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Comparative Study of Free Amino Acid Profiles in Underground Organs of Several Species of the Genus Rumex During Different Phases of the Vegetation Cycle

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Abstract

Introduction. Amino acids (AA), the primary metabolites in plants, play a crucial role in various physiological processes, including the synthesis of phenolic compounds. Drug products and dietary supplements made from medicinal plants can become a rich source of both nonessential and essential amino acids. High levels of free amino acids found in herbal raw material often indicate the presence of biotic and abiotic stress in the plants. Therefore, understanding the dynamics of bioactive compound accumulation in plants throughout their phenological phases of development is critical to optimizing their potential health benefits.

Aim. To compare qualitative composition and dynamics of AA accumulation in the underground organs of four representatives of the *Rumex* genus: *R. confertus*, *R. crispus* L., *R. obtusifolius* L., *R. aquaticus* L. of three different vegetative phases.

Materials and methods. Water extracts from underground organs of the studied plants were analyzed using two different methods for qualitative and quantitative analysis. Extracts were applied to the chromatographic plates TLS Silica gel 60 F254 (Merk, Germany) 20 × 20 cm with a micro-syringe (LLC "Tsvet", Russia). After elution, the plates were treated with a 2 % ninhydrin solution. Quantitative analysis was carried out using the SF-2000 spectrophotometer (LLC "OKB Spectr", Russia).

Results and discussion. The amino acid profile of the underground organs of *R. confertus*, *R. crispus* L., *R. obtusifolius* L., *R. aquaticus* L. during three vegetative phases was determined using the TLC method; the quantitative analysis performed using spectrophotometry.

Conclusion. The most diverse amino acid (AA) profiles were found in the withering phase in all *Rumex* species, with up to 9 amino adsorption zones. In contrast, the least diverse AA profiles were observed in the flowering phase, ranging from 2 to 4 adsorption zones. The quantitative content of AA was lowest in the flowering phase, increased during the regrowth phase, and peaked in the withering phase. However, *R. crispus* L. showed an unusual pattern, with the highest quantitative content of AA detected in the regrowth phase.

Keywords: TLC, UV spectrophotometry, phytochemistry, amino acids, Rumex

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 N. V. Bobkova invented and designed the experiment. Andrey M. Poluyanov, Anna Yu. Sokolova, Uliana A. Matvienko and Anna E. Savelyeva analyzed the objects using the TLC and spectrophotometry methods. Andrey M. Poluyanov, Uliana A. Matvienko, Anna Yu. Sokolova, Natalya A. Durnova and Natalya V. Bobkova participated in data processing. Andrey M. Poluyanov, Uliana A. Matvienko, Anna Yu. Sokolova and Anna E. Savelyeva participated in writing the text of the article. All authors participated in discussion of the results.

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Сравнительное изучение профиля свободных аминокислот в подземных органах нескольких видов рода Rumex на разных фазах вегетационного цикла

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Резюме

Введение. Аминокислоты (АК) являются первичными метаболитами в растениях. Одна из функций – синтез фенольных соединений. Препараты и биологически активные добавки на основе лекарственных растений могут быть доступным источником заменимых и незаменимых АК. Большое содержание АК в свободном виде в растительном сырье чаще всего указывает на наличие биотического и абиотического стресса. Помимо этого – важной задачей является определение динамики накопления биологически активных веществ в зависимости от фенологической фазы развития растения.

Цель. Сравнительное изучение качественного состава и динамики накопления АК в подземных органах четырех представителей рода Rumex: *R. confertus*, *R. crispus* L., *R. obtusifolius* L., *R. aquaticus* L. трех различных фаз вегетации.

Материалы и методы. В качестве анализируемых растворов использовались водные извлечения из подземных органов изучаемых видов рода Rumex, получаемые для качественного и количественного исследования по двум различным методикам. На хроматографические пластинки марки TLS Silica gel 60 F254 (Merk, Германия) 20 × 20 см наносили извлечения микрошприцем (ООО «Цвет», Россия). После элюирования пластинки обрабатывали 2%-м раствором нингидрина. Количественное определение проводилось на спектрофотометре СФ-2000 (ООО «ОКБ Спектр», Россия).

Результаты и обсуждение. Было проведено определение АК методом ТСХ и оценено количественное содержание методом спектрофотомерии в подземных органах четырех представителей рода Rumex: *R. confertus*, *R. crispus* L., *R. obtusifolius* L., *R. aquaticus* L. трех различных фаз вегетации.

Заключение. Наиболее разнообразные профили АК были обнаружены в фазу отмирания надземной части у всех видов Rumex (до 9 зон адсорбции), наименее разнообразные в фазу цветения (от 2 до 4 зон адсорбции). Количественное содержание АК наименьшим было в фазе цветения, возрастало в фазу отрастания и достигало пика в фазу отмирания, исключением является *R. crispus* L., у которого наибольшее количественное содержание АК было отмечено в фазу отрастания.

Ключевые слова: TLC, UV, фитохимия, аминокислоты, щавель

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

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INTRODUCTION

Amino acids (hereinafter referred to as AA) are the primary metabolites in plants. They play a vital role in protein synthesis, ion transport, enzyme activation, environmental resistance [1, 2], and phenolic compound synthesis [3].

Drug products and dietary supplements made from medicinal plants, such as those belonging to the Fabaceae, Urticaceae, or other families, can serve as a rich source of both nonessential and essential amino acids [4, 5]. Moreover, research has shown that AA can participate in nervous regulation, exhibit neuroprotective effects [6, 7], and are indispensable to the proper functioning of the cardiovascular system [8]. Of particular note is the importance of essential AA.

While both nonessential and essential amino acids can be synthesized in herbal raw materials, all AA can exist in either a free or bound form within peptides and other compounds. Notably, high concentrations of free-form AA in herbal raw materials often suggest the presence of biotic and abiotic stress [9–11].

Numerous studies report on the content of amino acids in representatives of such families as Nettle (Urticaceae) [12, 13], Oleaster (Elaeagnaceae) [14], Leguminosae (Fabaceae) [15, 16], and others [17, 18]. It has been proven that amino acids are capable of accumulating both in aboveground plant organs and in underground ones. [19–21] The data on the content of amino acids in plant organs is presented in Table 1.

AA can accumulate in different morphological organs of plants in free and bound forms. It is vital to determine the patterns of biologically active substances accumulation throughout phenological phases of plant development.

Table 1. **The content of amino acids in different morphological groups**

Morphological group	Total content of free amino acids, %	Content of essential amino acids, %	Publications
Leaves	11.65–20.21	0.7–7.2	[12, 18]
Herbs	11.26–15.88	4.0–4.17	[15, 16]
Roots	8.39–8.89	2.05–2.46	[16, 22]
Fructus	5.98–13.44	1.74–2.75	[12, 23]
Flowers	5.86–7.34	2.38–3.47	[24, 25]

MATERIALS AND METHODS

Objects of study

The underground organs of *Rumex confertus* Willd, *Rumex aquaticus* L., *Rumex crispus* L. and *Rumex obtusifolius* L. were harvested for this study during the 2021–2022. All the samples were harvested from the same population (from Rogovskoye settlement, Moscow, Russia, coordinates: 55°14′44.3″N 37°00′34.5″E). The underground organs of the studied species belong to the group of "roots" according to the morphological classification (in *R. confertus* and *R. crispus* L.) or "rhizomes and roots" (in *R. obtusifolius* L. and *R. aquaticus* L.). All of the samples were dried until the moisture content was less than 14 %.

Reagents and solutions

The following aqueous solutions were used as standard samples for the TLC: 0.1 % alanine ≥99 % (HPLC) (Sigma Aldrich, Germany), arginine ≥99.5 % (HPLC) (Sigma Aldrich, Germany), asparagine ≥99 % (TLC) (Sigma Aldrich, Germany), aspartic acid ≥99 % (HPLC) (Sigma Aldrich, Germany), glutamic acid ≥99 % (HPLC) (Sigma Aldrich, Germany), glutamine ≥99 % (HPLC) (Sigma Aldrich, Germany), glycine ≥99 % (HPLC) (Sigma Aldrich, Germany), histidine ≥99 % (TLC) (Sigma Aldrich, Germany), isoleucine ≥98 % (HPLC) (Sigma Aldrich, Germany), leucine ≥98 % (HPLC) (Sigma Aldrich, Germany), lysine ≥98 % (TLC) (Sigma Aldrich, Germany), methionine ≥99.5 % (NT) (Sigma Aldrich, Germany), phenylalanine ≥99.0 % (HPLC) (Sigma Aldrich, Germany), proline ≥99 % (HPLC) (Sigma Aldrich, Germany), serine ≥99 % (HPLC) (Sigma Aldrich, Germany), threonine ≥98% (HPLC) (Sigma Aldrich, Germany), tryptophan ≥98% (HPLC) (Sigma Aldrich, Germany) and valine ≥98% (HPLC) (Sigma Aldrich, Germany), Acetone (c.p., Merck KGaA, Germany), acetic acid (c.p., Vecton, Russia), *n*-butanol (analytically pure, Vecton, Russia), ninhydrin (≥98 % of LLC "Diaem", Russia), ascorbic acid (≥99 % Sigma Aldrich, USA).

Equipment

The moisture content of the crushed Rumex underground organs was determined using the OHAUS MB27 moisture analyzer (OHAUS, USA). The extract from the plant material was applied on the chromatographic plates TLS Silica gel 60 F254 (Merk, Germany) 20 × 20 cm using a microsyringe (LLC "Tsvet", Russia).

SF-2000 spectrophotometer (LLC "OKB Spectr", Russia); Stegler WB-4 water bath (Stegler, China), mechanical pipettes of 100-1000 µl, and of 1000–5000 µl (Sartorius, Germany), analytical balance R200D (Sartorius, Germany).

Sample preparation

To study the amino acid profile of the roots using thin-layer chromatography (TLC), aqueous extracts were prepared at a ratio of 1:5 (herbal raw material:extractant) from the roots of 12 analyzed samples. The extracts were prepared by crushing 1.0 g of herbal raw material into particles that passed through a 2 mm sieve, followed by mixing with 5 ml of distilled water and heating in a water bath with a reflux condenser for 30 minutes [19]. After extraction, the extract was filtered.

For spectrophotometric analysis, aqueous extracts were prepared according to the following method: 1.0 g of herbal raw material was placed in a 250 ml flask and 25 ml of distilled water was added. The mixture was then heated for 30 minutes in a boiling water bath with a reflux condenser. The hot extract was filtered through a small folded paper filter into a 100 ml flask; the filter contaminated with particles was placed in a flask as well for extraction. The extraction was repeated again under the conditions described above, into the same flask. After cooling, the extract was replenished to the volume of 100 ml.

To analyze the extracts, 2 ml of each extract or distilled water (for a blank solution) were combined with 4 ml of phosphate buffer solution (pH 6.4) and 2 ml of 1 % ninhydrin solution, with 2 ml of 0.05 % ascorbic acid solution in a separate 100 ml flask. The entire reaction mixture was heated for another 30 minutes. After rapid cooling, the mixture was replenished to its original volume and analyzed spectrophotometrically (Figure 1).

RESULTS AND DISCUSSION

Qualitative determination: method development

To determine the optimal sample size for the experiment, a series of tests was conducted using a microsyringe to apply analyzed extracts of varying volumes (ranging from 1 to 10 µL) to chromatographic plates. Our findings indicated that the visibility of spots was compromised at sample volumes below 4 µL, while separation efficiency was adversely affected at volumes exceeding 7 µL. Therefore, the optimum sample volume for the experiments is 5 µL.

Standard samples of AA were applied to chromatographic plates in a volume of 1 µL, and elution was performed twice in a pre-saturated chromatographic cham-

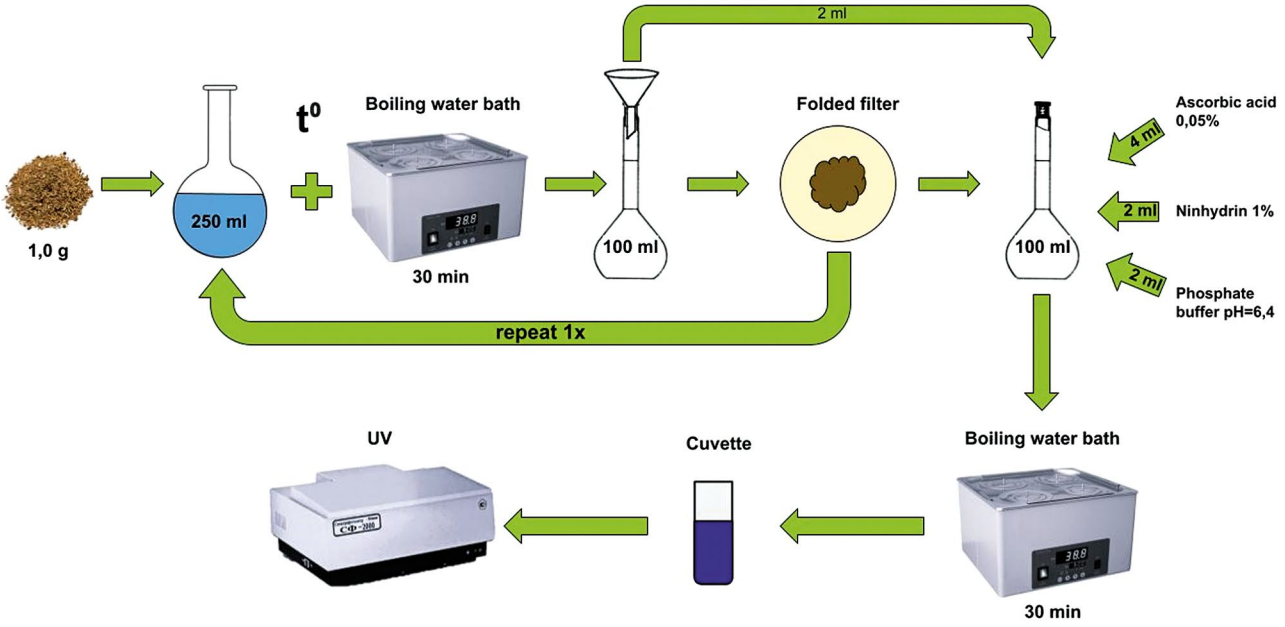

Figure 1. Sample preparation for quantitative analysis of AA

ber utilizing a solvent system composed of *n*-butanol, acetone, glacial acetic acid, and water (35:35:10:20) with a solvent front of 18 cm. Spots were visualized by spraying a 2 % solution of ninhydrin (acetone: *n*-butanol 1:1) onto the plate and drying it in a drying cabinet at a temperature of 105 °C for a period of 15 minutes. The AA in the studied extracts were identified by comparing the Rf values and spot colors to those of standard samples (Figure 2).

The results of the study of AA profiles in the extracts from *R. confertus, R. crispus* L., *R. obtusifolius* L., *R. aquaticus* L. via TLC are shown in Figure 3.

The chemical composition of different *Rumex* species in the flowering phase has been found to be non-identical. Data collected revealed the presence of 2–4 chromatographic zones, which can be classified as non-essential (alanine, serine) and essential (isoleucine, methionine) amino acids. Notably, the richest AA profile was observed in *R. obtusifolius* L., while the least diverse was found in *R. aquaticus* L. Furthermore, isoleucine was detected in the samples of *R. confertus*, *R. obtusifolius* L., and *R. aquaticus* L., while methionine was found in the samples of *R. confertus, R. crispus* L., and *R. obtusifolius* L. These findings imply that the examined types of herbal raw material possess considerable potential for utilization in different pharmaceutical domains.

The TLC profiles of *R. confertus, R. crispus L., R. obtusifolius L., and R. aquaticus* L. during the withering phase show an increase in the number of chromatographic zones, ranging from 5 to 8. The extract of *R. confertus* exhibits an unidentified adsorption zone with an Rf value of 0.91 ± 0.02, while the adsorption zone corresponding to aspartic acid is present in *R. obtusifolius* L. All the studied extracts display an unidentifiable zone with an Rf value of 0.730 ± 0.02.

In the regrowth phase, *R. confertus* displays five chromatographic zones, including an unidentified zone with an Rf value of 0.91 ± 0.02; however, the zone corresponding to alanine disappears.

In *R. crispus* L. two additional adsorption zones corresponding to aspartic acid and phenylalanine appear, meanwhile in *R. obtusifolius* L. the zone with an Rf value of 0.73 ± 0.02 disappears. Notably, *R. aquaticus* L. has the most diverse profile among the four species, featuring 8 chromatographic zones; compared with the withering phase, an adsorption zone corresponding to aspartic acid appears. Interestingly, only *R. obtusifolius* L. retains an unidentified adsorption zone with an Rf value of 0.73 ± 0.02 throughout the regrowth phase.

Isoleucine, methionine, alanine, serine and lysine were found in all of the analyzed samples; four of these AA are essential. The TLC profiles of the aqueous extracts of the roots of *R. crispus* L. and *R. aquaticus* L. during the withering phase are identical, whereas *R. confertus* has an additional adsorption zone during flowering, and *R. obtusifolius* L. has an additional zone attributed to aspartic acid.

The total content of AA was assessed using spectrophotometry after the ninhydrin reaction at a wavelength of 568 nanometers in a 10 mm cuvette (Figure 4).

The total content of free AA (%), expressed as the equivalent of glutamic acid, and the moisture-free herbal raw material (X), was calculated using the following formula:

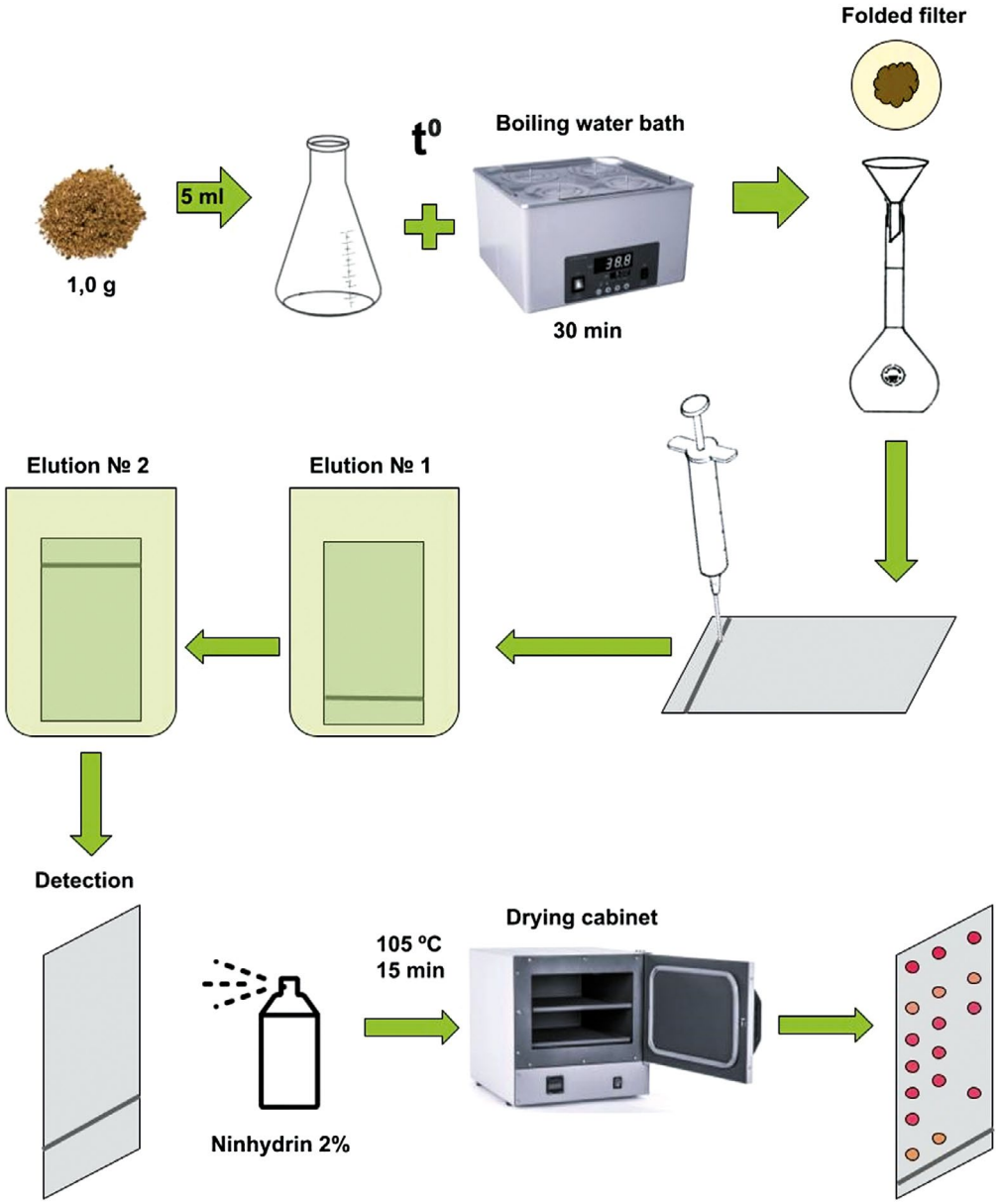

Figure 2. Scheme of TLC- method for the detection of AA

$$\frac{A \cdot 100 \cdot 100 \cdot a_0 \cdot 2 \cdot 100 \cdot 100}{A_0 \cdot a \cdot 2 \cdot 200 \cdot 100 \cdot (100 - W)} = \frac{A \cdot a_0 \cdot 50 \cdot 100}{A_0 \cdot a \cdot (100 - W)},$$

where A – optical density of the test solution; A_0 – optical density of the standard solution of glutamic acid; a – sample weight (raw materials), g; a_0 – standard sample weight (glutamic acid), g; W– weight loss during drying of raw materials, %.

The results of the spectrophotometric analysis are presented in Table 2 and graphically represented in Figure 5 below.

In *R. confertus*, *R. aquaticus* L., and *R. obtusifolius* L., the maximum total content of AA is observed during the withering phase. Conversely, *R. crispus* L. exhibits the highest total AA content during the regrowth phase, while R. *obtusifolius* has the highest total AA content across both the flowering and withering phases. Notably, R. *confertus* has the lowest total content of AA throughout all phases of vegetation. It is interesting to note that all four species share a common trend of having the minimum amount of AA during the flowering phase.

Previous studies [22, 23] have shown that members of the Rumex genus display the highest total content of phenolic compounds (anthracene derivatives and flavonoids) during the flowering phase. Given that AA act as material for synthesis of secondary metabolites, it can be assumed that the lowest content of AA during the flowering phase and accumulation of bioactive compounds display a negative correlation.

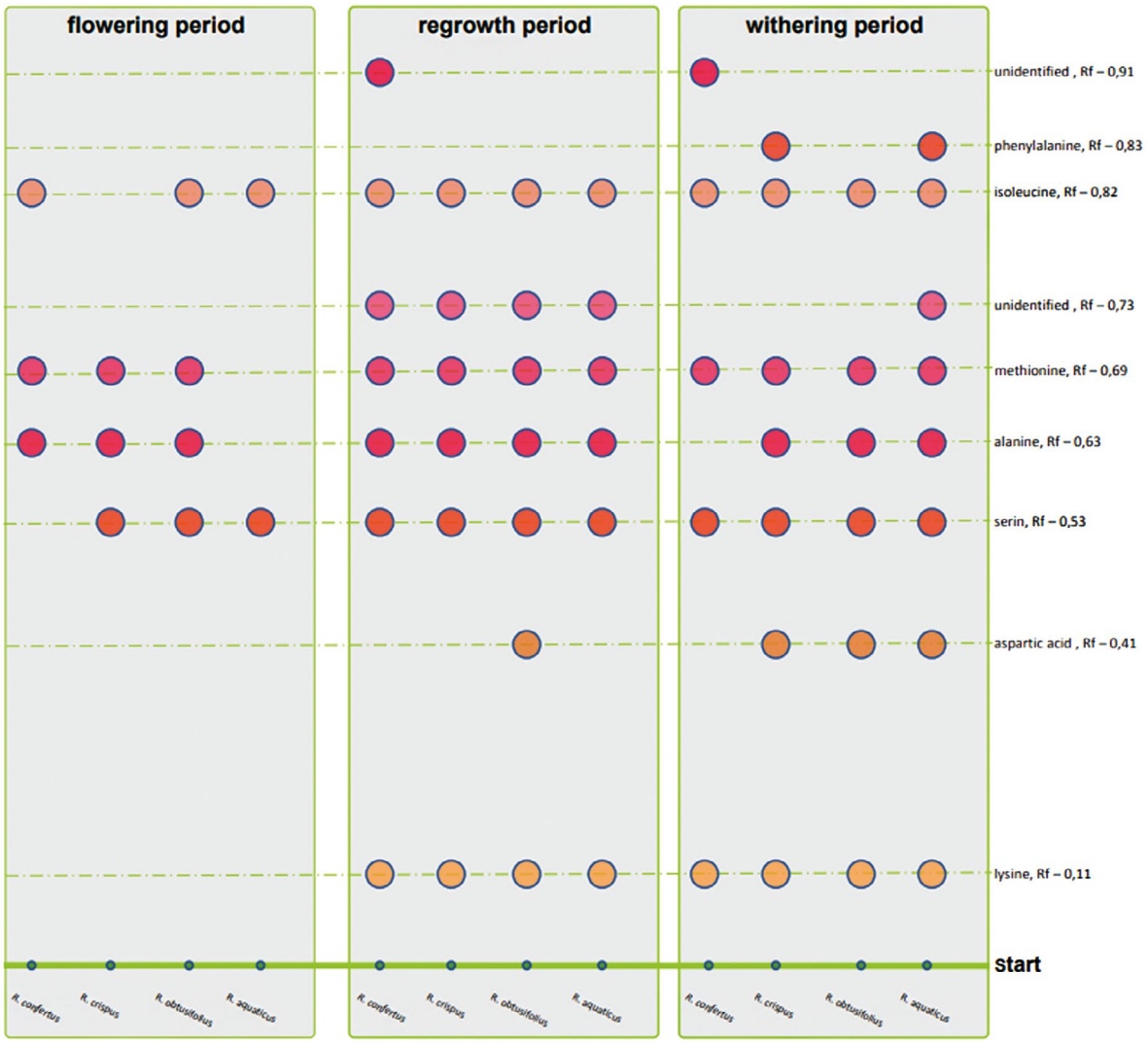

Figure 3. **TLC- profiles of AA for *R. confertus*, *R. crispus* L., *R. obtusifolius* L., *R. aquaticus* L. in the flowering period, regrowth period and withering period**

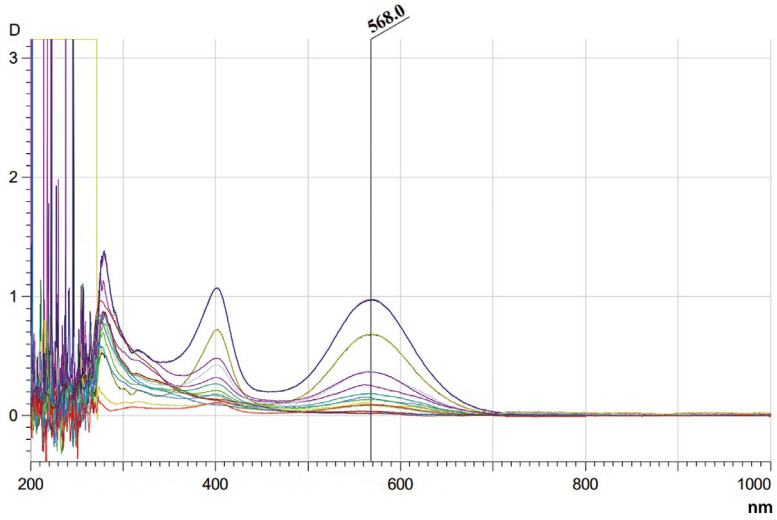

Figure 4. **Spectra of the analyzed objects after reaction with ninhydrin**

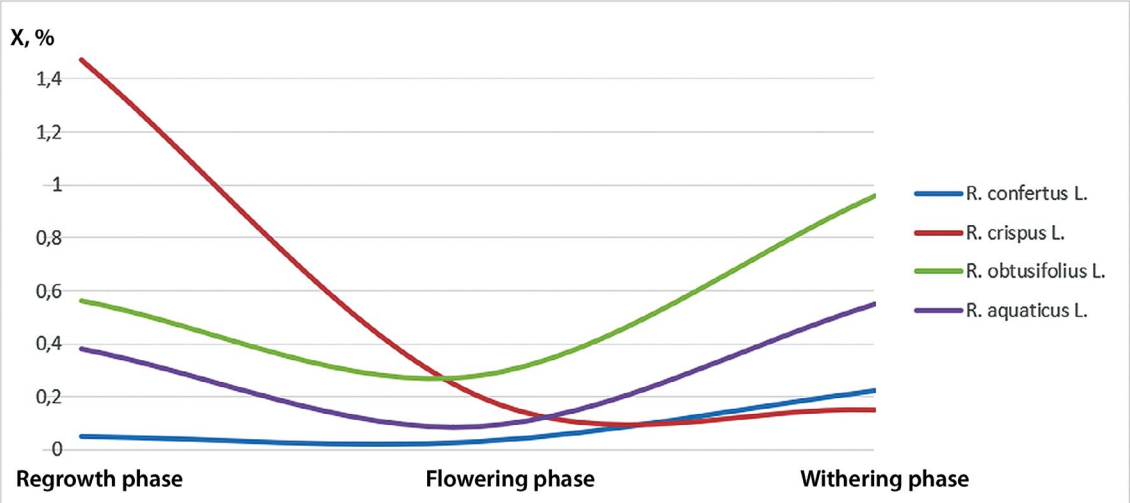

Figure 5. **Comparison of *R. confertus*, *R. crispus* L., *R. aquaticus* L. and *R. obtusifolius* L.**

Table 2. **Results of spectrophotometric analysis for *R. confertus*, *R. crispus* L., *R. aquaticus* L. and *R. obtusifolius* L in %**

Object	Average optical density	X, %
Regrowth phase object		
R. confertus	0.033	0.050 ± 0.004
R. crispus L.	0.969	1.469 ± 0.306
R. aquaticus L.	0.251	0.381 ± 0.046
R. obtusifolius L.	0.371	0.562 ± 0.051
Flowering phase object		
R. confertus	0.020	0.030 ± 0.001
R. crispus L.	0.134	0.203 ± 0.036
R. aquaticus L.	0.057	0.087 ± 0.420
R. obtusifolius L.	0.183	0.278 ± 0.016
Withering phase object		
R. confertus	0.147	0.222 ± 0.015
R. crispus L.	0.099	0.150 ± 0.003
R. aquaticus L.	0.363	0.550 ± 0.040
R. obtusifolius L.	0.632	0.957 ± 0.644

CONCLUSION

During the flowering phase, *R. obtusifolius* L. has the most diverse AA profile, and *R. aquaticus* L. the least. The highest total content of AA is in *R. obtusifolius* L., the lowest in *R. confertus*.

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