферол, мирицитин, (+)-катехин, (-)-эпикатехин, галлат эпикатехин, процианидин B<sub>2</sub> және кверцетин-3-D-глюкозид* сияқты *флавоноидтар* бар. Күрделі химиялық құрамы зауыттың әртүрлі салалардағы маңыздылығын атап көрсетеді.

*Rubus vulgaris* құрамын одан әрі талдау кезінде *ылғалдылық (6.71 %), күлділігі (3.48%), экстрактивті заттар (16.48 %), органикалық қышқылдар (1.43 %), флавоноидтар (0.23 %), таниндер (1.56 %), алкалоидтар (3.98 %), кумарин (2.18 %), сапониндер (3.80 %)* және *көмірсулар (2.12 %)* анықталды. Сонымен қатар, көп элементті атомдық эмиссиялық спектрлік талдауды қолдану өсімдік күлінде сегіз элементтің, ең алдымен *калий (162 мг/г), кальций (29 мг/г), фосфор (22 мг/г)* және *магнийдің (20 мг/г)* бар екенін анықтады. *Rubus vulgaris* химиялық құрамының бұл жан-жақты түсіндірмесі оның тағамдық құндылығын көрсетіп қана қоймайды, сонымен қатар оны фармацевтикадан тағам ғылымына дейін және одан тыс жерлерде әртүрлі салаларда қолдануға жол ашады.

**Кілт сөздер:** *Rubus vulgaris*, экстракция, биологиялық белсенді заттар, хроматография, минералды заттар және т.б.

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ИЗУЧЕНИЕ ХИМИЧЕСКОГО СОСТАВА РАСТЕНИЯ *RUBUS VULGARIS*

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**Резюме.** *Введение:* *Rubus vulgaris*, член семейства розоцветных рода *Rubus*, включает в себя разнообразное множество древесных колючих растений, характеризующихся травянистым разноцветным или часто вертикальным ростом, с вьющимися или удлиненными ветвями, украшенными простыми или сложными листьями. Среди этих видов *Rubus vulgaris* выделяется как кустарник, известный своими ценными плодами, обычно называемыми ежевикой. Эти фрукты имеют большое значение из-за их многогранной пользы в медицине, косметике и питании. *Rubus vulgaris* признан концентрированным источником ценных питательных веществ и биологически активных соединений с терапевтическим потенциалом и признан функциональным продуктом питания. Плоды и листья *Rubus vulgaris* содержат богатый резервуар фенольных кислот, включая эллаговую, галловую, кофейную и п-кумаровую кислоты, а также флавоноиды, такие как кверцетин, гиперозид, кемпферол, мирицитин, (+)-катехин, (-)-эпикатехин, галлат эпикатехина, процианидин В2 и кверцетин-3-D-глюкозид. Сложный химический состав подчеркивает значимость растения в различных отраслях промышленности. Дальнейший анализ состава *Rubus vulgaris* выявил в нем влажность (6.71 %), зольность (3.48 %), экстрактивные вещества (16.48 %), органические кислоты (1.43 %), флавоноиды (0.23 %), дубильные вещества (1.56 %), алкалоиды (3.98%), кумарин (2.18 %), сапонины (3.80 %) и углеводы (2.12 %). Кроме того, использование многоэлементного атомно-эмиссионного спектрального анализа выявило наличие в золе растений восьми элементов, в первую очередь калия (162 мг/г), кальция (29 мг/г), фосфора (22 мг/г) и магния (20 мг/г). Такое всестороннее объяснение химического состава *Rubus vulgaris* не только подчеркивает его пищевую ценность, но и открывает путь к его использованию в различных областях, от фармацевтики до пищевой науки и за ее пределами.

**Ключевые слова:** *Rubus vulgaris*, экстракция, биологически активные вещества, хроматография, минералы и т. д.

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