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Identification and Quantitative Determination of Flavonoids by HPLC-UV Method in the Raw Materials of Some Representatives of the Genus *Rumex* of Three Vegetation Time

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Abstract

Introduction. The study of the dynamics of accumulation of biologically active substances (hereinafter – BAS) in relation to the phenological phases of plant development is of great scientific and practical interest. The quantitative content of aglycones and glycosides of flavonoids depends on the life cycle of the plant and its vegetation phase.

Aim. To identify and quantify flavonoids in the underground organs of *Rumex confertus* Willd., *Rumex aquaticus* L., *Rumex crispus* L. and *Rumex obtusifolius* L. harvested during 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 analyzed. Chromatographic separation and detection were performed on a Nexera-i LC-2040 high-performance liquid chromatograph (Shimadzu Corporation, Japan). The chromatograph was equipped with a column thermostat, chromatography column Grace HPLC-COLUMN 250 × 4.6mm platinum C8-EPS 5 mm (Grace, США) and Guard column Phenomenex SecurityGuard™ Cartridges Widepore C18 4 × 3,0 mm, a degasser, an autosampler (injection volume: 10 µl), and an ultraviolet detector. Detection was carried out at a wavelength λ = 365 ± 2 nm. Mobile phase contains 0.1 % phosphoric acid in water (v/v) (eluent A); acetonitrile (eluent B) with flow rate: 0.9 ml/min.

Results and discussion. All studied objects were analyzed. The authenticity of the substances contained were confirmed using the external standard, and their quantitative content was determined. The discovered and quantified substances were: 3-O-rutinoside of quercetin (rutin), 3-O-rutinoside of isorhamnetin (narcissin), 3-O-glucoside of kaempferol (astragalin), luteolin, kaempferol and isorhamnetin. 7-O-glucoside of luteolin (cynaroside) and 7-O-beta-D-glucoside apigenin (cosmosiin) were not found. The aglycone luteolin had the biggest share in the total quantitative content of flavonoids. It is noted that this aglycone is contained in larger quantities in relation to other flavonoids in all studied objects, regardless of the phase of vegetation.

Conclusion. In the process of the research, a method for the quantitative determination of flavonoids in alcohol-water extracts was developed. Aglycones and glycosides of flavonoids were identified and quantified in the underground organs of *R. confertus* Willd., *R. aquaticus* L., *R. crispus* L. and *R. obtusifolius* L. of three different vegetations.

Keywords: flavonoids, HPLC-UV, underground organs, *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, Anna Yu. Sokolova and Anna-Daniela Koynova analyzed the objects by HPLC-UV. Andrey M. Poluyanov, Evgeniya A. Malashenko and Sofia D. Kulikova 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.

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Идентификация и количественное определение флавоноидов методом ВЭЖХ-УФ в сырье некоторых представителей рода Щавель (*Rumex*) трех сроков вегетации

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Резюме

Введение. Изучение динамики накопления биологически активных веществ (далее – БАВ) применительно к фенологическим фазам развития растений представляет большой научный и практический интерес. Количественное содержание агликонов и гликозидов флавоноидов зависит от жизненного цикла растения и фазы его вегетации.

Цель. Идентификация и количественное определение флавоноидов в подземных органах *Rumex confertus* Willd., *Rumex aquaticus* L., *Rumex crispus* L. и *Rumex obtusifolius* L., собранных в разные фазы вегетации.

Материалы и методы. Извлечения из подземных органов изучаемых растений были получены по методике из фармакопейной статьи на *R. confertus*. Хроматографическое разделение и детектирование осуществляли на высокоэффективном жидкостном хроматографе Nexera-i LC-2040 (Shimadzu Corporation, Япония). Хроматограф оснащён колоночным термостатом, хроматографической колонкой Grace HPLC-COLUMN 250 × 4,6 мм платина C8-EPS 5 мм (Grace, США) и колонкой Guard Phenomenex SecurityGuard™ Cartridges Widepore C18 4 × 3,0 мм, дегазатором, автосамплером (объем ввода: 10 мкл) и ультрафиолетовым детектором. Детектирование проводили при длине волны λ = 365 ± 2 нм. Подвижной фазой была смесь 0,1%-й фосфорной кислоты в воде (об./об.) (элюент А); ацетонитрил (элюент В), скорость потока: 0,9 мл/мин.

Результаты и обсуждение. Все исследуемые объекты были проанализированы, с помощью внешнего стандарта была подтверждена подлинность содержащихся веществ и рассчитано их количественное содержание. Для веществ, которые были идентифицированы – проведена количественная оценка, среди них: 3-О-рутинозид кверцитина (рутин), 3-О-рутинозид изорамнитина (нарциссин), 3-О-гликозид кемпферола (астрагалин), лютеолин, кемпферол и изорамнитин. 7-О-гликозид лютеолина (цинарозид) и 7-О-β-D-гликозид апигенина (космосиин) не обнаружены. Наибольший вклад в общее содержание флавоноидов вносит агликон – лютеолин. Отмечено, что этот агликон содержится в большем количестве по сравнению с другими флавоноидам во всех исследованных объектах, независимо от фазы вегетации.

Заключение. В процессе исследования разработана методика количественного определения флавоноидов в водно-спиртовых экстрактах. В подземных органах Щ. конского, Щ. водного, Щ. курчавого и Щ. туполистного трёх различных вегетаций были идентифицированы и количественно определены агликоны и гликозиды флавоноидов.

Ключевые слова: флавоноиды, ВЭЖХ-УФ, подземные органы, *R. confertus*, *R. crispus*, *R. obtusifolius*, *R. aquaticus*

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INTRODUCTION

The discovery of new medicinal plant raw materials sources becomes an important goal of scientific society. To incorporate a new plant species as a medicinal herb source, it is necessary to identify and quantify the substances contained in it using the cutting-edge technology.

Representatives of the *Rumex* genus of the *Polygonaceae* family, which are widespread throughout the world, contain a few biologically active substances (hereinafter- BAS): quinones, flavonoids, tannins, terpenes, terpene alkaloids, lignans, carotenoids, ascorbic acid, and anthracene derivatives [1]. According to meta-studies, raw materials from representatives of the genus *Rumex* (hereinafter – *R.*) can be used for: mild forms of diabetes, constipation, various infections, diarrhea, jaundice; as an antihypertensive agent and hepatoprotector [2].

Polyphenols are an important group of BAS, contained in the raw materials of representatives of the *Rumex* genus, famous for their antioxidant activity and the ability to form chelate complexes with metals [3]. The group of polyphenols includes, among others, such

substances as flavonoids and isoflavonoids, which therapeutic potential is being widely discussed in the context of their antioxidant activity, anti-aging properties, anti-inflammatory activity, immunomodulatory activity and cardioprotective effects [4]. The content of flavonoids is usually studied in such species of the *Rumes* genus, as: *R. acetosa, R. hastatus, R. aquaticus, R. chalepensis, R. crispus, R. dentatus, R. gmelini, R. japonicus, R. vesicarius, R. luminiastrum, R. patientia, R. pictus, R. rechingerianus, R. acetosaella, R. confertus* [5].

In Russia, the only representative of the genus *Rumex – R. confertus –* has been described as a medicinal plant raw material-a source of anthracene derivatives; the raw material being used are the roots. The information about the total flavonoids content (TFC) in the underground organs of *R. confertus* is scarce, however there is some data about the TFC in leaves of this species: 3.8 ± 0.2 % [6].

The study underground organs of some *Rumex* species, widespread in Russia, is of great practical and scientific interest, the species being: *R. crispus, R. obtusifolius, R. aquaticus*. The TFC in the leaves of these species is presented in the Table 1.

Table 1. TFC in the leaves of studied R.species, according to the literature

Rumex species	TFC, % [7]
R. crispus	9.2 ± 0.5
R. obtusifolius	9.2 ± 0.4
R. aquaticus	No data available

According to the literature, there is a discrepancy between the period of maximum content of various groups of BAS in underground organs and the generally accepted time of their harvesting [7, 8]. In this regard, it is especially important to conduct a comparative analysis of the dynamics of the accumulation of flavonoids in Rumex species, depending on their stage of vegetation.

Therefore, the purpose of this study is to identify and quantify flavonoids in the underground organs of *R. confertus Willd, R. aquaticus L., R. crispus L.* and *R. obtusifolius L.* harvested during different phases of vegetation.

MATERIALS AND METHODS

Materials

The following reagents were used during the research: acetonitrile, "for UHPLC" grade (PanReac, Spain); phosphoric acid, "for HPLC" grade (Scharlau, Spain); ethanol 95 % chemically pure grade (LLC TD "HIMMED", Russia); demineralized water, purity class I. Stock standard solutions were prepared by dissolving an exact sample weight of the substance in 95 % pure ethanol.

Equipment

The determination of the moisture content of the crushed underground organs was carried out on an Ohaus MB27 moisture content analyzer (Ohaus, USA).

Objects of study

The underground organs of *Rumex confertus* Willd., *Rumex aquaticus L., Rumex crispus L.* and *Rumex obtusifolius L.* were collected for this study. They were harvested during the spring regrowth period (April-May 2022) – (hereinafter – "regrowth"), during the flowering period (June-July 2021) – (hereinafter – "flowering"), during the withering period of the aerial part (October 2021) – (hereinafter – "overhead part dieback"). All the objects' data are presented in Table 2.

The harvested underground organs were washed with cold water. Before drying, the more massive underground organs were divided lengthwise into two parts. Drying was carried out at room temperature in a well-ventilated area, with no access to sunlight. The moisture content of the dried raw materials was determined using a moisture meter and did not exceed 13 %. Water-alcohol extracts were obtained from raw materials with a ratio of 1 g sample of medicinal plant materials in 50 ml of extractant.

Chromatographic separation and detection were performed on a Nexera-i LC-2040 high-performance liquid chromatograph (Shimadzu Corporation, Japan) equipped with a column thermostat, chromatography column Grace HPLC-COLUMN 250 × 4.6 mm platinum C8-EPS 5 mm (Grace, США) and Guard column Phenomenex SecurityGuard™ Cartridges Widepore C18 4 × 3,0 mm, a degasser, an autosampler (injection volume: 10 µl), and an ultraviolet detector. Detection was carried out at a wavelength λ = 365 ± 2 nm. Mobile phase contains 0.1 % phosphoric acid in water (v/v) (eluent A); acetonitrile (eluent B) with flow rate: 0.9 ml/min. The composition gradient of the mobile phase is shown in Figure 1.

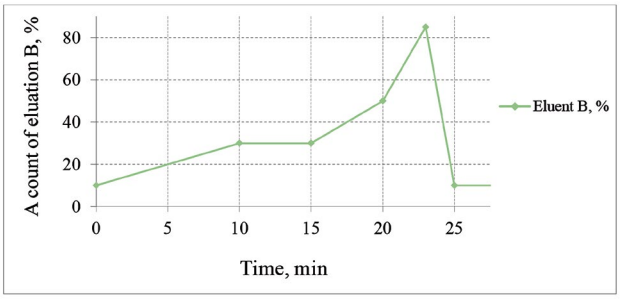


Figure 1. Gradient elution scheme

The primary data were processed using LabSolutions Single LC software (Shimadzu Corporation, Japan).

RESULTS AND DISCUSSION

Method development

To identify and determine the quantitative content of the BAS, the HPLC-UV method was chosen. The conditions for chromatographic separation were adjusted

Table 2. **Objects of research.**

Object of research	Photo of underground organs	Vegetative phase, harvesting period	Harvesting place
Rumex confertus Willd.		regrowth, April 2022	Rogovskoye, Moscow, Russia Coordinates: 55.245626, 37.009576
		flowering, June 2021	
		dieback, October 2021	
Rumex crispus L.		regrowth, April 2022	Krasnopakhorskoye settlement Moscow, Russia Coordinates: 55.384859, 37.225441
		flowering, June 2021	
		dieback, October 2021	
Rumex obtusifolius L.		regrowth, April 2022	Rogovskoye settlement, Moscow, Russia Coordinates: 55.245626, 37.009576
		flowering, June 2021	
		dieback, October 2021	
Rumex aquaticus L.		regrowth, April 2022	Rogovskoye settlement, Moscow, Russia Coordinates: 55.245626, 37.009576
		flowering, July 2021	
		dieback, October 2021	

experimentally, based on previous studies and publications [9]. The flow rate and gradient scheme were changed as well as the usage of phosphoric acid as a modifier was favored over the formic acid, Figure 2 shows chromatogramms of standard solutions of substances used in the experiment, Figure 3 shows chromatogramms of extracts from underground organs of the studied objects of various phases of vegetation.

For all the studied objects, the values of the quantitative content of flavonoids in terms of absolutely dry raw materials, in%, were calculated and presented in Table 3.

Cynaroside and cosmosiin were not found in any of the objects. The Table 4 compares the content of some flavonoids in different species.

The aglycone luteolin has the biggest share in the total quantitative content of flavonoids. It is noted that this aglycone is contained in larger quantities in relation to other flavonoids in all studied objects, regardless of the vegetation phase. Meanwhile, the luteolin glycoside was not found in any of the objects.

The total content of detected flavonoids in each species was calculated for each vegetation phase in Table 5 and shown in Figure 4.

The total value allows us to conclude that the content of substances from the group of flavonoids is quite low. For three representatives of the *R.* genus, the highest content of flavonoids was noted in the flowering phase, the exception being R. *confertus*. The content of the studied flavonoids in *R. aquaticus* in the flowering phase is twice as high as in the other studied species.

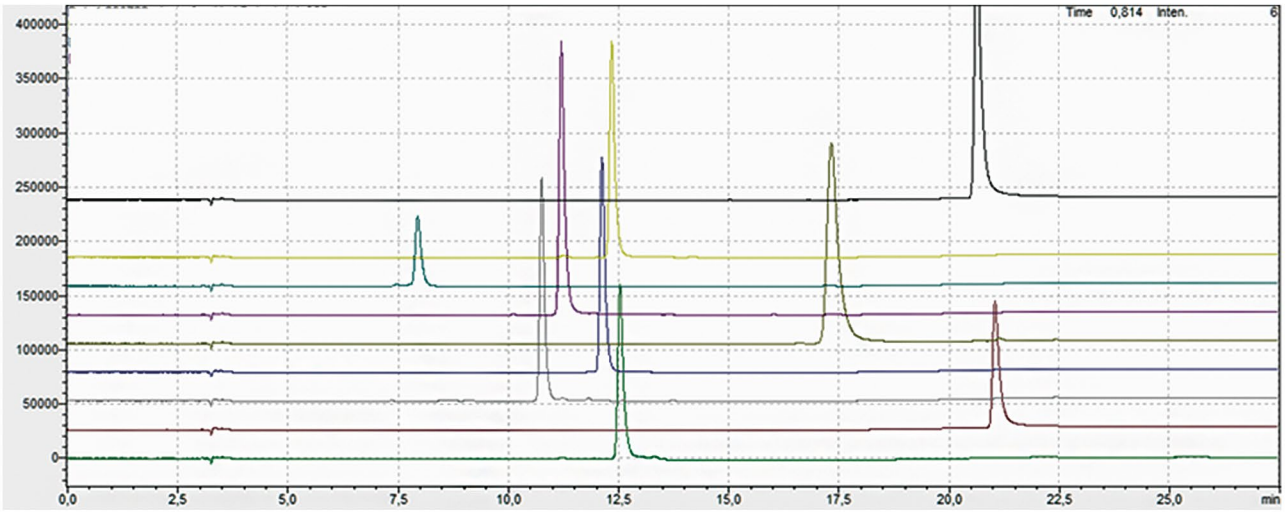
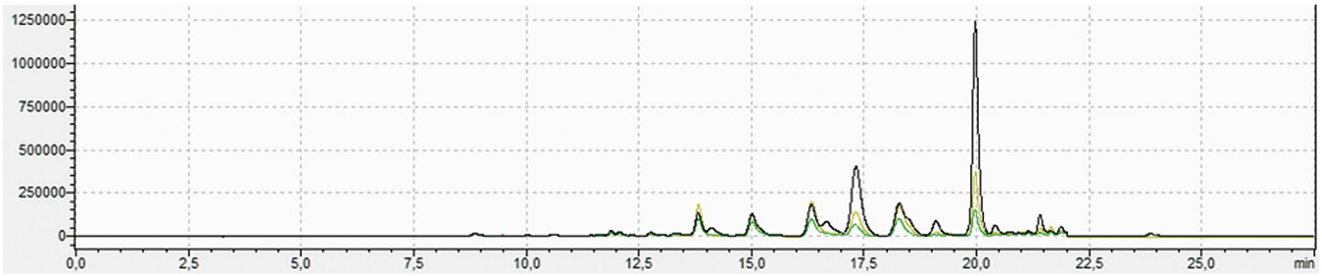


Figure 2. **Chromatogramms of standard solutions of substances**

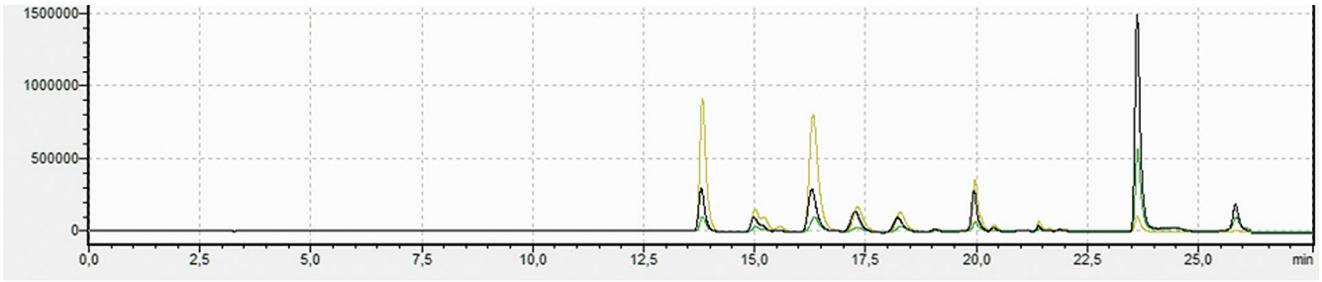
Table 3. **Content of the amount of certain flavonoids, in %**

Vegetation phase	The content of the amount of certain flavonoids, in %			
	R. confertus	***R. crispus***	***R. obtusifolius***	***R. aquaticus***
The content of narcissin, in %				
Regrowth	–	0.003 ±0.001	0.009 ±0.001	0.025 ±0.001
Flowering	0.008 ± 0.001	0.011 ±0.002	0.016 ±0.002	0.054 ±0.003
Dieback	0.002 ± 0.001	0.002 ±0.001	0.006 ±0.001	0.028 ±0.001
The content of rutin, in %				
Regrowth	–	0.004 ± 0.001	–	0.029 ± 0.001
Flowering	–	0.003 ± 0.001	–	0.023 ± 0.001
Dieback	–	0.002 ± 0.001	–	0.026 ± 0.001
The content of luteolin, in %				
Regrowth	0.409 ± 0.023	0.056 ± 0.004	0.138 ± 0.009	0.298 ± 0.029
Flowering	0.323 ± 0.011	0.305 ± 0.030	0.383 ± 0.018	0.888 ± 0.034
Dieback	0.059 ± 0.003	0.022 ± 0.001	0.018 ± 0.003	0.138 ± 0.012
The content of isorhamnetin, in %				
Regrowth	0.019 ± 0.002	0.027 ± 0.003	0.017 ± 0.001	0.065 ± 0.001
Flowering	0.008 ± 0.001	0.026 ± 0.001	0.020 ± 0.001	0.025 ± 0.001
Dieback	0.005 ±0.001	0.004 ± 0.001	0.005 ± 0.001	0.011 ± 0.001
The content of astragalin, in %				
Regrowth	–	0.026 ± 0.001	0.023 ± 0.001	0.005 ± 0.001
Flowering	–	0.002 ± 0.001	0.017 ± 0.001	0.004 ± 0.001
Dieback	–	0.004 ± 0.001	0.019 ± 0.001	0.004 ± 0.001
The content of kaempferol, in %				
Regrowth	0.003 ± 0.001	0.005 ± 0.001	0.005 ± 0.001	0.010 ± 0.001
Flowering	0.002 ± 0.001	0.006 ± 0.001	0.007 ± 0.001	0.013 ± 0.001
Dieback	–	–	0.002 ± 0.001	0.005 ± 0.001

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



A



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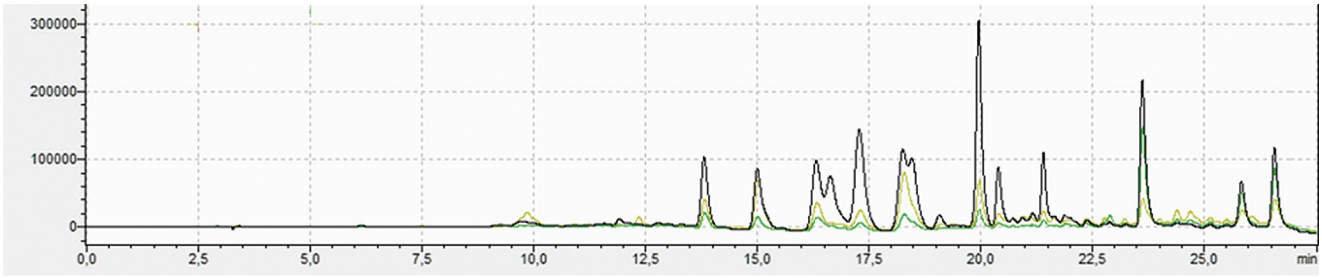

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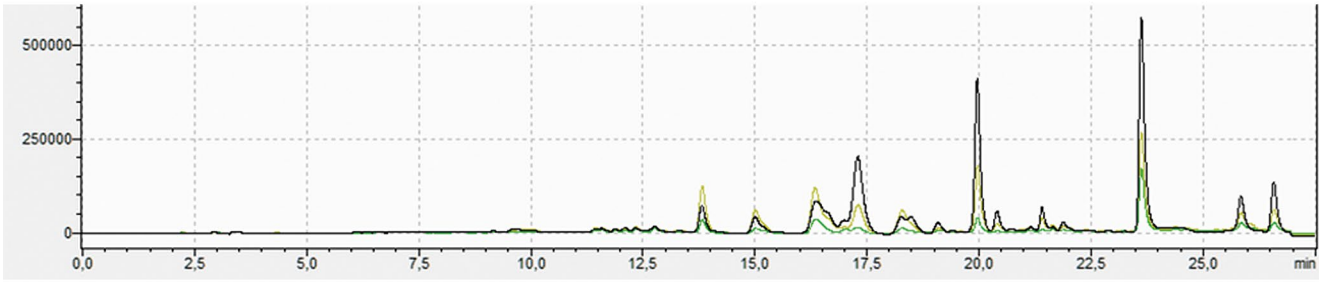


D

Figure 3. Chromatogramms of the extracts from the underground organs.

A – *Rumex aquaticus* L.; B – *Rumex confertus* Willd.; C – *Rumex crispus* L.; D – *Rumex obtusifolius* L. The black line is the flowering phase, the green line is the end of the growing season, the yellow line is the beginning of the growing season

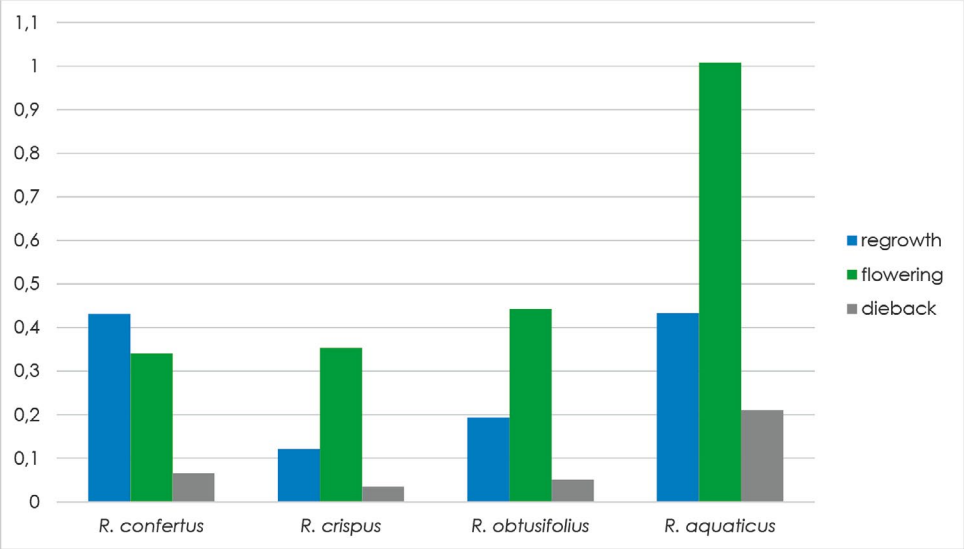
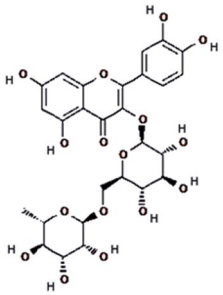
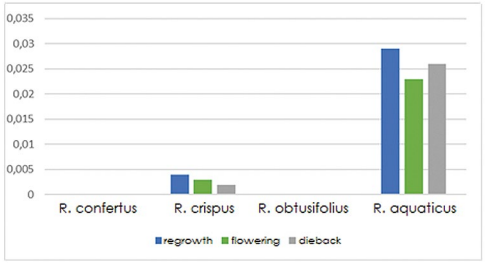
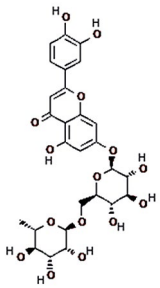
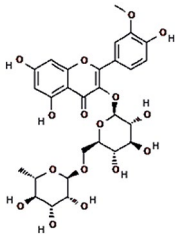
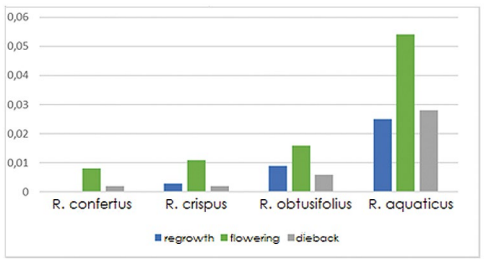


Figure. 4. **Diagram of the total quantitative content of flavonoids**

Table 4. **Physical and chemical properties of the analyzed substances**

Compound	Structural formula	Content
3-O-rutinoside of quercetin (rutin)		
7-O-glucoside of luteolin (cynaroside)		Not found in any of the objects
3-O-rutinoside of isorhamnetin (narcissin)		

End of table 4

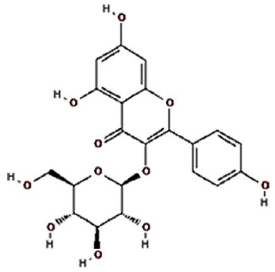
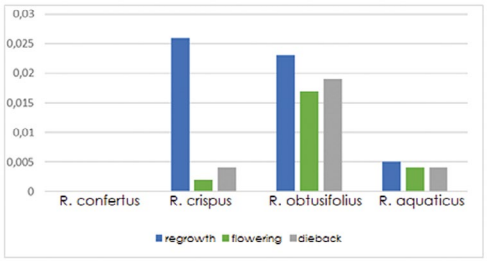
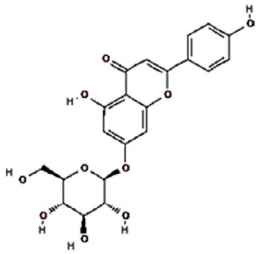
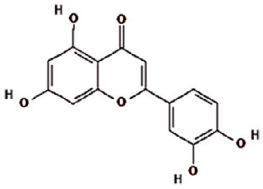
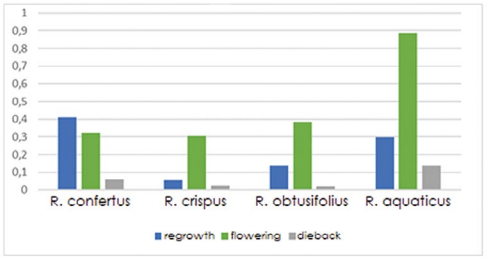
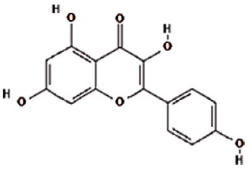
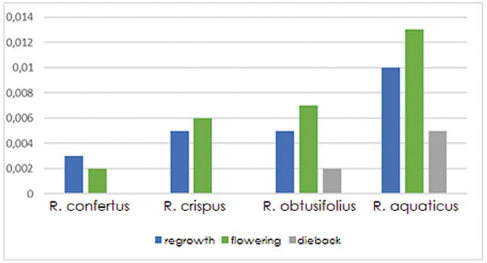
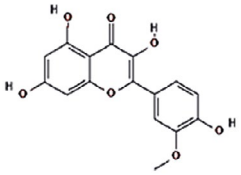
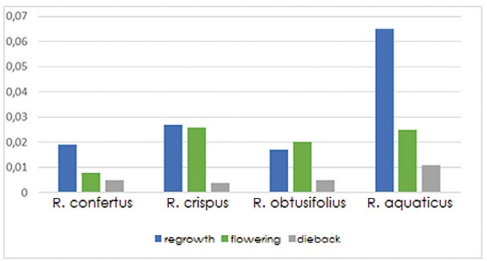
Compound	Structural formula	Content
3-O-glucoside of kaempferol (astragalin)		
7-O-beta-D-glucoside apigenin (cosmosiin)		Not found in any of the objects
Luteolin		
Kaempferol		
Isorhamnetin		

Table 5. The content of certain flavonoids, in %

Vegetation phase	*R. confertus*	*R. crispus*	*R. obtusifolius*	*R. aquaticus*
Spring regrowth	0,431	0,121	0,194	0,433
Flowering	0,341	0,353	0,443	1,008
Aboveground part dieback	0,066	0,035	0,051	0,211

It can be noted that there is a trend of low content in the overhead part dieback. The results obtained allow us to judge the prospects for further study of the dynamics of BAS accumulation in representatives of the genus Rumex in underground organs, depending on the phenological phase of the plant.

CONCLUSION

In the process of the research, a method for the quantitative determination of flavonoids in alcohol-water extracts was developed. The method allows to determine the presence of accurately and reliably one of the BAS groups – flavonoids. The discovered and quantified substances were: 3-O-rutinoside of quercetin (rutin), 3-O-rutinoside of isorhamnetin (narcissin), 3-O-glucoside of kaempferol (astragalin), luteolin, kaempferol and isorhamnetin. 7-O-glucoside of luteolin (cynaroside) and 7-O-beta-D-glucoside apigenin (cosmosiin) were not found.

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