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Alena V. Spirydovich[1], Polina S. Shabunya[2], Alena D. Ahabalayeva[1], Alla M. Deeva[1], Yulia K. Vinogradova[3], Nastassia B. Vlasava[1,4], Michael A. Kost[4], Academician Vladimir N. Reshetnikov[1]

[1]*Central Botanical Gardens of the National Academy of Sciences of Belarus, Minsk, Republic of Belarus*
[2]*Institute of Bioorganic Chemistry of the National Academy of Sciences of Belarus, Minsk, Republic of Belarus*
[3]*Tsitsin Main Botanical Garden of the Russian Academy of Sciences, Moscow, Russian Federation*
[4]*Matthaei Botanical Gardens, Ann Arbor, United States of America*

VARIABILITY OF SECONDARY METABOLITES IN THE BARK OF *RHAMNUS CATHARTICA* L. FROM NATIVE AND INTRODUCED POPULATION

Abstract. The European buckthorn (*Rhamnus cathartica* L.) – a shrub native to most parts of Europe and Western Asia – has been successfully naturalized in the Midwest and Northeast of the USA, dominating various habitats and displacing native species. It is known that *Rhamnus cathartica* L. plants contain a wide range of secondary metabolites with pharmacological effects. Permanent sample plots with *R. cathartica* – the species native for Belarus (two sites) and invasive in the USA (two sites) – were selected. This study investigated the level of anthraquinones, catechins, and leucoanthocyanins, and identified anthraquinones using HPLC with mass spectrometry and UV detection, showing a significant variation in these indicators depending on the conditions and the geographical area of growth. *Rhamnus cathartica* chemorases were identified, which will allow further isolation of samples with a required set of phenolic compounds and a directed selection for obtaining artificially introduced populations with an increased content of one or another valuable compound.

Keywords: European buckthorn (*Rhamnus cathartica* L.), Belarus, regions of the Upper Midwest, USA, anthraquinones, HPLC method with mass spectrometric and UV detector, catechins, leukoanthocyanins

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Е. В. Спиридович[1], П. С. Шабуня[2], Е. Д. Агабалаева[1], А. М. Деева[1], Ю. К. Виноградова[3], А. Б. Власова[1,4], М. А. Кост[4], академик В. Н. Решетников[1]

[1]*Центральный ботанический сад Национальной академии наук Беларуси, Минск, Республика Беларусь*
[2]*Институт биоорганической химии Национальной академии наук Беларуси, Минск, Республика Беларусь*
[3]*Главный ботанический сад имени Н. В. Цицина Российской академии наук, Москва, Российская Федерация*
[4]*Меттай Ботанический Сад Николз Арборетум, Университет Мичигана, Энн-Арбор, Соединенные Штаты Америки*

ИЗМЕНЧИВОСТЬ СОДЕРЖАНИЯ ВТОРИЧНЫХ МЕТАБОЛИТОВ В КОРЕ *RHAMNUS CATHARTICA* L. В ПРИРОДНЫХ И ИНТРОДУЦИРОВАННЫХ ПОПУЛЯЦИЯХ

Аннотация. Жостер слабительный (*Rhamnus cathartica* L.) – кустарник, произрастающий в большей части Европы и Западной Азии, успешно натурализовался в условиях Среднего Запада и Северо-Востока США, доминируя в различных местообитаниях и вытесняя природные виды. Известно, что растения *Rhamnus cathartica* L. содержат широкий спектр вторичных метаболитов, обладающих фармакологическим действием. Выбраны постоянные пробные площадки произрастания *R. cathartica* – аборигенного для Беларуси (2 площадки) и инвазионного в США вида (2 площадки). В данной работе был исследован уровень содержания антрахинонов (гидроксиантраценпроизводных), катехинов и лейкоантоцианов, а также проведена идентификация антрахинонов методом высокоэффективной жидкостной хроматографии (ВЭЖХ) с масс-спектрометрическим и УФ-детектором, показано значительное варьирование этих показателей в зависимости от условий и ареала. Выявлены хеморасы *Rhamnus cathartica*, что позволит в дальнейшем отбирать образцы с требуемым набором фенольных соединений и проводить направленную селекцию на получение искусственных интродукционных популяций с повышенным содержанием того или иного ценного соединения.

Ключевые слова: жостер слабительный (*Rhamnus cathartica* L.), Беларусь, регионы Среднего Запада, США, глюкофрангулины, метод ВЭЖХ с масс-спектрометрическим и УФ-детектором, катехины, лейкоантоцианы

Для цитирования. Изменчивость содержания вторичных метаболитов в коре *Rhamnus cathartica* L. в природных и интродуцированных популяциях / Е. В. Спиридович, П. С. Шабуня, Е. Д. Агабалаева [и др.] // Доклады Национальной академии наук Беларуси. – 2025. – Т. 69, № 1. – С. 40–47. https://doi.org/10.29235/1561-8323-2025-69-1-40-47

Introduction. The emergence of invasive species in various biocenoses of the world poses a challenge for the scientific community to study the mechanisms that ensure the survival, spread and sustainability of these species in the conditions of new habitats. The understanding of the mechanism behind the adaptation and interaction with other biocenoses participants will help develop effective measures to curb the spread of invasive species, while protecting and conserving the biodiversity of native biocenoses.

Rhamnus cathartica L. (European buckthorn) is a shrub growing in much of Europe and Western Asia, that has successfully naturalized in the conditions of the Midwest and Northeast of the USA, and also in the coastal provinces of Canada, having invaded many habitats, including open areas, forests, anthropogenic territories and wetland edges [1]. The European buckthorn prefers open, moist fertile areas, rich in calcium but it can also withstand both drought and semi-flooded conditions.

In the previous studies, we compared the following parameters on three sample plots for *Rhamnus cathartica* L.: in Belarus and two plots in the US Midwest – a full floristic description of the sample sites, the field enumeration of buckthorn plants, average height, and the projective cover of associated species. The obtained data confirmed the hypothesis of the increased competitiveness of invasive species in the introduced range (EICA). In the American populations, as compared to the Belarusian ones, *Rhamnus cathartica* L. plants on average are larger and have higher seed productivity. Higher numbers of juveniles and a higher overall number of individuals, up to the formation of monodominant thickets, were also recorded in the US Midwestern population. It has been noted that the introduction of buckthorn into natural communities in the USA reduces the number of species in the herb layer by eight times [2]. Plants of *R. cathartica* contain a wide range of organic substances (flavonoids, saponins, alkaloids, tannins, coumarins, starch, pectin) [1]. Despite their structural diversity, most phenolic compounds exhibit antioxidant, anti-inflammatory, and antimicrobial activities *in vivo* [1; 3–5], to which they largely owe the therapeutic potential in treating a range of diseases [6].

Characteristic of many *Rhamnus* L. species compounds of the secondary metabolism of the quinone series, particularly anthracene derivatives in the form of hydroxy derivatives of anthraquinone such as frangula emodin, aloe emodin, chrysophanol, rhein, physcion, etc. [7], may contribute to the invasiveness of this species in the ecosystem. These substances can repel herbivorous animals from eating the leaves, bark, and fruits, protect plants from pathogens and insect pests, produce allelopathic effects on nearby plants, affect soil microorganisms, and influence the consumption and digestion of fruits by birds [8]. It is well known that many birds in North America consume the ripe (mature) fruits of *R. cathartica* [9], because the concentration of emodin decreases as the fruits mature, as shown for *Rhamnus alaternus* [10]. Thus, the high concentration of emodin in immature fruit of Rhamnus species protects it from predation by birds, while its lower concentration in mature fruit ensures its dispersal by birds across the landscape. In addition, it should be noted that when plants are introduced into areas different from their natural ones in terms of weather and climatic conditions, species adaptation may involve not only phenotypic but also genetic changes [11], which are reflected in the secondary metabolism of plants. The overall resilience of *R. cathartica* to various habitats, as well as the accumulation of biologically active compounds that produce effects on surrounding plants and other participants of biocenoses, contributes to its widespread distribution [3].

To find a practical solution to the problem of controlling the number of invasive species, it is necessary to study the physiological and biochemical features of their increased competitiveness, and with that research should be conducted both in the primary and secondary ranges, preferably in a comparative aspect.

Based on the above, the aim of the study is the comparative analysis of bark extracts from buckthorn plants (*Rhamnus cathartica* L.) growing in different geographical and climatic regions, for the content of anthraquinones, catechins, leucoanthocyanins, with an assessment of anthracene derivatives of phenolic nature using HPLC-MS with a UV detector.

Materials and methods of research. For the biochemical analysis the samples of bark from the selected plants of *R. cathartica* from two permanent sample plots (PSPs) in two locations in Belarus were used: PSP 1 (Lake Balduk) – the shore of Lake Balduk: plot 100 m^2 = 50 × 2 m, PSP 2 (Cialiaki–Skory) – roadside of the R28 Miadziel–Narach resort area 100 m^2 = 25 × 4 m; and in 2 locations in the USA: PSP 3 (Minnesota, Carver County, private area near the University of Minnesota Landscape Arboretum (monodominant thickets), PSP 4 (Michigan, Central USA), described in detail in [1].

The content of anthraquinones, catechins and leucoanthocyanins was determined spectrophotometrically in accordance with standard methods with statistical data processing [11; 12].

For the extraction of phenolic compounds for HPLC, a triple extraction was carried out with a raw material to extractant (70 % ethyl alcohol) ratio of 1 g : 50 ml.

For the identification and quantitative analysis of secondary metabolites in the bark of *Rhamnus cathartica* L., the HPLC method with mass spectrometric and UV detector was used. Separation was carried out on an Agilent Zorbax XDB C18 column (4.6 × 150 mm; 5.0 μm) at a temperature of +40 °C on an Agilent 1200 liquid chromatograph. The mobile phase consisted of two solvents: A – 0.15 % acetic acid (v/v) in deionized water and B – 100 % acetonitrile. A stepwise elution mode from 15 % to 90 % of phase B over 30 minutes at a flow rate of 0.5 ml/min was used. Detection was carried out at λ = 435 nm. The injection volume was 20 μl. The temperature in the autosampler was +15 °C. The sample from the chromatograph was fed into the inlet of the Agilent 6410 Triple Quad tandem mass spectrometer (triple quadrupole). An Agilent G1948B API-ES electrospray was used as the ionization interface in negative ion mode. For identification, ion scanning mode in the mass range of 100 to 2000 Da was used. Mass detector operation parameters: temperature and flow rate of the drying gas +350 °C and 7 l/min; sprayer pressure 30 psi; capillary voltage 4000 volts; fragmentor voltage 135 volts. The analysis of chromatograms and mass spectra was performed using Agilent MassHunter Workstation Software version B.01.03 and Agilent ChemStation (Agilent Technologies Inc., USA). Calibrations and quantitative calculations were made using chromatograms from a UV detector. For calibration, the following standards were used: frangulin A (>95 % HPLC, Extrasynthese), aloe emodin (>98.5 % HPLC, Extrasynthese) and chrysophanol (99.63 % HPLC, Chem Scene). Peaks of substances with UV spectra similar to the spectra of available anthraquinone standards were integrated on chromatograms. Compounds 1, 2, 3–13, 14, 18 were calculated based on the calibration for frangulin A. Compounds 15–17, 19, and 20 were calculated based on the calibration for aloe emodin. Chrysophanol (compound 21) was calculated based on the calibration for chrysophanol.

Results and discussion. Currently, many published data support that invasiveness of a particular species is a result of complex interaction of various biotic and abiotic factors, influencing the accumulation of spectra of secondary metabolites by plants and the display of antioxidant activity [13]. Moreover, invasiveness is manifested only in specific ecological conditions. We have compared European buckthorn plants (*Rhamnus cathartica* L.) for the content of anthraquinones, catechins and leucoanthocyanins.

It has been shown that buckthorn bark is characterized by the presence of a wide range of anthracene derivatives – anthraquinones [4]. Many natural anthraquinones exhibit antimicrobial activity, which depends on their chemical structure. For chrysophanol, emodin, physcion, rhein, and aloe emodin, antimicrobial activity *in vitro* against many strains of microorganisms pathogenic to humans has been described in the majority of studies [6]. For instance, inhibitory properties exhibited by chrysophanol and other derivatives of 9,10-anthraquinone were shown against *Candida albicans*, *Cryptococcus neoformans*, *Trichophyton menagrophytes* and *Aspergillus fumigatus* (MIC 25250 mg/ml).

Bark extracts were analyzed using the HPLC-MS method. For the identification of anthracene derivatives, chromatograms from a diode array detector (435 nm) were used, as well as chromatograms of total ion current in negative ionization mode. The choice of the wavelength of 435 nm for chromatogram registration was due to the fact that anthracene derivatives have one of the characteristic absorption maxima in this area. Three standards (chrysophanol, frangulin A, aloe emodin) were used in the work both for plotting calibration curves for the quantitative analysis, and for the comparison of UV spectra of putative anthraquinones with the spectra of standards. Fig. 1 presents chromatograms of the standards and extracts from the plots in Belarus (*b*) and the USA (*c*). In the analyzed samples, 21 anthraquinone compounds were identified. Among them, two – frangulin A and chrysophanol – were identified by comparing their retention times and mass spectra with corresponding standards. According to the obtained data, compounds 15–17, 19, and 20 have presumed molecular masses that coincide with those of known anthraquinone aglycones. For precise identification, standard substances are required.

Based on the analysis, the possible structures of 21 chemical compounds extracted from the bark of buckthorn were established: 1–14 were identified as compounds of the anthraquinone series; compounds 15, 17 – as anthraquinone aglycones of alaternin or hydroxyemodin; compounds 16, 19 – as anthraquinone aglycones of pseudopurpurin; compound 20 – as an anthraquinone aldehyde of emodin, lucidin, or morindone. Compound 18 was identified as Frangulin A, and compound 21 as chrysophanol.

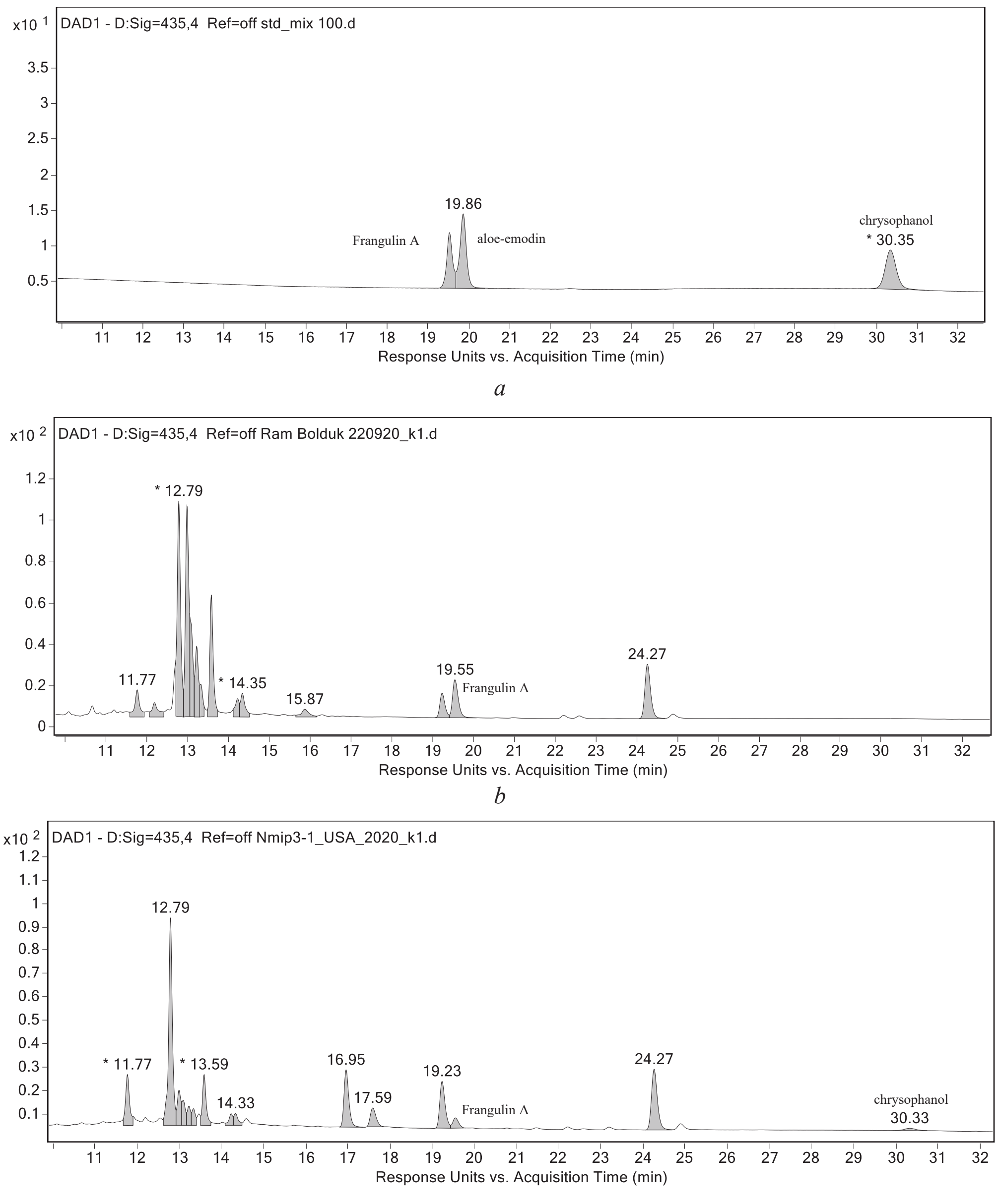

Fig. 1. UV chromatograms of standards (*a*) and samples of *Rhamnus cathartica* L., PSP 1 (*b*) and *Rhamnus cathartica* L., PSP 4 (*c*)

For all extracts, the content of each separate anthraquinone compound was determined; with that compounds 1, 2, 3–13, 14, 18 were calculated based on frangulin A, which is a glycoside (emodin rhamnoside), because these substances in the mass spectra were characterized by higher m/z values, close to possible glycosides, and had shorter retention times compared to frangulin A. For quantitative analysis of compounds 15–17, 19, and 20, aloe emodin calibration was used, as these substances had m/z values in the mass spectrum close to the molecular masses of anthraquinone aglycones. Table presents data on the content of anthracene derivatives in the studied extracts.

Concentration (mg/100 g) of detected compounds in the extracts of *Rhamnus cathartica*

RT, min (λ = 435 nm)	Sample name			
	PSP 1 Balduk, Belarus	PSP 2 Cialiaki–Skory, Belarus	PSP 3 Nmip3-1, Minnesota, USA	PSP 4 Nmb1-1, Michigan, USA
10.11	–	–	–	–
10.68	–	–	–	–
11.78	17.13	10.11	31.24	5.36
12.19	7.41	3.03	–	–
12.79	179.21	164.06	133.88	78.91
12.99	150.42	129.00	17.00	39.65
13.09	53.34	–	10.25	6.95
13.30	46.07	46.68	8.25	9.53
13.33	17.49	–	8.13	7.05
13.58	87.04	47.54	28.85	38.43
14.19	–	–	–	–
14.23	12.61	18.00 9.89	8.33 9.36	8.43
14.33	17.34	–	–	13.70
15.87	10.03	–	–	–
16. 95	–	–	37.75	4.19
17.59	–	–	16.26	3.82
19.23	21.49	11.37	34.65	2.49
19.55	47.74	160.83	7.06	3.83
21. 48	–	12.79	–	–
24.27	50.08	30.67	49.55	8.12
30.33	–	4.75	5.12	–

Analyzing the obtained data, it should be noted that the extracts of *Rhamnus cathartica* L. bark differed in the composition and content of anthraquinone compounds both between populations in Belarus and the USA and between populations from the native and the invasive ranges. For example, chrysophanol was found only in the samples of *Rhamnus cathartica* L. bark collected from PSP 2, Cialiaki–Skory, Belarus (4.75 mg/100 g) and PSP 3, Minnesota, USA (5.12 mg/100 g), whereas frangulin A was present in the samples from all testing sites. All examined *Rhamnus cathartica* L. samples in terms of frangulin A content in the bark can be arranged in descending order: PSP 2, Cialiaki–Skory (160.83 mg/100 g) > PSP 1, Lake Balduk (47.74 mg/100 g) > PSP 3, Minnesota (7.06 mg/100 g) > PSP 4 Michigan (3.83 mg/100 g). Thus, this indicator was the highest (160.83 mg/100 g) in *Rhamnus cathartica* L. samples collected from PSP 2, and the lowest (3.83 mg/100 g) in the samples from PSP 4.

The pharmacological value of plants is determined by the quantity of biologically active substances, their ratio, and their prevalence in the chemical composition of certain compounds. Among natural antioxidants, phenolic compounds have the highest activity. These include polyphenols, phenolic hydroxy acids, various types of flavonoids, vitamins, etc. The quantitative content of phenolic substances in plant raw materials can be indicative of their antioxidant properties.

Another important property of plant raw materials is its vitamin P activity, determined by the content of various phenolic compounds. P-active substances are represented by flavonols (rutin, quercetin, isoquercetin), anthocyanins, leucoanthocyanins, and catechins. In some plants, it is catechins that are prevalent, in others it is leucoanthocyanins.

According to the analysis, the content of leucoanthocyanins in buckthorn bark varies from 96.00 ± 2.4 mg/100 g (PSP 3, Minnesota) to 304.95 ± 2.9 mg/100 g (PSP 2, Cialiaki–Skory). The obtained data suggest that buckthorn bark accumulates leucoanthocyanins in amounts comparable to the content of this biologically active compound in the leaves of peppermint, common flax, safflower levzea, large-flowered foxglove, lovage, elecampane, marshmallow, and asparagus [13].

Leucoanthocyanins, being precursors of catechins, directly affect the concentration of these compounds in plants. Along with catechins, flavan-3,4-diols are also precursors of condensed tannins. It is worth noting that these condensed forms of compounds are capable of accumulation, and a higher content of leucoanthocyanins and catechins is indicative of the low activity of tannins formation processes. The largest amount of catechins was found (in descending order of their quantity): in the samples from sites PSP 1 < PSP 4 < PSP 2 < PSP 3. The content of catechins in the bark varied from 123.03 ± 3.7 mg/100 g (PSP 3, Minnesota) to 326.35 ± 4.0 mg/100 g (PSP 1, Balduk). Among the samples collected from the four studied locations, the maximum content of flavan-3-ols was observed in the samples from Belarus. The samples from PSP 3 and PSP 4 collected in the USA contained on average 50–60 % less catechins than the Belarusian samples with its maximum content.

The antioxidant properties of many plant products are significantly determined by the content of flavan-3-ols, whose antioxidant activity is 50 times greater than that of vitamin E and 20 times that of vitamin C. There are data on the effectiveness of using plant extracts containing catechins in the food industry. Catechins can have an inhibitory effect on hydrolytic and oxidative processes in lipids, reducing the rate of hydrolysis product formation, and stabilizing protein systems.

The analysis of the fractional composition of phenolic compounds in the studied samples of *Rhamnus cathartica* L. bark from PSP 1, PSP 2, PSP 3, PSP 4 showed that this complex is dominated by anthraquinone compounds (53 %, on average – 500.6 mg/100 g), followed by catechins (24.9 %, 224.7 mg/100 g – henceforward average value), with components of the anthocyanin complex – leucoanthocyanins – present to a slightly lesser extent (22.4 %, 211.9 mg/100 g). Fig. 2 shows the fractional composition of secondary metabolites of buckthorn bark in the studied samples.

Economically valuable wild plants, such as the European buckthorn (*Rhamnus cathartica* L.) in Belarus, usually reduce the content of biologically active substances when cultivated. Research on the reverse process is quite rare, and there is still little evidence on whether the level of secondary metabolites increases in plants "escaping" from cultivation and invading natural communities (invasive species), as was the case with this species in the USA. So far, single phytochemical studies have been carried out on representatives of the genera *Aronia*, *Echinocystis*, *Solidago*, and it is difficult to include them in a certain hypothesis. Referring to the "Enemy Release Hypothesis" proposed in several variants [13], which in general implies that many alien plants, after introduction or naturalization in a new territory, are freed from the pressure of specialized natural enemies (in particular, phytophagans and pathogens) that usually control the number of species or their populations within the primary range, this hypothesis needs further study in the case of a long-term invasion, as with *Rhamnus cathartica* in the USA. For

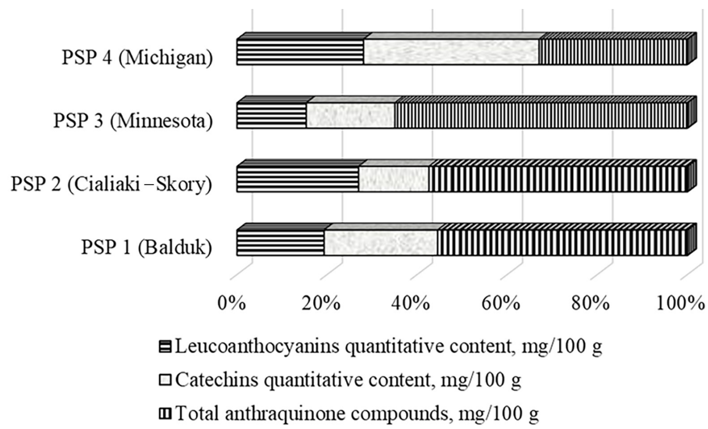

Fig. 2. Fractional composition of the secondary metabolites of buckthorn bark

example, monitoring of phytophagans and pathogenic organisms of *Ribes aureum* revealed that during naturalization, pathogens adapt to alien plants. The duration of this process is determined by environmental conditions, the influence of anthropogenic factors, and the age of plantations. Thus, the hypothesis about the influence of phytophagans and phytopathogens on the success of the invasion of alien species in the introduced range can only be considered at the initial stage of naturalization. Later on, phytophagans and pathogens of closely related species actively become part of the "alien species–pathogen" system [15].

Conclusion. As a result of HPLC analysis of anthraquinones in the bark of *Rhamnus cathartica* L., we identified 2 chrysacim derivatives: frangulin A and chrysophanol. Chrysophanol was found only in the samples of *Rhamnus cathartica* L. bark collected from PSP 2, Cialiaki–Skory, Belarus (4.75 mg/100 g) and PSP 3, Minnesota, USA (5.12 mg/100 g). Frangulin A was found in the samples from all testing sites. Thus, both in the primary and secondary habitats it is possible to differentiate specialized chemorases of *Rhamnus cathartica* based on set of phenolic compounds.

The analysis of the fractional composition of phenolic compounds in the studied samples of *Rhamnus cathartica* L. bark from PSP 1, PSP 2, PSP 3, PSP 4 showed that the dominant position in this complex is occupied by compounds of the anthraquinone series, in the second position are catechins, and in the third position are the components of the anthocyanin complex, leucoanthocyanins.

Currently, representatives of the genus *Rhamnus* in Belarus and the USA are not fully studied as potential medicinal plant raw materials containing hydroxy derivatives of anthraquinone and other secondary metabolites. Anthraquinone derivatives from *Rhamnus* bark have a wide range of biological action, however, in practice, Eurasian buckthorn preparations are used only as a laxative.

A more comprehensive study of the comparative qualitative and quantitative composition, as well as the biological action of anthraquinone derivatives of *Rhamnus cathartica*, will allow us to understand their role as metabolites and to reveal their potential applications. This article is the first attempt at comparative quantitative analysis of secondary metabolites in species of the genus *Rhamnus*, growing in the territory of Belarus, as a native species, and in the USA, as an invasive species.

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Information about the authors

Spirydovich Alena V. – Ph. D. (Biology), Associate Professor, Head of the Laboratory. Central Botanical Gardens of the National Academy of Sciences of Belarus (2v, Surganov Str., 220012, Minsk, Republic of Belarus). E-mail: a.spirydovich@gmail.com. ORCID: 0000-0002-9674-7648.

Shabunya Polina S. – Ph. D. (Biology), Senior Researcher. Institute of Bioorganic Chemistry of the National Academy of Sciences of Belarus (5/2, Kuprevich Str., 220141, Minsk, Republic of Belarus). E-mail: pshabunya@rambler.ru. ORCID: 0000-0002-5650-8538.

Ahabalayeva Alena D. – Ph. D. (Biology), Senior Researcher. Central Botanical Gardens of the National Academy of Sciences of Belarus (2v, Surganov Str., 220012, Minsk, Republic of Belarus). E-mail: plechischik@rambler.ru. ORCID: 0000-0003-3035-5040.

Deeva Alla M. – Ph. D. (Biology), Associate Professor, Head of the Department. Central Botanical Gardens of the National Academy of Sciences of Belarus (2v, Surganov Str., 220012, Minsk, Republic of Belarus). E-mail: alladzeeva@gmail.com. ORCID: 0000-0001-9075-185X.

Vinogradova Yulia K. – D. Sc. (Biology), Chief Researcher. N. V. Tsitsin Main Botanical Garden of the Russian Academy of Sciences (4, Botanicheskaya Str., 127276, Moscow, Russian Federation). E-mail: gbsad@mail.ru. ORCID: 0000-0003-3353-1230.

Vlasava Nastassia B. – Ph. D. (Biology), Senior Researcher. Central Botanical Garden of the National Academy of Sciences of Belarus (2v, Surganov Str., 220012, Minsk, Republic of Belarus). E-mail: nastassia_vlasova@yahoo.com. ORCID: 0000-0003-0990-3532.

Kost Michael A. – Associate Curator. Matthaei Botanical Gardens and Nichols Arboretum; Lecturer. School for Environment and Sustainability of the University of Michigan (1800 N Dixboro Rd, Ann Arbor, MI 48105, United States of America). E-mail: michkost@umich.edu.

Reshetnikov Vladimir N. – Academician, D. Sc. (Biology), Professor, Head of the Department. Central Botanical Garden of the National Academy of Sciences of Belarus (2v, Surganov Str., 220072, Minsk, Republic of Belarus). E-mail: V.Reshetnikov@cbg.org.by. ORCID: 0000-0002-1067-4346.

Информация об авторах

Спиридович Елена Владимировна – канд. биол. наук, доцент, заведующий лабораторией. Центральный ботанический сад НАН Беларуси (ул. Сурганова, 2в, 220012, Минск, Республика Беларусь). E-mail: a.spirydovich@gmail.com. ORCID: 0000-0002-9674-7648.

Шабуня Полина Станиславовна – канд. биол. наук, ведущий научный сотрудник. Институт биоорганической химии НАН Беларуси (ул. Купревича, 5/2, 220141, Минск, Республика Беларусь). E-mail: pshabunya@rambler.ru. ORCID: 0000-0002-5650-8538.

Агабалаева Елена Дмитриевна – канд. биол. наук, ст. науч. сотрудник. Центральный ботанический сад НАН Беларуси (ул. Сурганова, 2в, 220012, Минск, Республика Беларусь). E-mail: plechischik@rambler.ru. ORCID: 0000-0003-3035-5040.

Деева Алла Михайловна – канд. биол. наук, доцент, заведующий отделом. Центральный ботанический сад НАН Беларуси (ул. Сурганова, 2в, 220012, Минск, Республика Беларусь). E-mail: alladzeeva@gmail.com. ORCID: 0000-0001-9075-185X.

Виноградова Юлия Константиновна – д-р биол. наук, гл. науч. сотрудник. Главный ботанический сад им. Н. В. Цицина РАН (ул. Ботаническая, 4, 127276, Москва, Российская Федерация). E-mail: gbsad@mail.ru. ORCID: 0000-0003-3353-1230.

Власова Анастасия Борисовна – канд. биол. наук, ст. науч. сотрудник. Центральный ботанический сад НАН Беларуси (ул. Сурганова, 2в, 220012, Минск, Республика Беларусь). E-mail: nastassia_vlasova@yahoo.com. ORCID: 0000-0003-0990-3532.

Кост Майкл А. – заместитель куратора. Меттай Ботанический Сад и Николз Арборетум; преподаватель Школы окружающей среды и устойчивого развития Мичиганского университета (1800 N Dixboro Rd, Энн-Арбор, Мичиган 48105, США). E-mail: michkost@umich.edu.

Решетников Владимир Николаевич – академик, д-р биол. наук, профессор, заведующий отделом. Центральный ботанический сад НАН Беларуси (ул. Сурганова, 2в, 220012, Минск, Республика Беларусь). E-mail: v.reshetnikov@cbg.org.by. ORCID: 0000-0002-1067-4346.