https://doi.org/10.33380/2305-2066-2022-11-4-216-225
UDC 615.072

Research article / Оригинальная статья

Isolation, Identification and Quantitative Determination of Anthracene Derivatives by HPLC-UV Method in the Raw Materials of Some Representatives of the Genus *Rumex* of Three Vegetation Times

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Received: 06.10.2022 **Revised:** 00.11.2022 **Published:** 25.11.2022

Abstract

Introduction. The creation of new highly effective drugs requires a thorough study of the metabolome of plant raw materials and a comparative phytochemical study of the underground organs of closely related species of Rumex, such as: *R. crispus*, *R. obtusifolius* and *R. aquaticus*, ubiquitous in Russia. It was noted that they have a metabolome like the official *R. confertus*, which in turn confirms the potential for studying these species. Of scientific and practical interest is the study of the dynamics of accumulation of the leading group of biologically active substances – anthracene derivatives, depending on the phenological phases of plant development.

Aim. Identify and quantify anthracene derivatives in the underground organs of *R. confertus*, *R. crispus*, *R. obtusifolius* and *R. aquaticus* harvested in three different phases of vegetation.

Materials and methods. Extracts from the underground organs of the studied plants obtained according to the method from the pharmacopoeial article on *R. confertus* were used as the analyzed solutions. The solutions were analyzed on a Nexera-i LC-2040 chromatograph (Shimadzu Corporation, Japan) equipped with a column and sample thermostat, a degasser, and an autosampler using an individually selected mobile phase elution gradient (0.1 % phosphoric acid/acetonitrile solution). Primary data were processed using LabSolutions Single LC software (Shimadzu Corporation, Japan). Compounds from the group of anthracene derivatives were identified by retention times. Detection was carried out using a UV detector with a dynamic change in the absorption wavelength during analysis from 365 ± 2 nm to 254 ± 2 nm.

Results and discussion. Alcohol-water extracts were obtained from the underground organs of *Rumex*. An elution gradient was selected for the simultaneous determination of 5 anthracene derivatives with a single analysis time of 40 minutes. These chromatographic conditions made it possible to identify and quantify the content of emodin, 8-O-β-D-glucoside of emodin, and chrysophanol in the underground organs of *R. confertus*, *R. crispus*, *R. obtusifolius* and *R. aquaticus* in three different vegetations. Glycosides of anthracene derivatives: glucofrangulin A and frangulin A were not found in the studied objects.

Conclusion. Anthracene derivatives were isolated from the underground organs of different vegetations, a method for the quantitative determination of anthracene derivatives in alcohol-water extracts was developed, emodin, 8-O-β-D-glucoside of emodin and chrysophanol were found and quantified.

Keywords: anthracene derivatives, emodin, HPLC-UV, roots, *R. confertus*, *R. crispus*, *R. obtusifolius*, *R. aquaticus*

Conflict of interest. The authors declare that they have no obvious and potential conflicts of interest related to the publication of this article.

Contribution of the authors. Andrey M. Poluyanov and Natalia V. Bobkova invented and designed the experiment. Andrey M. Poluyanov and Anna Yu. Sokolova analyze on HPLC-UV. Andrey M. Poluyanov, Anna Yu. Sokolova, Evgeniya A. Malashenko and Ekaterina V. Sergunova participated in data processing. Andrey M. Poluyanov, Evgeniya A. Malashenko and Natalia V. Bobkova participated in writing the text of the article. All authors participated in discussion of the results

For citation: Poluyanov A. M., Sokolova A. Yu., Malashenko E. A., Sergunova E. V., Bobkova N. V. Isolation, identification and quantitative determination of anthracene derivatives by HPLC-UV method in the raw materials of some representatives of the genus *Rumex* of three vegetation times. *Drug development & registration*. 2022;11(4):216–225. (In Russ.) https://doi.org/10.33380/2305-2066-2022-11-4-216-225

Выделение, идентификация и количественное определение антраценпроизводных методом ВЭЖХ-УФ в сырье некоторых представителей рода Щавель (*Rumex*) трех сроков вегетации

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Статья поступила: 06.10.2022 **Статья принята в печать:** 00.11.2022 **Статья опубликована:** 25.11.2022

Резюме

Введение. Создание новых высокоэффективных лекарственных препаратов требует тщательного изучения метаболома растительного сырья и проведения сравнительного фитохимического изучения подземных органов, повсеместно распространенных на территории России близкородственных видов Щавелей, таких как: Щ. курчавый, Щ. туполистный и Щ. водный. Отмечено, что они обладают сходным с официнальным щавелем конским метаболомом, что в свою очередь подтверждает потенциал изучения данных видов. Научный и практический интерес представляет изучение динамики накопления ведущей группы биологически активных веществ – антраценпроизводных в зависимости от фенологических фаз развития растения.

Цель. Выделение, идентификация и количественное определение антреценпроизводных в подземных органах Щ. конского, Щ. курчавого, Щ. туполистного и Щ. водного, заготовленных в три различные вегетации.

Материалы и методы. В качестве анализируемых растворов использовались извлечения из подземных органов изучаемых растений полученные по методике из фармакопейной статьи на щавель конский. Растворы анализировали на хроматографе Nexera-i LC-2040 (Shimadzu Corporation, Япония), оснащенном термостатом колонок и образцов, дегазатором и автосамплером по индивидуально подобранному градиенту элюирования подвижной фазы (0,1%-й раствор ортофосфорной кислоты/ацетонитрил). Обработку первичных данных проводили при помощи программного обеспечения LabSolutions Single LC (Shimadzu Corporation, Япония). Соединения из группы антраценпроизводных идентифицировали по временам удерживания. Детектирование проводилось с помощью УФ-детектора с динамическим изменением длины волны поглощения в ходе анализа от 365 ± 2 нм до 254 ± 2 нм.

Результаты и обсуждение. Были получены спирто-водные извлечения из подземных органов Щавелей. Подобран градиент элюирования для одновременного определения 5-ти антраценпроизводных со временем единичного анализа 40 минут. Данные хроматографические условия позволили идентифицировать и определить количественное содержание эмодина, 8-O-β-D-глюкозида эмодина и хризофановой кислоты в подземных органах Щ. конского, Щ. водного, Щ. курчавого и Щ. туполистного трёх различных вегетаций. Гликозиды антраценпроизводных: глюкофрангулин А и франгулин А в изучаемых объектах обнаружены не были.

Заключение. Из подземных органов Щ. конского, Щ. водного, Щ. курчавого и Щ. туполистного были выделены антраценпроизводные, разработана методика количественного определения антраценпроизводных в спирто-водных извлечениях, обнаружены и количественно определены эмодин, 8-O-β-D-глюкозид эмодина и хризофановая кислота.

Ключевые слова: антраценпроизводные, эмодин, ВЭЖХ-УФ, корни, *R. confertus*, *R. crispus*, *R. obtusifolius*, *R. aquaticus*

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Вклад авторов. А. М. Полуянов и Н. В. Бобкова придумали и разработали эксперимент. А. М. Полуянов и А. Ю. Соколова провели исследования методом ВЭЖХ-УФ. А. М. Полуянов, А. Ю. Соколова, Е. А. Малашенко и Е. В. Сергунова участвовали в обработке данных. А. М. Полуянов, Е. А. Малашенко и Н. В. Бобкова участвовали в написании текста статьи. Все авторы участвовали в обсуждении результатов.

Для цитирования: Полуянов А. М., Соколова А. Ю., Малашенко Е. А., Сергунова Е. В., Бобкова Н. В. Выделение, идентификация и количественное определение антраценпроизводных методом ВЭЖХ-УФ в сырье некоторых представителей рода Щавель (*Rumex*) трех сроков вегетации. *Разработка и регистрация лекарственных средств*. 2022;11(4):216–225. https://doi.org/10.33380/2305-2066-2022-11-4-216-225

INTRODUCTION

The main goal of modern pharmacognosy is to search for new sources of biologically active substances (BAS) in order to expand the raw material base, which, in turn, will allow producing highly effective domestic drugs.

Rumex species widely used in our country, have a number of various groups of BAS: anthracene derivatives (AD), tannins, phytoalexins, flavonoids, organic acids, saponins, amino acids and polysaccharides [1, 2], which potentially causes a wide range of pharmacological action [3].

Currently, only one Rumex species is allowed for medical use in the Russian Federation: *Rumex* (hereinafter R), *Rumex confertus* Willd., its chemical composition has been quite known [4]. The pharmacopoeial monograph for *Rumex confertus* Willd. in raw materials – underground organs showed a method for AD quantification equivalent to 8-O-β-D-glucoside emodin by spectrophotometry (SPM).

Of particular interest is the comparative phytochemical study of the underground organs of closely related Rumex species, such as: *Rumex confertus* Willd., *Ru-*

mex obtusifolius and *Rumex aquaticus*. It has been found that they have a similar metabolome, which, in turn, confirms the potential for investigation of these species [5, 6].

The literature sources report that the metabolome of the tested Rumex species contain such substances of glicoside structure from the AP group as: chrysophanol-1-O-β-D-glucoside, 8-O-β-D-glucoside emodin, frangulin A, glucofrangulin A [7, 8]. Aglycones for these substances are chrysophanoic acid and emodin. The articles are predominantly devoted to the quantitative analysis of BAS in flowers, fruits and leaves. Information on the quantification of anthracene derivatives by modern instrumental methods in the underground organs of the tested species is not available, with the exception of Article [9], which indicates the total content of AP in methanol extracts from underground organs in *Rumex confertus* Willd. 163.2 mg/g, *Rumex crispus* 25.22 mg/g and *Rumex obtusifolius* 14.71 mg/g, where as the method for quantification, method of high-performance liquid chromatography with ultraviolet detection (HPLC-UV) has been selected. This method is also often used in the analysis of other morphological groups [10, 11].

Of scientific and practical interest is the investigation of the dynamics of the accumulation of biologically active substances depending on the phenological phases of plant development. According to the regulatory documentation for underground organs of medicinal plants (General pharmacopeia monograph 1.5.1.0006.15 "Roots, rhizomes, bulbs, tubers, corms"), medicinal plant raw materials are harvested in the phase of wilting of the above-ground part. The contents AP aglycones depend on the life cycle of the plant and the vegetation phase [12].

The purpose of this study is to isolate, identify and quantify AP in the underground organs of Rumex confertus Willd, Rumex Crispus, Rumex Obtusifolius, Rumex Aqueticus harvested in various phases of vegetation.

MATERIALS AND METHODS

Reagents and solutions

During the study, the following reagents were used: acetonitrile (class "for UHPLC", PanReac, Spain); formic acid (class "for analysis", PanReac, Spain); ethanol 95 % (class "chemically pure", TH "HIMMED", LLC, Russia); demineralized water (purity class I). For the preparation of reference samples, the following substances were used: emodin, a powder substance, an emodin content of 98 % (HPLC, Sigma-Aldrich, USA, batch 043K35051, valid until 06.2028); chrysophanoic acid, powder substance, chri-zophanic acid content 98.3 % (Supelco Inc., USA, bcCG0998 series, valid until 05.2024); 8-O-β-D-glucoside emodin, substance powder, content of emodin glucoside 92.04 % (PhytoLab GmbH & Co. KG, Germany, batch 120413929, valid until 09.2026); glucofrangulin A, substance powder, glucofrangulin A content of 98.72 % (PhytoLab GmbH & Co. KG, Germany, batch 111810966, valid until 08.2024); franulin A, powder substance, content of franguline A of 98.87 % (PhytoLab GmbH & Co. KG, Germany, batch 114031178, valid until 12.2024).

Stock reference solutions were prepared by dissolving the exact weigh of the substance in ethanol 95 % "c.p" class. The working reference solutions were prepared by diluting the aliquot of the stock reference standard solution with the same solvent.

Equipment

Moisture of crushed underground organs was performed on the moisture analyzer OHAUS MB27 (OHAUS, USA). Chromatographic separation and detection were carried out on a high-performance liquid chromatograph Nexerai LC -2040 (Shimadzu Corporation, Japan), equipped with a column thermostat and samples, a degasser, an autosampler and an ultraviolet detector. Raw data was processed using LabSolutions Single LC software (Shimadzu Corporation, Japan).

Chromatographic separation and detection conditions

Chromatographic column: Grace HPLC Column Platinum C8-EPS, 250 × 4.6 mm, 5mm (Grace, USA).

Precolumn: Phenomenex SecurityGuardTM Cartridges Widepore C18, 4 × 3.0 mm.

Thermostat temperature: 27 °C.

Mobile phase: 0.1 % solution of formic acid water (by volume) (eluent A); 0.1 % solution of formic acid in acetonitrile (by volume) (eluent B).

Flow rate of the mobile phase: 1.0 ml/min.

Gradient of the mobile phase composition phase is shown in figure 1.

Injection volume: 10 µl.

Retention time of 8-O-β-D-glucoside emodin: about 16.6 min.

Retention time of Emodin: about 28.5 min.

Retention time of chrysophanoic acid: about 29.5 min.

Chromatogram recording time: 0.0–40.0 min.

Detection: A UV detector with a dynamic change in the absorption wavelength during the analysis from 365 ± 2 nm to 254 ± 2 nm, shown in figure 1.

Study objects

For this study, underground organs of the following species were collected: *Rumex confertus* Willd., *Rumex aquaticus* L., *Rumex crispus* L., *Rumex obtusifolius* L. harvested during spring regrowth (April-May

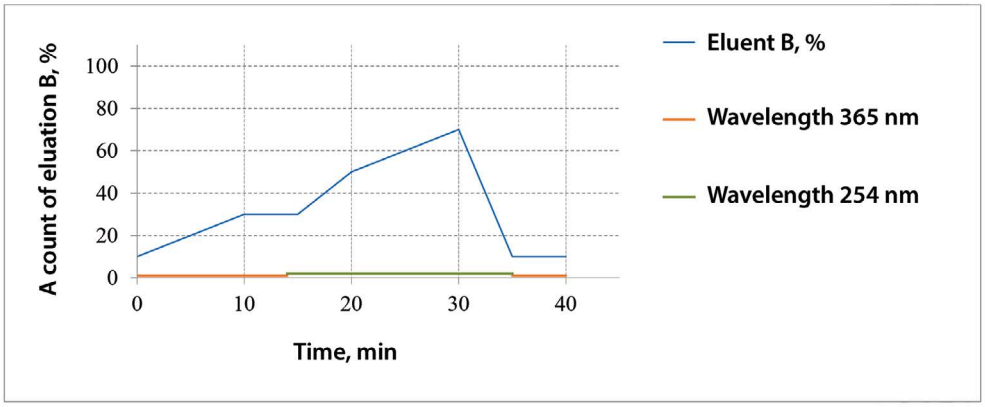


Figure 1. **Elution's gradient**

2022) – "regrowth", during the flowering period (June-July 2021) – flowering, during the wilting of the above-ground part (October 2021) – "wilting". Table 1 shows the study objects and the place and date of their harvesting.

The collected underground organs were washed from the ground with running cold water. Before drying, the most massive underground organs were divided lengthwise into two parts. They were dried at room temperature in a well-ventilated room, out of sunlight. The moisture content of the dried raw materials was determined using a moisture meter and did not exceed 13 %.

Sample preparation

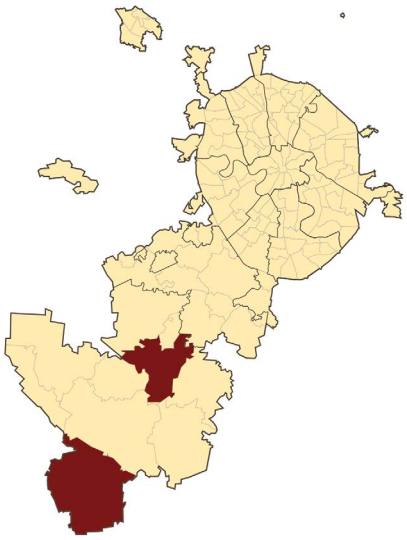
From the raw materials, alcohol extracts were obtained according to the method of quantification from the pharmacopoeial monograph for the raw materials of *Rumex confertus* Willd. roots (GPM 2.5.0052.15).

RESULTS AND DISCUSSION

Method development

To identify and quantify BAS, the HPLC-UV method was chosen. This method is more selective and sensitive than SPM and allows to detect and quantify compounds of the BAS group under consideration.

Table 1. **Investigated objects**

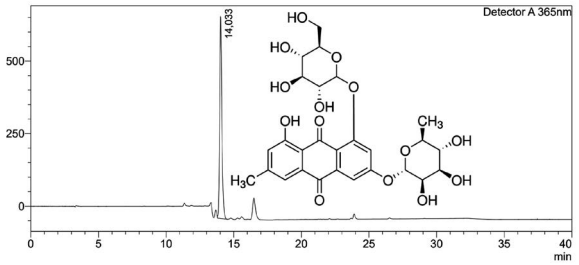
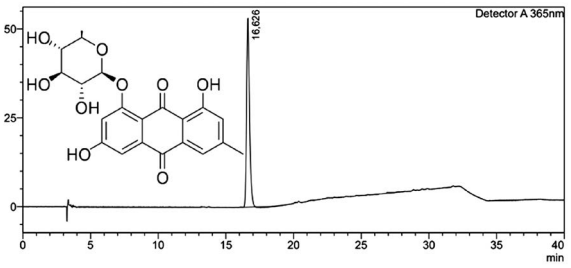
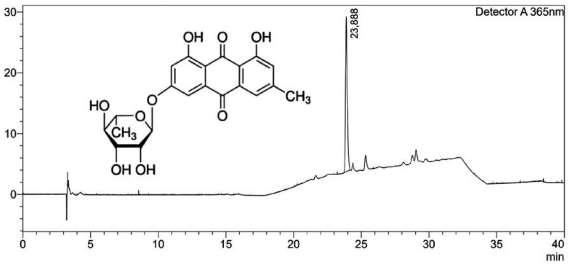
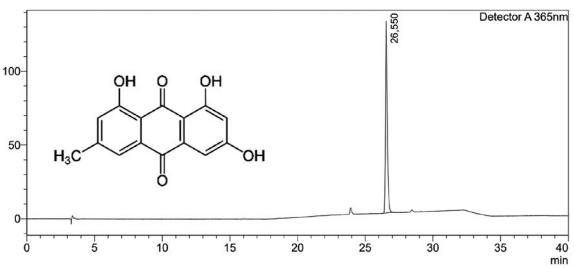
Object	Location of growth	Vegetation phase, harvesting period	
Rumex confertus Willd.	Rogovskoye, Moscow, Russia Coordinates: 55.245626, 37.009576	Regrowth, April 2022	
		flowering, June 2021	
		dieback, October 2021	
Rumex crispus L.	Krasnopakhorskoye settlement Moscow, Russia Coordinates: 55.384859, 37.225441	regrowth, April 2022	
		flowering, June 2021	
		dieback, October 2021	
Rumex obtusifolius L.	Rogovskoye settlement, Moscow, Russia Coordinates: 55.245626, 37.009576	regrowth, April 2022	
		flowering, June 2021	
		dieback, October 2021	
Rumex aquaticus L.	Rogovskoye settlement, Moscow, Russia Coordinates: 55.245626, 37.009576	regrowth, April 2022	
		flowering, July 2021	
		dieback, October 2021	

The conditions for chromatographic separation were selected experimentally based on the analysis in plant raw materials of emodin [13], chrysophaneic acid [14], frangulin A and glucofrangulin A [15], then modified for the study objects, due to a number of features: poor separation of components of different nature (glycosides and aglycones) within one method, close values of lipophilicity of most APs (log P from 3.5 to 2) and the presence of many other components contained in plant raw materials, which influenced analytes and distorted the shape of peaks. Frangulin A and glucofrangulin were analyzed the study objects by the HPLC-UV method for the first time.

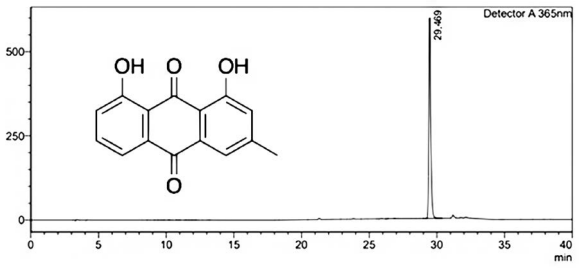
For the detection of analytes, an ultraviolet detector was used which records substances due to the presence of chromophore groups in their structure (table 2).

The presence of 8-O-β-D-glucoside emodine, chrysophanoic acid and emodin in all analyzed samples was confirmed. At the end of the analysis, the peak areas of two aglycones were measured: emodin and chryso-

Table 2. **Physical and chemical properties of the analyzed substances**

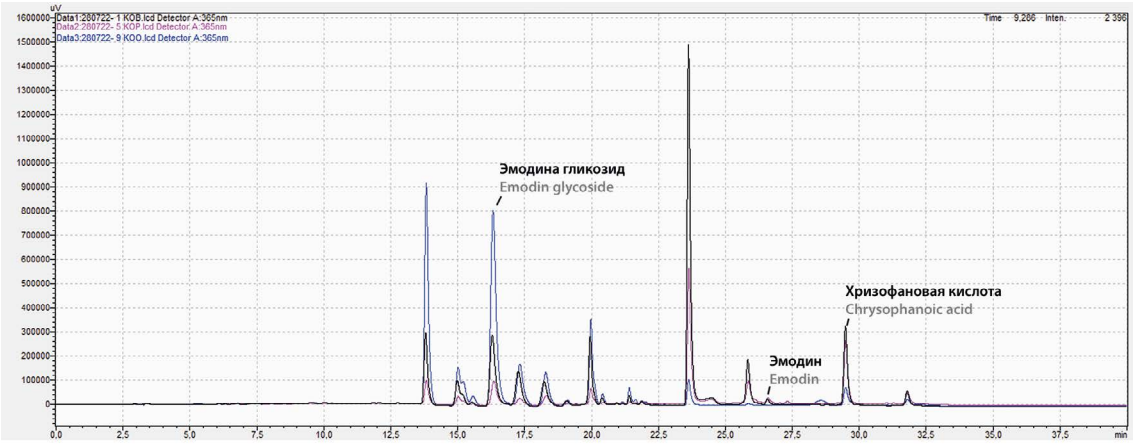
Compound	log P	log S	Retention time, min	Structural formula
Glucofrangulin A	–0,4	–2,2	14,03	
8-*O*-*β*-*D*-emodin glucoside	0,9	–2,3	16,63	
Frangulin A	1,4	–2,4	23,86	
Emodin	2,7	–3.09	26,55	

End of table 2

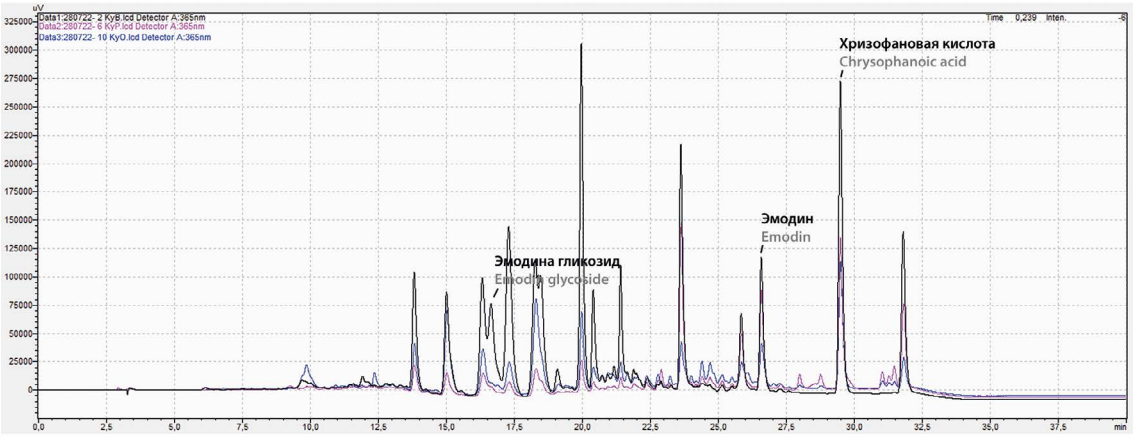
Compound	log P	log S	Retention time, min	Structural formula
Chrysophanic acid	3.1	–3.31	29,47	

phanoic acid, and also one glycoside: 8-O-β-D-glucoside emodin in solutions of the corresponding reference samples and in extracts of the study objects.

The analyzed objects did not contain any peak of the substance with the retention time corresponding to the one of reference sample for frangulin A and glucofrangulin, which suggests that these substances in the study objects were not detected. Figure 2 shows chromatograms of extracts from the underground organs of the study objects of various vegetations.

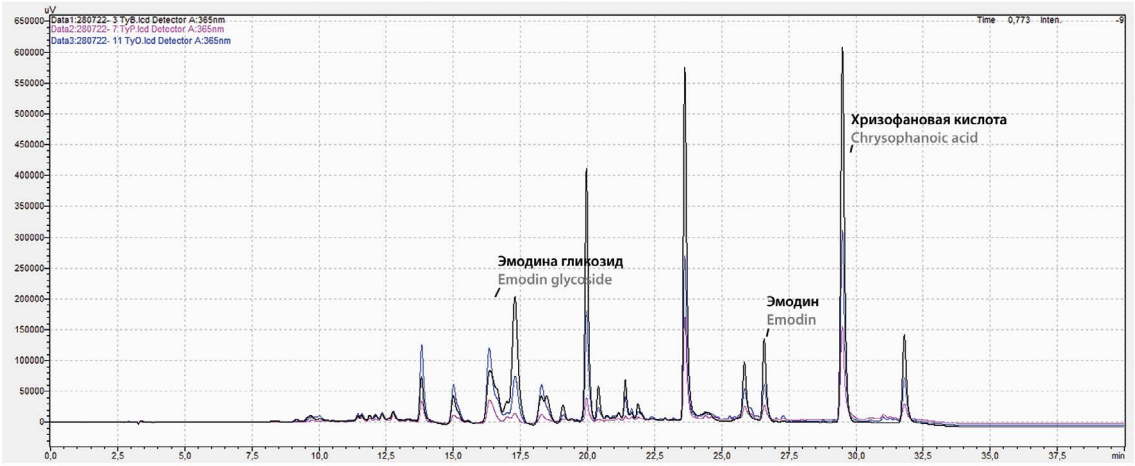


A

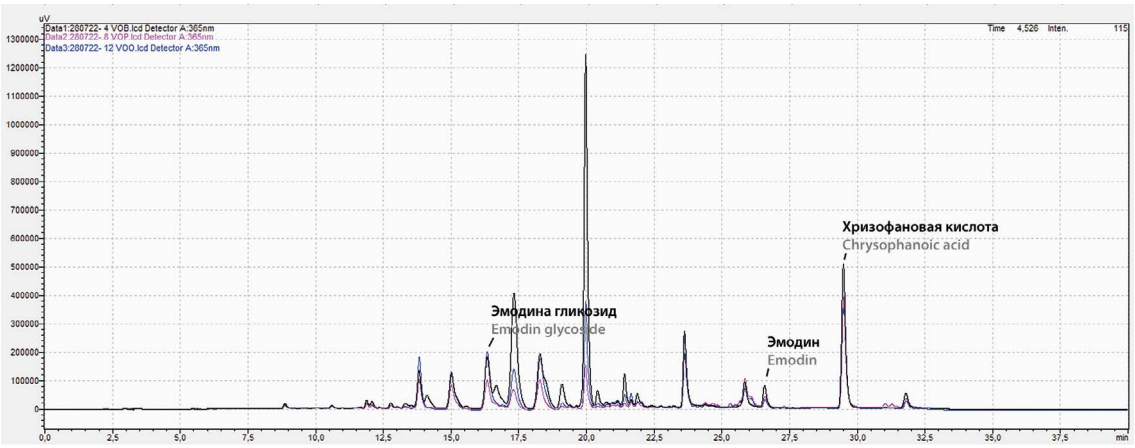


B

Figure 2. Chromatogramms of alcohol-water extracts.
The black line is the flowering phase; the pink line is the end of the growing season; the blue line is the beginning of the growing season



C



D

Continuation of Figure 2

For the developed method, the system suitability was checked, and values of the suitability parameters are determined (table 3).

The results of the statistical processing of the measurements show that the relative error of a single determination with the confidence level of 0.95 does not exceed ±3.48 % (table 4).

For all study objects, values of quantification of emodin, chrysofanic acid and 8-O-β-D-glucoside emodin equivalent to absolutely dry raw materials, in % and presented in table 5, are calculated.

The leading peak in the region of the chromatogram corresponding to the retention time of AP aglycones was often the peak of chrysophanoic acid. The highest content of chrysophanoic acid was found in *Rumex obtusifolius* during the flowering period. It should be also noted that the highest content of chrysophanic acid during the flowering period in all 4 species.

Table 3. Chromatographic system suitability parameters.

Compound	Retention time, min	Resolution	Number theoretical plates	Symmetry factor
8-O-β-D-glucoside emodin	16,62	–	33609	1,29
Emodin	26,59	34,49	199106	1,06
Chrysophanic acid	29,50	11,93	224100	1,14

The highest content of emodin was found in *Rumex obtusifolius* during flowering, the lowest in *Rumex confertus* Willd. during regrowth.

Table 4. Metrological characteristics of the procedure for the quantitative determination of 8-O-β-D-glucoside of emodin, emodin and chrysophanol in alcohol-water extracts during flowering ($n = 5$, $f = 4$, $P = 95$ %, $T(f, P) = 2,7764$)

Specie	Compound	$\bar{X}$, %	S_x^2	S_x	$S_{\bar{x}}$	ΔX	E, %
R. confertus	8-O-β-D-glucoside emodin	1,327	$1,48 \times 10^{-4}$	$1,22 \times 10^{-2}$	$5,45 \times 10^{-3}$	$1,51 \times 10^{-3}$	1,14
	Emodin	0,029	$6,44 \times 10^{-7}$	$8,02 \times 10^{-4}$	$3,59 \times 10^{-4}$	$9,96 \times 10^{-4}$	3,48
	Chrysophanic acid	0,321	$2,34 \times 10^{-5}$	$4,83 \times 10^{-3}$	$2,16 \times 10^{-3}$	$6,00 \times 10^{-3}$	1,87
R. crispus	8-O-β-D-glucoside emodin	0,451	$1,73 \times 10^{-6}$	$1,32 \times 10^{-3}$	$5,88 \times 10^{-4}$	$1,63 \times 10^{-3}$	0,36
	Emodin	0,161	$1,22 \times 10^{-5}$	$3,50 \times 10^{-3}$	$1,56 \times 10^{-3}$	$4,34 \times 10^{-3}$	2,69
	Chrysophanic acid	0,281	$2,40 \times 10^{-6}$	$1,55 \times 10^{-3}$	$6,93 \times 10^{-4}$	$1,92 \times 10^{-3}$	0,69
R. obtusifolius	8-O-β-D-glucoside emodin	0,187	$1,02 \times 10^{-5}$	$3,20 \times 10^{-3}$	$1,43 \times 10^{-3}$	$3,97 \times 10^{-3}$	2,13
	Эмодин Emodin	0,180	$1,04 \times 10^{-6}$	$1,02 \times 10^{-3}$	$4,56 \times 10^{-4}$	$1,27 \times 10^{-3}$	0,71
	Chrysophanic acid	0,622	$4,88 \times 10^{-5}$	$6,99 \times 10^{-3}$	$3,12 \times 10^{-3}$	$8,67 \times 10^{-3}$	1,40
R. aquaticus	8-O-β-D-glucoside emodin	0,792	$3,56 \times 10^{-5}$	$5,97 \times 10^{-3}$	$2,67 \times 10^{-3}$	$7,41 \times 10^{-3}$	0,94
	Emodin	0,102	$1,44 \times 10^{-6}$	$1,20 \times 10^{-3}$	$5,37 \times 10^{-4}$	$1,49 \times 10^{-3}$	1,47
	Chrysophanic acid	0,531	$8,24 \times 10^{-6}$	$2,87 \times 10^{-3}$	$1,28 \times 10^{-3}$	$3,56 \times 10^{-3}$	0,67

Note. $\bar{X}$, % – mean value; S_x^2 – variance; S_x – standard deviation; $S_{\bar{x}}$ – standard deviation of the mean result; ΔX – confidence interval; E, % – relative error of determination.

Table 5. The quantitative content of emodin, chrysophanoic acid and emodin 8-O-β-D-glucoside

Vegetation phase	The content of emodin, in %			
	R. confertus	***R. crispus***	***R. obtusifolius***	***R. aquaticus***
Spring regrowth	0,002 ± 0,001 %	0,054 ± 0,002 %	0,080 ± 0,002 %	0,040 ± 0,001 %
Flowering	0,029 ± 0,001 %	0,161 ± 0,004 %	0,180 ± 0,001 %	0,102 ± 0,001 %
Overhead part dieback	0,028 ± 0,001 %	0,119 ± 0,001 %	0,033 ± 0,001 %	0,043 ± 0,001 %
The content of chrysophanic acid, in %				
Spring regrowth	0,097 ± 0,002	0,119 ± 0,003	0,334 ± 0,002	0,403 ± 0,010
Flowering	0,321 ± 0,005	0,281 ± 0,002	0,622 ± 0,007	0,531 ± 0,003
Overhead part dieback	0,162 ± 0,001	0,087 ± 0,001	0,100 ± 0,001	0,236 ± 0,001
The content of 8-o-β-d-glucoside emodin, in %				
Spring regrowth	3,732 ± 0,022	0,169 ± 0,001	0,670 ± 0,016	0,907 ± 0,003
Flowering	1,327 ± 0,010	0,451 ± 0,001	0,187 ± 0,003	0,792 ± 0,006
Overhead part dieback	0,453 ± 0,002	0,063 ± 0,001	0,239 ± 0,003	0,498 ± 0,002

Note. The percentage value is indicated as the arithmetic mean ± standard deviation.

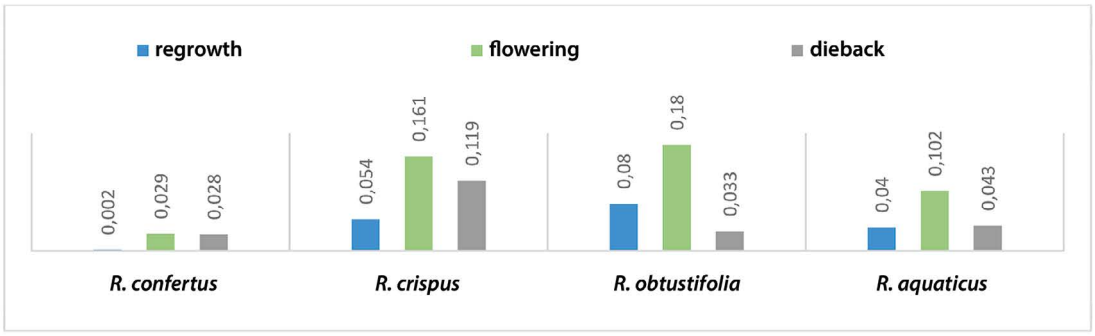
8-O-β-D-glucoside emodin was found in all objects, its quantitative content in % is shown in figure 3. The diagram shows the lack of correlation of the vegetation phase – the content of 8-O-β-D-glucoside emodin between different species.

For each vegetation, the total content of detected PAs in each species was calculated (table 6).

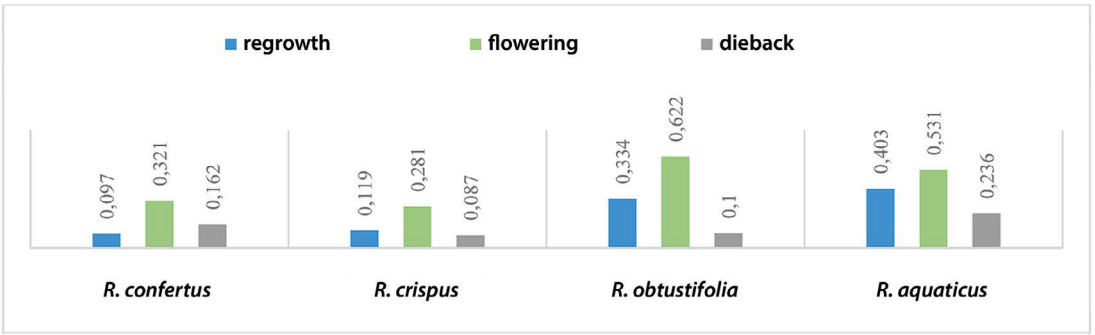
As the table shows, the highest content of the sum of AP is found in *Rumex confertus* L. in the phase of spring regrowth, the lowest – in *Rumen crispus* L. at the end of

Table 6. The content of the amount of certain AP, in %

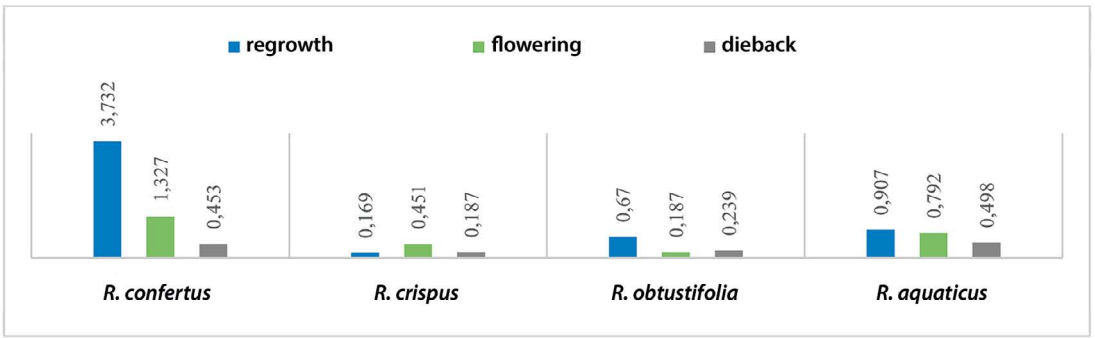
Vegetation phase	*R. confertus*, %	*R. crispus*, %	*R. obtusifolius*, %	*R. aquaticus*, %
Spring regrowth	3,901	0,342	1,084	1,350
Flowering	1,677	0,893	0,989	1,425
Overhead part dieback	0,643	0,269	0,372	0,777



A



B



C

Figure. 3. Diagram of the quantitative content of AP.
A – emodin; B – chrysophanoic acid; C – emodin 8-O-β-D-glucoside

vegetation. It is of interest that *Rumex crispus* L. differs from other study objects: it has a higher content of substances during the flowering period. All other species tend to reduce the content of AP from the phase of regrowth to the phase of withering.

This information gives grounds to believe that, in addition to *Rumex confertus* L., investigation of *Rumex aquaticus* L., *Rumex crispus* L., *Rumex obtusifolius* L., is rather promising based on the data on the content of chrysophaneic acid, emodin and its glycoside. There is a significantly higher content of each substance during the flowering period for most plants. The data obtained contradict the accepted standards of harvesting raw materials, since the official raw materials of the *Rumen confertus* L. roots of are harvested in autumn or spring.

CONCLUSION

During the study of *Rumex confertus* L., *Rumex aquaticus* L., *Rumex crispus* L., *Rumex obtusifolius* L. of three different vegetation periods, a method for isolation of anthracene derivatives according to GPM for *Rumen confertus* L. was tested, a method for quantification of APs in alcohol-water extracts was developed that allows to determine objectively and reliably the presence of the dominant group of BAS – anthracene derivates. Emodin, 8-O-β-D-glucoside emodin and chrysophanoic acid in all study objects were detected and quantified, the dynamics of their accumulation depending on the vegetation phase was analyzed.

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