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Investigation of organic acids of great burnet (*Sanguisorba officinalis* L.) rhizomes with roots and herb

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Abstract
This study aimed to investigate and determine qualitative and quantitative content of free organic acids, including ascorbic acid (vitamin C) of *Sanguisorba officinalis* rhizomes, roots and herb using thin layer chromatography method. Citric, tartaric and succinic acids had been found in *Sanguisorba officinalis* herb, oxalic, tartaric, citric and benzoic acids in *Sanguisorba officinalis* rhizomes and roots.

Keywords: Thin layer chromatography, organic acids, ascorbic acid, *Sanguisorba officinalis* rhizomes with roots and herb

1. Introduction
Great burnet (*Sanguisorba officinalis* L., *Sanguisorba major* Gilib.) – perennial plant of the rose family (Rosaceae), which is used as astringent, painkiller and hemostatic agent.
This is one of the important medicinal plants in official and folk medicine. First of all, due to its antibiotic and phytoncidic properties.
Galen drugs of this medicinal plant have vasoconstrictive (local application), astringent, anti-inflammatory, analgesic and hemostatic properties, demonstrate an inhibitory effect on many pathogenic bacteria such as Escherichia coli, typhoid, paratyphoid (A and B types) and dysentery bacillus.
Liquid extract and decoction of great burnet rhizomes and roots is used internally as a hemostatic agent in various bleeding - pulmonary, gastrointestinal, intestinal, hemorrhoids, uterus, bloody vomit, bloody diarrhea, in the case of hemorrhagic metropathy, in complex treatment of cholecystitis, enterocolitis, chronic dysentery, different pathology of the intestine, accompanied by flatulence. *Sanguisorba officinalis* is successfully used for the treatment of lambliosis and trichomonas colpitis [7].
In Korea, the roots of Greater burnet are traditionally used as anti-inflammatory, anti-infectious and analgesic agents [10]. Chinese scientists have studied antitumor, immunomodulating [9] and antioxidant [8] properties of polysaccharides from the roots of this plant.
Studies by scientists at the Bashkir State Medical University have shown that *Sanguisorba officinalis* extracts with high levels of tannins have a high total antioxidant activity, which allows to recommend this object as an additional source of antioxidants in plant collections and biologically active additives with antioxidant action [3, 4].
Sanguisorba officinalis is a valuable medicinal plant, which has a sufficient raw material base, is cultivated on the territory of Ukraine and has many years' experience of using in folk and scientific medicine. However, currently phytochemical studies of this species are insufficient, so the aim of our research was to study the content of organic acids, including ascorbic acid (vitamin C) of great burnet rhizomes with roots and herb.

2. Materials and methods
Plant materials of great burnet (*Sanguisorba officinalis* L.) were collected on research grounds of I. Horbachevsky Ternopil State Medical University (Druzhba village, Ternopil region). The aerial parts were collected when the plants were in bloom in 2014-2016, the rhizomes and roots – after the death of the above-ground part of the plant.

The raw material was dried using conventional methods in a heat convection dryer at a temperature 40°C; rhizomes and roots were washed previously in a stream of cold water and cut longitudinally.

Determination of the qualitative composition of free organic acids was carried out by thin layer chromatography using chromatographic plates "Sorbfil". Pre-obtained extracts of medicinal plant raw material [5] and samples of organic acids (salicylic, oxalic, benzoic, citric, succinic, tartaric and malic) were used. Investigations were carried out in the solvent system 95% ethanol R - concentrated ammonia solution R (16:4, 5) [4, 5]. The chromatograms after chromatography were well dried and treated with bromocresol green solution, dried in a drying cabinet, and observed the appearance of substances in the form of yellow stains on a blue background. As a developer also was used a 0.1% solution of 2, 6-dichlorophenolindophenol in 95% ethanol R with observing the appearance of pink stains on a blue background.

Determination of the quantitative content of free organic acids was carried out by the titrimetric method in recalculation on malic acid in accordance with the method described in State Pharmacopoeia XI of the USSR [1].

The content of free organic acids (X) normalized by malic acid in absolutely dry raw materials was calculated by the formula (in percent):

$$X = \frac{V \times 0{,}0067 \times 250 \times 100 \times 100}{m \times 10 \times (100 - W)},$$

Where: V – volume of 0, 1 M sodium hydroxide solution, that was spent on titration, ml;
0,0067 – the amount of malic acid corresponding 1 ml 0,1 M sodium hydroxide solution;
m – a mass of a plant material, g;
W – weight loss during drying, %.

Quantitative content of ascorbic acid was determined by spectrophotometric method on a Lambda 25 Perkin Elmer spectrophotometer.

Test solution. 0.500 g of freshly powdered raw material was placed in a round- bottomed flask, a solution of 1.0 g of oxalic acid R in 50.0 ml of methanol R was added, boiled with reflux for 10 min, cooled in an ice bath to a temperature of 15-20°C and filtered. 2.0 ml of the filtrate was transferred to a conical flask of 50 ml capacity, successively added 2.0 ml of dichlorophenolindophenol standard solution R, then, exactly after 60 s, 0.5 ml of solution of 100 g / l of thiourea R in ethanol (50%, v / v) R and 0.7 ml of dinitrophenylhydrazine-sulfuric acid solution R was heated with reflux at temperature 50°C for 75 minutes and immediately placed in an ice bath for 5 minutes. 5.0 ml of a mixture of 12 ml of water R and 50 ml of sulfuric acid R were added drop by drop during the period at least 90 seconds and no more than 120 seconds, shaking the flask in an ice bath. Stirred for 30 min at room temperature and measured optical density at 520 nm wavelength, using solution A as a compensatory liquid.

Solution A. 2.0 ml of the filtrate, obtained during the preparation of the test solution, was treated as described above, adding dinitrophenylhydrazine-sulfuric acid solution R directly before measuring the optical density.

Comparison solution. 40.0 mg of ascorbic acid R was dissolved in a freshly prepared solution of 20 g/l oxalic acid R in methanol R, and the volume of the solution was adjusted to 100.0 ml with the same solvent. 5.0 ml of the resulting solution was brought up with freshly prepared solution of 20 g/l oxalic acid R in methanol R to 100.0 ml. 2.0 ml of the resulting solution was treated as described above for the filtrate, obtained during the preparation of the test solution. The optical density was measured at a wavelength of 520 nm, using solution B as a compensatory liquid.

Solution B. 2.0 ml of the comparison solution was treated as described above for solution A.

The content of ascorbic acid was calculated by the formula (in percent):

$$X = \frac{2{,}5 \times A_1 \times m_2}{A_2 \times m_1}$$

Where: A_1 — optical density of the test solution;
A_2 — optical density of the solution of comparison;
m_1 — mass of the tested raw material, g;
m_2 — mass of ascorbic acid, g [2].

The quantitative content of ascorbic acid in the investigated parts of *Sanguisorba officinalis* was also determined by titrimetric method using 0,001 mole/l solution of sodium 2, 6-dichlorophenolindophenolate as a titrant, according to the method of the State Pharmacopoeia XI [1].

3. Results and Discussion

The determination of free organic acids was carried out using thin layer chromatography with previously obtained extracts in the following solvent system:

- 95% ethanol R - concentrated ammonia solution (16: 4.5).

Organic acids were represented in the form of yellow stains on a blue background when the chromatograms were treated with bromocresol green solution. We observed the appearance of pink stains on a blue background when 0.1% solution of 2, 6-dichlorophenolindophenol in 95% ethanol R was used as a developer.

The results of the research are presented in Figure 1.

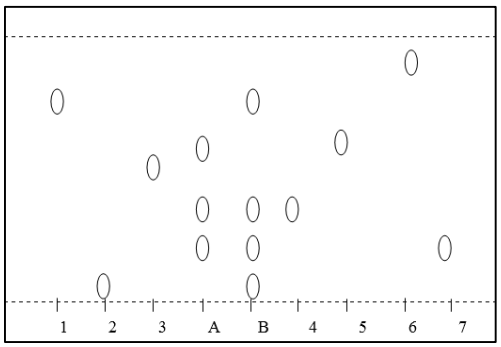

Fig 1: Scheme of chromatogram of free organic acids of *Sanguisorba officinalis*:

A - Extract of *Sanguisorba officinalis* herb, B - extract of *Sanguisorba officinalis* rhizomes and roots, 1 - benzoic acid, 2 - oxalic acid, 3 - malic acid, 4 - tartaric acid, 5 - succinic acid, 6 - salicylic acid, 7 - citric acid.

Solvent System: 95% ethanol R - concentrated ammonia solution (16:4.5).

Citric, tartaric and succinic acids had been found in *Sanguisorba officinalis* herb, oxalic, tartaric, citric and benzoic acids in *Sanguisorba officinalis* rhizomes and roots.

The results of determining the quantitative content of organic acids in rhizomes and roots and herb of the studied medicinal plant are given in Table 1.

The quantitative content of organic acids in *Sanguisorba officinalis* herb was (5.80 ± 0.22)%, in rhizomes and roots - (2.55 ± 0.10)% in recalculation on absolutely dry raw material.

It was carried out a quantitative determination of ascorbic acid in aqueous extracts of the investigated types of raw material of the medicinal plant. Quantitative content of ascorbic acid in the investigated raw material was determined by titrimetric (State Pharmacopoeia of USSR) and spectrophotometric method according to State Pharmacopoeia of Ukraine. The results of the quantitative content of ascorbic acid, in terms of totally dry raw materials, are given in Table 2.

Table 1: Quantitative content of the sum of organic acids in *Sanguisorba officinalis* raw material (titrimetric method)

Raw material	Quantitative content of the sum of organic acids in recalculation on absolutely dry raw material (%) (m = 5)
Rhizomes and roots	2.55±0.10
Herb	5.80±0.12

Note. Probability of error P <0.05

Table 2: Quantitative content of ascorbic acid in *Sanguisorba officinalis* rhizomes and roots and herb (%)

Raw material	State Pharmacopoeia of USSR	State Pharmacopoeia of Ukraine
Herb	0.05±0.01	0.32±0.0001
Rhizomes and roots	0.04±0.02	0.12±0.005

The results of our study showed that the content of ascorbic acid in *Sanguisorba officinalis* herb (titrimetric method) was (0.05 ± 0.01)%, in rhizomes and roots - (0.04 ± 0.02)% in terms of dry raw material. It was found out that content of ascorbic acid in herb of the investigated medicinal plant was (0.32 ± 0.0001)%, in rhizomes and roots - (0.12 ± 0.005)% in terms of dry raw material under the spectrophotometric determination.

4. Conclusions

1. It was found out a qualitative composition of the organic acids in the raw material of *Sanguisorba officinalis* using thin layer chromatography method. Citric, tartaric and succinic acids were determined in *Sanguisorba officinalis* herb, oxalic, tartaric, citric and benzoic acids in *Sanguisorba officinalis* rhizomes and roots.
2. The quantitative content of free organic acids and ascorbic acid in *Sanguisorba officinalis* herb and rhizomes with roots was determined (5.80 ± 0.12)% and (2.55 ± 0.10)%) and (0.05 ± 0.01)% and (0,04 ± 0,02)% (State Pharmacopoeia XI of USSR) and (0.32 ± 0.0001)% and (0.12 ± 0.005)% (State Pharmacopoeia of Ukraine) respectively.

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