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CONTENT OF ORGANIC ACIDS IN *PORTULACA OLERACEA* L. HERB

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Medicinal herbs are popular medicines because they are available, cheap, effective and low-toxic in most cases. People have been using them since ancient times to obtain medicines, food, insecticides, etc. One of these plants is Portulaca oleracea L. which is well known in folk medicine and widely used in food. Free organic acids are one of the important biologically active groups as a part of this plant, which are known for their numerous medicinal properties.

The purpose of this work is to study the content of the sum of free organic acids in the Green Purslane herb (Portulaca oleracea L.) using the pharmacopoeial procedure for determining the sum of organic acids in fresh viburnum berries, for which it is necessary to carry out qualitative confirmation of the content of free organic acids; to select the conditions and to develop a procedure for determining the sum of free organic acids as well as to provide the validation evaluation of the developed procedure in accordance with compendial requirements.

The scope of this study includes the qualitative and quantitative determination of the sum of organic acids in aqueous extracts from the Portulaca oleracea L. herb using the titration and TLC method; the main metrological characteristics are evaluated.

The procedures of qualitative and quantitative determination of the sum of organic acids in the Portulaca oleracea L. herb are proposed. The content of the sum of organic acids in the studied form of medicinal plant raw materials has been established. The relative error in determining the sum of organic acids is not higher than 5%. The content of the sum of organic acids in the Portulaca oleracea L. herb is at least 2%.

Conditions have been selected and methods have been developed for qualitative (using TLC) and quantitative (by titrometry) determination of the sum of organic acids in the Portulaca oleracea L. herb. The developed methods for determining the sum of organic acids in the Portulaca oleracea L. herb can be recommended for inclusion into the regulatory documentation for this type of raw material. The statistical characteristics obtained when determining repeatability correspond to the established criteria for the acceptability of the analytical procedure for determining the sum of free organic acids, which allows us to conclude that it is valid.

Keywords: standardization, titration, *Portulaca oleracea* L. herb, free organic acids, TLC

Green Purslane herb (*Portulaca oleracea* L.) is an herbaceous annual plant, which is a Eurasian species. It is found in Eastern and Western India, China, Japan, Ascension Island, as well as

in the British Isles [1]. On the territory of Russia it grows in the European part, the Caucasus, the Far East [2].

Since ancient times, the *Portulaca oleracea* L. herb was used for food in raw form in salads. The whole plant is edible. It is considered a valuable salad-spinach vegetable in most of Europe and Asia, in many parts of the United States, in developing countries. Over time, several different varieties of *portulaca* have been bred. It was noted that feeding of pets and birds with the leaves of *Portulaca oleracea* L. are useful for their immune system and as a prevention of diarrhea [3]. The widespread use of this plant in food can serve as a confirmation of the safety of its use. The possibility of cultivation will provide a sufficient raw material base.

Portulaca oleracea L. is used in folk medicine in many countries of the world as an antipyretic, antiseptic, anthelmintic agent and is included in the PRC SP [4,5]. It has a wide range of pharmacological activities, including antibacterial, anti-ulcer, anti-inflammatory, antioxidant and wound healing ones [6–10]. Russian scientists estimate it as a high vitamin plant [11].

Portulaca oleracea L. contains 3.5% lipids in terms of dry weight, 25% of which are free fatty acids. *Portulaca oleracea* L. is one of the richest sources of omega-3 polyunsaturated fatty acids at a level of 4 mg/g of wet weight [12–14]. When studying atherosclerosis, a proposal was made to use *Portulaca oleracea* L. as an alternative to fish oil in relation to omega-3 fatty acids [15]. *Portulaca* has an anti-atherosclerotic effect, increases blood clotting and lowers blood pressure [16]. According to other data, *Portulaca* contains nutrients in high percentages of recommended dietary intake such as alpha-linolenic acid, beta-carotene, tocopherol, magnesium and potassium [17–18].

Phenolic components, namely scopoletin, bergapten, isopimpinellin, lonchocarpic acid, lonchocarpenin, robustin and genistein, which have antimicrobial activity, were isolated from *Portulaca oleracea* [19].

Carotenoids are present in an amount of 89 mg/g. Beta-carotene is contained in significant amounts, but is lost up to 43% due to incorrect processing methods [20–21]. The level of a-tocopherol in the leaves of *Portulaca oleracea* L. is seven times higher than in spinach (1.71 mg per 100 g) [22]. Phylloquinone, or vitamin K1, is present in an amount of 381 mg per 100 g and is quite resistant to cooking [23].

A polysaccharide complex in the form of transparent and viscous mucus, having physicochemical properties suitable for industrial use as food fillers and thickeners, was extracted from the leaves of *Portulaca oleracea* L. It has been previously established that it is a neutral arabinogalactan and a polydisperse pectin-like polysaccharide [24]. We have identified a significant content of the sum of reducing sugars (at least 9%) in the Green Purslane herb [25].

Malic and citric acids, coumarins, flavonoids, alkaloids and saponins are also noted as ingredients of the Green Purslane (*Portulaca oleracea* L.) herb [26–27]. *Portulaca oleracea* L. contains 3-quinoline carboxylic acid, p-coumaric acid, ferulic acid, catechol, caffeic acid and oxalic acid [28–30]. The content of the sum of flavonoids in the aboveground part of *Portulaca oleracea* L. is at least 0.3% [31]. Rutin was found to be the main leaf flavonoid, and the highest myricetin content was in flowers and stems [32]. Both of these flavonoids are powerful antioxidants and have been found to have antimutagenic properties in laboratory studies [33].

The anti-inflammatory activity of *Portulaca oleracea* L. was confirmed in the Common Use Center (CUC) (REC) of the RUDN on the model of "acute formalin paw edema" in rats in comparison with carprofen. In more detail, the chemical composition, pharmacological properties and application of the Green Purslane herb were presented earlier [34].

Thus, *Portulaca oleracea* L. is a potentially valuable medicinal plant raw material with a sufficient raw material base. It is actively studied

abroad in various directions, which is due to the diversity of its chemical composition and the manifestation of a whole range of activities. However, this plant is not pharmacopoeial, there is no standardization of raw materials. The latter makes it much more difficult to obtain medicines based on this medicinal plant raw material. In this regard, it was interesting to study the chemical composition of the *Portulaca oleracea* L. herb for the purpose of its further standardization and introduction into medical practice in the Russian Federation and Syria. Previously, we studied the reducing sugars and flavonoids that provide anti-inflammatory, antioxidant and expectorative properties of the studied raw materials [25,31].

Organic acids are pharmacologically active substances (citric, nicotinic, ascorbic acids). They delay the growth of bacteria, exhibit anti-inflammatory properties and have a positive effect on functioning the gastrointestinal tract and other body systems [11,37]. Since the literature data indicate that the herb of the Green Purslane contains a significant amount of organic acids, their study in the herb of this type of plant was also interesting.

Volumetric, enzymatic, colorimetric, spectrophotometric, microfluorimetric, polarographic, chromatographic methods are used to determine the content of organic acids. One of the classical methods included in the SP is titration [35].

The purpose of this work is to study the content of the sum of free organic acids in the Green Purslane (*Portulaca oleracea* L.) herb using a Compendial Procedure for determining the sum of organic acids in fresh viburnum berries [36].

To achieve this purpose, it is necessary to provide qualitative confirmation of the content of free organic acids; selection of conditions and development of a procedure for determining the sum of free organic acids; validation evaluation of the developed procedure in accordance with compendial requirements.

MATERIALS AND METHODS

The object of the study was samples of the *Portulaca oleracea* L. herb harvested in different districts of the Voronezh region in the period from July to September and in Syria (Latakia province).

Water was used as an extractant for the extraction of acids from plant material [8].

In [9], a titer of 0.0067 was established that is the amount of malic acid corresponding to 1 ml of caustic soda solution (0.1 mol/l), in grams.

The following reference standards (RS) were used in the TLC research process:

- citric acid, substance – powder, citric acid content 99%, Sigma-Aldrich, USA, series 77-92-9, valid until 02/19/2022;
- oxalic acid, substance – powder, oxalic acid content 99%, chemically-pure, Himmed, Russia, series 392/10, valid until 02.03.2021.

Qualitative analysis procedure

TLC analysis was used to prove the presence of free organic acids. During the analysis, an aqueous extraction from the *Portulaca oleracea* L. herb was used. Extracts were prepared according to the following procedure: 5 g of crushed raw materials to the size of particles passing through a sieve with holes of 1 mm, placed in a flask with a slice with capacity of 100 ml, 50 ml of extractant (water) was added. The flask was attached to a backflow condenser and heated in a water bath for 60 minutes, with periodical shaking to wash off the raw material particles from the walls. The extract was then cooled to room temperature and filtered through cotton wool and a folded paper filter. In the analysis the "Sorbfil PTSH AF-UV" plates, on which 5 μl of each extract was applied, and aqueous 0.1% solutions of reference standards of citric acid and oxalic acid were used. Chromatography was carried out by an ascending method in the "ethyl acetate – acetic acid – formic acid – water" solvent system (100:11:11:25). Saturation time of

the chamber: 30–40 min. After chromatography, the plate was removed from the chamber, dried in air for 5 min., detection was performed at a wavelength of 365 nm. Solutions of reference standards of citric acid and oxalic acid were used as "witnesses".

Quantitative analysis procedure

The content of sum of free organic acids in *Portulaca oleracea* L. was determined using a compendial procedure for determining the sum of organic acids in fresh viburnum berries [34].

The analytical sample of raw materials is crushed to the size of particles passing through a sieve mesh with a diameter of 2 mm. Then 2.5 g of crushed raw materials are placed in a flask with capacity of 250 ml, is poured with 200 ml of water and kept for 2 hours in a boiling water bath, cooled and quantitatively transferred to a measuring flask with capacity of 250 ml. After cooling to room temperature, the mixture is filtered through a paper filter "red ribbon", brought the volume of extract with water to the mark and mixed. 10 ml of extract is taken and placed in a flask with capacity of 100 ml, then, 40–50 ml of freshly boiled water, 0.2 ml of 1% alcohol solution of phenolphthalein, 0.4 ml of 0.1% methylene blue solution are added and the mixture is titrated with a solution of caustic soda (0.1 mol/l) until a purple-red color appears in the foam.

The content of free organic acids in terms of malic acid in absolutely dry raw materials as a percentage (X) is calculated by the formula:

$$X = \frac{v \cdot 0{,}0067 \cdot 250 \cdot 100}{m \cdot 10 \cdot (100 - W)} \cdot 100 = \frac{v \cdot 1675}{m \cdot (100 - W)},$$

where 0.0067 is the amount of malic acid corresponding to 1 ml of caustic soda solution (0.1 mol/l), in grams; v – volume of caustic soda solution (0.1 mol/l) used for titration, in milliliters; m – mass of raw materials in grams; W – mass loss during drying of raw materials as a percentage.

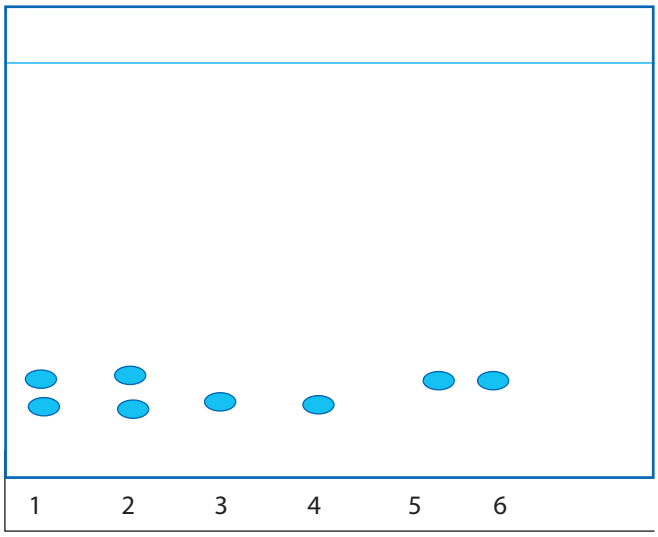
Statistical analysis

To determine the repeatability, the coefficient of variation was calculated based on the results of quantitative determination of the sum of free organic acids (n=6) in the test solution.

RESULTS AND DISCUSSION

As a result of the TLC analysis, the content of free organic acids corresponding to the reference standard was established (Fig. 1): reference standard of citric acid (Rf= 0.24±0.03); reference standard of oxalic acid (Rf=0.21±0.02).

In order to develop a procedure for quantifying the sum of organic acids in this work, the influence of the degree of grinding of raw materials, the ratio "raw material: extractant", the multiplicity of extraction on the yield of free organic acids was studied. The results are presented in Table 1.



Chromatography conditions:
Stationary phase: "Sorbfil PTSH AF-UV" plate
Mobile phase: ethyl acetate – acetic acid – formic acid – water (100:11:11:25)
Reference Standards: solutions of reference standards of oxalic and citric acids
1–2 water extraction
3–4 reference standard of citric acid, Rf=0.24±0.03
5–6 reference standard of oxalic acid, Rf=0.21±0.02

FIG. 1. *Chromatogram diagram for determination of organic acids*

Table 1

THE INFLUENCE OF VARIOUS FACTORS ON THE COMPLETENESS OF THE EXTRACTION OF THE SUM OF FREE ORGANIC ACIDS FROM THE *PORTULACA OLERACEA* L. HERB (N=3, P=0,95)

Unchangeable parameter	Variable parameter	Content of free organic acids, %
Grinding degree, mm		
	7	3.2±0.1
	5	3.1±0.1
	3	3.1±0.1
	2	**3.3±0.1**
	1	2.7±0.1
The ratio of the weight of raw materials to the volume of the extractant		
Grinding degree 2 mm	1:10	0.9±0.2
	1:20	2.1±0.1
	1:40	2.4±0.1
	1:80	**2.8±0.1**
	1:100	2.6±0.1
Extraction time (min)		
Ratio of Raw material: extractant 1:80, grinding degree 2 mm	30	2.9±0.1
	60	3.1±0.2
	90	3.2±0.1
	120	**3.5±0.1**
	180	3.4±0.1
Multiplicity of Extraction		
Ratio of Raw material: extractant 1:80, grinding degree 2 mm, extraction time 120 min	1	**2.7±0.1**
	2	2.6±0.1
	3	2.6±0.2

From the data presented in Table 1, it can be seen that the greatest completeness of the extraction of the sum of free organic acids can be achieved by a single extraction with purified water in 120 minutes when the degree of grinding of raw materials is 2 mm and the ratio of "raw material: extractant" is 1:80.

As a result of the conducted research, a procedure for quantifying the amount of organic acids of the *Portulaca oleracea* L. herb has been developed.

Validation of the procedure was carried out in accordance with the requirements of the OFS.1.1.0012.15 "Validation of analytical procedures" according to the parameters: specificity, linearity, correctness, repeatability, precision, stability of solutions. This study was conducted within the framework of the verification

Table 2

METROLOGICAL CHARACTERISTICS OF THE PROCEDURE OF QUANTITATIVE DETERMINATION OF THE SUM OF FREE ORGANIC ACIDS IN THE *PORTULACA OLERACEA* L. HERB

n	f	P	t (P,f)	Xav, %	S^2	S	ΔX	E, %
5	4	0.95	2.78	2.97	0.01	0.1	0.13	3.23

requirements for the following parameters: specificity and repeatability, since the compendial procedure was used [11]. The metrological characteristics of the procedure are presented in Table 2.

The error in quantifying the content of organic acids in (n= 5) is not higher than 5.0%.

Using the developed procedure, the content of the sum of free organic acids in samples of the Green Purslane (*Portulaca oleracea* L.) herb harvested in Syria and Voronezh region was determined. The results are presented in Table 3.

Thus, it was found that the Green Purslane (*Portulaca oleracea* L.) herb contains at least 2% of the sum of free organic acids. At the same time, the highest content of the studied substances is observed in samples harvested in Voronezh region in 2017, 2018 and 2019, and the lowest content is in the samples harvested in Syria in 2019.

Table 3

CONTENT OF THE SUM OF FREE ORGANIC ACIDS IN THE *PORTULACA OLERACEA* L. HERB HARVESTED IN SYRIA AND VORONEZH REGION

Place of harvesting of *Portulaca oleracea* / year of harvesting	Content of the sum of free organic acids
Voronezh region, 2017	2.2±0.2
Voronezh region, 2018	2.9±0.2
Voronezh region, 2019	2.3±0.1
Syria, Latakia, 2019	2.5±0.3

The amount of organic acids found is comparable to the amount established in medicinal plant raw materials containing organic acids as the main active substances (cranberry berries (2–5%), raspberry berries (up to 2%), fresh viburnum berries (at least 6%), rosehip fruits (at least 2.6%), mountain ash fruits (at least 3.2%) [11,36].

Specificity

The color of the test solution corresponded to the color of the standard solution, which is specified in the reference standard procedure after titration with a solution of caustic soda (0.1 mol/l) when the end point of titration is reached. The color of the placebo solution indicated that there was no placebo effect on the results of determining the quantitative content of organic acids at the titration point corresponding to the color change after adding one drop of caustic soda solution (0.1 mol/l).

Repeatability

The results are shown in Table 4.

CONCLUSIONS

1. Conditions have been selected and procedures have been developed for qualitative (TLC method) and quantitative determination (titrometry method) of the total content of organic acids in the *Portulaca oleracea* L. herb.

2. The content of the sum of organic acids in the *Portulaca oleracea* L. herb is at least 2%.

Table 4

EVALUATION OF THE REPEATABILITY OF THE PROCEDURE OF QUANTITATIVE DETERMINATION OF THE SUM OF FREE ORGANIC ACIDS

Name	1	2	3	4	5	6
Sample volume, ml	10.00	10.00	10.00	10.00	10.00	10.00
V, ml	0.30	0.35	0.35	0.30	0.35	0.35
Content, mg/ml	0.216	0.252	0.252	0.216	0.252	0.252
V_0, ml	0					
Xav, mg/ml	0.240					
Standard deviation, S	0.01					
Standard deviation of the average result, S_0	0.007					
Coefficient of variation, S_0, %	7.75					
Confidence interval (P=0.95), μg/ml	0.02 (от 0.22 до 0.26)					

Relative error in determining the sum of organic acids in the *Portulaca oleracea* L. herb is not higher than 5%.

3. Verification of the developed procedure for the quantitative determination of the sum of organic acids in terms of specificity and repeatability was carried out. The statistical characteristics obtained in determining repeatability correspond to the established criteria for the acceptability of the analytical procedure for determining the sum of free organic acids, which allows us to conclude that it is valid.

4. Developed procedures of qualitative and quantitative determination of the sum of organic acids in the *Portulaca oleracea* L. herb may be recommended for inclusion in the regulatory documentation for this type of medicinal plant raw materials.

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