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## **STUDY OF CARBONIC ACIDS OF ROOTS *COMARUM PALUSTRE* L.**

**Actuality.** According to the literature, the underground organs of *Comarum palustre* L. contain organic acids, and there is no information about their component composition.

**The purpose of the study** is to study the component composition of organic acids in the roots of *Comarum palustre* L. and determine the quantitative content of the sum of organic acids in 7 series of raw materials present on the pharmaceutical market of Ukraine.

**Material and methods.** Plant raw materials for research were purchased in the spring of 2024 in online markets of Ukraine specializing in the sale of medicinal plant raw materials: series 1 – “Chlorophytum”, series 2 – “Sednivski travy”, series 3 – “Svit trav”, series 4 – TM “Organic herbs”, series 5 – “PbayMarket”, series 6 – “Narod pharma”, series 7 – “Dary Karpat”. The component composition of organic acids was studied by gas chromatography-mass spectrometry. The quantitative content of the sum of organic acids was determined by the method of the monograph of the State Pharmacopoeia of Ukraine 2.1 “Rosehips Fruits<sup>N</sup>”.

**Research results.** In the roots of *Comarum palustre*, for the first time, the presence of 6 dicarboxylic acids was established by the method of chromatography-mass spectrometry and the quantitative content of each of them was determined. Their total content was 1462.49 µg/g. Three carboxylic acids were dominant in content: levulinic (almost 47% of the total content of identified acids), citric and malic acids (28.49% and 15.41%, respectively).

For the first time, in 7 batches of raw materials purchased in Ukraine, the quantitative content of the sum of organic acids in terms of malic acid was determined in the roots of *Comarum palustre*, using the modified method of the monograph of the State Pharmacopoeia of Ukraine 2.1 “Rosehips Fruits<sup>N</sup>”. The quantitative content of organic acids varies more than 1.2 times – from 1.98% (series 1) to 2.41% (series 6). The validation characteristics of the method were established for the first time and tested on five batches of raw materials.

**Conclusions.** For the first time, the component composition of organic acids was established in the roots of *Comarum palustre* by the method of gas chromatography-mass spectrometry. For the first time, the content of organic acids in 7 series of *Comarum palustre* roots purchased in specialized marketplaces of Ukraine was determined. The obtained data will be used in further pharmacognostic research of *Comarum palustre* roots.

**Key words:** *Comarum palustre* L., roots, series, organic acids, component composition, quantitative content, gas chromatography-mass spectrometry.

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## ДОСЛІДЖЕННЯ КАРБОНОВИХ КИСЛОТ КОРЕНІВ *COMARUM PALUSTRE* L.

**Актуальність.** За даними літератури, підземні органи *Comarum palustre* L. містять органічні кислоти, а інформація про їх компонентний склад відсутня.

**Мета дослідження.** Вивчити компонентний склад органічних у коренях *Comarum palustre* L. та визначити кількісний вміст суми органічних кислот у 7 серіях сировини, яка присутня на фармацевтичному ринку України.

**Матеріали та методи.** Рослинну сировину для дослідження було придбано навесні 2024 року в internet-маркетах України, які спеціалізуються на продажу лікарської рослинної сировини: серія 1 – «Хлорофітум», серія 2 – «Седнівські трави», серія 3 – «Світ трав», серія 4 – TM Organic herbs, серія 5 – PbayMarket, серія 6 – «Народ фарма», серія 7 – «Дари Карпат». Компонентний склад органічних кислот вивчали методом газової хромато-мас-спектрометрії. Кількісний вміст суми органічних кислот визначали за методикою монографії ДФУ 2.1 «Шипшини плоди».

**Результати дослідження.** У коренях *Comarum palustre* вперше методом хромато-мас-спектрометрії встановлено присутність 6 дикарбонових кислот та визначено кількісний вміст кожної з них. Їх загальний вміст становив 1462,49 мкг/г. Домінуючими за вмістом виявилися три карбонові кислоти: левулінова (майже 47% від загального вмісту ідентифікованих кислот), лимонна та яблучна кислоти (28,49% та 15,41% відповідно).

Уперше в 7 серіях придбаної в Україні сировини, коренях *Comarum palustre*, визначено кількісний вміст суми органічних кислот у перерахунку на яблучну за модифікованою методикою монографії ДФУ 2.1 «Шипшини плоди»<sup>1</sup>. Кількісний вміст органічних кислот коливається більше ніж у 1,2 раза – від 1,98% (серія 1) до 2,41% (серія 6). Уперше встановлено валідаційні характеристики методики її апробовано її на 7 яких серіях сировини.

**Висновки.** Уперше методом газової хромато-мас-спектрометрії в коренях *Comarum palustre* L. встановлено компонентний склад органічних кислот. Уперше визначено вміст органічних кислот 7 серій коренів *Comarum palustre*, придбаних у спеціалізованих маркетплейсах України. Отримані дані будуть використані в подальшому фармакогностичному дослідженням коренів *Comarum palustre*.

**Ключові слова:** *Comarum palustre* L., корені, серії, органічні кислоти, компонентний склад, кількісний вміст, газова хромато-мас-спектрометрія.

**Introduction.** Marsh cinquefoil or Potentilla marsh (*Comarum palustre L. = Potentilla palustris (L.) Scop.*) is a perennial herbaceous plant, a member of the Rosaceae family. Its distribution area includes the USA, Canada, Mongolia, Japan, China and Europe (Who plant list, 2024). In Ukraine, it grows mainly in sphagnum bogs, along river banks, and in wet meadows in Polissya (World Flora Online, 2025; Roslynny Ukrayny, 2011).

Roots produce gallic, ellagic, quinic acid and their derivatives, condensed tannins, flavophenes, flavonoids (kaempferol derivatives, quercetin, catechin, anthocyanins), hydroxycinnamic acids, essential oil and polysaccharides (Serafin, 2022).

Experimental studies have proven that extracts from the grass and roots of the *Comarum palustre* have astringent, anti-inflammatory, antiseptic, antiexudative, antioxidant, hepatoprotective, desensitizing, nephroprotective, diuretic effects, and accelerate epithelial healing (Serafin, 2022; Garna, 2016). Root-based medicines are mainly used to treat inflammatory diseases of the musculoskeletal system, relieve pain in joints and tissues, treat dislocations and sprains, as well as for general strengthening of the body and are extremely popular in Ukraine.

But regarding organic acids, we found information only about their presence in the raw materials and the total quantitative content (Serafin, 2022). We did not find data on the component composition of this group of compounds in the available literature. Organic acids participate in the regulation of acid-base balance, contribute to improving digestion, and have antibacterial, antioxidant, and tonic properties (Smoljovska, 2022). Their significant content in medicinal plant raw materials emphasizes its multifaceted therapeutic effect.

Therefore, establishing the qualitative composition of organic acids in the roots of the *Comarum palustre* and determining the quantitative content in series of this type of raw material available on the pharmaceutical market of Ukraine is relevant.

**The purpose of the work** is to study the component composition and establish the quantitative content of organic acids in the roots of the *Comarum palustre*.

**Material and methods of research.** The roots of the *Comarum palustre* were chosen as the objects of research. Plant raw materials for research were purchased in the spring of 2024 in Ukrainian online markets specializing in the sale of medicinal plant raw materials: series 1 – “Chlorophytum”, series 2 – “Sednivski travy”, series 3 – “Svit trav”, series 4 – TM “Organic herbs”, series 5 – “PbayMarket”, series 6 – “Narod pharma”, series 7 – “Dary Karpaty”.

*Extraction of organic acids.* Extraction of organic acids was carried out from a portion of plant raw mate-

rials (0.06–0.2 g) by adding 1.0 ml of methyl alcohol and 1.0 ml of 0.5 N hydrochloric acid, the mixture was thoroughly mixed. Extraction was carried out in an ultrasonic bath at 45 °C for 3 h. After the end of extraction, the mixture was centrifuged at 3000 rpm for 5 min. An aliquot of the extract (1000 µl) was evaporated to dryness on a rotary evaporator at 40 °C.

To the dry residue were added 600 µl of methyl alcohol and 300 µl of 50% sulfuric acid, mixed thoroughly. Methylation of organic acids was carried out overnight at a temperature of 60 °C. After methylation, the mixture was cooled to room temperature, 500 µl of chloroform and 500 µl of 6.0% potassium carbonate solution were added, mixed thoroughly. The chloroform phase was used for chromatographic analysis.

*Chromatography conditions.* Chromatographic separation was performed on an Agilent 6890N/5973 insert gas chromatography-mass spectrometry system (Agilent technologies, USA) (Agius, 2018; Marchyshyn, 2021). Capillary column HP-5ms (30 m × 0.25 mm × 0.25 mkm, Agilent technologies, USA). Evaporator temperature 250 °C, interface temperature 280 °C. Separation was performed in temperature programming mode – the initial temperature of 70 °C was maintained for 1 min, raised with a gradient of 5 °C/min to 220 °C, maintained for 1 min, raised with a gradient of 10 °C/min to 300 °C. The final temperature was maintained for 5 min. A sample volume of 1 µl was introduced in the flow splitting mode 1:50. Detection was performed in SCAN mode in the range (38–400 m/z). The carrier gas flow rate through the column was 1.0 ml/min. (Budniak, 2021; Slobodianik, 2021).

*Acid identification.* Standard pharmacopoeial samples of organic acids from Sigma-Aldrich Chemical Company (St. Louis, MO, USA) with analytical purity ≥95% were used for compound identification. Organic acids were identified by comparing retention times of standards (oxalic, maleic, succinic, malic, alpha-keto-glutaric, citric, and isocitric acids) and the NIST database 17 (Budniak, 2021).

Statistical processing of the results was carried out in accordance with the requirements of the State Pharmacopoeia of Ukraine 2.0 5.3.N.1 “Statistical analysis of the results of chemical experiment N” using the program “SPSS Statistics 26.0”. The non-parametric Mann-Whitney test was used, and when comparing statistical indicators, a significance level of  $p < 0.05$  was adopted (Derzhavna Farmakopeia Ukrayny, 2015).

The quantitative content of the sum of organic acids in the raw material batches was determined by the titrimetric method according to the method of the monograph of the State Pharmacopoeia of Ukraine 2.0

"Rosehip Fruits" in terms of malic acid (Derzhavna Farmakopeia Ukrayny, 2016). For the correctness of the obtained results, it is necessary to validate the method. For this, the validation characteristics were determined: linearity, accuracy and precision (Derzhavna Farmakopeia Ukrayny, 2015).

**Research results and their discussion.** The obtained results of the chromatographic-mass spectrometric analysis of the component composition and content of individual carboxylic acids in the roots of the *Comarum palustre* (series 5) are presented in Table 1 and Fig. 1.

As can be seen from Table 1, the presence of 6 dicarboxylic acids was established in the roots *Comarum palustre*. Their total content was 1462.49 µg/g. The dominant in terms of content were three carboxylic acids: levulinic (almost 47% of the total content of identified acids), citric and malic acids (28.49% and 15.41%, respectively). The remaining acids are present in insignificant amounts.

Of particular interest is the presence of levulinic acid, which is widely used in pharmacy as an antiseptic and preservative, acidity regulator. In cosmetology, it is added to creams, ointments and serums as a moisturizing and emollient ingredient. Levulinic acid and its derivatives are used in the synthesis of anti-inflammatory and analgesic drugs (Bhardwaj, 2024). Therefore, the identification of this acid in the raw material to some extent explains its anti-inflammatory activity. It is also used to

Table 1  
Component composition of organic acids of roots  
of *Comarum palustre* (n = 5)

№	Holding time, min	Compound	Content, µg/g	Content, % of total quantity
1	3.06	Oxalic acid	30.32 ± 0.6	2.07
2	4.34	Malonic acid	37.58 ± 0.7	2.57
3	5.30	Levulinic acid	686.15 ± 11.2	46.92
4	6.39	Succinic acid	66.39 ± 1.5	4.54
5	8.64	Malic acid	225.44 ± 8.4	15.41
6	18.15	Citric acid	416.62 ± 9.3	28.49

produce gamma-aminolevulinic acid, which is used in photodynamic therapy of cancer (Liao, 2024).

The results of titrimetric quantitative determination of the amount of organic acids in the roots of the *Comarum palustre* of different series are given in Table 2. As can be seen from Table 2, the quantitative content of organic acids differs significantly – from 1.98% (series 1) to 2.41% (series 6), which is a difference of almost more than 1.2 times. Such a difference in content can be explained primarily by the origin of the raw material, the conditions of procurement and storage throughout the entire shelf life. However, for all studied samples of raw materials, the content of organic acids is at a sufficiently high level – not less than 1.9%.

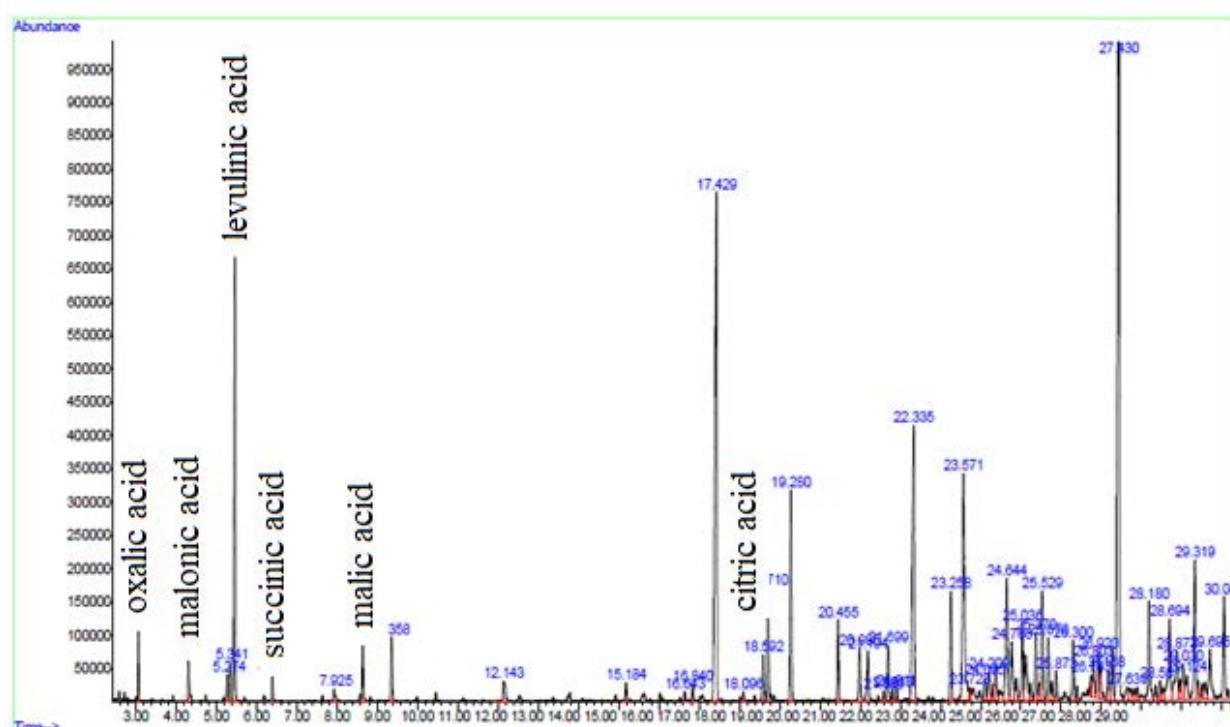


Fig. 1. GC/MS chromatogram of organic acids of *Comarum palustre* L. roots (example)

The determination of validation characteristics was carried out in accordance with the requirements of the State Pharmacopoeia of Ukraine 2.0, Vol. 1, using normalized coordinates and universal acceptance criteria. For the study, 5 samples were prepared, in which the amount of the sum of organic acids was evenly distributed in the range of the method: 80%, 90%, 100%, 110%, 120%. To determine the precision, 3 parallel titrations were performed for each concentration (Table 3). The uncertainty of the analysis results is expressed as a one-sided relative confidence interval for a confidence level of 95%, and should not exceed 6.4% (SPhU 2.0, Vol. 1). Based on the data obtained, the parameters of linear dependence (Table 4), accuracy and precision (Table 5) were calculated.

Table 2

**Quantitative content of the sum of organic acids in the studied series of *Comarum palustre* ( $n = 5$ , calculated as malic acid and dry matter)**

№ series	Producer	Contents, %
1	Chlorophytum	$1.98 \pm 0.06$
2	Sednivski travy	$2.20 \pm 0.007$
3	Svit trav	$2.13 \pm 0.06$
4	TM Organic herbs	$2.24 \pm 0.07$
5	PbayMarket	$2.41 \pm 0.05$
6	Narod pharma	$2.07 \pm 0.06$
7	Dary Karpaty	$2.12 \pm 0.06$

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

The methodology is correct because the criteria of statistical insignificance and practical acceptability are

Table 3

**Validation tests to determine normalized coordinates and calculate linear dependence**

Weight of the sample	Xi fact, %	V 0,1 M solution NaOH, ml	Sum of organic acids, %	Yi found, %	Relationship found / conducted Zi, %	Linear dependence
10.1064	80	1.25	2.07	80.13	100.16	79.70
	80	1.24	2.06	79.49	99.36	
	80	1.24	2.06	79.49	99.36	
11.3682	90	1.4	2.06	89.74	99.72	90.17
	90	1.41	2.08	90.39	100.44	
	90	1.41	2.08	90.39	100.44	
12.6401	100	1.56	2.07	100	99.94	100.32
	100	1.57	2.08	100.48	100.42	
	100	1.57	2.08	100.48	100.42	
13.9014	110	1.72	2.07	110.26	100.18	109.83
	110	1.71	2.06	109.62	99.6	
	110	1.71	2.06	109.62	99.6	
15.1609	120	1.89	2.09	121.15	100.94	120.94
	120	1.88	2.08	120.51	100.4	
	120	1.89	2.09	121.15	100.94	

Table 4

**Parameters of the linear dependence of the method for quantitative determination of the amount of organic acids in the roots of the *Comarum palustre***

Validation characteristic	Resulting value
A	-1.63
$S_a$	0.73
Criterion of statistical non-significance	$-1.63 \leq 4.91$
B	1.01913
$S_b$	0.007
$S_0$	0.39
Residual standard deviation requirements	$0.39 \leq 3.82$
R	0.9997
Correlation coefficient requirements	$0.9997 \geq 0.9965$

Table 5

**Results of the study of the correctness and precision of the method for quantitative determination of the amount of organic acids in the roots of the *Comarum palustre* in terms of malic acid**

Validation characteristic	Resulting value
Average ratio found/entered, $Z_{\text{average}}$ , %	100.13
Relative standard deviation, $S_z$ , %	0.51
One-sided confidence interval, $\Delta z$ , %	0.91
Critical value for convergence of results $\frac{1}{3}\Delta as$ , %	2.13
Systematic error $\delta$	0.13
Criterion of statistical non-significance	0.23
Practical Acceptability Criterion	4.27

greater than the systematic error and precision (one-sided confidence interval is less than the critical value for the convergence of the results  $\frac{1}{2}\Delta\alpha$ ).

The obtained data are the first stage in a comprehensive phytochemical study of the medicinal plant raw materials of the *Comarum palustre* available on the pharmaceutical market of Ukraine and allow for further comparative research of the composition and content of organic acids in the roots in samples of raw materials from cultivated and wild *Comarum palustre* plants in Ukraine.

## Conclusions

**1. For the first time, 7 series of roots of the *Comarum palustre* purchased in specialized market-places of Ukraine were studied.**

**2. For the first time, 6 carboxylic acids were identified in the roots of the *Comarum palustre* by the method of chromatography-mass spectrometry. Their total content was 1462.49 µg/g.**

**3. It was established that the following acids are significantly dominant in terms of content (µg/g): levulinic ( $686.15 \pm 11.2$ ), citric ( $416.62 \pm 9.3$ ), malic ( $225.44 \pm 8.4$ ) acids.**

**4. By the titrimetric method, in terms of malic acid, the content of the sum of organic acids in 7 series of raw materials was determined. The quantitative content is within 1.98–2.41%.**

**5. The obtained data will be used in further pharmacognostic research of the roots of the *Comarum palustre*.**

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**Authors' contribution:**

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**Khvorost O.P.** – idea, research design, conclusions, summary, correction of the article;  
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