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# Study of the component composition of seven *Berberis* species

**Abstract.** The qualitative and quantitative composition of fruit extracts of 23 forms of 7 barberry species (*Berberis amurensis, B. heteropoda, B. iliensis, B. integerrima, B. oblonga, B. sibirica* and *B. thunbergii*) collected on the territory of Kazakhstan and Uzbekistan has been studied. The extracts were obtained by adding 70% ethanol to the crushed fruits in a ratio of 1:5 (raw material-reagent) and subsequent separation with organic solvents, namely n-hexane, chloroform and ethyl acetate. Chromatographic analysis was conducted on Agilent 7890 A GC gas chromatography with Agilent 5975 inert MSD quadrupole mass spectrometer as a detector. The number of components found in the fruits of the 7 barberry species studied varies from 10 to 61. The identified components belong to various classes of chemical compounds as fatty acids, fatty acid esters, alkanes, alkadienes, aromatic hydrocarbons, aldehydes, fatty alcohols, phenols, triterpenoids, ketones, and silicones. Squalene, phylloquinone, pyridine-3-carboxymidamide and propanamide were also found in all the samples studied, except *B. oblonga*. Also, in the all investigated *Berberis* species free monosaccharides as glucose, galactose, xylose and arabinose were found. The obtained information on the component composition of barberry can be used for the production of new medicines of a wide spectrum of action.

Keywords: Berberis, component composition, fruits, extracts.

## Introduction

Barberry is a unique plant that is a huge source of vitamins and has many medicinal qualities. About 500 plant species of this genus are known, distributed in Europe, Asia and North America. The following species grow in Kazakhstan: Berberis iliensis M. Pop. in the gorges of the eastern part of the Zailiysky Alatau, the Ketmensky Range, the Dzungarian Alatau, at the mouths of the Chilik and Charyn rivers flowing into the Ili River; Berberis heteropoda Schrenk (synonyms Berberis sphaerocarpa Kar. Et Kir.) - from Altai to Kirghiz Alatau; Berberis integerrima Bunge - in Tien Shan, Pamir-Altai, Karatau; Berberis oblonga (Regel) C.K. Schneid. - in the gorges of the Tien Shan; Berberis sibirica Pall. -Altai and Dzungarian Alatau [1]. In Uzbekistan, B. oblonga grows mainly in the Chatkal, Pskem, Ugam and Kuraminsky ranges, and B. integerrima grows in the territory of the Zarafshan National Natural Park, in the floodplain of the Zeravshan River [2].

For thousands of years, people have known the beneficial properties of barberry. There are a sufficient number of articles published in different countries on the phytochemical composition of its various species [3-4]. The authors present the results obtained on the positive effect of pharmaceutical preparations based on barberry, which are used in the treatment of cancer, diabetes mellitus, cerebral ischemia, cardiovascular diseases, bacterial, viral, parasitic lesions, and also have antispasmodic properties in cholecystitis, have a hypotensive effect, enhance contractility of the uterus, stimulate the action of the blood coagulation system [5-9].

Given all the beneficial properties of the barberry, it is important to study and preserve its unique species, already now such barberry species as the *B. iliensis* and *Berberis karkaralensis* Kornil. & Potapov are listed in the Red Book of Kazakhstan, in the near future other species may be endangered [10-11]. Work on the preservation of barberry is carried out at the Institute of Plant Biology and Biotechnology. An *in vitro* collection and a cryogenic bank of apical mer-

istems and seeds have been created [12-13]. There is no meaningful summary of Kazakh barberry species reflecting the current state of their phytochemical knowledge, except for our earlier published article on the study of *B. iliensis* and *B. integerrima* [14]. There are data on the composition of B. oblonga growing in neighboring Kyrgyzstan [15]. The articles provide data on the component composition rich in organic compounds and high antioxidant activity of several forms of three species of barberry. The authors refer to the fact that the obtained scientific information can serve as the basis for the development of the Kazakh pharmaceutical industry, for the production of new drugs with a wide therapeutic spectrum, because at the moment there is not a single Kazakh-made drug based on barberry, except for syrup used as a vascular and fortified with vitamin C.

The purpose of this work was to study the qualitative and quantitative composition of fruit extracts of 23 forms of 7 *Berberis* species, collected on the territory of Kazakhstan and Uzbekistan, as well as obtained from the collections of botanical gardens and nurseries. The analysis of the revealed components is given.

## Materials and methods

Plant material. The objects of the study were 23 accessions of barberry: *Berberis amurensis* Rupr., 7 forms of *B. heteropoda*, 4 forms of *B. iliensis*, 7 forms of *B. Integerrima*, 2 forms of *B. oblonga*, *B. sibirica*, and *B. thunbergii*. Data on the collection points of *Berberis* plant material is presented in Table 1.

Accession	Collection point, year				
B. amurensis	From the collection of the Karaganda National University, collected in the Altai Botanical Garden, 2013	819			
B. iliensis					
Form 2	Floodplain of the Ili River, v. Bakanas, 2014	388			
Forms 3-4	Ili river floodplain, Kerbulak gorge, 2014-2015	428-488			
Form 9	JSC "Lesnoy pitomnik", Almaty region, Aktogay settlement. 2015	410			
<i>B.integerrima</i> Forms 9, 11, 13, 16, 20 Forms 23-25	Floodplain of the Zeravshan River, Zarafshan National Natural Park, 2014 Turkestan region, Sairam-Ugam National Park, Kaskasu gorge, 2015	837-848 1426-1433			
<i>B. oblonga</i> Forms 1-2	Floodplain of the Zeravshan River, Zarafshan National Natural Park, 2014	712-723			
B. sibirica	JSC "Lesnoy pitomnik", Almaty region, Aktogay settlement. 2015	1410			
<i>B. heteropoda</i> Form 1	Floodplain of the Bolshaya Almatinka River: Almarasan Gorge, 2015	1403			
Form 10	Altai Botanical Garden, 2015	815			
Form 11-15	Zailiysky Alatau. Issyk Gorge, 2015	1689-1770			
B. thunbergii	Altai Botanical Garden, 2014	822			

 Table 1 – List of Berberis accessions with description of collection points

Preparation of extracts. Fruit pulp was separated from seeds, dried in air, crushed and extracted with 70% ethanol in a ratio of 1:5 (raw material-reagent, w/v) 4 times within 24 hours at room temperature. Then, the extracts were concentrated by using a rotary evaporator and diluted with water 1:1, and subsequent separation with organic solvents, namely n-hexane (Sigma-Aldrich, USA, chloroform (Sigma-Aldrich, USA) and ethyl acetate (Sigma-Aldrich, USA).

ples of gGenuine monosaccharide and extracts were applied to chromatographic paper. To determine free

Thin Layer Chromatographic Separation. The

water extract of barberry fruit was dried and, in or-

der to determine the carbohydrate composition, pa-

per chromatography was carried out on paper of the Filtrak No. 11 brand in n-butanol-acetic acid-water

solvent systems (Sigma-Aldrich, USA) 4:1:5 (v/v)

(system 1), n-butanol-ethanol-pyridine-water (Sig-

ma-Aldrich, USA) 6:4:3 (v/v) (system 2). The sam-

monosaccharides were sprayed with aniline phthalate (Merck, USA). The yield of monosaccharides was determined by the weight of the dried water extract; the size of the spots was used to determine their percentage.

*Chromatographic analysis*. Chromatographic analysis was performed on Agilent 7890 A GC gas chromatograph (Agilent Technologies, USA) with Agilent 5975 inert MSD quadrupole mass spectrometer (Agilent Technologies, USA) as a detector.

The components of the 23 samples extracts were separated on HP-SMS quartz capillary column (30 m  $\times$  250  $\mu$ m  $\times$  0.25  $\mu$ m) with grafted stationary phase of 5% phenylmethyltrisiloxane at 50°C (2 min) –

10°C/min up to 200°C (6 min) – 15°C/min to 290°C (15 min).

The volume of the injected sample was 1  $\mu$ L, the flow rate of the mobile phase was 1.3 mL/min. The identification of the components was based on the comparison of the characteristics of mass spectra with the data of W8NO5ST.L electronic libraries [14].

# **Results and discussion**

The qualitative and quantitative composition of fruit extracts of 23 barberry accessions (7 species) was determined. Data on the component composition of fruits are given in Table 2.

Table 2 – Compound composition of	f Barberry's fruit pulp
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B. thun-В. В. В. B. amu-RT\* Compound B. iliensis B. oblonga integerrima heteropoda rensis bergii sibirica 3.691 0.02-0.04 p-Xylene \_ 0.62 -\_ -\_ Ethane 6.471 0.03-0.04 \_ \_ \_ \_ \_ \_ 7.515 Cyclopentasiloxane 0.38-0.59 0.28 \_ \_ --\_ Cyclohexasiloxane 9.932 0.02-0.03 3.94 \_ \_ \_ \_ Cycloheptasiloxane 12.146 0.09-0.22 2.98-3.30 1.98 \_ \_ \_ \_ Cyclooctasiloxane 14.144 0.08-0.11 1.76-2.13 \_ ---\_ Cyclononasiloxane 15.874 \_ \_ 0.03-0.18 1.65 \_ \_ \_ 1,2-Benzenedicarboxylic 16.364 0.25 0.12 0.02-0.04 \_ acid 1-Cyclohexylnonene 0.03-0.05 17.131 \_ \_ \_ \_ Dibutyl phthalate 17.306 0.16-0.27 0.13 0.11-0.22 0.12-0.26 0.26 -\_ Cyclodecasiloxane 17.427 \_ 0.05-0.07 \_ \_ \_ \_ \_ Hexadecanoic acid 17.535 0.02-0.03 1.42-6.61 \_ 0.19 \_ \_ -9-Octadecenoic acid 18.559 \_ 1.10-1.29 0.08-0.18 \_ \_ \_ \_ \_ 2-Nonadecanone 18.597 0.04 \_ \_ \_ \_ \_ 3,4-Dihydroxyphenylglycol 18.839 0.28 0.03-0.05 -0.28 --\_ n-Propyl 19.113 0.02-0.04 \_ 9,12-octadecadienoate 19.999 1,19-Eicosadiene 0.39-0.52 0.35-1.17 0.26 0.34 \_ \_ \_ Cyclohexanecarboxylic acid 20.124 0.18 \_ \_ \_ \_ Hexacosane 20.277 0.19 \_ -\_ \_ -\_ 0.10-0.37 0.18-0.91 Eicosane 20.283 0.36-9.70 0.04-0.32 0.58 0.51 \_ Phenol, 2,2'-methylenebis[6-0.07-0.19 0.48 (1,1-dimethylethyl)-4-21.377 0.06 0.36 \_ methyl-Glycidyl oleate 21.747 0.23 0.11-0.18 0.56 -\_ \_ \_ 9-Octadecenal \_ 0.25-1.28 \_ 21.747 \_ \_ \_ \_ 9.12.15-Octadecatrienoic 21.791 0.13 0.08 \_ \_ \_ \_ acid

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Table	continuation

Compound	RT*	B. integerrima	B. iliensis	B. heteropoda	B. oblonga	B. amu- rensis	B. thun- bergii	B. sibirica
Octacosanol	21.855	0.12	-	-	-	-	-	-
Octadecane	21.899	0.11-07.06	0.46	0.25-0.66	0.28	0.24	-	7.32
Heptadecane	21.899	1.20	0.49	0.34	-	-	-	2.66
Pentacosane	21.899	1.20	0.42-0.61	-	0.74	-	-	-
5-Dodecyne	21.899	0.24	_	-	-	-	-	-
Phthalic acid	22.338	0.13-0.23	0.13	0.06-0.42	0.21	0.19	-	-
Bis(2-ethylhexyl) phthalate	22.338	0.16-0.77	0.18	0.19-0.29	-	-	0.37	0.27
Diisooctyl phthalate	22.338	0.14-0.17	-	0.20-0.29	-	-	-	-
Octadecanoic acid	22.627	-	0.08-1.70	-	-	-	-	-
3(2H)-Furanone	22.663	-	1.12	-	-	-	-	-
Docosanal	22.936	0.17	_	0.14	-	-	-	-
E-11 (13-methyl) tetradecene 1-ol acetate	22.942	0.32	0.36	0.04	-	-	-	-
Tetracosanal	22.943	0.20-0.23	-	0.11-0.26	-	-	2.17	1.78
1-Hexacosene	23.305	0.49	-	-	-	-	-	-
1-Tetracosene	23.401	4.15	-	0.13-5.87	-	-	-	-
1-Eicosene	23.407	-	-	3.12-5.50	-	-	-	-
1-Nonadecene	23.411	3.33-4.21	3.96	2.54	-	-	-	-
Cyclotetracosane	23.420	-	2.18-5.41	-	-	-	-	-
Tetradecanoic acid	23.626	-	0.03-0.07	0.02	-	-	-	-
Eicosanoic acid	24.005	2.00	-	0.12-0.15	-	-	1.19	-
Octacosane	24.119	1.40-2.90	3.36	0.82	-	-	-	-
Hydroxymethanoic acid	24.120	0.06-0.11	1.15	0.04	-	-	-	0.08
Heptacosyl acetate	24.120	0.45	0.65-1.37	-	-	-	-	-
3-Methyldotriacontane	24.120	2.14	-	0.82-1.02	-	-	-	-
1-Docosene	24.202	0.19-6.84	-	0.20-3.83	-	-	-	-
Heptacosanal	24.317	-	-	0.08	-	-	-	-
Squalene	24.380	0.77-3.19	0.08-0.22	0.13-0.24	-	-	0.62	-
2-octadecyl-propane-1,3-diol	24.387	0.27	-	-	-	-	-	-
Pentadecanal	24.393	0.60-0.88	-	-	-	0.84	-	-
Oxirane	24.400	0.14-0.49	0.18-0.80	0.33-1.06	-	-	-	0.45
Propanamide	24.813	-	-	0.10	-	-	-	-
Nonacosane	24.826	5.65-12.99	10.08- 12.22	3.44-8.25	8.38	5.60	20.58	-
Tetracosane	24.832	0.20-14.03	1.20-4.01	0.12-8.36	5.26	0.05	-	0.43
Heneicosane	24.838	0.26-2.28	2.07	11.62	-	-	3.42	-
Heptacosan	24.838	9.51	-	-	-	-	-	-
Tetrapentacontane	25.023	1.23	-	0.28	-	-	-	-
Hexacosanoic acid	25.036	-	0.14-0.20	0.08	-	-	-	-
Heptacos-1-ene	25.131	1.09	0.18-0.24	-	-	-	-	-
Octasiloxane	25.385	-	-	0.16-0.20	-	-	-	-
Triacontane	25.500	-	1.04-1.73	-	-	-	-	-
13-Methyl-Z-14-nonacosene	25.538	0.17	-	-	-	-	-	-

Int. j. biol. chem. (Online)

Compound	RT*	B. integerrima	B. iliensis	B. heteropoda	B. oblonga	B. amu- rensis	B. thun- bergii	B. sibirica
Tetracosyl heptofluorobutyrate	25.545	-	0.18-0.24	-	-	-	-	-
9-Tricosene	25.545	0.34-1.90	-	-	-	0.28	-	-
Nonacos-1-ene	25.589	0.64	0.16	0.18-0.62	-	-	-	-
Hexacosanal	25.818	-	-	0.46	-	-	-	-
2-Chloropropionic acid	25.824	1.05	-	-	-	-	-	-
Octacosanal	25.825	0.44-0.98	-	0.74	-	-	-	-
Nonacosan-10-one	26.187	0.17-2.68	0.49-2.90	0.41-1.87	0.21-0.30	0.18	-	0.43
Phylloquinone	26.275	0.02	1.84-5.04	2.16-3.45	-	0.04	-	-
Octacosanoic acid	26.562	-	0.48-0.56	-	-	-	-	-
Triacontyl acetate	26.664	0.41-0.67	0.41-0.57	0.72	-	0.34	-	-
Z-14-Nonacosane	26.664	0.63-1.37	-	0.40-1.53	0.55-0.72	-	-	-
28-Nor-17-α	27.040	0.31	-	-	-	-	-	-
Pyridine-3-carboximidamide	27.224	0.05	1.08-1.29	5.01	-	-	-	0.02
Cyclohexane	27.237	0.36	0.62	0.33	-	0.22	-	-
Triacontanal	27.600	-	-	0.46	-	-	-	-
1,30-Triacontanediol	27.606	-	0.24-0.76	0.11-0.16	-	-	-	-
Cyclooctacosane	28.255	0.80-1.38	0.56	0.42-1.03	-	-	-	-
Fumaric acid	28.248	-	-	-	2.15	-	-	-
1-Hexacosanol	28.267	-	-	1.25-1.36	-	-	-	-
1-Heneicosyl formate	28.414	-	-	-	1.83	-	-	-
γ-Sitosterol	28.789	0.65-1.45	0.32	0.28-2.21	-	0.56	1.64	-
β-Sitosterol	28.796	-	0.64	0.46	-	-	-	1.25
2-Methyltriacontane	32.454	1.93	-	0.29-0.49	-	-	-	-
Number of identified components	53	43	61	19	13	10	16	

Table continuation

\*RT – Retention time

The largest number of components was identified in three species: 61 components in *B. heteropoda*, 53 in *B. integerrima*, and 43 in *B. iliensis*. Perhaps the reason for the small number of identified components in fruit extracts of 3 species of barberry (*B. amurensis* – 13 components, *B. thunbergii* – 10 components, *B. sibirica* – 16 components) is the earlier collection of plant material (2013-2014), because other authors noted a rich component composition of fruits in these species (Table 2) [16-17]. Some accessions of *B. thunbergii* and *B. heteropoda* (forms 1, 12, and 14) contain a squalene. In *B. amurensis* and in 11 forms of *B. heteropoda*, phylloquinone. *B. sibirica* and *B. sphaerocarpa* form 13 have pyridine-3-carboximidamide, while form 15 has propenamide. It was revealed that in all barberry accessions there are such classes of chemical compounds (Figure 1) as fatty acids, fatty acid esters, alkanes, alkenes, alkadienes (with the exception of *B. oblonga*), aromatic hydrocarbons (with the exception of *B. thunbergii* and *B. sibirica*), phenols, alcohols and aldehydes (with the exception of *B. oblonga*). Ketones were found in the fruits of only two species of barberry *B. iliensis* and *B. heteropoda*, while *B. iliensis* had a high content (4.02%). Silicones were found in 4 species of barberry, while in *B. heteropoda*, *B. amurensis* and *B. sibirica* their content varied from 0.28% to 1.98%, only *B. oblonga* had a high content of 11.61%.



Figure 1 - Classes of chemical compounds found in the fruits of 7 Berberis species

As the main classes of chemical compounds in fruit extracts of all 7 *Berberis* species there are alkanes (from 5.89% to 28.70%), alkenes and alkadienes (from 0.28% to 7.57%), as well as fatty acids and their esters (from 0.72% to 11.60%). It has been established that 3 species of barberry are leaders in terms of quantitative (*B. iliensis* – 61.77%, *B. integerrima* – 46.25%, *B. heteropoda* – 36.38%) and qualitative (*B. iliensis* – 63 components, *B. integerri*  ma - 53 components, *B. heteropoda* - 61 components) composition.

Qualitative and quantitative composition of fruit extracts of *B. integerrima* and *B. heteropoda*, *B. amurensis* and *B. sibirica* are similar.

All the *Berberis* species studied contained free monosaccharides (glucose, 4.6%, galactose, 0.5%, xy-lose, 0.6%, and arabinose, 0.9%), as well as unknown components with different RT and percentages. Ana-

lyzing the results obtained and the scientific literature, it can be concluded that a wide range of organic substances have been found in the fruits of barberry, which in one way or another have something in common with the published data [18-21]. In the most papers, the component composition of *Berberis vulgaris* L. fruits, leaves, bark, and roots has been studied. In the roots, for the most part, among the components, alkaloids prevail, which are also found in unripe fruits. The fruits are rich in the main classes of organic compounds listed above. Differences can be noted only in the number of some components [7-8].

Analyzing the scientific literature, we can conclude that the activity of barberry is mainly due to the action of phenolic substances, which have pronounced anti-allergic, anti-carcinogenic, anti-inflammatory and antiviral properties, reduce the risk of cardiovascular diseases and reduce mortality from stomach cancer. In addition, recently, natural phenolic compounds have attracted the general attention of researchers not only as an object of chemical study, but also as promising substances for the production of biologically active drugs and medicines [3, 9, 16, 22].

The search for new drugs is the most important direction in the development of organic and bioorganic chemistry. Heterocyclic compounds, in particular, pyridine and its condensed analogs, such as pyridine-3-carboximidamide discovered by us, have various types of biological activity and are part of many drugs used in medicine. It has been established that this compound is an extractant of some radioactive metals and is also used for the treatment and prevention of cardiovascular diseases [23].

The most important function of phylloquinone is that it is involved in the regulation of blood clotting, as it is necessary for the synthesis of proteins that provide a sufficient level of coagulation. In addition, it plays many other important roles in maintaining life and protecting the body from diseases, including very dangerous ones, for example, in the metabolism of bones and connective tissue, as well as in the healthy functioning of the kidneys, participates in the absorption of calcium and in ensuring the interaction of calcium and vitamin D. The importance of phylloquinone is extremely high, the scientists who discovered it were awarded the Nobel Prize [24].

The identified carotenoid squalene is an intermediate in the biological synthesis of steroids, including cholesterol, and is also involved in metabolism. Squalene was first discovered in shark liver at the beginning of the last century by Japanese scientists led by Tsujimoto Mitsumaro. The study of the many beneficial properties of squalene continues successfully to this day, it has been established that it fights cancer cells, preserves youth, supports immunity, improves the functioning of the heart and hormones, and also functions as antioxidants in the human body. It is also non-toxic and safe in any concentration. From a biochemical point of view, squalene is a natural unsaturated hydrocarbon; hydrogen atoms are necessary for its stable state. In the human body, squalene reacts with water, releasing oxygen and saturating cells with it. Therefore, it is often called the "vitamin of oxygen" [25].

Organic compounds of the amide group (like propanamide) can react in many different organic processes to form other useful compounds for synthesis, may interact with proteins and phospholipids of neuronal membranes and disrupts their permeability to sodium, reduce the excitability and automatism of the neurons of the epileptogenic focus and block the generalization of convulsive discharges. Most often, propanamide is used as an anticonvulsant [26].

## Conclusion

For thousands of years, plants have been used by humans to treat many diseases. For the development of the modern pharmaceutical industry, it is important to study the phytochemical compounds of plants in order to determine the medicinal properties of a particular component.

In this study, the component composition of 7 Berberis species – B. amurensis, B. heteropoda, B. iliensis, B. integerrima, B. oblonga, B. sibirica and B. Thunbergii was analyzed, a quantitative analysis of the components was also carried out. It became known that the component composition of these Berberis species is represented by such classes of chemical compounds as fatty acids, fatty acid esters, alkanes, alkenes, alkadienes, aromatic hydrocarbons, phenols, alcohols, aldehydes, ketones, silicones. The most common chemical compounds in the extracts were alkanes, alkenes and alkadienes, as well as fatty acids and their esters. Fruit extracts of 7 species of barberry differed from each other in chemical compounds. As a result, it was found that the fruits of 3 species of barberry (B. heteropoda, B. integerrima and B. iliensis) have a rich component composition, which makes these species an important raw material for the pharmaceutical industry, as well as useful food sources with a lack of vitamins and minerals in the human diet.

It should be noted that in this study, the component composition of *B. iliensis* was studied for the first time. In addition, *B. heteropoda*, *B.integerrima* and *B. sibirica* growing in Kazakhstan also were studied for the first time. Given the lack of knowledge of Kazakh *Berberis* species, the authors believe that the new information obtained on the component compounds of the studied species complements the scientific data in this area, and can be used for the production of new broad-spectrum drugs, as well as for the development of the pharmaceutical industry in Kazakhstan as a whole.

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