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PHENOLIC COMPOUNDS IN BARBERRY AND WILD ROSE FRUITS

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The subjects of the study are samples of fruits of several species of barberry and wild rose. The aim of work is studying the chemical composition of phenolic compounds (PC) and comparative assessing the quantitative content of flavonoids as the most valuable PC group in different species of fruits. PC composition research was completed applying HPLC method in reverse-phase mode. Different concentrations of ethyl alcohol were employed to extract flavonoids while determining their total content. The extraction was carried out with the use of three methods: method of infusion (1, MI), in an ultrasonic extractor (2, US) and under the influence of super high frequencies electromagnetic field (3, SHF). Species differences were assessed on UV spectra of the colored complexes of PC extracts with aluminum chloride. 13 PC components content was quantified for fruits collected in 2014-2016. Dominating components were revealed: they are chlorogenic acid and hyperoside. Species of barberry with the most valuable PC sets were determined: Dark purple barberry (*Berberis vulgaris f. atropurpurea Regel*), Thunberg barberry (*Berberis thunbergii* DC) – samples of 2015 year of collection, Regel barberry (*Berberis regeliana Kochne*) – those of 2016. The total flavonoids (F) content measured by spectrophotometric method (with quercetin as a reference solution) while extracting them by infusion in three species of barberry fruits varies from 1,30 to 1,41 %. While defining the optimum extraction method maximum extent of F extraction from barberry fruits reached 39%, from wild rose fruits – 51,5 % (SHF-extraction). According to the results of the research there are recommendations given on using extracts as substances for producing medical and pharmaceutical herbal remedies and food additives with antioxidant properties.

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Introduction

During the past 10 years Russian and foreign scientists have actively been exploring biologically active substances (BAS) of plants including phenolic compounds (PC), improving the methods of their extraction from vegetable raw materials and broadening the spheres of their application in different industries (food, medical, pharmaceutical, cosmetic) [7, 11, 19]. Interest to phenolic compounds is not occasional, it can be explained by the wide range of their biological activity and low toxicity (except phenol itself) [1].

PC of medical raw materials, fruits and berries include mainly hydroxybenzoic, hydrocynnamic acids (phenolic acids) and their derivatives; flavonoids and glycosides which aglycons are subdivided into flavones, isoflavones, flavanols, flavanones, flavanonols, flavans, flavan-3,4-diols and catechins and proanthocyanidins as well. All PC have aromatic (benzoic) nucleus with hydroxyl group in the molecule [3, 19, 20].

Chromatographic methods are employed to explore PC [7, 9, 14, 17]. Flavonoids quantification is completed applying spectrophotometric method [6, 8, 18]. Different methods such as sorption combined with flash-chromatography are used to purify PC [16].

Objects and Methods of Research

Objects of research are samples of three species of barberry fruits that are grown in NARFU's Dendrological Garden and were collected for three years from the same bushes in autumns (years 2014, 2015, 2016). All the fruits correspond to the type morphological features considered. Samples of two species of wild rose (rose) fruits were explored: those of the hybrid species (*Rosa hybrida*) grown in the Dendrological Garden (2 samples) and forest one (*Rosa canina* L.) (1 sample) laid in in the Primorsky district of the Arkhangelsk region. The samples were collected in September 2016. All the samples were refrigerated, stored at the temperature of 18 °C above zero, reduced to the fragments of the same size before exploring.

40 %, 70 % and 96 % concentrations of ethyl alcohol (EA) were used in order to extract flavonoids (F) in the process of determining their total content. The extraction was carried out applying three methods: infusion at the boiling point of the extragent (MI), in an extractor with ultrasound treatment in pulsating mode at a frequency of 27 kHz at a temperature of 70 degrees above zero and under the influence of the super high frequencies (SHF) electromagnetic field at short-term boiling of the extract. Such a choice of extraction methods is due to the elaboration of recommendations on express-method of F extraction. Extraction terms correspond to the literary data, for instance, to the method used to extract polyphenols from rose, described in [4].

F quantitation is based on the formation of colored F complexes with aluminum chloride III and comparison of their colorings with the coloring of standard quercetin and rutin samples in similar conditions [11, 12]. For quercetin: working solution contained 2,5 ml of extract, 5 ml of 2 % $AlCl_3$ and 17,5 ml of 96 % EA. Reference solution – 2,5 ml of extract and 22,5 ml of EA without diluting. For rutin: working solution – 5 ml of extract, 1 ml of 2 % $AlCl_3$, 1 drop of glacial acetic acid and 19 ml of 70 % EA. Reference solution – 5 ml of extract and 20 ml of 70 % EA without diluting.

UV-spectra of barberry and wild rose fruits extracts were obtained on the spectrograph model Unico 2800 UV/Vis in the range of 200500 nm.

Chromatographic analysis of the fruits on 13 components was conducted with the use of HPLC system LC-30 «Nexera» (Shimadzu, Japan) in reverse-phase mode with the column Zorbax SB-Aq (Agilent, USA), particle size 3,5 mcm, size 150×3,0 mm. Volume of the sample injected into the column – 5 mcl, eluent flow-rate – 0,7 ml/min. Thermostat temperature – 40 °C above zero. Detection was carried out at a wavelength 280 nm. Managing chromatograph, collecting and processing the information included the use of software LabSolutions.[1]

Water with 0,5 % formic acid (solution A) and acetonitrile with 0,5 % formic acid (solution B) were used as eluent for chromatographic division. Gradient elution

[1] Analyses completed in the Shared Use Equipment Center "Arktika" for Collective Usage of NARFU

was conducted according to the following program: 0-20 min – 5 % B, 25-30 min – 20 % B, 35 min – 40 % B. Total analysis time – 35 min.

Extractions obtained by the method of infusion of fruits reduced to fragments with 96 % EA were used for HPLC analysis. Preventive calibration of the mixed-standard PC solution in methanol was conducted. Limits of spotting were set to the level 0,01…0,05 and detection limits were in general from 0,05 to 0,10 mg/ml. Standards of the company «Sigma-Aldrich» with content of main substance not less than 97 % were conformed to. PC analytes choice is completed according to the literary data of the barberry, wild rose and other bushes fruits chemical composition [13]. An example of an extraction chromatogram of barberry fruits is given in the fig. 1, wild rose fruits – in the fig. 2.

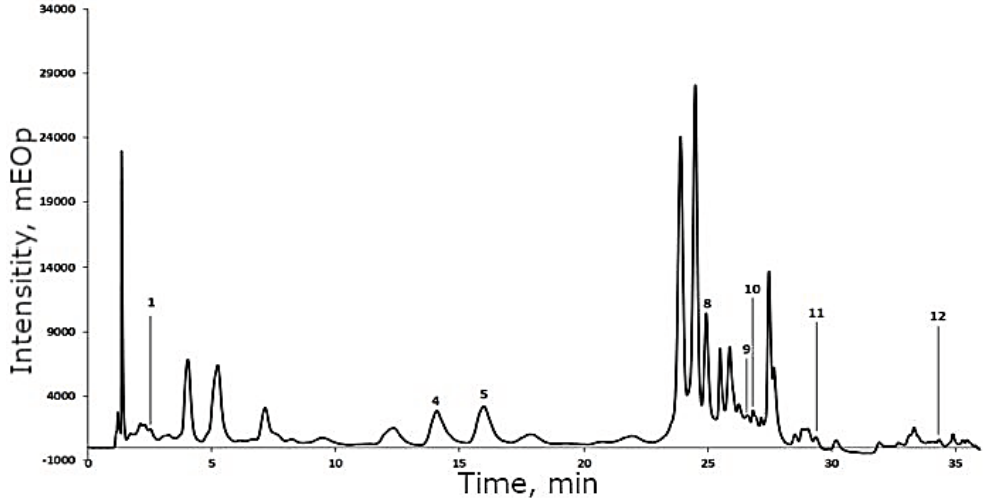


Fig. 1. Barberries extract chromatogram: 1-gallic acid, 4-chlorogenic acid, 5-epicatechin, 8-ferulic acid, 9-rutin, 10-hyperoside, 11-hesperidin, 12-quercetin

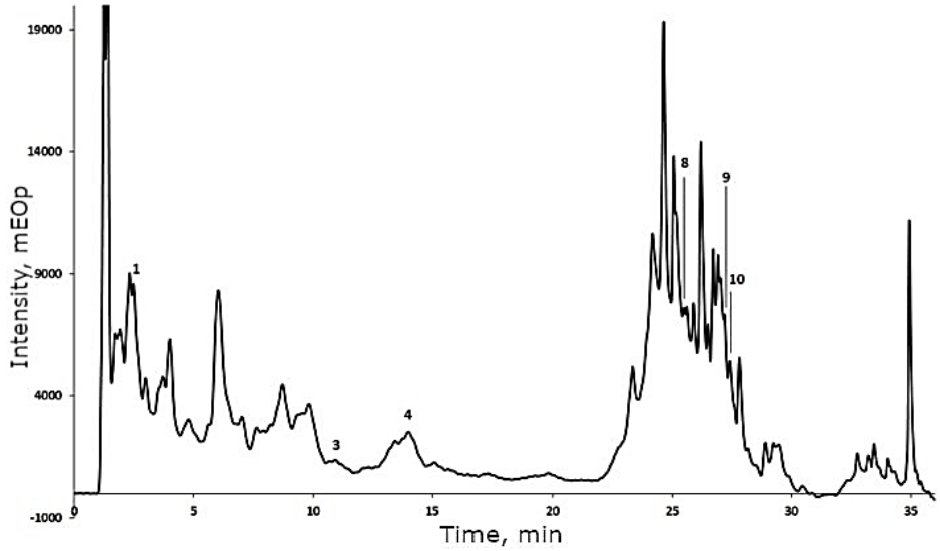


Fig. 2. Wild rose hips extract chromatogram: 1-gallic acid, 3-caffeic acid, 4-hlorogenic acid, 8-ferulic acid, 9-rutin, 10-hyperoside

RESULTS OF RESEARCH AND DISCUSSION

The majority of detectable components in different quantities are present in the fruits of Thunberg, Regel and Dark purple barberry of 2014 and 2015 years of collection, the total content of the components is shown in the table 1. Quantitation of PC content in the material is completed regarding the humidity of the fruits and the concentration of obtained extracts extractives considered. PC content barberry, wild rose hybrid and forest fruits of 2016 year of collection is shown in the table 2.

Table 1

PC content in barberry fruits of 2014-2015 years of collection, g/kg

Component	Thunberg barberry 2014	Regel barberry 2015	Dark purple barberry 2015
Gallic acid	0,024	0,085	0,109
Vanillic acid	*	–	–
Caffeic acid	0,481	1,067	0,913
Chlorogenic acid	50,714	72,990	120,737
Epicatechin	3,460	–	6,608
Syringic acid	–	–	–
Coumarin	0,730	2,218	2,851
Ferulic acid	0,319	0,376	3,574
Rutin	0,069	0,204	1,808
Hyperoside	0,815	8,696	10,455
Hesperidin	0,440	0,620	0,921
Quercetin	0,226	-	-
Luteolin	0,177	0,226	0,061

*Below detection limits.

In barberry fruits extracts chlorogenic acid is dominating, it exceeds 1-2 times other phenolic acids content (up to 120 g/kg). In flavonoids group hyperoside and rutin (both refer to glycosides) prevail in quantity reaching up to 10 g/kg. Species diversity of barberry based on PC composition in fruits becomes apparent both from the set of the components and from their quantity. Content of chlorogenic acid, epicatechin, caffeic acid in dark purple barberry fruits is at least twice higher than their content in Thunberg barberry fruits. Rutin, hyperoside, ferulic acid, coumarin content differences in fruits of these two types of barberry are shown even clearer. Regel barberry fruits contain more than 1g/kg of caffeic acid and flavonoid luteolin. Luteolin is one of the most effective anticarcinogenic agents [19].

A high content of chlorogenic acid and hyperoside was also noticed in barberry fruits collected in 2016, vanillic and syringic acids are absent. In these samples contrary to those of 2014-2015, ferulic acid, epicatechin, coumarin, hesperidin are absent, content of quercetin is lowered against the background of increasing in gallic and caffeic acids content.

Phenolic compounds set in wild rose fruits corresponds to the one in barberries. In the group of phenolic acids chlorogenic and gallic acids were detected in quantities from 0,43 to 4,21 g/kg, while other acids content remained under 0,10 g/kg; hyperoside prevails in the group of flavonoids. Significant differences between explored samples on the grounds of quality and quantity content were not revealed, but forest wild rose hyperoside content is twice higher. In the work [2] there are results presented that confirm species differences in BAS substances in wild rose fruits.

Table 2

PC content in barberry and wild rose fruits of the year 2016, g/kg

Component	Barberry			Rose		
	Thunberg	Regel	Dark-purple	Hybrid 1	Forest	Hybrid 2
Gallic acid	0,801	1,518	1,100	0,636	0,434	0,698
Vanillic acid	–	–	–	–	–	–
Caffeic acid	2,010	2,798	1,280	0,052	0,106	0,069
Chlorogenic acid	110,95	127,27	112,963	4,211	0,858	1,891
Epicatechin	–	–	–	–	–	–
Syringic acid	–	–	–	–	–	–
Coumarin	–	–	–	–	–	–
Ferulic acid	–	–	–	0,021	–	0,021
Rutin	0,062	0,281	2,641	0,091	0,075	0,333
Hyperoside	4,182	16,545	7,784	0,618	1,298	0,329
Hesperidin	–	–	–	–	–	–
Quercetin	0,059	0,020	0,148	0,008	0,412	–
Luteolin	0,024	–	0,063	–	–	–

Phenol carboxylic acids including gallic, vanillic, syringic acids are widely spread in plants [17], particularly as a component of tannins. The majority of acids refer to phenolic compounds of C_6–C_1 structure, are contained in plant tissues in bound form and are released after hydrolysis. Gallic acid is capable of self-condensation and forms depsides (esters) [20]. High biological activity, antiseptic and keratolytic properties are typical for all representatives of hydroxycinnamic acids. They have tonic, immunostimulating, anti-inflammatory, choleretic, antiallergic, vasodilatory and antioxidant impact on organism [10]. Caffeic and chlorogenic acids are the most vivid representatives of cinnamic acids [20]. Peculiarity of caffeic acid is its capability to form esters [14]. Analyzed barberry samples correspond to the literary data [5] caffeic acid content compared, are in the lead chlorogenic acid content compared.

Considering PC of C_6–C_3–C_6 structure (flavonoids) and their glycosides quercetin should be noted as one of the most wide spread flavanols as well as quercetin glycoside rutin (3-rutinoside quercetin) [20]. Namely these substances are used as standards when determining total F content in flowers and fruits of different plant species [9]. F have crucial properties: antitumor and anti-inflammatory effects, ability to reduce the risk of ischemia and blood clots [18].

BAS of phenolic content have antidepressant and anti-inflammatory effect that is why they are widely used in pharmacology [6, 8]. Particularly barberry and wild rose fruits show physiological activity of such action spectrum.

Barberry fruits are not included in Pharmacopoeia, therefore F total content was determined by available spectrographic method (quercetin was used as a standard) [13]. The method is based on the methods of detecting flavonoids in haws described in GOST 3852–93 [21].

As it is known, EA of different concentrations can be used to extract F. We were using 96 % EA to quantify F total content in fruits. To define optimum extraction method 40 % and 70 % concentrations of EA were used as the most widespread ones to make potions in pharmaceutics. Extraction duration is accepted to be 120 min

for MI, US and 5 min for SHF extraction basing on the results of previously conducted experiments. Results of F quantitation in several samples of fruits and their extracts received by different methods with quercetin as a standard reference sample are presented in the table 3 (% from a. d. r. m.). Sample choice for research is connected with the results of phenolic compounds detection (tables 1, 2).

Table 3

Flavonoids content in barberry and wild rose fruits and in their extracts

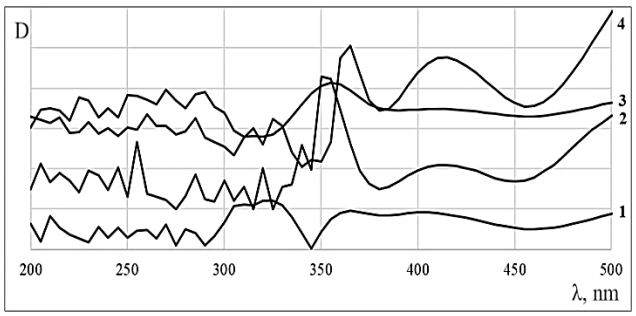
Species	Fruits	Extracts obtained in conditions			
		SHF, 40 % EA, 5 min	SHF, 70 % EA, 5 min	US, 70 % EA, 120 min	MI, 70 % EA, 120 min
Regel barberry, year 2015	1,32	0,41	0,17	0,23	0,24
Dark purple barberry, year 2015	1,30	0,51	0,36	0,53	0,43
Regel barberry, year 2016	1,41	–	0,61	0,54	0,45
Forest wild rose, year 2016	1,36	–	0,70	0,49	0,36

F total content in barberry fruits fluctuates from 1,30 to 1,41%. In extracts F content composes from 0,17 to 0,61 % counting from a. d. r. m. Extraction extent of all extraction variants does not exceed 39 %. F extraction from rose fruits by US treatment was more effective than by infusion method and SHF. For Regel barberry and forest rose fruits SHF method with 70 % EA on the contrary showed the best results. Extent of F-extraction from rose fruits SHF-method applied is 51,5 %. This result is indicative of the prospects of short term SHF-extraction realization in order to extract PC. Difference in F output from different species of barberry fruits is not significant. Years of collection compared, 2016 was more favorable for F biosynthesis, their content increased.

Obtained spectra of colored products are depicted in fig. 3,4. Peaks of UV-spectra of colored complexes of extracts of barberry fruits with aluminum chloride III with quercetin as a standard are situated mainly in diapason 350-365 nm which is typical exactly for flavonoids (fig. 3), species differences are displayed by peaks displacement. Optical density of Regel barberry fruits and Dark purple barberry fruits extracts obtained by infusion method is much higher than other barberry species fruits extracts optical density (fig. 3, *a*).

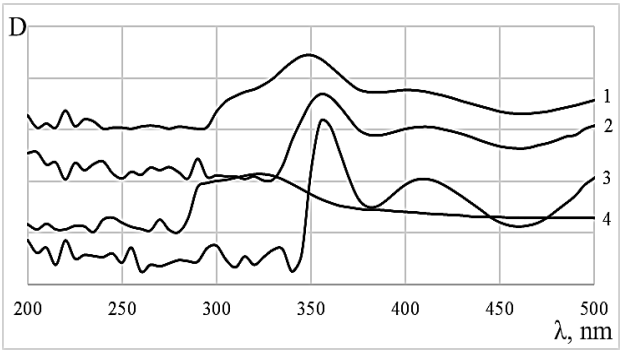
UV-spectra of barberry and rose fruits of 2016 year of collection extracts were obtained with 2 reference solutions: quercetin and rutin. Peaks of UV-spectra of colored extract products are also situated in the diapason typical exactly for flavanoids (fig. 4).

Peak of 355 nm and wide peak in the diapason 410–455 nm were revealed in reference solution spectrum of rutin (not shown). In the fig. 4, *a* spectra peaks of Dark purple barberry fruits extracts – 350, Regel barberry – 350, Thunberg barberry – 360 nm, i.e. in the same diapason. Those for extract spectra of wild rose hybrid (1) – 280, hybrid (2) – 275 and wild rose forest – 260 nm (fig. 4, *a*). Peak in reference solution spectrum of quercetin – 350 nm, it is low and smooth. For forest wild rose fruits extracts for the same standard spectra peaks are 325 nm (MI) and 325 nm (US) (fig. 4, *b*). For Regel barberry fruits extracts spectra – 340 nm (MI) and 340 nm (US) (not shown).



1 – Dark purpure barberry, SHF 70 % EA
2 – Regal barberry, SHF 40 % EA
3 – Regal barberry, SHF 70 % EA
4 – Dark purpure barberry, SHF 40 % EA

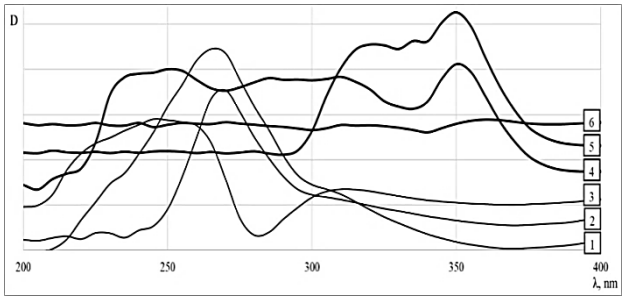
a



1 – Dark purpure barberry, MI
2 – Regal barberry, US
3 – Regal barberry, MI
4 – Dark purpure barberry, US

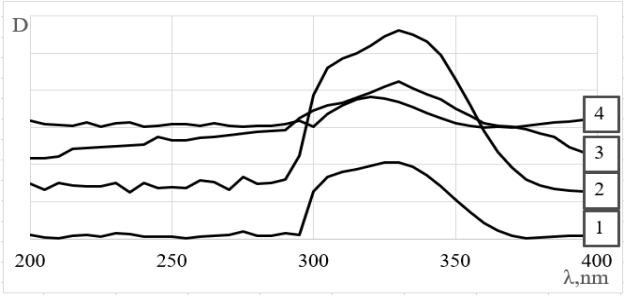
b

Fig. 3. Spectra of barberry fruits extracts with $AlCl_3$ obtained in different extraction conditions: SHF, 40 and 70 % EA (*a*); MI и US, 70 % EA (*b*)



1 – Rose hybrid 2, MI
2 – Rose hybrid 1, M
3 – Forest rose, MI
4 – Regal barberry, MI
5 – Dark purpure barberry, MI
6 – Thunberg barberry, MI

a



1 – Regal barberry, US
2 – Regal barberry, MI
3 – Forest rose, MI
4 – Forest rose, US

b

Fig. 4. Spectra of barberry and rose fruits extracts obtained with $AlCl_3$ with different reference solutions: rutin (*a*); quercetin (*b*)

Considering the spectra the following conclusion can be made. To determine total flavanoids content in terms of rutin the reommended interval is λ_{max} 320–330 nm. For the extracts which maximum absorbation is in the diapason 325–415 nm, quercetin should be used as a standard.

While studying flavonoids method of qualitative reactions with different reagents was approbated [11,12]. Qualitative color reactions show the presence of certain flavonoids groups. Basing on the results of these qualitative reactions the conclusion comes that main flavonoid groups (flavanols, flavanones, flavones, anthocyanin pigments, chalcones or aurones, anthocyanins, leucoanthocyanidins, catechins) are present in all samples explored. The only difference is in the catechins presence in Thunberg barberry and dark-purple barberry collected in 2015, 2016.

Conclusion

Biologically active substances are vital and necessary compounds, each of them plays irreplaceable and very important role in the organism's lifecycle. It is a scientific fact that barberry and wild rose fruits grown in the north are rich in chlorogenic acid and flavones. Spectrophotometric method of flavonoids total content detection in fruits should be applied only with the standard substance of which is dominating in phenolic compounds. It was revealed that dark-purple and Regel barberry fruits are of maximum valuable components content, they should be recommended to be spread in the north conditions. Obtained extracts can be used as pharmaceutical substances or food additives with antioxidant properties.

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ФЕНОЛЬНЫЕ СОЕДИНЕНИЯ ПЛОДОВ БАРБАРИСА И ШИПОВНИКА

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Предметом исследования являются образцы плодов барбариса и шиповника (розы) нескольких видов. Цель работы – изучение состава фенольных соединений и сравнительная оценка количественного содержания флавоноидов как наиболее ценной группы фенольных соединений в плодах растений различных видов. Исследования выполнены методом ВЭЖХ в обращенно-фазовом режиме. Для извлечения флавоноидов при определении их суммарного содержания использовали этиловый спирт различной концентрации. Экстракция проведена тремя методами: методом настаивания, в экс-

тракторе с обработкой ультразвуком и под воздействием электромагнитного поля сверхвысоких частот. Оценка видовых различий выполнена по УФ-спектрам окрашенных комплексов фенольных соединений экстрактов с хлоридом алюминия. Определено количественное содержание 13 компонентов фенольных соединений в плодах, собранных в 2014–2016 гг. Выявлены доминирующие компоненты – хлорогеновая кислота и гиперозид. Установлены виды барбариса с наиболее ценным набором фенольных соединений в плодах: барбарис темно-пурпуровый (*Berberis vulgaris f. atropurpurea Regel*) и барбарис Тунберга (*Berberis thunbergii* DC) – образцы 2015 г., барбарис Регеля (*Berberis regeliana Kochne*) – 2016 г. сбора. Суммарное содержание флавоноидов, определенное спектрофотометрическим методом (раствор сравнения – кверцетин) при их извлечении методом настаивания, составляет от 1,30 до 1,41 %. Максимальная степень извлечения флавоноидов из плодов барбариса наиболее эффективным методом составила 39,0 %, из плодов шиповника – 51,5 % (СВЧ-экстракция). На основании полученных данных подготовлены рекомендации по использованию экстрактов в качестве субстанций для получения фармацевтических фитопрепаратов и пищевых добавок с антиоксидантными свойствами.

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Ключевые слова: экстрактивные вещества, фенольные соединения, флавоноиды, УФ-спектры, ВЭЖХ, кверцетин, рутин.

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