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Phytochemical Analysis of the Aerial Part of Golden Dock (*Rumex Maritimus* L.)

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Abstract

Introduction. Golden dock (*Rumex maritimus* L., *Polygonaceae*) is used as a medicinal and food plant in Asian countries. The plant contains phytochemicals of various classes: flavonoids, tannins, anthraquinones etc. Plant extracts exhibit antibacterial, antioxidant, anti-inflammatory, astringent activity, and have antidiabetic potential. The plant is annual, and most of the biologically active substances accumulate in its aboveground organs. An important problem is the standardization of *Rumex maritimus* and the development of regulatory documentation for its the introduction to medical practice.

Aim. To conduct phytochemical analysis of the aerial part of golden dock.

Materials and methods. Air-dried aerial part of *Rumex maritimus* collected at flowering and beginning of fruiting stage, as well as individual above-ground organs (leaves, flowers, fruits, stems), were used for obtaining the extracts. Qualitative analysis of the extracts was carried out using reverse phase HPLC. The relative content of the components in the mixture was calculated by the method of simple normalization. Total content of free anthraquinones and anthraglycosides in terms of chrysophanic acid was determined using spectrophotometric method after acid hydrolysis. Total tannin content was calculated by titrimetric method.

Results and discussion. Flavonoids isoquercetin and avicularin were first discovered in the aerial part of *Rumex maritimus*. The dominant component of the plant is rutin. Chrysophanol predominates among anthraquinones. The highest concentration of anthraquinones (2.80 ± 0.04 %) was found in flowers. Tannins accumulate mainly in leaves (9.97 ± 0.02 %). A significant amount of tannins (6.60 ± 0.03 %) and anthracene derivatives (1.96 ± 0.03 %) is contained in the whole aerial part.

Conclusion. Phytochemical analysis of the aerial part of *Rumex maritimus* showed the presence of a significant amount of anthraquinones. As a plant raw material it is proposed to use the herb of *Rumex maritimus*. Standardization is recommended for anthraquinones in terms of chrysophanic acid (at least 1.5 %).

Keywords: anthraquinones, flavonoids, saponins, tannins, spectrophotometry, gravimetry

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. Vera V. Podgurskaya and Elena A. Luksha conducted the literature research and the experiments, analysed and interpreted the data, prepared the article, reviewed the text. Irina N. Korneeva, Irina A. Savchenko, Evgenia V. Ivanova conducted the experiments, analysed and interpreted the data, reviewed the text.

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Фитохимический анализ надземной части щавеля приморского (*Rumex maritimus* L.)

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Резюме

Введение. Щавель приморский (*Rumex maritimus* L., *Polygonaceae*) используется как лекарственное и пищевое растение в странах Азии. Растение содержит биологически активные вещества (БАВ) различных классов: флавоноиды, дубильные вещества, антрахиноны и др. Извлечения из растения проявляют антибактериальную, антиоксидантную, противовоспалительную, вяжущую активность, обладают противодиабетическим потенциалом. Растение является однолетником, поэтому большая часть биологически активных веществ

накапливается в надземных органах. Важной задачей является стандартизация сырья щ. приморского и разработка нормативной документации для его внедрения в медицинскую практику.
Цель. Провести фитохимический анализ надземной части щ. приморского.
Материалы и методы. Для получения извлечений использовали высушенную надземную часть щ. приморского, а также отдельные высушенные надземные органы (листья, цветки, плоды, стебли). Качественный анализ извлечений проводили с использованием метода обращенно-фазовой ВЭЖХ. Относительное содержание компонентов смеси вычисляли методом простой нормировки. Суммарное содержание свободных антрахинонов и антрагликозидов определяли спектрофотометрическим методом в пересчете на хризофановую кислоту после проведения кислотного гидролиза. Количественное определение танинов проводили титриметрическим методом.
Результаты и обсуждение. В надземной части щ. приморского впервые обнаружены флавоноиды изокверцетин, авикулярин. Доминирующим компонентом сырья является рутин. Среди антраценпроизводных преобладает хризофанол. Наиболее высокая концентрация антрахинонов (2,80 ± 0,04 %) обнаружена в цветках. Дубильные вещества накапливаются преимущественно в листьях (9,97 ± 0,02 %). Значительное количество дубильных веществ (6,60 ± 0,03 %) и антраценпроизводных (1,96 ± 0,03 %) содержится в траве.
Заключение. Фитохимический анализ надземной части щ. приморского показал присутствие значительного количества антрахинонов. В качестве сырья предлагается использовать траву щ. приморского в стадии цветения. Предлагается проводить стандартизацию сырья по антраценпроизводным в пересчете на хризофановую кислоту (не менее 1,5 %).

Ключевые слова: антрахиноны, флавоноиды, дубильные вещества, спектрофотометрия, ВЭЖХ

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

Вклад авторов. В. В. Подгурская и Е. А. Лукша выполнили литературный поиск и провели эксперименты, анализировали и интерпретировали данные, подготовили статью. И. Н. Корнеева, И. А. Савченко, Е. В. Иванова проводили эксперименты, анализировали и интерпретировали данные, редактировали текст. Все авторы участвовали в обсуждении результатов.

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INTRODUCTION

Rumex maritimus L. (golden dock) is an erect annual herbaceous plant from the family Polygonaceae Juss. with a height of 10 cm to 0.8–1.2 m [1]. The plant grows in the European part of Russia, Ciscaucasia, Western and Eastern Siberia, and the Far East (Primorsky Territory, Kamchatka, and Amur Region) [1].

Currently, the plant is used by the population of Southeast Asia as astringent, antidiarrheal, laxative, antiflatulent and aphrodisiac agents [2–5]. The seeds are used to treat flatulency and notalgia [3, 4], and the paste from leaves is applied to cure burns, wounds, and itchy areas [5, 6].

It has been experimentally established that the methanol extract of the plant has astringent properties and antibacterial, antioxidant, neuropharmacological [2–4, 7], anti-inflammatory and analgetic characteristics [8]. The ethanolic plant extract is characterized by an antidiabetic potential due to its ability to inhibit the formation of glycation end-products [9].

R. maritimus is an annual plant; therefore, biologically active substances (BAS) accumulate mostly in its aboveground organs. The plant contains flavonoids (rumarin, hyperin, and rutin), tannins, alkaloids [2], anthraquinones (chrysophanol, emodin, and physcion), and chromones [3, 4].

The development of quality specifications for plant raw materials is one of the important tasks to implement the plant into medical practice. It is necessary to conduct a qualitative and quantitative analysis of the aboveground organs of *R. maritimus* and to determine the BAS groups to provide the standardization procedure.

The aim of the study was to perform the phytochemical analysis of the aerial part of golden dock (*Rumex maritimus* L.).

MATERIALS AND METHODS

The objects of the study were both the aerial part of the plant and the plant components, such as leaves, stems, and flowers, collected in the flowering period (July 2021), and fruits, gathered in the period of full ripening (August 2021). Plant raw materials were collected in Omsk Oblast (Lyubinsky Rayon, the Avlukha River). Plant raw materials were air-shade dried.

Chemical compounds analysis was performed using a reverse-phase HPLC method: 2.0 g of the plant raw materials were placed in a round-bottom flask, 50 ml of 80 % ethanol was added and the mixture was heated in a boiling water bath for 40 min. The obtained extract was refined through a paper filter into a 50 ml volumetric flask and adjusted to the mark with 80 % ethanol.

Chromatography conditions: LC-20 Prominence chromatograph (Shimadzu) with a photodiode array detector; isocratic mode; PerfectSil Target HD ODS-3 5 µm 4.6 × 200 mm analytical column; 254 nm wavelength detection; column temperature – room temperature; mobile phase rate of 0.5 ml/min; 20 µl injection volume. The following mixtures were used for the mobile phase: acetonitrile / water (70:30) (for anthraquinone analysis); acetonitrile / 5 % acetic acid (20:80) (for the analysis of other phenolic compounds).

Compounds were identified using the standard samples (Sigma Aldrich, USA: chrysophanol, 98 % purity, the expiry date 02.2024; physcion, 98 % purity, the expiry date 09.2023; emodin, 97 % purity, the expiry date 09.2023; vanillic acid, 97 % purity, the expiry date 06.2026; 2-hydroxycinnamic acid, 97 % purity, the expiry date 08.2024; 3-hydroxycinnamic acid, 99 % purity, the expiry date 04.2024; chlorogenic acid, 95 % purity, the expiry date 02.2025; syringic acid, 95 % purity, the expiry date 02.2025; *p*-coumaric acid, 98 % purity, the expiry date 08.2023; ferulic acid, 99 %, the expiry date 07.2025; quercetin, 95 % purity, the expiry date 06.2026; avicularin, 90 % purity, the expiry date 03.2025; resveratrol, 99 % purity, the expiry date 08.2025; catechin, 99 % purity, the expiry date 11.2023; SupelcoInc, USA: caffeic acid, 99.3 % purity,the expiry date 01.2025; isoquercetin, 98% purity, the expiry date 04/2024; gallic acid, 95 % purity, the expiry date 11.2024; (-)-epigallocatechingallate, 98% purity, the expiry date 12.2023; Acros Organics B.V.B.A., Belgium: rutin, 97 % purity, the expiry date 08.2025. The relative compound ratio (%) was calculated by a simple normalization method from the chromatographic peak area ratio.

Total tannin content was measured by permanganometric titration according to the Guidelines of the Russian State Pharmacopoeia XIV edition (GPM.1.5.3.0008.15 «Tannins ratio determination in medicinal plant raw materials and herbal medicinal products»).

To quantify free anthraquinones and anthraglycosides ratio, the method described in [10] was used, based on the acid hydrolysis of anthraglycosides, including aglycones extraction with chloroform, and their further re-extraction with the alkaline ammonia solution.

BAS content in plant organs was calculated as $g \cdot 100\ g^{-1}$ DW (dry weight) of the plant raw materials and expressed as per cent (%) equivalent. Statistical processing was carried out according to the Russian State Pharmacopoeia, GPM.1.1.0013.15 «Statistical processing of the chemical experiment results». Each experiment was performed five times.

To obtain data, the following metrological characteristics were specified: minimum value (x_{min}), maximum value (x_{max}), variance (S^2), standard deviation (S), relative standard deviation (RSD), and a mean relative error. The results were described as the mean ± standard deviation.

Independent sample t-test was used to compare the BAS ratio in different organs. The significance level was set at $p \le 0.05$.

RESULTS AND DISCUSSION

It has been detected by HPLC method that rutin is a dominant component of the aerial part of *R. maritimus*, 28.15 % in a sample (Figure 1, Table 1).

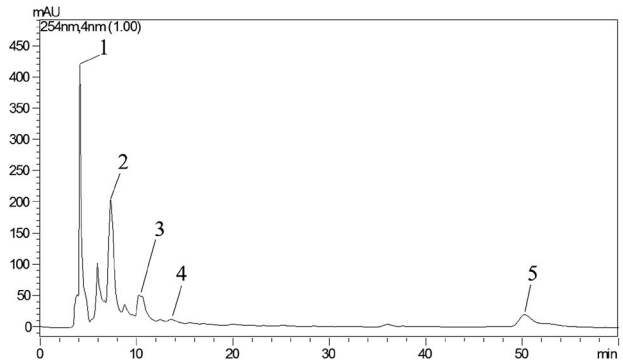

Figure 1. HPLC-chromatogram of the extract from the aerial part of Rumex maritimes (mobile phase: 20 % acetonitrile – 80 % acetic acid 5 %).

1 – Gallic acid; 2 – rutin; 3 – isoquercetin; 4 – avicularin; 5 – quercetin

Chrysophanol is a key component among anthraquinonesis, 8 % relative content in the sample (Figure 2, Table 1).

Qualitative analysis of the ethanol extract fractions from the aerial part of the *R. maritimus* has been studied earlier [11]. In addition to the compounds listed above, catechins (catechin, epigallocatechin-3-gallate, and epicatechin-3-gallate), phenolic acids (syringic, *p*-coumaric, ferulic, and caffeic), stilbenes (resveratrol) were detected in the plant raw materials.

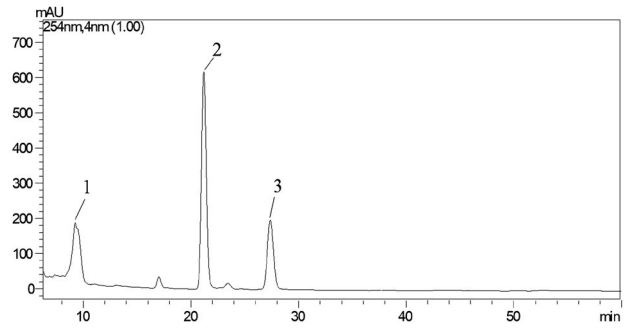

Figure 2. HPLC-chromatogram of the extract from the aerial part of Rumex maritimus (mobile phase: 70 % acetonitrile – 30 % water).

1 – emodin; 2 – chrysophanol; 3 – physcion

Table 1. HPLC-analysis of the extract from the aerial part of Rumex maritimis

Peak #	Phytochemical	Retention time, min	Spectral characteristics (λ_{max}), nm	Content in sample, %
Flavonoids, phenolic acids				
1	Gallic acid	4.09	239, 279	24.7
2	Rutin	7.29	254, 354	28.15
3	Isoquercetin	10.25	255, 263, 352	11.31
4	Avicularin	12.90	239, 265, 341	2.82
5	Quercetin	47.15	253, 368	4.61
Anthraquinones				
1	Emodin	9.23	253, 265, 288, 439	4.96
2	Chrysophanol	21.18	256, 277, 287, 429	7.99
3	Physcion	27.36	253, 266, 285, 429	3.29

According to the results of spectrophotometry (Table 2), it has been found that a large rate of anthracene derivatives is contained in the aerial part (1.96 ± 0.03 %) of the plant. That is comparable to senna leaves, in which, according to PM.2.5.0038.15 of the Russian State Pharmacopoeia, must contain at least 1.35 % of the total amount of anthracene aglycones equivalent to chrysophanic acid. The highest concentration of anthraquinones was detected in flowers (2.80 ± 0.04 %). Antibacterial activity of *R. maritimus* is stipulated by the presence of anthraquinones in high ratio. Moreover, the extracts and individual anthraquinones derived from the plant are considered promising in the treatment of oncological, inflammatory, fungal, viral (including SARS-COVID-19 infection) and other diseases [12, 13].

Table 2. Total content of anthraquinones in terms of chrysophanic acid in the aerial organs of Rumex maritimus

Plant organs	Quantitative content $\bar{x}$, %	Metrological characteristics	Relative standard deviation, %	Relative error of the mean, %
Aerial part	1.96	$x_{min} = 1.94$ $x_{max} = 1.99$ $S^2 = 0.0006$ $S = 0.03$	1.28	1.45
Leaves	0.99	$x_{min} = 0.96$ $x_{max} = 1.00$ $S^2 = 0.0004$ $S = 0.02$	2.12	1.20
Flowers	2.80	$x_{min} = 2.77$ $x_{max} = 2.84$ $S^2 = 0.0012$ $S = 0.04$	1.25	2.03
Fruits	1.55	$x_{min} = 1.52$ $x_{max} = 1.57$ $S^2 = 0.0006$ $S = 0.03$	1.63	1.45
Stems	0.67	$x_{min} = 0.65$ $x_{max} = 0.69$ $S^2 = 0.0004$ $S = 0.02$	2.99	1.15

Table 3 presents the results of the quantitative determination of tannins. Tannins occupy in the plants from 5 to 10 % of the dry weight [14] and accumulate in bark, stems, fruits and seeds, roots, flower buds and leaves [15]. A very high content of tannins (6.60 ± 0.03 %) has been detected in the aerial part of *R. maritimus*, explaining the antidiarrheal effect of *R. maritimus* extracts. The highest content of tannins has been found in the leaves (9.97 ± 0.02 %).

Table 3. Total content of tannins in terms of tannic acid in the aerial organs of Rumex maritimus

Plant organs	Quantitative content $\bar{x}$, %	Metrological characteristics	Relative standard deviation, %	Relative error of the mean, %
Aerial part	6.60	$x_{min} = 6.58$ $x_{max} = 6.64$ $S^2 = 0.0012$ $S = 0.03$	0.52	2.00
Leaves	9.97	$x_{min} = 9.96$ $x_{max} = 9.99$ $S^2 = 0.0002$ $S = 0.02$	0.15	0.88
Flowers	7.10	$x_{min} = 7.07$ $x_{max} = 7.13$ $S^2 = 0.0009$ $S = 0.03$	0.42	1.73
Fruits	5.19	$x_{min} = 5.14$ $x_{max} = 5.23$ $S^2 = 0.002$ $S = 0.05$	0.87	2.60
Stems	0.71	$x_{min} = 0.69$ $x_{max} = 0.72$ $S^2 = 0.0003$ $S = 0.02$	2.44	1.00

The conducted studies show that the aerial part of *R. maritimus* should be considered as a source of anthraquinones and tannins. It is proposed that *R. maritimus* raw materials are to be standardized in anthracene derivatives equivalent to chrysophanic acid (not less than 1.5 %).

Due to the fact that a large number of anthraquinones and flavonoids accumulate in flowers, it is recommended to collect plants at the flowering stage. At the same time, it is not advisable to use flowers as the plant raw materials, since their specific weight in the total mass of the aerial part is less than 3 %.

The stems of *R. maritimus* do not accumulate significant amounts of biologically active substances; therefore, while collecting the plant raw materials, it is suggested to remove large stems from the aerial part.

The quantitative determination of the dominant components (flavonoids) was not carried out due to the fact that that at present there is no method for determining flavonoids in this raw material.

CONCLUSION

As a result of the studies flavonoids: isoquercetin and avicularin were first detected in the ethanol extract from the aerial part of *R. maritimus*. It has been established that rutin is a dominant component of the plant. A new method is required to be developed for flavonoids quantitative determination (equivalent to rutin) in the plant raw materials.

In *R. maritimus* herb, the dominant presence of anthraquinones (1.96 ± 0.03 %) and tannins (6.60 ± 0.03 %) has been found. Thus, the aerial part of *R. maritimus* (herb) is to be considered as a source of BAS.

Based on the conducted studies, the aerial part of the plant (herb) in the flowering stage is proposed to use as the plant raw materials. The plant raw materials are to be standardized in anthraquinones equivalent to chrysophanic acid. The data obtained will be applied to develop quality specifications for "Golden Dock herb ("*Rumicis maritimi* herba")".

REFERENCES

1. Lomonosova M. N., Bolshakov N. M., Krasnoborov I. M., Kashina L. I., Tupitsyna N. N., Geltman D. V., Shemberg M. A. Flora of Siberia. *Salicaceae – Amaranthaceae*. Novosibirsk: Nauka; 1992. 312 p. (In Russ.)
2. Hossain M. S., Rashid A. H. M. A., Rahman M. M., Sadhu S. K. Antioxidant, Antimicrobial and Antidiarrhoeal Activity of Methanolic Extract of *Rumex maritimus* L. (Polygonaceae). *Journal of Applied Pharmaceutical Science*. 2015;5(3):056–060. DOI: 10.7324/JAPS.2015.510.S10.
3. Islam M. S., Iwasaki A., Suenaga K., Kato-Noguchi H. 2-Methoxystypandrone, a potent phytotoxic substance in *Rumex maritimus* L. *Theoretical and Experimental Plant Physiology*. 2017;29(4):195–202. DOI: 10.1007/s40626-017-0095-9.
4. Islam M. S., Iwasaki A., Suenaga K., Kato-Noguchi H. Evaluation of phytotoxic potential and identification of phytotoxic compounds in *Rumex maritimus*. *Plant Biosystems*. 2018;152(4):804–809. DOI: 10.1080/11263504.2017.1338630.
5. Li J. J., Li Y. X., Li N., Zhu H. T., Wang D., Zhamg Y. J. The genus *Rumex* (Polygonaceae): an ethnobotanical, phytochemical and pharmacological review. *Natural Products and Bioprospecting*. 2022;12(1):21. DOI: 10.1007/s13659-022-00346-z.
6. Topno S. C., Sinha M. R. Study of medicinal plants used to heal skin diseases by tribes of west Singhbhum district of Jharkhand (India). *Journal of Pharmacognosy and Phytochemistry*. 2018;7(1):371–376.
7. El-Amier Y. A., El-Halawany E. F., Soliman H. M., El Hayyany L. Y. Primary GC-MS chemical analysis of alcoholic extract of *Emex spinosa* (L.) Campd. and screening of their antioxidant, antibacterial, and cytotoxic characteristics. *Bulletin of the Chemical Society of Ethiopia*. 2023;37(1):101–114. DOI: 10.4314/bcse.v37i1.9.

8. Hinna H., Abdullah S. T., Ansari S. H., Alam M. S. Anti-inflammatory and analgesic activity of *Rumex maritimus*. *Hamdard Medicus*. 2007;50(3):31–33.
9. Thida M., Nyein C. M., Chan K. N., New M. T., Ei S. L., Mya K. M. In Vitro study on Antidiabetic Potential of Crude Plant Extracts by Attenuating Oxidative-Stress and Advanced Glycation End-Product. *International Journal of Pharmaceutical Research*. 2021;13(2): 282–291. DOI: 10.31838/ijpr/2021.13.02.063.
10. Grinkevich N. I., Safronich L. N. The Chemical Analysis of Medicinal Plants. Moscow: Vysshaya Shkola; 1983. 176 p. (In Russ.)
11. Podgurskaya V. V., Luksha E. A., Savchenko I. A., Korneeva I. N., Ivanova E. V., Kalinichenko A. M. The study of the chemical composition of the aerial part of golden dock (*Rumex maritimus* L.) by high-performance liquid chromatography. *Journal of Siberian Medical Sciences*. 2022;6(1):38–45. (In Russ.) DOI: 10.31549/2542-1174-2022-6-1-38-45.
12. Malik E. M., Müller C. E. Anthraquinones as pharmacological tools and drugs. *Medicinal Research Reviews*. 2016;36(4):705–748. DOI: 10.1002/med.21391.
13. Ntemafack A., Singh R. V., Ali S., Kuiate J. R., Hassan Q. P. Antiviral potential of anthraquinones from Polygonaceae, Rubiaceae and Asphodelaceae: potent candidates in the treatment of SARS-COVID-19, A comprehensive review. *South African Journal of Botany*. 2022;151:146–155. DOI: 10.1016/j.sajb.2022.09.043.
14. Barbehenn R. V., Constabel C. P. Tannins in plant–herbivore interactions. *Phytochemistry*. 2011;72(13):1551–1565. DOI: 10.1016/j.phytochem.2011.01.040
15. Das A. K., Islam M. N., Faruk M. O., Ashaduzzaman M., Dungani R. Review on tannins: Extraction processes, applications and possibilities. *South African Journal of Botany*. 2020;135:58–70. DOI: 10.1016/j.sajb.2020.08.008.