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COMPONENT COMPOSITION AND QUANTITATIVE CONTENT OF ORGANIC ACIDS IN RHIZOMES WITH ROOTS OF *RUMEX CONFERTUS* WILLD

Actuality. *According to the literature, the underground organs of Rumex confertus contain oxalic and caffeic acids and there is no information about the content of fatty acids.*

The aim *is to study the component composition of organic including fatty acids in rhizomes with roots of Rumex confertus and to determine the quantitative content of the amount of organic acids in the series of plant raw materials.*

Material and methods. *The plant raw materials were harvested in 2015 in Kharkiv region to study component composition of organic including fatty acids and in 2019 in Vinnytsia, Ternopil, Kharkiv, Poltava, and Khmelnytskyi regions to determine quantitative content of the amount of organic acids. The component composition of organic, including fatty, acids was studied by gas chromatography-mass spectrometry. The quantitative content of the sum of organic acids was determined according to the methodology of the State Pharmacopoeia of Ukraine monograph 2.1 «Rose fruits [N]».*

Research results. *For the first time 6 organic and 10 fatty acids were identified in rhizomes with roots of Rumex confertus by gas chromatography-mass spectrometry and the quantitative content of each of them was determined. For the first time it was modified the pharmacopoeial methodology (monograph «Rose Fruits[N]») for rhizomes with roots of Rumex confertus: determination of the quantitative content of the amount of organic acids was carried out in conversion not to malic, but to oxalic acid. The validation characteristics of the method were established and it was tested on 5 series of plant raw materials.*

Conclusion. *For the first time, the component composition of organic and fatty acids in the rhizomes with the roots of Rumex confertus was determined with using the gas chromatography-mass spectrometry method. For the first time it was modified the pharmacopoeias method of determining the quantitative content of the amound of organic acids in terms of oxalic acid for rhizomes with roots of Rumex confertus and determined its validation characteristics. The obtained results were applied in the development of the project of quality control methods «Rumex confertus rhizome with roots».*

Key words: *Rumex confertus, organic acids, fatty acids.*

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КОМПОНЕНТНИЙ СКЛАД ТА КІЛЬКІСНИЙ УМІСТ ОРГАНІЧНИХ КИСЛОТ У КОРЕНЕВИЩАХ ІЗ КОРЕНЯМИ *RUMEX CONFERTUS* WILLD

Актуальність. *За даними літератури, підземні органи Rumex confertus з органічних кислот містять щавлеву і кавову кислоти, а інформація про вміст жирних кислот відсутня.*

Мета дослідження *– вивчити компонентний склад органічних, у тому числі жирних, кислот у кореневищах із коренями Rumex confertus та визначити кількісний уміст суми органічних кислот у серіях сировини.*

Матеріал і методи. *Сировину заготовляли в 2015 р. у Харківській області для вивчення компонентного складу органічних, у тому числі жирних, кислот та в 2019 р. у Вінницькій, Тернопільській, Харківській, Полтавській та Хмельницькій областях для визначення кількісного вмісту органічних кислот. Компонентний склад органічних, у тому числі жирних, кислот вивчали методом газової хромато-мас-спектрометрії. Кількісний уміст суми органічних кислот визначали за методикою монографії ДФУ 2.1 «Шипшини плоди^N».*

Результати дослідження. *Уперше у кореневищах із коренями Rumex confertus методом газової хромато-мас-спектрометрії було ідентифіковано 6 органічних та 10 жирних кислот та визначено кількісний уміст кожної з них. Уперше для кореневищ із коренями Rumex confertus модифіковано фармакопейну методику (монографія «Шипшини плоди^N» ДФУ 2.1): визначення кількісного вмісту суми органічних кислот проводили у перерахунку не на яблучну, а на щавлеву кислоту. Установлено валідаційні характеристики методики та апробовано її на п'яти серіях сировини.*

Висновок. *Уперше методом газової хромато-мас-спектрометрії в кореневищах з коренями Rumex confertus установлено компонентний склад органічних та жирних кислот. Уперше для кореневищ із коренями Rumex confertus модифіковано фармакопейну методику визначення кількісного вмісту суми органічних кислот у перерахунку на щавлеву кислоту та визначено її валідаційні характеристики. Отримані результати застосовані під час розроблення проєкту методів контролю якості «Щавлю кінського кореневище з коренями».*

Ключові слова: *Rumex confertus, органічні кислоти, жирні кислоти, газова хромато-мас-спектрометрія.*

Introduction. The genus *Rumex* includes 152 species of perennial herbaceous plants (Who plant list, 2024). Plants of the genus are found in Europe, Asia, and China (Who plant list, 2024). According to the literature, rhizomes with roots and leaves of plants of the genus *Rumex* accumulate the largest amount of anthracene derivatives, compounds of phenolic nature, tannins and oxalic acid, due to which the plants have a sour taste (Li, 2022, pp.1-29). 268 substances were identified in the plant raw materials of 35 plant species, of which anthracene derivatives (56), flavonoids (57) and tannins (31) were found in the largest amount (Li, 2022, pp. 1–29). *Rumex acetosa* L. (*R. acetosa*), *Rumex japonicas* Houtt. *(R. japonicas)*, *Rumex nepalensis* Spreng. (*R. nepalensis*) and *Rumex patientia* L. (*R. patientia*) are the most studied in terms of quality composition (Li, 2022, pp. 1–29).

Underground organs and leaves of *R. acetosa*, *Rumex dentatus* L. (*R. dentatus)*, *Rumex vesicarius* Linn. (*R. vesicarius*,) *R. japonicus*, *R. nepalensis*, *Rumex crispus* L. (*R. crispus*) and *Rumex hanus by* (*R. hanus)* are widely used in medicine as antioxidant, anti-inflammatory, antimicrobial, antifungal, hepatoprotective, antiplatelet, hypotensive, cardioprotective and anticancer agents (Kim, 2022; Mohammadhosseinpour, 2023; Nguengang, 2023; Piao, 2022; Jeong, 2020; Khan, 2022; Riffat, 2019). This plant activity is due to the multicomponent chemical composition: compounds of phenolic nature, organic and fatty acids, vitamins and minerals.

Despite on such a well-studied qualitative composition of the plants of the genus p-coumaric, oxalic and tartaric acids were identified from the organic acids in the plant raw material of *R. acetosa*, succinic – in *R. dentatus* and *R. nepalensis*, ferulic – in *R. nepalensis*, caffeic – in *Rumex confertus* Willd. (*R. confertus*) (Li, 2022; Khaliq, 2023; Prakash Mishra, 2018).

Our attention was paid to *R. confertus* whose underground organs are used in scientific (monographs are included in some pharmacopoeias) and folk medicine as an antitumor, anti-inflammatory and antimicrobial agent (Prakash Mishra, 2018; Oproshanska, 2023). In addition, *R. confertus* is an invasive species that is widely distributed on the territory of Ukraine.

There are data from the last century about the content of the amount of organic acids in the underground organs of *R. confertus* which depend on the harvesting period (up to 3%). Quantitative content of total oxalic acid (up to 13%) and free acid (up to 0.77%) was determined by the modified volumetric method (Benzel, 1995; Kos I. O., 1999). We did not detect any information about the identification and quantitative content of fatty acids in plant raw materials of *R. confertus* or other plants of the genus *Rumex* at all. Therefore, the study of the qualitative composition of organic including fatty acids in the rhizomes with roots of *R. confertus* and the determination of the quantitative content of the amount of organic acids in the series of plant raw materials is actual.

The aim is to study the component composition of organic including fatty acids in rhizomes with roots of *R. confertus* and to determine the quantitative content of the amount of organic acids in the series of plant raw materials.

Materials and methods. Underground organs of *R. confertus* were used for the research. Plant raw materials were harvested in 2015 in Kharkiv region to study component composition of organic including fatty acids and in 2019 in Vinnytsia (series 1), Ternopil (series 2), Kharkiv (series 3), Poltava (series 4), and Khmelnytskyi (series 5) regions to determine quantitative content of the amount of organic acids. The component composition of organic including fatty acids was studied by gas chromatography-mass spectrometry (GC/MS). Methyl ethers were obtained for identification and quantification of components (Carrapiso, 2000, pp. 1167–1177).

The quantitative content of the amount of organic acids was determined according to the methodology in the monograph «Rose fruits^{N}» of the State Pharmacopoeia of Ukraine 2.1 (SPhU). (Derzhavna Farmakopeia Ukrainy, 2016). Validation of the quantification method and statistical processing of the results were carried out in accordance to the requirements of the SPhU 2.0 (Derzhavna Farmakopeia Ukrainy, 2015).

Research results and their discussion. For the first time, the component composition of organic acids including fatty acids was determined in rhizomes with roots of *R. confertus* by the GC/MS method. The results of the study are given in table 1, a sample of GC/MS chromatogram of compounds – in fig. 1.

6 organic and 10 fatty acids were identified in *R. confertus* plant raw materials and the quantitative content of each of them was determined. Oxalic acid has the highest content from the identified organic acids (8335.9±21.8 μg/g) which is 64% of the total content of identified organic acids. Also, the plant raw material contains a significant amount of citric and malic acids (2047.4±1.4 μg/g and 1978.4±2.4 μg/g, respectively). The smallest content is characteristic of *para*-coumaric acid (32.6±0.6 μg/g).

4 unsaturated and 6 saturated fatty acids were identified in underground organs of *R. confertus*. At the same time, the content of unsaturated fatty acids is 2 times higher than the content of saturated fatty acids. Of the unsaturated fatty acids, the content of linolenic (5684.9±2.6 μg/g) and linoleic acid (2875.6±1.7 μg/g)

dominated, which accounted for 60% and 30% respectively of the amount of unsaturated fatty acids. The quantitative content of linolenic acid is 2 times higher than that of linoleic acid. From saturated fatty acids in rhizomes with roots of *R. confertus* the content of palmitic acid is dominated 3145.3±2.0 μg/g which is 75% of the amount of saturated fatty acids. The lowest content was established for cerotic acid (58.5±0.4 μg/g).

Table 1

The component composition of organic including fatty acids in rhizomes with roots of *R. confertus*, (n=5, μg/g)

No	The name of acid	Quantitative content
Organic acids		
1	Oxalic	8335.9±21.8
2	Malonic	241.9±1.4
3	Succinic	314.7±1.0
4	Malic	1979.4±2.4
5	Citric	2047.4±1.4
6	para-coumaric	32.6±0.6
Fatty acids		
7	Palmitic	3145.3±2.0
8	palmitoleic	537.2±1.1
9	Margaric	100.4±0.6
10	Stearic	587.7±0.6
11	oleic	358.4±1.0
12	linoleic	2875.6±1.7
13	linolenic	5684.9±2.6
14	arachinic	113.2±0.3
15	lignoceric	174.5±0.6
16	cerotic	58.5±0.4

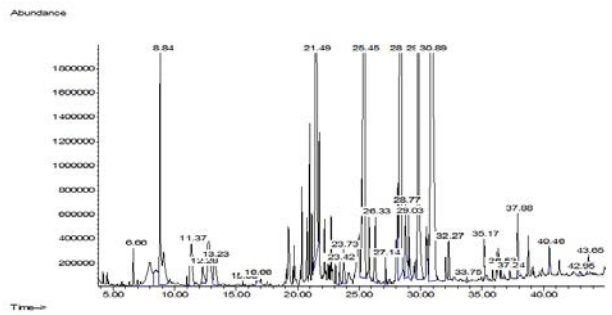

Fig. 1. GC/MS chromatogram of organic including fatty acids of rhizomes with roots of *R. confertus* (sample)

Underground organs of *R. confertus* contain the highest content of oxalic acid from the identified organic acids therefore it is advisable to convert to oxalic acid when quantifying the amount of organic acids in the plant raw material (according to the methodology in monograph «Rose fruits[N]» of the SPhU 2.1 the conversion is carried out to malic acid) (Derzhavna Farmakopeia Ukrainy, 2016). The conversion factor for oxalic acid is 0.0045.

The results of quantitative determination of the amount of organic acids in series of rhizomes with roots of *R. confertus* are shown in fig. 2.

As evidenced by the data of fig. 2 the quantitative content of the amount of organic acids in different series of underground organs of *R. confertus* fluctuated slightly and amounted to at least 2.3%.

The method from the monograph of «Rose fruits[N]» was used for the quantitative determination of the amount of organic acids, but it was applied for a new type of plant raw material with the use of a new compound for calculation – oxalic acid. Therefore, according to the recommendation of SPhU 2.0, it requires experimental confirmation for the correctness of the obtained data by studying validation characteristics (Derzhavna Farmakopeia Ukrainy, 2015). According to the recommendations for the validation of volumetric titration methods, such validation characteristics as linearity, correctness and precision are determined (Derzhavna Farmakopeia Ukrainy, 2015).

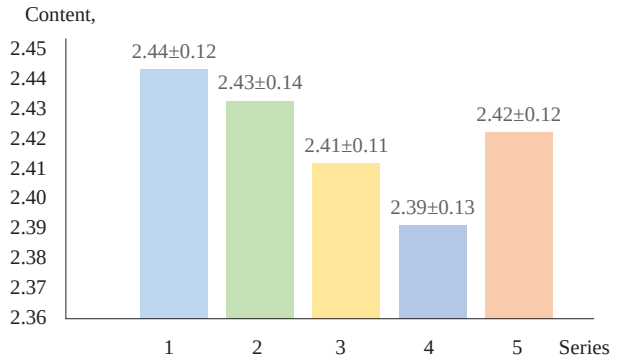

Fig. 2. Quantitative content of the amount of organic acids in different series of rhizomes with roots of *R. confertus* in terms of oxalic acid (%, n=5)

Determination of validation characteristics was carried out according to normalized coordinates and universal acceptance criteria. 5 samples were prepared for the study in which the amount of organic acids is evenly distributed in the range of the method: 80%, 90%, 100%, 110%, 120%. 3 parallel titrations were performed for each concentration to determine the precision (table 2). The uncertainty of the analysis results expressed as a one-sided relative confidence interval for the level of confidence probability of 95% should not exceed 6.4% (Derzhavna Farmakopeia Ukrainy, 2015). On the basis of the obtained data, the parameters of linear dependence (table 3), correctness and accuracy (table 4) were calculated.

Table 2

Validation tests for determining normalized coordinates and calculating linear dependence

The weight of plant raw materials	Xi fact, %	V 0,1 M solution of NaOH, ml	Amount of organic acids, %	Yi found, %	Relationship found / entered Zi, %	Linear dependence
0.9142	80	0.95	2.36	79.17	98.96	80.67
	80	0.97	2.41	80.83	101.04	
	80	0.97	2.41	80.83	101.04	
1.0254	90	1.1	2.43	91.67	101.86	90.67
	90	1.09	2.41	90.83	100.92	
	90	1.09	2.41	90.83	100.92	
1.1391	100	1.21	2.41	100.83	100.83	100.67
	100	1.23	2.45	102.5	102.5	
	100	1.22	2.43	101.67	101.67	
1.2534	110	1.31	2.37	109.17	99.25	110.67
	110	1.3	2.35	108.33	98.46	
	110	1.31	2.37	109.17	99.25	
1.3672	120	1.46	2.42	121.67	101.39	120.670
	120	1.45	2.41	120.83	100.69	
	120	1.46	2.42	121.67	101.39	

Table 3

Parameters of the linear dependence of the method of quantitative determination of the amount of organic acids in the rhizomes with roots of *R. confertus* in terms of oxalic acid

Validation characteristic	The resulting value
A	0.66
S_a	2.27
Criterion of statistical insignificance	0.66≤4.91
B	1.00067
S_b	0.02
S_0	1.23
Requirements to residual standard deviation	1.23≤3.82
R	1.000067
Requirements for the correlation coefficient	1.000067≥0.9965

Table 4

The results of the study of the correctness and precision of the method of quantitative determination of the amount of organic acids in the rhizomes with roots of *R. confertus* in terms of oxalic acid

Validation characteristic	Resulting value
Average ratio found / entered $Z_{average}$, %	100.68
Relative standard deviation, Sz,%	1.2794
One-sided confidence interval, Δz, %	2.08
Critical value for convergence of results ⅓Δas, %	2.13
Systematic error δ	0.68
Criterion of statistical insignificance	1.00
Criterion of practical acceptability	4.27

The obtained results indicate that the method meets all the requirements for parameters of linear dependence.

The method is correct, because the criteria of statistical insignificance and practical acceptability are greater than the systematic error and precision (one-sided confidence interval is less than the critical value for the convergence of results ⅓Δas).

Conclusions

1. For the first time, the component composition of organic (at least 6 compounds) and fatty acids (at least 10) was determined in rhizomes with roots of *R. confertus* with using the GC/MS method. The highest quantitative content is characteristic of oxalic acid (8335.9±21.8 μg/g).

2. For the first time for rhizomes with roots *R. confertus* modified the pharmacopoeial methodology (monograph «Rosehip Fruits» SPhU 2.1): determination of the quantitative content of the amount of organic acids was carried out in conversion not to malic, but to oxalic acid.

3. To confirm the accuracy and correctness of the method its validation characteristics were determined for the first time: linearity, precision, and its correctness was proven. The method was tested on 5 series of *R. confertus* plant raw materials.

4. The obtained results were applied in the development of the project of quality control methods «Horse sorrel rhizome with roots» and will be used in the future in the standardization of phytoremedies based on rhizomes with roots of *R. confertus*.

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