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DETERMINATION OF STANDARDIZATION PARAMETERS OF OXYCOCCUS MACROCARPUS (AIT.) PURSH AND OXYCOCCUS PALUSTRIS PERS. LEAVES

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The aim. *Extracts of cranberry large leaves have prospects when used to correct insulin-resistant conditions. Therefore, to create new drugs based on cranberry leaves, you need to develop instructions for cultivating the plant, harvesting raw materials and methods of quality control. Therefore, the aim of the research was to determine the parameters of standardization of large cranberry leaves* (*Oxycoccus macrocarpus* (Ait.) Pursh) *and swamp cranberries* (*Oxycoccus palustris* Pers.).

Materials and methods. *Macro- and microscopic studies of raw materials were performed according to the method of SPhU 2.8.23 "Microscopic examination of medicinal plant raw materials". Macroscopic examinations were performed using a magnifying glass and MBS-9 binocular microscope. Identification of basic substances was performed by TLC, testing and quantification of flavonoids according to SPhU methods.*

Results*. The morphological and anatomical features of the leaves of common cranberry and large cranberry were determined. The general features of the structure of stems and leaves species and different differences for each species are were revealed. TLC identification of the main BAS of raw materials was developed and standardization parameters were determined.*

Conclusions*. The parameters of cranberry leaf standardization are determined by the following indicators: macro-and microscopic features, TLC identification of the main BAS raw materials (hyperoside, rutin and caffeic acid), impurities (not more than 2 %), brown stems not more than 5 %, weight loss during drying (not more than 10 %), total ash (not more than 7 %) and not less than 1 % of flavonoids, in terms of hyperoside*

Keywords*: large cranberry, common cranberry, leaves, morphological and anatomical features, standardization*

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1. Introduction

Common cranberry (*Vaccinium oxycoccos, Oxycoccus palustris* Pers.; *Oxycoccus quadripetalus* Gilib.) Is an evergreen plant of the genus Vaccinium of the heather family (*Ericaceae*). Occurs in northern and central Europe, northern Asia, and North America [1, 2]. Cranberries grow in swampy pine and mixed forests, in oligotrophic and mesotrophic swamps. Distributed in the western right-bank Polissya, the north-eastern part of the left-bank Forest-Steppe, occasionally in the Carpathians and Prykarpattya. Procurement is possible in Volyn, Rivne, Zhytomyr, Chernihiv regions and in the Carpathians [3]. The small cranberry (*Oxycoccus microcarpus* Turcz. Ex Rupr.) is listed in the Red Data Book of Ukraine [4]. There are certain logistical difficulties in harvesting raw cranberries in the natural environment, so for use in the pharmaceutical industry and in accordance with the requirements of Good practice of growing and collecting raw materials of plant origin [5], it is advisable to cultivate this plant.

Cranberries are one of the main commercial crops in America, but most of them cultivate large cranberries (*Oxycoccus macrocarpus* (Ait.) Pursh) and various varieties. In Ukraine, this crop is grown in Rivne, Volyn, Poltava, Zhytomyr, Kyiv, and Cherkasy regions. In Ukraine, cranberries occupy only 312 hectares, and the area of plantations is constantly growing [6].

In the food and pharmaceutical industries, cranberries are mainly used, which contain micro- and macroelements, vitamins, flavonoids, phenolic acids, anthocyanins, organic acids, and other substances. Cranberry fruits are widely used in the confectionery industry. Most of this raw material is processed into juice, fruit juice, syrup, sauce, jam and dried sweetened berries, and the rest is sold fresh. Cranberry juice has a bactericidal effect against staphylococci, streptococci, Escherichia coli, and Proteus, so it is used in the treatment of purulent wounds and burns. Cranberry fruits stimulate the secretion of gastric juice, so they are often used to treat gastritis with low acidity and inflammation of the pancreas [7].

In the Ukrainian pharmaceutical market such drugs as "Uromax", "Nefrokea", "Uroxin", "Uronorm", "Urinal", etc. are presented, and are used in diseases of the urinary tract [8, 9]. But all these drugs and functional supplements are made based on biologically active substances (BAS) cranberry fruit, although the leaves and shoots of this plant also contain valuable BAS and are promising raw materials for further study and development of new drugs.

Previous studies have shown that plants of the genus *Vaccinium* are promising sources for the creation of hypoglycemic and hypolipidemic agents, namely extracts from bilberry leaves [10, 11], blueberry leaves [12] and bearberry leaves [13, 14]. Studies have shown that extracts from the leaves of cranberries are also promising when used to correct insulin-resistant conditions [15].

In view of this, in order to create new medicines based on cranberry shoots and leaves, it is necessary to develop instructions for cultivating the plant, harvesting raw materials and methods of quality control. For analysis, common cranberry leaves were chosen as the most common wild raw material, and large cranberry leaves as the most common cultivated raw material, as they are the most promising types of medicinal plant raw materials for introduction into pharmaceutical and medical practice.

The aim of the research was to determine the standardization parameters of the leaves of large cranberry (*Oxycoccus macrocarpus* (Ait.) Pursh) and common cranberry (*Oxycoccus palustris* Pers.).

2. Planning (methodology) of the research

In Fig. 1 a graphical representation of the research planning process is shown.

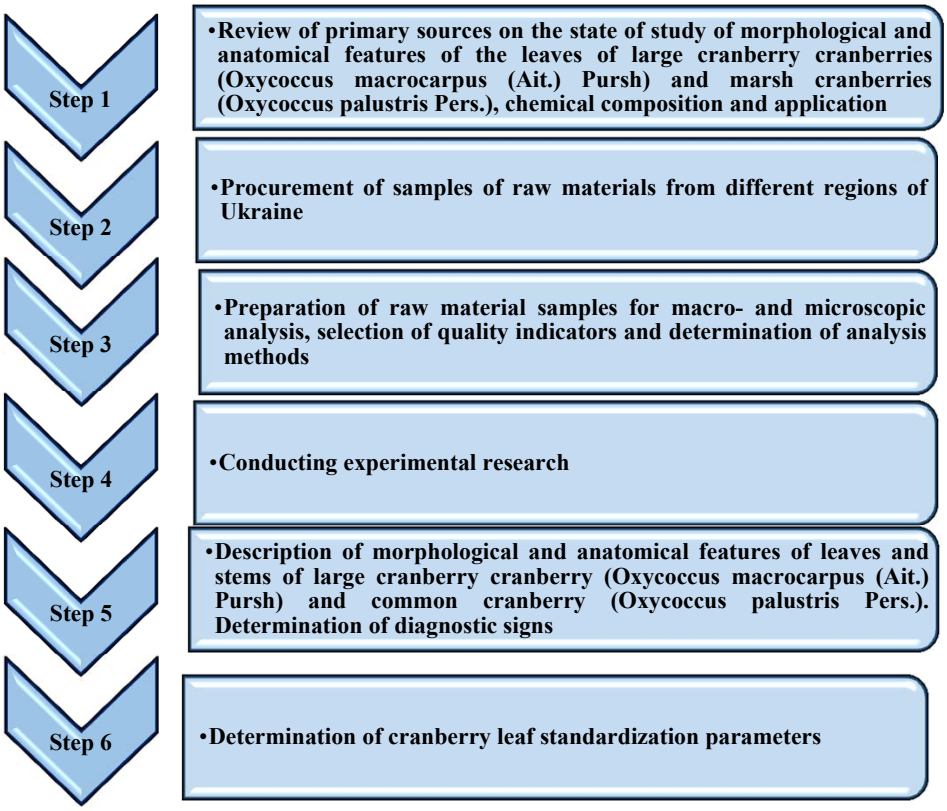

Fig. 1. Planning of the research

3. Materials and methods

The objects of the study were samples of large cranberry leaves and common cranberries, which were harvested in August 2020 in Kyiv (Pereyaslav suburbs, 50.10314334026342, 31.46151900698126) and Volyn (Manevychi district, Nova Ruda village, 51.4963673941) regions. The identity of the plant was established by professor Tetiana Gontova, D.Sc. [16]. Voucher specimens were deposited at the Department of Pharmacognosy (National University of Pharmacy, Kharkiv, Ukraine, No. 592–594). The raw material was dried at room temperature in a well-ventilated area for ten days and stored in paper bags [17].

Macro- and microscopic studies of raw materials were performed according to the method of State Pharmacopoeia of Ukraine 2.8.23 "Microscopic examination of medicinal plant raw materials" [17]. Macroscopic examinations were performed using a magnifying glass and MBS-9 binocular microscope. The study of the anatomical structure of large and common cranberry shoots was performed on samples of whole and cut raw materials in accordance with the requirements of SPhU. Shoots were fixed in a mixture of 96 % *ethanol P-glycerol P-purified water P* (1:1:1). The structure of stems and leaves was studied in cross sections. The epidermis of the organs was examined from the surface according to conventional methods [17]. The powdered raw material was ground in accordance with the requirements of SPhU monograph 2.9.12 "Sieve analysis" and clarified with chloral hydrate P [17]. Investigations of transverse and longitudinal sections, epidermis and preparations from the surface were performed using microscopes MBS 9, MS 10 (glasses x5, X10, 15, lenses x10, X40), Micromed XS-4130 (eyepiece WF15X, lenses x40/0,65, x10/0,25) with a photomicro nozzle (China). The results of the study were recorded using a Canon IXUS 220 HS camera.

To develop a method for identifying the main BAS in the cranberry leaf used the TLC method. Merck Silica gel F254 blades were used for chromatography. Solvents for the preparation of chromatographic systems used the qualifications of p.f.a. or ch. p.; ratios of solvents, denoted by numbers, taken in volumetric units.

Determination of the content of impurities was performed in accordance with the requirements of SPhU 2.0, 2.8.2 [17]. Determination of weight loss during drying was performed in accordance with the requirements of SPhU 2.0, 2.2.32 [17]. Determination of the total ash content was performed in accordance with the requirements of SPhU 2.0, 2.4.16 [17].

The content of flavonoids was determined in accordance with the monograph SPhU spectrophotometric method in terms of hyperoside according to the method described in the monograph SPhU 2.2 "Blueberry leaves N" [17].

4. Research results

The raw material is dried whole of large cranberry (*Oxycoccus macrocarpus* (Ait.) Pursh) or common cranberry (*Oxycoccus palustris* Pers.). Sometimes there are remnants of stems.

According to the results of macroscopic analysis of samples of whole raw materials of large cranberries (Fig. 2) fragments of woody plagiotropic (horizontal) shoots 7.0–42.0 cm long, 0.15–0.20 cm in diameter and orthotropic (ascending) shoots were considered. 3.0–25.0 cm long, up to 0.1 cm in diameter. The stems of horizontal shoots are covered with dark brown periderm, often with longitudinal crinkle, ascending - light brown or brown periderm. Leaf arrangements alternate, internode short (0.3–0.9 cm). Leaves (Fig. 3, *a–c*) are simple, short-petiolate. The leaf blade is entire, leathery, shiny on the upper side, from dark green to brown-green and brown, from the bottom – from bluish green to light brown. The blade is narrow to oval in shape, occasionally round and ovoid, 0.3–0.6 cm wide and 0.8–1.8 cm long, the apex and base are rounded, the edge is solid, curved downwards. Veining pinnate, on the upper side well visible main and lateral veins, on the lower – well visible main vein, occasionally – lateral.

1 a 2 b c

Fig. 2. Macroscopic signs of large cranberry shoots: 1 – general view, 2 – leaves: *a* – mixture, *b* – size range of width; *c* – size range of length

Fragments of horizontal woody shoots of common cranberry (Fig. 3) 7.0–12.0 cm long, up to 0.1 cm in diameter and ascending shoots 4.0–23.0 cm long, up to 0.1 cm in diameter. The surface of the stems is light brown, the periderm easily peels off into thin strips. Leaf arrangements alternate, internode short (0.4–1.2 cm). Leaves (Fig. 3, *a–c*) are simple, short-petiolate. Leaf blade leathery, shiny on the upper side, green (occasionally single leaves are brownish green), on the lower – bluish-green or almost white from the wax layer, shape – narrowly oval or lanceolate, 0.2–0.5 cm wide and 0.6–1.3 cm long, the tip is pointed or sharp, the base is rounded, the edge of the blade is solid, bent down. Veining pinnate, on the lower and upper sides are clearly visible main and lateral veins.

When studying the microscopic features of the raw material, it was determined that the top of the horizontal shoot of a large cranberry (Fig. 4.1) is not woody, has a triangular shape with rounded corners in the cross section. The surface of the stem is very unevenly tuberous (Fig. 4.1, *a*), covered with epidermis with a layer of well-developed cuticle. Epidermal cells with unevenly thickened membranes, compressed, with brown content. The cells of the primary cortex are parenchymal, compressed on the sides, mainly with thickened brown walls, filled with a homogeneous substance (Fig. 4.1, *b*).

In the area of the primary cortex there are rounded cavities. The type of structure is the axial non-fascicular cylinder. Outside is a wide (3–5 layers) ring of mechanical tissues with a wide lumen, unevenly compressed cells (Fig. 4.1, *c*), below the solid ring contains 2–3 layers of phloem cells (Fig. 4.1, *d*) with brown content. The xylem is dominated by leading elements, represented by porous and ladder vessels, which differ slightly in diameter. Vessels have a radial arrangement and are interspersed with 1-row core beams (Fig. 4.1, *e*). The core is considerably compressed and consists of the squeezed cells containing a brown secret (Fig. 4.1, *f*).

The stem in the middle part of a horizontal shoot has a round shape, covered with a layer of tissue that peels in places. This layer consists of a thin epidermis with cutin (Fig. 4.2, *a*), a narrow homogeneous brown zone of the primary cortex (Fig. 4.2, *b*), the cells of which are compressed and do not differ in structure and rings of densely large lumen fibers (Fig. 4.2, *c*), which in cross section have a shape from polygonal to oval.

Below is a thin periderm, which borders the phloem (Fig. 4.2, *f*). Phellogen has a protophloem origin, still characteristic of some heather [18, 19].

Cork cells have a dense homogeneous brown content, phelloderma cells accumulate anthocyanin (Fig. 4.2, *e*). The phloem area is narrow (Fig. 4.2, *f*), the cells have slightly thickened walls, sieve tubes are small in diameter. The xylem ring (Fig. 4.2, *g*) is wide, the vessels dominate, approximately the same in diameter, are located more often by 1-row rays and alternate with 1-(2)-row core rays. The type of vessels is porous and ladder-shaped (Fig. 4.2, *h, i*). In the primary xylem there is almost no libriform.

1 2 a b c

Fig. 3. Macroscopic features of cranberry shoots. 1 – general view, 2 – leaves: *a* – mixture, *b* – size range of width; *c* – size range of length

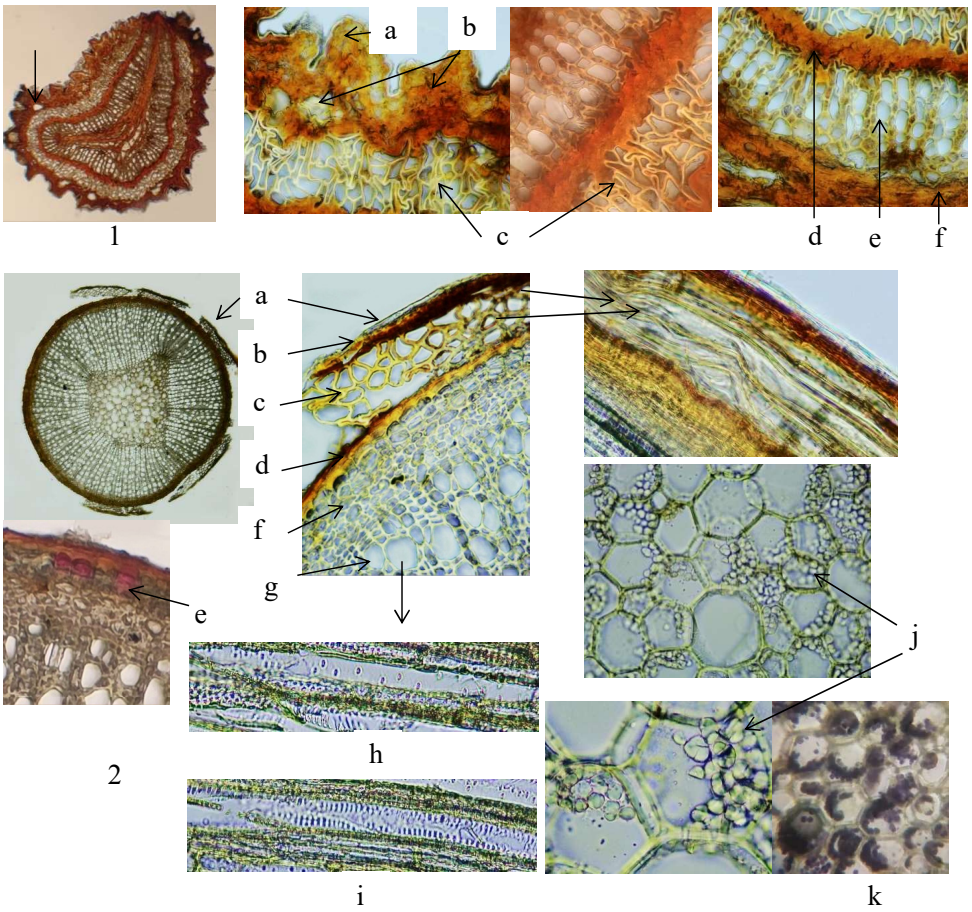

Fig. 4. Microscopic features of the horizontal stem of large cranberries: 1 – apex: *a* – epidermis with cutinized walls; *b* – bark parenchyma with cavities; *c* – ring of mechanical elements; *d* – phloem; *e* – xylem; *f* – core; 2 – middle part: *a* – epidermis; *b* – zone of primary cortex; *c* – ring of mechanical elements in transverse and longitudinal sections; *d* – periderm; *e* – phelloderma with anthocyanin; *f* – phloem; *g* – xylem; *h* – porous vessels in longitudinal section; *i* – spiral vessels; *j* – starch grains in core cells; *k* – the reaction of starch with Lugol's solution

The areas of the primary xylem opposite the corners of the core are darker because the vascular rays are centered. The core is well developed, in outline – 4–5-angular, its cells are parenchymal, with slightly thickened walls and straight pores, containing starch (Fig. 4.2, *j*), differ in size. Starch grains are small, simple and complex, with Lugol's solution giving a dark blue colour (Fig. 4.2, *k*).

In general, the anatomical structure of the ascending stems of large cranberries is like the structure of horizontal stems [20]. But there are some differences. At the top of the stem is rounded in outline, but with significantly wavy contours (Fig. 5.1, 5.2). In the epidermis there are single simple covering hook-shaped hairs (Fig. 5.3); cells of the cortex parenchyma contain large druses (Fig. 5.2, *c*); the type of structure of the axial cylinder is transitional; xylem is less developed, the xylem area contains many large cavities (Fig. 5.1, 5.2, *a*); core rounded, well defined, does not contain starch. In the middle part of the stem under the periderm (Fig. 5.4, *a*) the ring has thick-walled elements of the phloem, the size of these cells varies considerably, the membranes are evenly thickened, there are small-diameter cavities (Fig. 5.4, *b*); the area of the phloem on the border with the cambium is brown (Fig. 5.4, *c*), which is determined by the content of cells and the color of cell membranes; in a xylem the libriform is better developed and therefore the accurate radial arrangement of vessels is not observed; vessels differ in diameter.

The general anatomical structure of horizontal and ascending stems of common cranberries in the upper and middle part is similar to the structure of large cranberry stems (Fig. 6.1, 2).

The tops of the ascending stem are covered with epidermis with simple covering upright curved to the surface trichomes with a rounded top (Fig. 6.1, *a*). Large druses and single prismatic crystals of calcium oxalate were found in the cells of the cortex parenchyma (Fig. 6.2, *b*, *c*). The vessels of the xylem are surrounded by well-developed areas of libriform.

The petiole of a large cranberry leaf is oval in cross-section (Fig. 7.1). The adaxial side is somewhat flattened, slightly wavy, the abaxial side is rounded. Beneath the epidermis is a ring of angular collenchyma: 2-5-layered on the adaxial side, multilayered on the abaxial side, and 1-layered on the sides (Fig. 7.1, *a*). Parenchyma cells are round, thin walled, of different diameters with frequent large druses (Fig. 7.1, *b*) and rare cavities. The bundle is single, rounded, shifted to the adaxial side (Fig. 7.1, *c*), phloem and xylem are well developed, surrounded by non-lignified thick-walled cells.

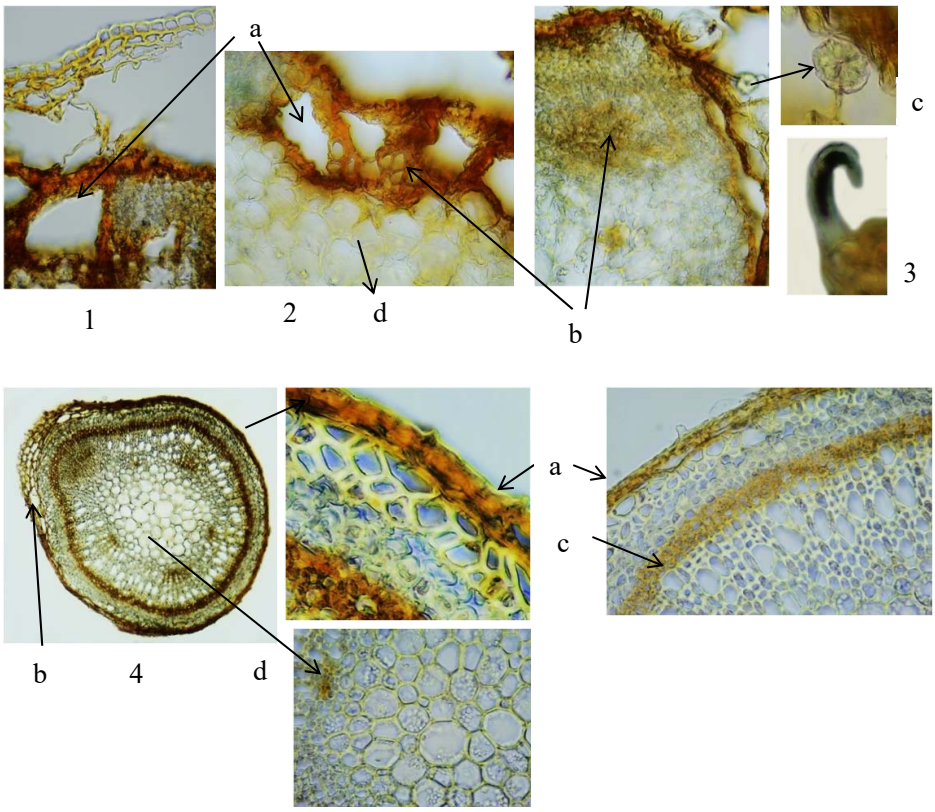

Fig. 5. Microscopic signs of lateral ascending stem of large-fruited cranberry. *Top fragment*: 1 – l/m; 2 – h/m; *a* – cavities; *b* – xylem; *c* – druse; *d* – core cells; 3 – simple hook-shaped hair; 4 – middle part: *a* – periderm; *b* – cavity; *c* – ring of phloem with brown content; *d* – core with starch grains

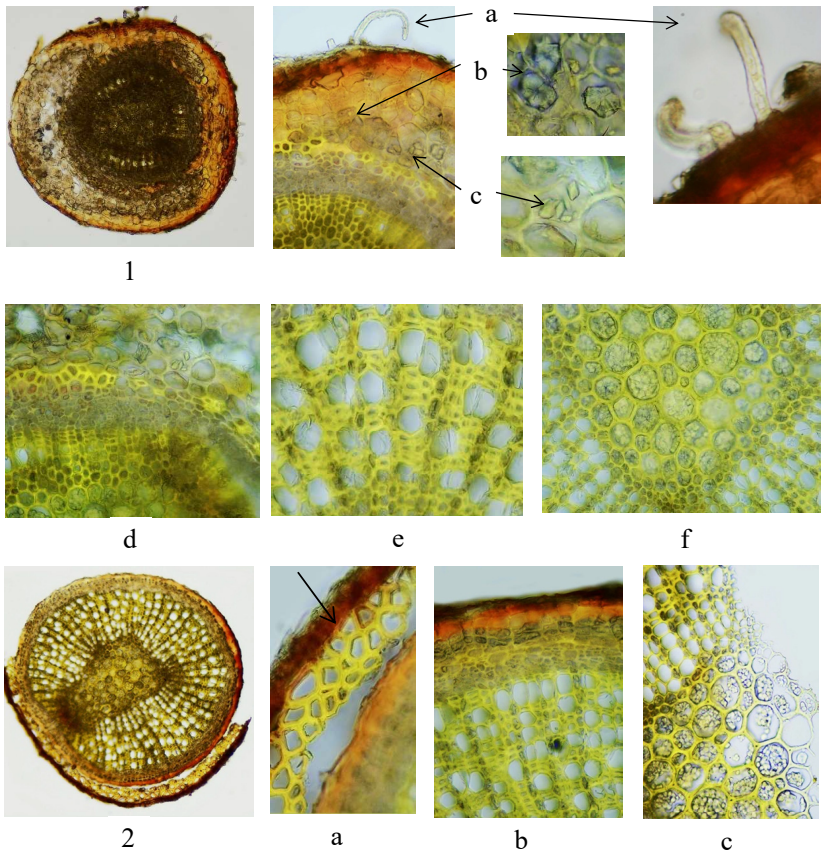

Fig. 6. Microscopic features of the stem of the swamp cranberry: 1 – the top of the ascending stem (plan on l/m); *a* – simple covering trichomes; *b* – druses; *c* – prismatic crystals; *d* – phloem; *e* – xylem; *f* – core with starch grains; 2 – the middle part of the horizontal stem on l/m: *a* – periderm; *b* – xylem fragment; *c* – core with starch grains

The leaf blade has a dorsiventral type of structure (Fig. 7.2) [17, 23]. Palisade chlorenchyma is 1-2-rowed, cells are small, thin-walled, spongy chlorenchyma is 3-4-rowed, cells are round, thin-walled, with small intercellular spaces (Fig. 2.3). The main vein is single-bundle, and does not protrude from the abaxial side. The central bundle is rounded, similar in structure to the petiole bundle. Small-cell thick-walled sclerenchyma from the xylem is better developed. A crystalliferous facing is formed around the side bundles (Fig. 7.2, 7.5, *a*), which is clearly visible from the surface. The lower epidermis of the surface consists of two types of cells. Along the veins, the cells are prosenchymal, straight walled, with weakly thickened membranes and straight pores. Between the veins, the cells are parenchymal, the walls are from almost straight to weakly sinuous, slightly thickened (Fig. 7.4). The stomata are frequent, oval, the type of the stomatal apparatus is paracytic and anomocytic. Simple prismatic and cubic crystals of calcium oxalate accumulate around the veins in the cells, forming a crystalline facin. The upper epidermis consists of parenchymal cells of the same shape and size with sinuous walls (Fig. 7.6). There are no stomata. The cuticle is barely visible, intermittently folded [24]. Cells along the edge of the leaf blade are smaller, elongated, quadrangular, with slightly thickened rectangular membranes.

The petiole of common cranberry is similar in structure to the petiole of large cranberry [24]. Distinctive features include the following features: in cross section the shape of the petiole is close to triangular (Fig. 8.1), the adaxial side is slightly emarginate, abaxial – rounded.

The epidermis is lined with 1–2 layers of the collenchyma, the parenchyma is small-celled, filled with large druses (Fig. 8.1, *b*), among the parenchyma there are large cavities (Fig. 8.1, *c*). The rounded bundle occupies a radial position, the elements of the xylem and phloem are small-cell (Fig. 8.1, *a*).

The anatomical structure of the leaf blade of cranberry is like the structure of the leaf of large cranberry (Fig. 8.2, *a–g*). The cells of the lower epidermis along the vein are sinuous-walled, prosenchymal and parenchymal, contain prismatic crystals that form a crystalliferous facing (Fig. 8.3, *a*). Cells between the veins are parenchymal, lobed, with slightly thickened membranes (Fig. 8.3), stomata apparatus, large, oval. Types of respiratory system – paracytic and anomocytic [25, 26]. The cells of the upper epidermis are parenchymal, with sinuous walls, covered with a striate-folded cuticle (Fig. 8.4, *a*).

Identification by thin layer chromatography was performed using PhRS SPhU hyperoside, caffeic acid and rutin in the system *ethyl acetate P – water P – formic anhydrous acid P – anhydrous acetic acid P* (72:14:7:7). After spraying the blade with solutions of aminoethyl ester of *diphenylboronic acid P*, *macrogol 400 P* when viewing the blade in UV light identifies zones at the level of the zones of hyperoside, rutin and caffeic acid.

According to the literature, it is flavonoids that provide hypoglycemic action, because the parameter of quantitative standardization was chosen as the content of flavonoids [10, 12].

The results of determination of impurities and particles, indicators *"Loss on drying"* and *"Total ash"* and quantitative determination of the quantitative content of flavonoid compounds are given in Table 1.

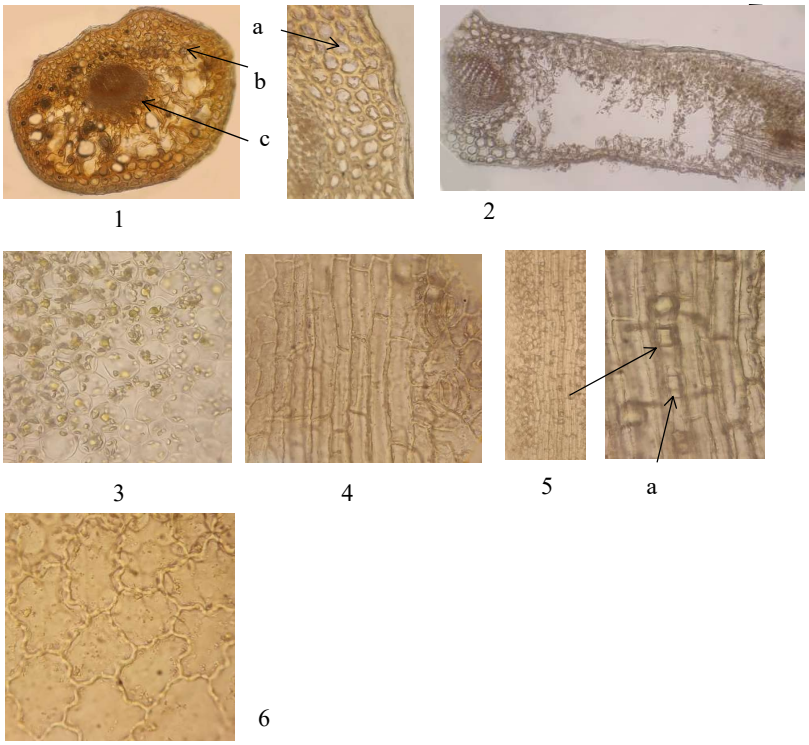

Fig. 7. Microscopic signs of a large cranberry leaf: 1 – petiole; *a* – collenchyma; *b* – druse; *c* – conductive bundle; 2 – transverse section of a blade; 3 – spongy chlorenchyma (top view); 4 – lower epidermis; 5 – crystalline facing of single crystals (l/m); *a* – on h/m; 6 – upper epidermis

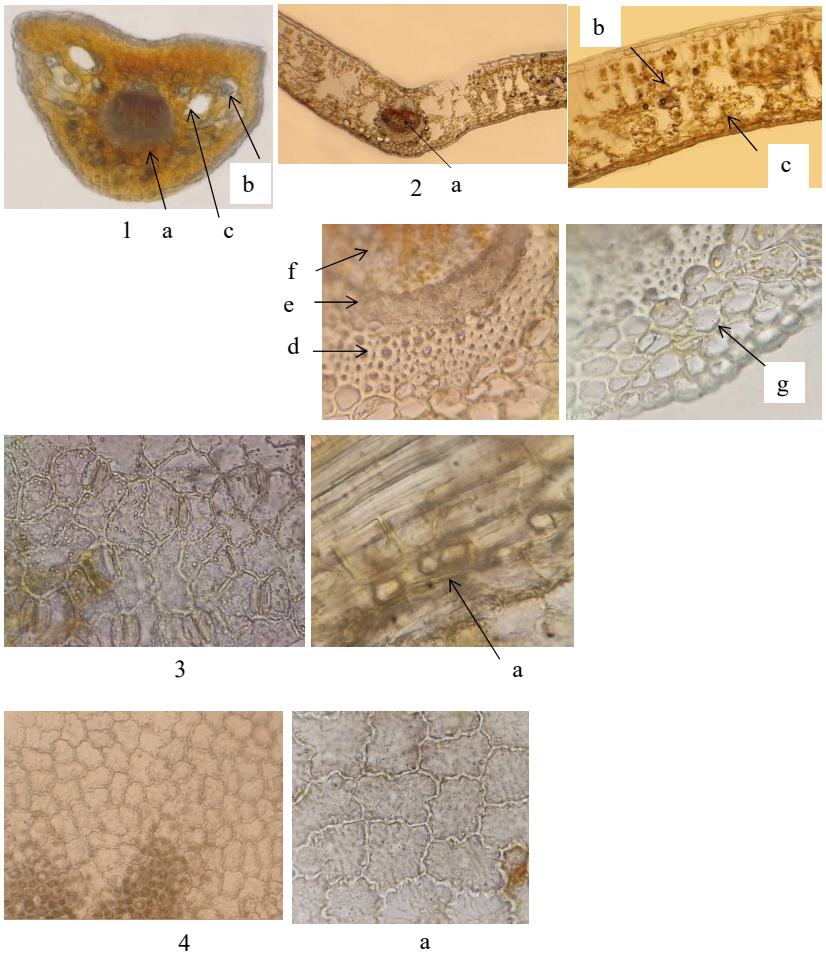

Fig. 8. Microscopic signs of a cranberry leaf: 1 – petiole, *a* – conductive bundle, *b* – druses, *c* – cavities, 2 – cross section of the blade: *a* – main vein, *b* – columnar chlorenchyma, *c* – spongy chlorenchyma, *d* – sclerenchymal cover, *e* – phloem, *f* – xylem , *g* – collenchyma, 3 – lower epidermis, *a* – crystalline fascing of a vein of single crystals, 4 – upper epidermis with folded cuticle, *a* – on h/m

Table 1

The results of the analysis of samples of cranberry leaves

Indicators	Graduation	Raw material samples					
		O. palustris			*O. macrocarpus*		
		1	2	3	4	5	6
Identification	According to the developed parameters	+	+	+	+	+	+
External signs – macroscopy	According to the developed parameters	+	+	+	+	+	+
Microscopy	According to the developed parameters	+	+	+	+	+	+
TLC identification	According to the developed parameters	+	+	+	+	+	+
Impurities	Brown stems – no more than 5 %	4.1 %	4.2 %	3.6 %	2.1 %	4.5 %	0 %
	No more impurities than 2 %	1.5 %	1.0 %	1.0 %	0.5 %	1.0 %	0.2 %
Weight loss during drying	No more than 10,0 %	6.7 %	7.3 %	7.2 %	8.7 %	7.5 %	7.0 %
Total ash	No more than 7 %	4.2 %	6.1 %	4.6 %	4.3 %	3.2 %	3.9 %
The content of the amount of flavonoids, in terms of hyperoside	Not less than 1 %	1.15 %	1.11 %	1.06 %	1.22 %	1.36 %	1.25 %

Note: "+" – meets the requirements

5. Discussion of the results

The literature provides data on the microscopic characteristics of cranberries [21, 27], but these data are covered more from a botanical point of view and can be the basis for the development of identification methods A and B for quality control methods for leaves and shoots of this raw material but requires take into account the specifics according to the requirements of SPhU [17]. However, this species in Ukraine is listed in the Red Book [4], so the procurement of these raw materials in nature for the needs of the pharmaceutical industry is prohibited. At the same time, there is no data on cultivated of cranberries grown in Ukraine, and they are a promising alternative raw material for common cranberries. These species, depending on varietal characteristics, may differ in polymorphism, so the data obtained

revealed common and diagnostic features between wild cranberries and cultivated samples of large cranberries, which are most widely grown in Ukraine.

As a result of the conducted researches it is established that the common morphological features of large cranberry and common cranberry are: stems long, thin, woody horizontal and ascending, covered with periderm; leaves simple, short-petiolate, with a single leaf blade, oval or lanceolate, leathery with a curved downward edge, the underside is lighter than the wax layer, the veins are pinnate.

For the first time, distinctive morphological features of large and common cranberry shoots were determined. Thus, for large cranberry shoots, these stems are covered with a dark brown periderm, often with longitudinal crinkle; internodes short, up to 0.9 cm, leaves oval, rounded and ovate, the colour of the upper side – from dark green to brown green, the lower – from bluish green to light brown. Distinctive morphological features of cranberry shoots are: stems are covered with light brown periderm, which easily peels off in thin strips; internodes longer, up to 1.2 cm, lanceolate leaves, smaller in size, apex pointed or sharp, the colour of the upper side is green, from the bottom – bluish-green to almost white.

As a result of microscopic analysis, common anatomical features of large cranberries and common cranberries were determined, namely: for stems: non-fascicular type of structure in the middle part, the top is covered with epidermis with cuticle, there are simple covering hairs of hook-shaped shape; cells of the primary cortex are compressed, with a brown colour; the middle part – a thin periderm, phellogen has a protophloem origin, outside the axial central cylinder contains a wide ring of mechanical tissue of large lumen, unevenly compressed cells, in the primary cortex there are large druses; type of vessels – porous and ladder, core cells parenchymal, with slightly thickened walls, accumulate simple and complex starch grains; for leaves: leaves hypostomatic, dorsoventral, main vein and petiole single-bundle, around the bundles are simple prismatic single crystals that form a crystalliferous facing, among the cells of the parenchyma there are cavities.

For the first time, distinctive anatomical features of stems and leaves of large cranberries and common cranberries were determined. Distinctive anatomical features of large cranberry stems are: at the apex the horizontal stem has a triangular shape, the non-fascicular type of structure of the central cylinder, and the ascending – rounded, with significant wavy contours of the surface and the transitional type of structures; between the elements of the xylem there are many large cavities. Also, in the ascending stems in the middle part under the periderm contains a ring of thick-walled elements of the phloem, in the xylem is better developed libriform. Distinctive anatomical features of the stems of common cranberries are: the epidermis of the lateral stem is covered with simple covering upright curved to the surface trichomes with a rounded tip; in the cells of the cortex parenchyma found, in addition to druse, single prismatic crystals of calcium oxalate. Distinctive features of the leaves are: petiole of large cranberry in the outlines of an oval shape, adaxial side slightly wavy, abaxial – rounded, and common cranberries – triangular, adaxial side slightly emarginate, abaxial – rounded.

These data are the basis for describing the macroscopic and anatomical features of raw materials in the identification of A and B for quality control methods.

As a result of the conducted research the method of identification of C for cranberry leaves with use of TLC is offered. Conditions for chromatography and detection of substances were proposed, like the method described in the monograph SPhU 2.2 “Blueberry leaves N”.

The sequence of zones on the chromatograms of the test solution and the reference solution is given in Fig. 9. Other fluorescent zones may also be detected on the chromatogram of the test solution.

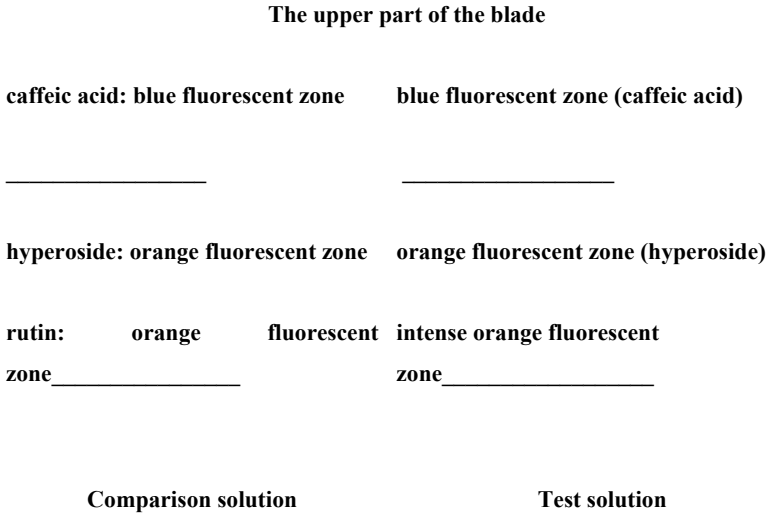

Fig. 9. The sequence of zones on the chromatograms of the test solution and the reference solution

In the middle part of the chromatogram of the reference solution is found an orange fluorescent zone corresponding to the routine, as well as above it an orange fluorescent zone corresponding to the hyperoside. A blue fluorescent zone corresponding to caffeic acid is found at the top of the chromatogram. The chromatogram of the test solution at the level of the routine comparison solution should show an intense orange fluorescent zone covered with a light blue zone. An orange fluorescent zone should be detected at the level of the hyperoside comparison solution and a blue fluorescent zone should be detected at the level of the caffeic acid comparison solution. Other fluorescent zones may also be detected on the chromatogram of the test solution.

TESTS are proposed to be carried out according to the following indicators: impurities (2.8.2), namely brown stems – not more than 5 %; other impurities – not more than 2 %; weight loss on drying (2.2.32) – not more than 10.0 % (1,000 g of crushed to powder raw materials

are dried at a temperature of 105 °C for 2 h); total ash (2.4.16) – not more than 7 %.

QUANTITATIVE DETERMINATION should be based on the content of flavonoids – not less than 1 %, in terms of hyperoside ($C_{21}H_{20}O_{12}$; M.w. 464.4) and dry raw materials.

The prospects for further research. The obtained results will be used in the development of instructions for harvesting leaves and shoots of cranberries, approval of quality control methods for these types of raw materials. Further phytochemical and pharmacological studies of these raw materials will show whether they can be combined into one type of raw cranberry leaves or shoots, or whether they will be two separate types of raw materials. This in turn will significantly expand the raw material base for the creation of new drugs based on cranberry raw materials.

6. Conclusions

The parameters of standardization of cranberry leaves according to the following indicators: macro-and microscopic features, TLC identification of the main BAS raw materials (hyperoside, rutin and caffeic acid), impurities (not more than 2 %), brown stems not more than 5 %, weight loss during drying (not more than 10 %), total ash (not more than 7 %) and not less than 1 % of flavonoids, in terms of hyperoside.

For the first time, a comparative analysis of morphological and anatomical features of stems and leaves of common cranberries and large cranberries, considering the requirements of SPhU. The general features of the structure of stems and leaves of both species and diagnostic features for each species, which can be used as key quality indicators in the standardization of the studied species and identification of raw materials.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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References

1. Lauber, K., Wagner, G., Gygax, A. (2018). Flora Helvetica. Illustrierte Flora der Schweiz. Haupt Verlag.

2. Vander Kloet, S. P. (1983). The taxonomy of Vaccinium section Oxycoccus (Rhodora) 85:30. New England Botanical Club, Inc.

3. Hrodzinskyi, A. M. (1992). Likarski roslyny. Kyiv:«Ukrainska Entsyklopediia» im. M. P. Bazhana, 542.

4. Zhuravlyna dribnoploda Oxycoccus microcarpus Turcz. ex Rupr. (O. quadripetalus Gilib. subsp. microcarpus (Turcz. ex Rupr.) Braun-Blanq., O. palustris Pers. subsp. microcarpus (Turcz. ex Rupr.) Nyman, Vaccinium microcarpum (Turcz. ex Rupr.) Schmalh., V. oxycoccus L. subsp. Micro. Available at: http://redbook-ua.org/item/oxycoccus-microcarpus-turcz-ex-ruprschmalh-v-oxycoccus-l-subsp-micro/

5. Good practice of cultivation and collection of raw materials of plant origin (2012). Guidelines СТ-Н МОЗУ 42–4.5: 2012.

6. Ukrainske sadivnytstvo: posadyty y zarobyty (2020). Ukrainskyi tyzhden, 36 (668).

7. Deinichenko, L. G. (2016). Cranberries as a promising raw material for the production of special purpose products. Food additives. Nutrition of healthy and sick people. Kryvyi Rih: DonNUET, 58–59.

8. Kolontarev, K. B. (2013). The use of cranberry preparations in patients with recurrent urinary tract infections. Effective pharmacotherapy. Urology and Nephrology, 3 (26), 42–46.

9. Kovalenko, V. N. (2020). Compendium 2020 – Medicines. Kyiv: MORION, 2700.

10. Koshovyi, O. M., Zagayko, A. L., Kolychev, I. O., Akhmedov, E. Yu., Komissarenko, A. N. (2016). Phytochemical study of the dry extract from bilberry leaves. Azerbaijan Pharmaceutical and Pharmacotherapy Journal, 16 (1), 18–23.

11. Zagayko, A. L., Kolisnyk, T. Y., Chumak, O. I., Ruban, O. A., Koshovyi, O. M. (2018). Evaluation of anti-obesity and lipid-lowering properties of Vaccinium myrtillus leaves powder extract in a hamster model. Journal of Basic and Clinical Physiology and Pharmacology, 29 (6), 697–703. doi: http://doi.org/10.1515/jbcpp-2017-0161

12. Koshovyi, O., Granica, S., Piwowarski, J. P., Stremoukhov, O., Kostenko, Y., Kravchenko, G. et. al. (2021). Highbush Blueberry (Vaccinium corymbosum L.) Leaves Extract and Its Modified Arginine Preparation for the Management of Metabolic Syndrome – Chemical Analysis and Bioactivity in Rat Model. Nutrients, 13 (8), 2870. doi: http://doi.org/10.3390/nu13082870

13. Chaika, N., Mazen, M., Koshovyi, O., Kravchenko, G., Goryacha, O., Kireyev, I. et. al. (2021). Research in phytochemical composition and hypoglycemic activity screening of the dry extracts from bearberry leaves. ScienceRise: Pharmaceutical Science, 3 (31), 42–50. doi: http://doi.org/10.15587/2519-4852.2021.235939

14. Chaika, N., Koshovyi, O., Ain, R., Kireyev, I., Zupanets, A., Odyntsova, V. (2020). Phytochemical profile and pharmacological activity of the dry extract from Arctostaphylos uva-ursi leaves modified with phenylalanine. ScienceRise: Pharmaceutical Science, 6 (28), 74–84. doi: https://doi.org/10.15587/2519-4852.2020.222511

15. Koshovyi, O. M., Vlasova, I. K., Brukhanova, T. O., Krasilnikova, O. A., Kravchenko, G. B., Zagayko, A. L., Komisarenko, M. A. (2021). Pat. No. 147975 UA. A method of obtaining a therapeutic and prophylactic agent from the leaves of large-fruited cranberries for the correction of insulin-resistant conditions. No. u 2021 00821, declareted: 02/22/2021; published: 23.06.2021, Bul. No. 25/2021.

16. Dobrochaeva, D. N., Kotov, M. I., Prokudin, Y. N., Barbarich, A. I. (1999). Key to Higher Plants of Ukraine. Kyiv: Naukova dumka.

17. State Pharmacopoeia of Ukraine. (2015). Kharkiv SO «Ukrainian Scientific Pharmacopoeial Center of Drugs Quality».

18. Boyko, V. I. (1993). Anatomy of the bark of some species of cranberries M. Dep. in SHSTI 5.1) 5.93. I176-VEZ.

19. Best, V. M., Vasanthakumar, A., McManus, P. S. (2004). Anatomy of Cranberry Stem Gall and Localization of Bacteria in Galls. The American Phytopathological Society, 94 (11), 1172–1177. doi: http://doi.org/10.1094/phyto.2004.94.11.1172

20. Cesoniene, L., Daubaras, R., Areskeviciute, J., Viskelis, P. (2006). Evaluation of Morphological Peculiarities, Amount of Total Phenolics and Anthocyanins in Berries of European Cranberry (Oxycoccus palustris). Baltic Forestry, 12 (1), 59–63.

21. Palser, B. F. (1961). Studies of Floral Morphology in the Ericales. V. Organography and Vascular Anatomy in Several United States Species of the Vacciniaceae. Botanical Gazette, 123 (2), 79–111. doi: http://doi.org/10.1086/336134

22. Pukauskiene, J., Paulauskas, A., Eesoniene, L., Daubaras, R. (2009). Genetic structure of isolated Vaccinium oxycoccus populations in Lithuania. Proceedings of The Latvian academy of sciences. Section B, 63 (1/2 (660/661)), 33–36. doi: http://doi.org/10.2478/v10046-009-0018-5

23. Schweingruber, F. H., Kucerova, A., Adamec, L., Dolezal, J. (2020). Anatomic Atlas of Aquatic and Wetland Plant Stems. Cham: Springer. doi: http://doi.org/10.1007/978-3-030-33420-8

24. Nemzer, B. V., Al-Taher, F., Yashin, A., Revelsky, I., Yashin, Y. (2022). Cranberry: Chemical Composition, Antioxidant Activity and Impact on Human Health: Overview. Molecules, 27 (5), 1503. doi: http://doi.org/10.3390/molecules27051503

25. Cesoniene, L., Daubaras, R.; Simmonds, M. S. J., Preedy V. R. (Eds.) (2016). Phytochemical composition of the large cranberry (Vaccinium macrocarpon) and the small cranberry (Vaccinium oxycoccos). Nutritional Composition of Fruit. Cambridge: Academic Press, 173–194. doi: http://doi.org/10.1016/b978-0-12-408117-8.00008-8

26. Cesoniene, L., Daubarus, R., Paulauskas, A., Zukauskiene, J., Zych, M. (2013). Morphological and genetic diversity of European cranberry (Vaccinium oxycoccos L., Ericaceae) clones in Lithuanian reserves. Acta Societatis Botanicorum Poloniae, 82, 211–217. doi: http://doi.org/10.5586/asbp.2013.026

27. Usmanov, Iy., Yumagulova, E., Ovechkina, E., Ivanov, V., Shcherbakov, A., Aleksandrova, V., Ivanov, N. (2016). Fractal Analysis of Morpho-Physiological Parameters of Oxycoccus Palustris Pers in Oligotrophic Swamps of Western Siberia. Vegetos- An International Journal of Plant Research, 29 (1), 1. doi: http://doi.org/10.5958/2229-4473.2016.00002.1

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