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# STUDY OF QUALITATIVE COMPOSITION AND QUANTITATIVE CONTENT OF FREE ORGANIC ACIDS IN LINGBERRY LEAVES

Actuality. Organic acids are aliphatic and aromatic compounds with carboxyl group that play crucial roles in plant metabolism. It's worth noting that all plant species, regardless of their family, contain varying amounts of organic acids, which are biologically active compounds.

Aim – to identify and quantify free organic acids in lingberry leaves (Vaccinium vitis-idaea L.).

# Біологія. Фармація

Materials and methods. Grinded dry lingberry leaves were used for the study. Thin layer chromatography (TLC) was employed to detect the organic acids, while the alkalimetric method was used for their quantification.

Results and discussion. A TLC technique was utilized to identify the presence of oxalic, citric, malic and tartaric acids. The total content of free organic acids is 2.03±0.04%, 2.08±0.01% u 3.62±0.13% for potentiometric, conductometric and indicator titration methods, respectively.

Conclusions. Qualitative analysis and determination of the quantitative content of organic acids in lingberry leaves were performed. The possibility of using conductometric and potentiometric titration methods in preliminary studies for the quantitative analysis of free organic acids in the studied raw materials is shown, and their advantages over the indicator titration method are shown.

**Key words:** organic acids, lingberry leaves, alkalimetric titration, TLC, analysis.

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## ДОСЛІДЖЕННЯ ЯКІСНОГО СКЛАДУ ТА КІЛЬКІСНОГО ВМІСТУ ВІЛЬНИХ ОРГАНІЧНИХ КИСЛОТ У ЛИСТІ БРУСНИЦІ

**Актуальність.** Органічні кислоти— це аліфатичні та ароматичні сполуки з карбоксильними групами, які відіграють вирішальну роль у метаболізмі рослин. Варто відзначити, що всі види рослин, незалежно від родини, містять різну кількість органічних кислот, які є біологічно активними сполуками.

**Мета дослідження** — провести ідентифікацію та визначити кількісний вміст органічних кислот у листі брусниці (Vaccinium vitis-idaea L.).

Матеріали та методи. Для дослідження використовували подрібнене сухе листя брусниці. Для виявлення органічних кислот використовували тонкошарову хроматографію (ТШХ), а для їх кількісного визначення – алкаліметричний метод.

**Результати та їх обговорення.** Метод ТШХ використовувався для виявлення оксалатної, лимонної, яблучної та винної кислот. Вміст суми вільних органічних кислот становить  $2,03\pm0,04\%$ ,  $2,08\pm0,01\%$  і  $3,62\pm0,13\%$  для потенціометричного, кондуктометричного та індикаторного методів титрування відповідно.

**Висновки.** Проведено якісний аналіз та визначено кількісний вміст вільних органічних кислот у листі брусниці. Показано можливість використання кондуктометричного та потенціометричного титрування для кількісного аналізу вмісту вільних органічних кислот у досліджуваній сировині та показано їхні переваги перед індикаторним методом титрування.

Ключові слова: органічні кислоти, листя брусниці, алкаліметричне титрування, ТШХ, аналіз.

Introduction. Organic acids are biologically active substances that can have an aromatic or aliphatic structure containing one or more carboxyl groups (Maslov, 2021, pp. 304–311). In the biochemical processes of mammals, organic acids have a significant part in the Krebs cycle for the formation of the main source of cell energy, adenosine triphosphate (Umarov, 2020, pp. 3874–3883). Also, organic acids are involved in the biochemical metabolism of plants. They are involved in the mechanisms of resistance and adaptation of plants to the action of heavy metals (Panchal, 2021, pp. 4038–4052). At present, organic acids are applied in the food and pharmaceutical industries as preservatives and pH regulators (Maslov, 2020, pp. 53–58).

Organic acids have antioxidant (Quiroga, 2019, pp. 267–272), anti-inflammatory (Kuda, 2016, pp. 2580–2590), antimicrobial (Shrivanova, 2007, pp. 70–72) and immunomodulatory activity (Umarov, 2020, pp. 3874–3883). In addition, they create favorable conditions for the vital activity of intestinal microorganisms (Maslov, 2020, pp. 53–58).

Lingonberry is a low (up to 20 cm) shrub of the lingonberry family, with a creeping, thin rhizome. The leaves are alternate, elliptical. On the underside of the leaf there are black punctate glands. The flowers are white or pink, collected in dense, drooping racemes. The fruits are red, round in shape, fruit type – polyspermous berries. It grows mainly in deciduous and coniferous forests, forming in places continuous thickets (Bujor, 2018, pp. 356–365).

Lingonberry leaves are rich sources of hydroquinone derivatives, flavonols, phenylcarboxylic acids, and flavan-3-ols (Komisarenko, 2012, pp. 24–26). Lingonberry leaves not only contain phenolic compounds, but also organic acids. According to the literature data, the chemical composition of lingonberry leaves is represented by the following organic acids: malic, citric, benzoic, lactic and salicylic acids (Komisarenko, 2014, pp. 291–295).

An analysis of recent studies has shown that ion-exchange (GU, 2014, pp. 204), gas-liquid (Umarov, 2020, pp. 56–58) and high-performance chromatography (Mortera, 2018, pp. 15–23) and capillary electrophoresis (Nogueira, 2011, pp. 267–272) are often applied for the identification and quantitative analysis of organic acids. There is no doubt that chromatographic methods are accurate and reliable, but chromatographic methods require expensive equipment and reagents. Thus, we will use potentiometric, conductometric and indicator titration methods.

# Materials and methods.

### Plant material

The lingonberry leaves (*Vaccinum vitis-idaea L.*) was collected in October 2021 in the Kostivtsi village, Zhutomyr region, Ukraine.

### Equipment

To measure the levels of free organic acids, a pH meter (Hanna 2550, FRG) equipped with a combined glass electrode (HI 1131P, FRG) and a conductometric electrode (76310, FRG) was utilized for titration. A microburette with class A accuracy was used for titration. The thin-layer chromatography plate Sorbfil ("PTSH-AF-A-UV") was used for detection organic acids.

## Reagents

Malic acid ( $\geq$  99.0%), citric acid ( $\geq$  99.0%), oxalic acid ( $\geq$  99.0%), tartaric acid ( $\geq$  99.0%) and sodium hydroxide ( $\geq$  98.0%) were analytical grade and purchased

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in Kharkiv Reachem (Ukraine). The distilled and bidistilled water were used as solvents for titration. All organic solvents were analytical grade.

## Preparation solutions

The standard solutions of malic, citric, oxalic and tartaric acids were prepared according to the following way: 250 mg of each acid was transferred in a separated measuring flasks with volume 25 mL and dissolved by 96% ethanol.

The solution of bromocresol green was prepared according to the following way: 100 mg of bromocresol green was transferred in measuring flask with volume 50 mL and dissolved by 96% ethanol.

A 0.05 mol/L NaOH solution was prepared as follows: 4.0 g (exact mass) NaOH was liquefied in distilled water and made up to 1 L with distilled water and standardized.

## Extraction procedure

5.0 g (exact mass) of dried leaves of lingonberry leaves were grinded in the 1-2 mm. The extraction of free organic acids was provided by distilled water on water bath in a flask with a reflux condenser and extracted at the ratio raw material/solvent 1/20 (m/v), during the 1 hour. The obtained extract was filtrated (State Pharmacopoeia of the USSR).

### Detection of free organic acids by TLC method

4.0 g of dry lingonberry leaves was mixed with 40.0 mL of distilled water and heated with a reflux condenser on a boiling water bath for 1 h. The obtained extract was filtered through a filter.

To detect organic acids, a mobile phase consisting of water ethyl acetate, glacial acetic acid, formic acid, and ethyl acetate (26/11/11/100) was employed. The test solu-

tions and standard solutions were spotted on the sample plates using a  $10~\mu L$  micro-pipette, with  $30~\mu L$  of each solution. Detection of organic acids was conducted under UV light at a wavelength of 254 nm. Finally, the dried plates were treated with a 0.2% solution of bromocresol green in 96% ethanol to determine the presence of organic acids.

## Quantitative analysis

An aliquot of 5.0 mL obtained extract was transferred in a 100 mL flask and 45 mL of freshly boiled distilled water (a bidistilled water was used in conductometric titration was added and titrated with 0.05 mol/L NaOH solution potentiometrically and conductometrically.

This equivalent volume was ascertained using a constructed differential curve plotted on the coordinates  $\Delta E/\Delta V - V$ . (Fig. 1).

The sum of free organic acids (X, %) in equivalent of citric acid in completely dry raw materials was found by the expression:

$$X(\%) = \frac{(V_{eq} - V_{x}) \cdot 0.0032 \cdot K \cdot 100 \cdot 100 \cdot 100}{m \cdot 5 \cdot (100 - W)},$$

where 0.0032 – the amount of citric acid, which is equivalent to NaOH solution, g/mL;  $V_{eq}$  is the equivalent volume of NaOH solution, mL;  $V_{x}$  – the blank volume of NaOH solution, mL; m – the mass of the raw materials, g; K – correction coefficient; W – the loss in mass upon drying, %.

The sum of free organic acids was also determined by conductometric and indicator titration methods. The method of obtaining the extract and the calculation of the sum of free organic acids were as described above. In the conductometric titration method, the equivalence

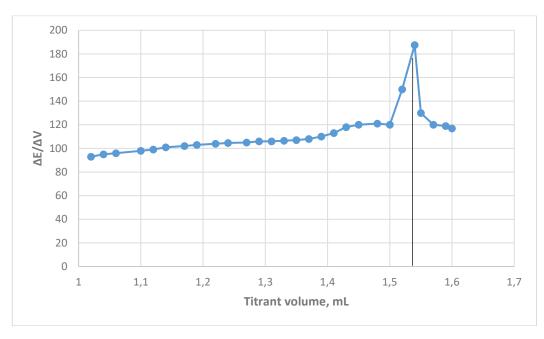


Fig. 1. The potentiometric titration curve

point was determined using the constructed integral titration curve in the coordinates  $\chi$ ,  $\mu S - V$  (Fig. 2) of the amount of free organic acids were as described above. In the conductometric titration method, the equivalence point was determined using the constructed integral titration curve in the coordinates  $\chi$ ,  $\mu S - V$  (Fig. 2).

The collected data was analyzed using Microsoft Excel 2010 and STATISTICA 6.0 software. The results were presented as the mean value  $\pm$  confidence interval, based on five measurements, with a significance level of p<0.05.

**Results and discussions.** Organic acids were identified by TLC in the mobile phase water/glacial acetic acid/formic acid/ethyl acetate (26/11/11/100). Under these conditions, organic acids are clearly separated. For the detection of organic acids, UV light at a wavelength of 254 nm was used. Dominant bands were observed on the chromatogram with Rf = 0.40 (succinic acid), Rf = 0.51 (citric acid), Rf = 0.58 (malic acid) and Rf = 0.30 (oxalic acid). During development of the chromatogram with a 0.2% bromcresol green solution, yellow bands were detected on a blue background with Rf = 0.40, Rf = 0.51, Rf = 0.58 and Rf = 0.30 (at the level of similar succinic, citric, malic and oxalic acids, respectively).

M.A. Komisarenko (Komisarenko et al., 2014, pp. 291–295) identified organic acids in lingonberry leaves by chromato-mass spectrometry with an esterification stage. A total of 34 organic acids were identified, of which 18 were fatty acids (caproic, lauric, myristic,

9-oxo-nonanoic, azelaic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, arachidic, 2-hydroxypalmitic, cheneicosanoic, behenic, tricosanoic, gentisic, tetracosanoic acids), 8 aromatic acids (phenylacetate, salicylic, benzoic, vanillic, m-hydroxybenzoic, p-coumaric, ferulic, α-furan acids), 5 saturated dicarboxylic acids (oxalic, malonic, succinic, 3-hydroxy-2-methylglutaric, malic acids), 1 saturated tricarboxylic acid (citric acid), 1 saturated oxy-monocarboxylic acid (levulinic acid) and 1 unsaturated dicarboxylic acid (fumaric acid). Compared to our results, we were only able to identify 4 organic acids. Fatty acids were not identified due to our study of water extraction, and the chosen chromatographic system was not capable of separating fatty acids, since the solvent system is polar.

According to the results shown in the table 1, the sum of free organic acids in equivalent of citric acid in lingonberry leaves was  $2.03\pm0.04\%$ ,  $2.08\pm0.01\%$  and  $3.62\pm0.13\%$  by potentiometric, conductometric and indicator titration methods, respectively. The results obtained showed the advantage of using the methods of conductometric and potentiometric titration in the quantitative analysis of organic 'acids due to their high sensitivity and accuracy. The relative error of conductometric and potentiometric titration did not exceed  $\pm1.83$  and  $\pm0.67\%$ , respectively, which is notably lower than the titration error when indicators are used, equal to  $\pm3.62\%$  (table 1).

M.A. Komisarenko (Komisarenko et al., 2014, pp. 291–295) found that lingonberry leaves contain

Table 1 Results of quantitative analysis of the total content of organic acids in lingonberry leaves

Titration method	$\overline{X}$	$S^2$	S	$S_{ar{ extbf{x}}}$	Δx	ε,%	$\bar{x} + \Delta x$
Potentiometry	2,03	0,00450	0,067	0,03	0,04	1,83	2,03±1,83
Conductometry	2,08	0,00062	0,025	0,01	0,01	0,67	2,08±0,67
with Indicator	3,62	0.05200	0.228	0.10	0.13	3,50	3,62±3,50

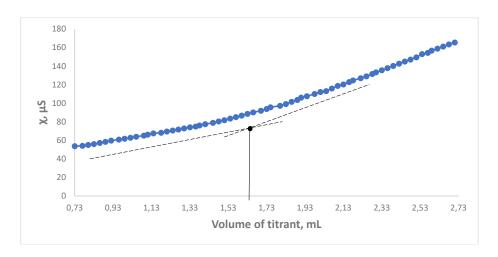


Fig 2. The conductometric titration curve

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2.43% organic acids. The main organic acid compounds identified were oleic acid (0.49%), palmitic acid (0.34%), linoleic acid (0.48%), linolenic acid (0.48%), stearic acid (0.16%), citric acid (0.11%), levulinic acid (0.09%), and p-coumaric acid (0.05%). Compared with our study results, the difference in sum organic acid content was 16.46%, 14.40% and 48.97% for potentiometric, conductometric and indicator titration methods. The difference in the values of the content of organic acids is quite significant, and especially in the case of the indicator method of titration. You need to understand that, firstly, in the studies of M.A. Komisarenko et al., methylene chloride was used for extractions; secondly, the extraction technique was also different. Thus, it is impossible to determine the content of each organic acid

by the titrimetric method, but the amount of organic acids can be determined quite accurately.

#### **Conclusions:**

- 1. A thin layer chromatography technique was utilized to identify the presence of oxalic, citric, malic and tartaric acids.
- 2. Quantitative content of the sum of free organic acids in lingonberry leaves was 2.03±0,04%, 2.08±0,01% and 3.62±0,13% for potentiometric, conductometric and indicator titration method, respectively.
- 3. The possibility of using the methods of conductometric and potentiometric titration in preliminary studies for the quantitative analysis of the sum of organic acids in lingonberry leaves and their advantages over the indicator titration method is shown.

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