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Original Article

Some Pharmacognostic Studies of the Bird Cherry (*Padus avium Mill.*) and Mountain Ash (*Sorbus aucuparia* L.) Fruits Collected from Moscow (Russia)

Ekaterina Vyacheslavovna Sergunova[1,*], Dmitry Olegovich Bokov[1,2,3]

Ekaterina Vyacheslavovna Sergunova[1,*], Dmitry Olegovich Bokov[1,2,3]

[1]Department of Pharmaceutical and Natural Sciences, Sechenov First Moscow State Medical University, 8 Trubetskaya St., bldg. 2, Moscow, 119991, RUSSIAN FEDERATION.
[2]Department of Analytical, Physical and Colloid Chemistry, Sechenov First Moscow State Medical University, 8 Trubetskaya St., bldg. 2, Moscow, 119991, RUSSIAN FEDERATION.
[3]Laboratory of Food Chemistry, Federal Research Center for Nutrition, Biotechnology and Food Safety, 2/14, Ustyinsky pr., Moscow, 109240, RUSSIAN FEDERATION.

Correspondence

Ekaterina Vyacheslavovna Sergunova

Department of Pharmaceutical and Natural Sciences, Sechenov First Moscow State Medical University, 8 Trubetskaya St., bldg. 2, Moscow, 119991, RUSSIAN FEDERATION.

E-mail: nakasl@mail.ru

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ABSTRACT

Background: Light microscopic analysis is appropriate pharmacopoeial method for quality control of fresh and fast-frozen *Rosaceae* family plants fruits. It is widely used for crude herbal drugs analysis and helps to identify significant anatomical and diagnostic signs. The aim of the research was to establish the identity characteristics of the dried and frozen fruits of mountain ash and bird cherry and to perform phytochemical analysis of studied objects harvested in Moscow. **Materials and methods**: Light microscopic analysis was used for *Rosaceae* fruits identity estimation. The fruits of mountain ash (*Sorbus aucuparia L.*) and bird cherry (*Padus avium Mill.*) were collected in the fruiting phase on the territory of the Botanical Garden of Sechenov First Moscow State Medical University. Fruits samples were dried at the temperature of 60-80°C and frozen at the temperature of -18-20°C. The epidermis surface preparation and the fruit pulp squash preparation were prepared for microscopic examination. The photographs were obtained from an AXIO IMAGER D1 biological laboratory microscope (Carl Zeiss Microscopy) with the help of a Canon Power shot A 650 IS camera. The elemental composition of the *P. avium* and *S. aucuparia* fruits was determined by X-ray fluorescence analysis (XFA) using an S4 Pioneer X-ray spectrometer. Simple sugar analysis was performed by using reverse phase HPLC with refractometric detection, analysis of organic acids – by using ion-pair HPLC with UV-detection (210 nm) in the isocratic elution mode (Agilent 1260 Infinity LC). **Results:** Characteristic signs of the anatomical structure of dried and frozen *S. aucuparia, P. avium* fruits were determined. Diagnostic anatomical signs of *S. aucuparia, P. avium* fruits are: the form of epidermal cells, stone cells, inclusions of calcium oxalate (prismatic crystals and druses), vessels, parenchyma with biologically active substances (anthocyanins, carotenoids). The presence and content of Na, Mg, Al, Si, P, S, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Rb, Sr were evaluated. Organic acids profile of *S. aucuparia, P. avium* fruits is presented by citric, malic, oxalic, succinic, tartaric, fumaric, quinic, ascorbic, sorbic acids. Total content of simple sugars, presented by glucose, fructose, sucrose, is in the interval of 8-9%. **Conclusion:** The results of a comparative microscopic analysis of bird cherry and mountain ash dried and frozen fruits were obtained. They allowed to identify diagnostically significant elements of the anatomical structure that can be used to establish the fruits identity. The obtained data is of great value for *S. aucuparia, P. avium* fruits pharmacopoeial analysis in the Russian Federation.

Key words: *Padus avium Mill., Sorbus aucuparia L., Rosaceae,* Fruits, Anatomical Signs, Organic Acids, Simple Sugars, Mineral Composition.

INTRODUCTION

Today, the issues of quality control of crude herbal drugs (CHD) and medicinal herbal remedies remain principal for a modern pharmacy. Microscopic analysis is a main method for determining the identity characteristics and good quality of crude herbal drugs.[1-5] Along with the study of dried CHD, this method is used in the standardization of fresh and frozen CHD.[6,7] Very little has been written in the scientific literature about the influence of low (negative) temperatures on the presence and variability of anatomical signs in determining the CHD identity characteristics.[8-10] Frozen CHD is studied to investigate the possibility of use of an alternative processing method for including in State Pharmacopoeia of Russian Federation.

The *Rosaceae* family numbers 3000-3500 species (111 genera). It is widely distributed in Russia and throughout the temperate zone of the Northern Hemisphere.[11] Among the plants of this family, there are both woody and herbaceous forms. These plants are mainly perennial herbs with the alternate leaf arrangement; leaves are simple or complex, with stipules. Medicinal plants include the genus *Rosa*, *Padus*, *Crataegus*, *Sorbus*, etc. Crude herbal drugs are the "Fruits" morphological group generally.[12-15] In present study we investigate two of them: bird cherry (*Padus avium* Mill.) and mountain ash (*Sorbus aucuparia* L.). According to literature its CHD contain different groups of biologically active substances (BAS): sugars, polysaccharides, organic and hydroxycinnamic acids, amino acids, flavonoids, carotenoids.[16-22] The whole complex of these BAS

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defines the pharmacological effect of drugs produced from *P. avium* and *S. aucuparia* fruits.

The aim of the research was to establish the identity characteristics of the dried and frozen fruits of *P. avium* and *S. aucuparia* by studying the anatomical diagnostic signs and give phytochemical characteristics of dried fruits that are used as a crude herbal drug in Russian Federation.

MATERIALS AND METHODS

We studied the fruits of bird cherry (*P. avium*) and mountain ash (*S. aucuparia*) (Figure 1) harvested in the Botanical Garden of Sechenov First Moscow State Medical University (55°44'46.615" N 37°31'48.886 "E) in September 2016. Plant samples were identified at the Department of Pharmaceutical Natural Science of the Institute of Pharmacy of Sechenov First Moscow State Medical University (Sechenov University) and a voucher specimen was placed in the department herbarium and caprological collection. Part of the fruits was subjected to drying at a temperature of 60-80°C, the other part – freezing at a temperature of -18-20°C.

The study of microscopic signs was carried out in accordance with the monograph "Technique of microscopic and microchemical study of crude herbal drugs"[23] and "Technique of microscopic and microchemical research of crude herbal drugs and medicinal plant preparations".[24] Epidermis surface preparation and fruit pulp squash preparation were prepared. Anatomical signs of frozen fruits were studied in comparison with dried CHDs. Before making microscopic preparations from frozen fruits, they were not thawed; when thawed, the fruits change their shape and it makes difficult to prepare samples. The softening of frozen fruits by boiling water complicates visualization of anatomical signs. Therefore, a pharmacopoeial technique was used for the analysis of frozen CHDs (CHD boiling in a sodium hydroxide solution 5%).

Epidermis surface preparation

2-3 fruits were boiled in a test tube with a sodium hydroxide solution 5% for 2-3 minutes and thoroughly washed with water. The object was placed on a glass slide, dissection needles were used to separate the tissues of the pericarp, then small pieces were placed on another glass slide in a drop of glycerin, covered with a cover glass and examined under a microscope at different magnifications.

Fruit pulp squash preparation

The fruit was boiled in a test tube with a sodium hydroxide solution 5% for 2-3 minutes and thoroughly washed with water. Small pieces of pulp were transferred by a dissecting needle into a drop of glycerol on a slide. They were covered with a cover glass, lightly pressed on the back of the dissecting needle to evenly distribute mesocarp tissue under the glass and examined under a microscope. All experiments were performed in triplicate. The photographs of the preparations were obtained from an AXIO IMAGER D1 biological laboratory microscope (Carl Zeiss Microscopy) with a help of a Canon Power shot A 650 IS camera. The signs of the anatomical structure of the dried and frozen fruits of mountain ash and bird cherry were determined.

Chemical studies

Reference standards of simple sugars (glucose, fructose, sucrose) and organic acids (citric, malic, oxalic, succinic, tartaric fumaric, quinic, ascorbic, sorbic acids) were purchased from Sigma-Aldrich Company Ltd (St. Louis, USA).

Simple sugar analysis was performed by reverse phase HPLC with refractometric detection. We used chromatograph Agilent 1260 Infinity LC with autosampler, column thermostat, refractometric detector (RID); software – ChemStation (ver. A.09.03). Separation was carried out with chromatographic column Sugar-Pak HPLC (WATERS, USA), 300 mm × 6.5 mm. The elution mode was isocratic, mobile phase – purified water with the addition of Ca-EDTA (concentration – 0.05 mg/ml). The flow rate – 0.5 ml/min, column temperature – 80 °C. The injected sample volume – 10 µl.

Analysis of organic acids was performed by ion-pair HPLC with UV-detection at 210 nm wavelength in the isocratic elution mode. Separation conditions are illustrated in our previous work.[25]

The elemental composition of the *P. avium* and *S. aucuparia* fruits was determined by X-ray fluorescence analysis (XFA) using an S4 Pioneer X-ray spectrometer (Bruker, Germany).

Figure 1: Fresh (left) and dried (right) of *P. avium*(A) *and S. aucuparia* (B).

RESULTS AND DISCUSSION

Microscopic analysis of dried and frozen mountain ash fruits

The epidermal cells of the dried fruits of the mountain ash are fenestrate, differ in size; the outer wall is strongly thickened (Figure 2). The cuticle is smooth, thin. The underlying tissue of epidermis is presented by 2-4 lines of collenchyma, together they form epicarp. There are small droplets of yellow carotenoids in the epidermal and collenchymal cells. Mesocarp cells are of various shapes and sizes, thin-walled with numerous orange-yellow chromoplasts. Conducting bundles are located in the mesocarp, their xylem consists of narrow spiral vessels with crystal-bundle sheath; there are druses and prismatic crystals (Figure 3). Fusiform and isodiametric stony cells are located near the endocarp.

When conducting an anatomical study of frozen fruits, diagnostic signs were detected. Epidermis cells with unevenly thickened walls, sometimes penetrated by pores, yellow-orange drops (carotenoids) were found. The mesocarp contains stony cells, calcium oxalate druses, or prismatic crystals that form a crystal-bundle sheath along the conducting bundles (Figure 4).

Microscopic analysis of the dried and frozen bird cherry fruits

When examining the epidermis of the dried fruit, there are polygonal cells with evenly thickened walls, covered with a thin layer cuticle. Over the entire fruit surface there are oval stomata surrounded by an indefinite number of cells (usually 7-8 cells of the epidermis), not differing in shape and size from other cells of the epidermis (anomocytic type stomatal complex). The epidermal cells are filled with brown contents, the color is associated with the anthocyanins accumulation (Figure 5). The mesocarp is presented by a parenchyma; it is consisted of polygonal, thin-walled cells with conducting bundles. The xylem conducting bundles consists of narrow spiral vessels. The

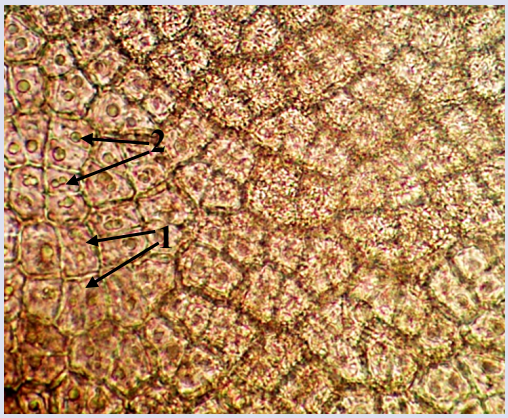

Figure 2: The epidermis of the dried fruits of mountain ash (× 200). 1 – epidermis cells; 2 – carotenoid drops.

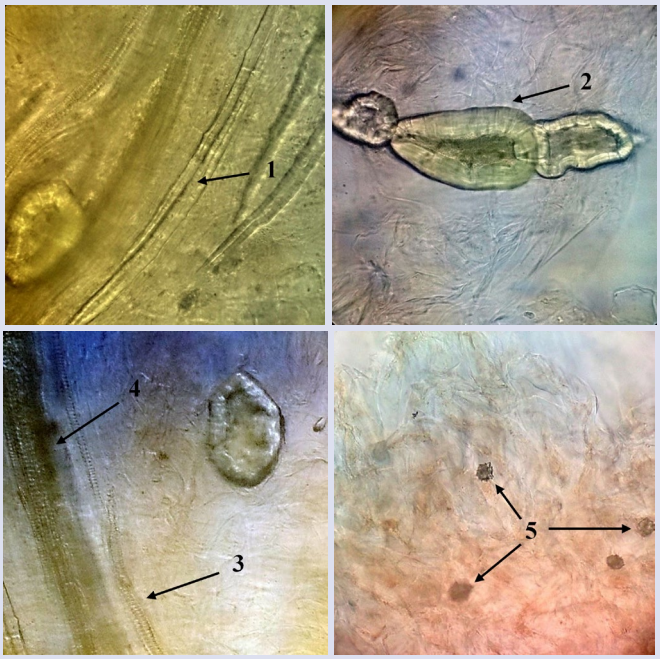

Figure 3: Mesocarp of mountain ash dried fruits (squash preparation) (× 400; × 1000) 1 – fusiform stony cells; 2 – isodiametric stony cells; 3 – vessels of the conducting bundle; 4 – crystal-bundle sheath; 5 –calcium oxalate druses.

endocarp is composed of two layers of sclerenchyma tissue. The outer layer includes stony cells of a rounded or slightly elongated shape with thickened woody shells. The inner layer includes sclerenchymatous, tangentially elongated fibers (Figure 6). Diagnostic signs of bird cherry fruits are established when studying the anatomical structure of frozen fruits. They are straight-sided epidermal cells of a polygonal shape, anomocytic type of stomatal complex (Figure 7), cells with brown contents, vessels of conducting bundles, stony cells (Figure 8).

Chemical composition of Rosacea fruits

The results of X-ray fluorescence determination of the content of elements in samples of plants are presented in Table 1. We also studied the composition and content of carbohydrates (Table 2) and organic acids (Table 3) of fruits.

CONCLUSION

There were no significant and fundamental differences in the anatomical structure of dried and frozen *S. aucuparia, P. avium* fruits. The results of a comparative microscopic analysis of bird cherry and mountain ash dried and frozen fruits allowed identifying diagnostically significant elements of the anatomical structure that can be used to establish the fruits identity. These elements include: the shape of epidermal cells; stony cells; calcium oxalate inclusions (prismatic crystals and druses); vessels; parenchyma with biologically active compounds (anthocyanins, carotenoids). Presence and content of Na, Mg, Al, Si, P, S, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Rb, Sr were sucseccfully evaluated in *P. avium* and *S. aucuparia* fruits. Organic acids profile of *S. aucuparia, P. avium* fruits is presented by citric, malic,

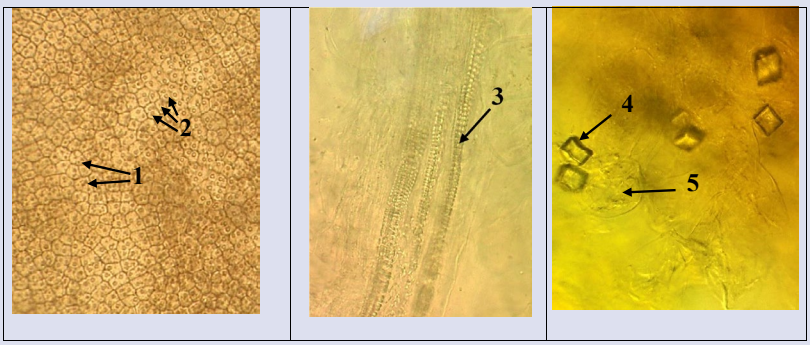

Figure 4: The preparation of the mountain ash frozen fruits (× 100; × 400). 1 – cells of the epidermis; 2 – carotenoids; 3 – vessels of the conducting bundle; 4 – prismatic crystals; 5 – stony cells.

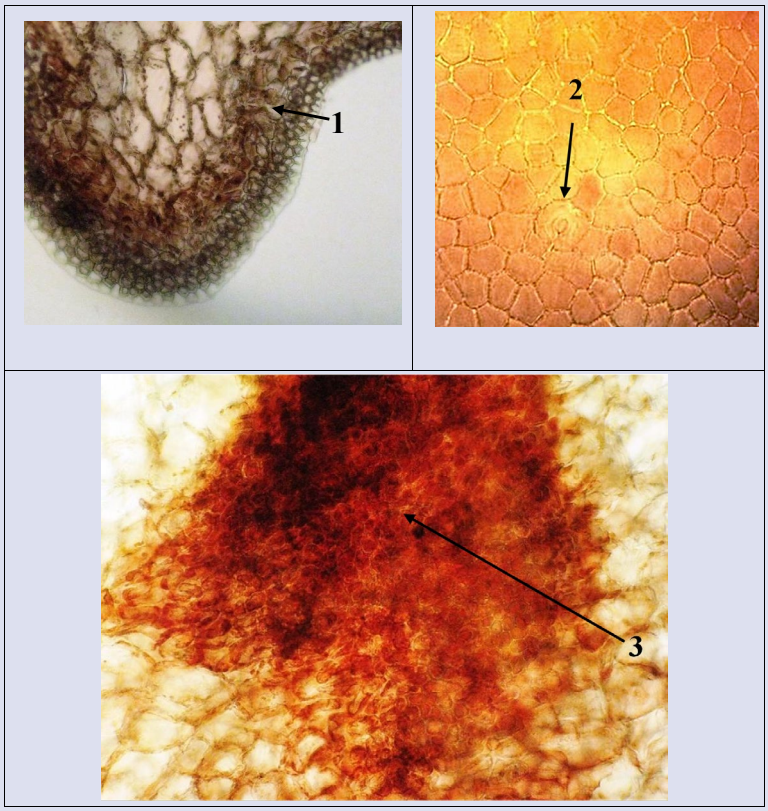

Figure 5: Epidermis of the bird cherry dried fruits (× 400). 1 – epidermis cells with polygonal cells; 2 – anomocytic stomatal complex; 3 – clusters of cells with brown contents.

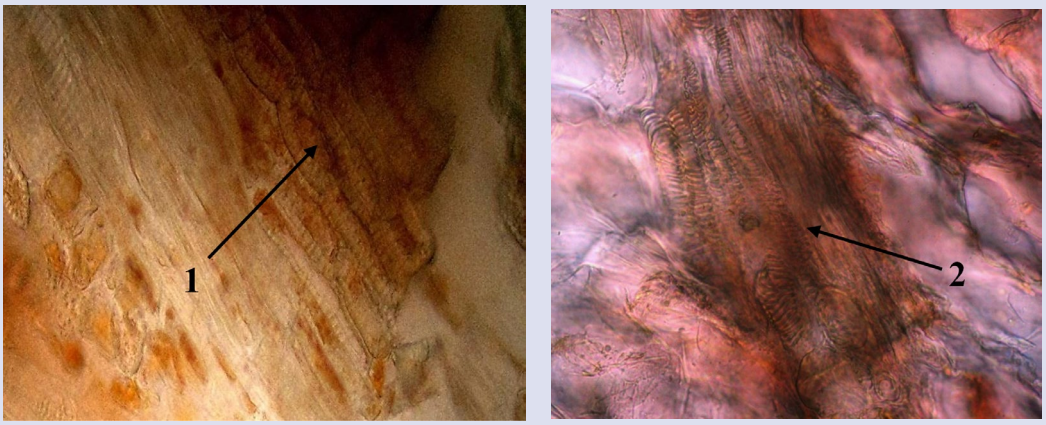

Figure 6: Fragment of the bird cherry dried fruits mesocarp (squash preparation) (× 1000). 1 – stony cells; 2– vessels of conducting bundles.

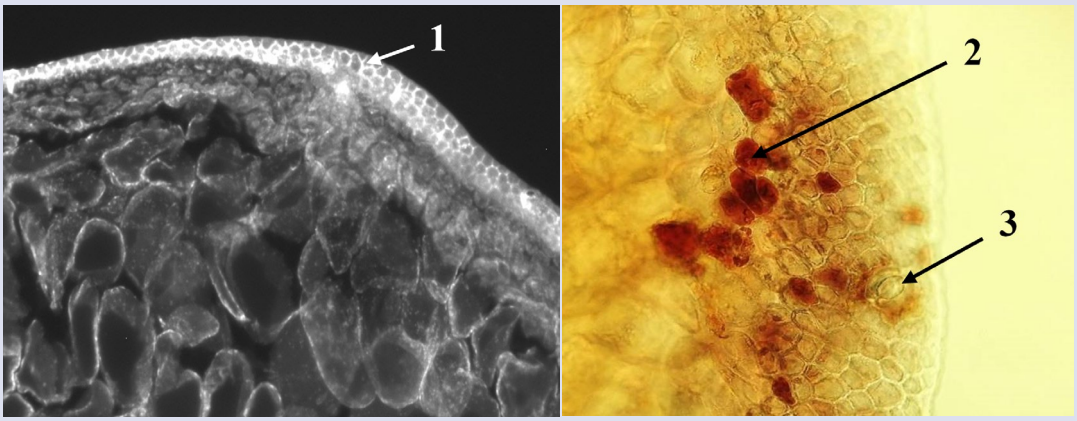

Figure 7: Epidermis of frozen bird cherry fruit (× 200). 1 – epidermis cells; 2 – cells with brown contents; 3 – anomocytic stomatal complex.

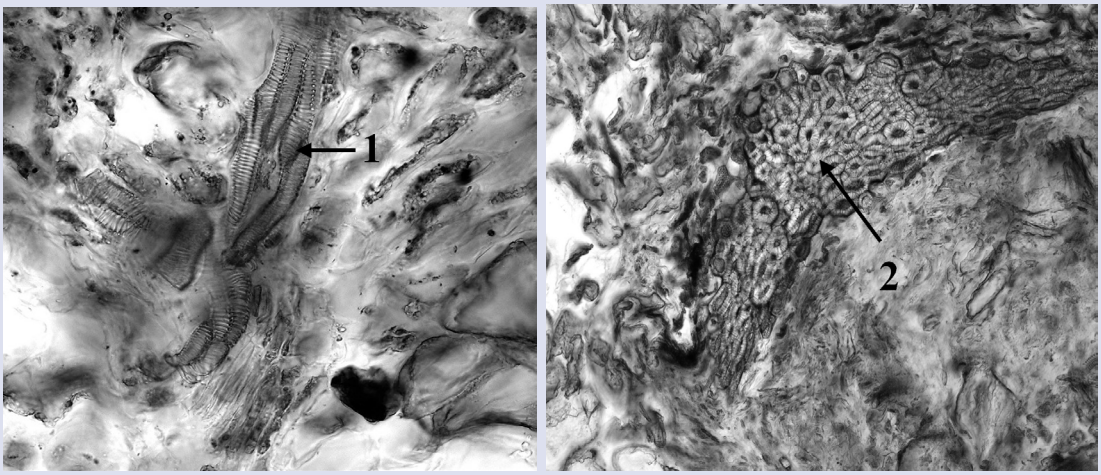

Figure 8: Mesocarp of frozen bird cherry (squash preparation) (× 200). 1– vessels of conducting bundles; 2 – stony cells.

Table 1: Mineral composition of *P. avium* and *S. aucuparia* fruits.

Object/Element	Na (%)	Mg (%)	Al (%)	Si (%)	P (%)	S (%)	Cl (%)	K (%)	Ca (%)	Ti (PPM)	Cr (PPM)
S. aucuparia	0.0056	0.095	0.0041	0.0233	0.139	0.053	< 0.0300	1.288	0.249	4.4	1.8
P. avium	0.0054	0.061	< 0.0030	< 0.0100	0.102	0.029	< 0.0300	1.042	0.241	< 4	1.9
Object/Element	**Mn (%)**	**Fe (%)**	**Ni (PPM)**	**Cu (PPM)**	**Zn (PPM)**	**Br (PPM)**	**Rb (PPM)**	**Sr (PPM)**	**Zr (PPM)**	**Pb (PPM)**	**Ba (PPM)**
S. aucuparia	0.0062	0.0079	< 1	4.1	26	< 2	7.0	12	< 1	< 3	18
P. avium	0.0013	0.0063	2.9	4.9	27	< 2	6.3	10	< 1	< 3	< 5

Table 2: Composition and content of simple sugars in *P. avium* and *S. aucuparia* fruits.

Sugar/object	Glucose	Fructose	Sucrose	Total content
S. aucuparia	3.76 ± 0.45	4.16 ± 0.72	0.81 ± 0.06	8.73
P. avium	2.48 ± 0.34	5.91 ± 0.81	0.25 ± 0.03	8.64

Table 3: Composition and content of organic acids in *P. avium* and *S. aucuparia* fruits.

Organic acid/object	Citric	Malic	Oxalic	Succinic
*S. aucuparia**	103.73 ± 1.06	301.28 ± 1.90	38.4 ± 0.63	37.91 ± 0.82
P. avium	223.15 ± 3.11	-	15.29 ± 0.87	-
Organic acid/object	**Tartaric**	**Fumaric**	**Quinic**	**Ascorbic**
S. aucuparia	1.23 ± 0.16	27.4 ± 0.67	15.9 ± 0.54	52.73 ± 1.45
P. avium	-	-	356.76 ± 7.31	7.87 ± 0.32

*Sorbic acid conten in *S. aucuparia fruits is* 75.19 ± 0.92.

oxalic, succinic, tartaric, fumaric, quinic, ascorbic, sorbic acids. Total content of simple sugars, presented by glucose, fructose, sucrose, is in the interval of 8-9%.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATIONS

BAS: Biologically active substances, CHD: Crude herbal drugs.

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GRAPHICAL ABSTRACT

ABOUT AUTHORS

Sergunova Ekaterina Vyacheslavovna: is doctor of Pharmaceutical Sciences, Associate Professor, Professor of Department of Pharmaceutical Natural Sciences, Institute of Pharmacy, Sechenov University. Research Interests: are influence of conservation methods on the composition and content of biologically active substances in crude herbal drugs.

Bokov Dmitry Olegovich: is candidate of Pharmaceutical Sciences (Ph.D.), Associate Professor, Department of Pharmaceutical Natural Sciences, Department of Analytical, Physical and Colloid Chemistry, Institute of Pharmacy, Sechenov University; Laboratory of Food Chemistry, Federal Research Center for Nutrition, Biotechnology and Food Safety. Research Interests: are pharmacognosy, analytical chemistry, investigation of the composition and content of biologically active compounds in crude herbal drugs using modern instrumental methods of analysis (HPLC with RID, UV, MS-MS detection, GC-MS).

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