Investigation of the morphological and anatomical features of the raw material Vaccinium myrtillus L.

G. Ya. Mechikova[1], T.A. Kopoteva[2], O.V. Smirnova[3]
([1]Far Eastern State Medical University, Khabarovsk, [2]Institute of Water and Environmental Problems FEB RAS, Khabarovsk, [3]FSBI "IMTSEUAOSMP" of Roszdravnadzor, Moscow)

Morphoanatomic study of raw material Vaccinium myrtillus L.

GY Mechikova[1], TA Kopoteva[2], OV Smirnova[3]
[1]Far Eastern State Medical University (Khabarovsk, Russia),
[2]Institute of Water and Ecological Problems FEB RAS (Khabarovsk, Russia),
[3]Federal State Budget Institution “Information and Methodological Center for Expertise, Stocktaking and Analysis of Circulation of Medical Products "(Moscow, Russia)

SUMMARY

The anatomical and diagnostic features of the stem and biometric parameters of microscopic features of leaves of Vaccinium myrtillus L. were established. Microscopic studies of the crushed raw material and powder of shoots were carried out. The obtained results are included in the section "Microscopy" of the draft pharmacopoeial monograph for the State Pharmacopoeia 12th ed. regulating the quality of V. myrtillus L.

Key words: common blueberry, microscopy, authenticity, diagnostic signs.

RESUME

Anatomical and diagnostic features of the stem and biometric microscopic features of the leaves of Vaccinium mirtillus L. are specified. Microscopic study of milled herb and sprouts powder were completed. The results are included in the section "Microscopy" of draft pharmacopeial description for State Pharmacopoeia of Russia XII regulating the shoot quality.

Keywords: Vaccinium mirtillus L., microscopy, authenticity, diagnostic features.

Vaccinium myrtillus L. (common blueberry) is a representative of the family. Ericaceae Juss. (Heather) has been widely used in our country for a long time.

V. myrtillus has an official status. The quality of the shoots of this plant is regulated pharmacopoeial monograph FS-42-2948-93.

The current regulatory document provides a description of the micro-characteristics of V. myrtillus raw materials in accordance with the requirements that were in force at the time of approval of this document. The content and design of this section today no longer meet modern requirements for the level of determining the authenticity of medicinal plant materials. In particular, illustrations (photographs) of micro-features are not provided and their quantitative characteristics are not indicated. Modern requirements for the section "Microscopy" imply the inclusion in the article of a description of the microscopic features of all the main parts of the morphological group of raw materials. For the species under discussion, these are the stem and leaf. In the existing regulatory documentation, only microdiagnostic signs of the sheet are presented.

To create a normative document in a modern format, there is a need for a number of additional anatomical studies of V. myrtillus raw materials, including ground and in powder form.

Thus, the purpose of our work was to establish the main anatomical and diagnostic signs of shoots (whole, crushed, powder) V. myrtillus, necessary for a complete modern diagnosis of the raw material of this medicinal plant.

MATERIALS AND METHODS

The objects of the study were samples of 5 batches of V. myrtillus raw materials collected in 2003–2012. in the Bryansk, Irkutsk and Arkhangelsk regions, as well as in the Republic of Sakha (Yakutia) and the Altai Territory. Shoots were harvested during the period recommended by the instructions for the procurement of raw materials [1].

The preparation of material for research was carried out in compliance with regulatory requirements and generally accepted methods in accordance with the articles of the State Pharmacopoeia of the 11th edition:

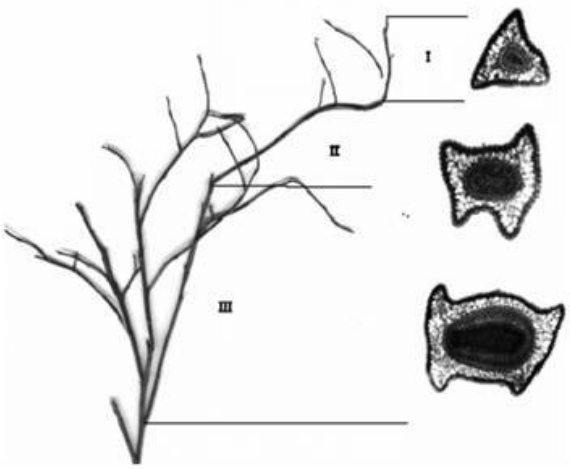
"Methods of analysis of medicinal plant raw materials" Trava "and" Technique of microscopic and microchemical research of medicinal plant materials "[2]. Anatomical studies were carried out according to the method of preparation of preparations from whole, crushed raw materials of herbs and herbal powder. For microscopic examination of the powder, the methodological technique proposed in [3] was used. To determine the characteristic microdiagnostic signs of V. myrtillus shoots, we studied the cross section of the stem: in the lower, middle, and upper parts of the shoot — the growth of the last growing year, as well as the epidermis of the longitudinal section of the stem and the leaf blade from the surface.

Work with microscope preparations was carried out on a Nikon Eclipse LV 100 Pol digital microscope (Japan) and on Micros MC400A and Mikmed-6 light microscopes. Computer processing of images of micropreparations for measuring biometric parameters and obtaining micrographs was carried out using a complex for hardware-software determination of morphometric parameters of cells "Mekos-C".

Statistical analysis of experimental data was performed using Microsoft Office Excel 2010.

RESULTS AND ITS DISCUSSION

Whole raw materials. On the cross section of the stem, a tendency to change its shape from top to bottom is clearly visible (Fig. 1). The stem at the base and middle zone of the shoot is tetrahedral with elongated ribbed corners. The stalk of the growth of the 1st order (the upper part of the shoot) is trihedral, also sharp-ribbed. Thus, a rather strongly pronounced ribbing is characteristic of the blueberry stem.



Rice. 1. Escape of annual growthV. myrtillus. I - cross section of the stem of the first order increment, taken away. 100 ×; II - a cross section of the stem of the second order growth, taken away. 100×; III - cross section stalk of growth of the third order, took away. 40×.

The epidermis of the stem is single-layered, consists of rectangular cells of almost the same size; at all three levels of the stem, it is covered with a thick cuticle. The epidermis of the stem of V. myrtillus is characterized by unicellular trichomes (more abundant in growths of the 1st order), defined in [4] as acicular. The shape of the trichomes is an important diagnostically significant characteristic of the stem of the species under discussion. Collenchyma is located under the epidermis, in 2-3 layers in the ribs, in 1-2 layers between the ribs.

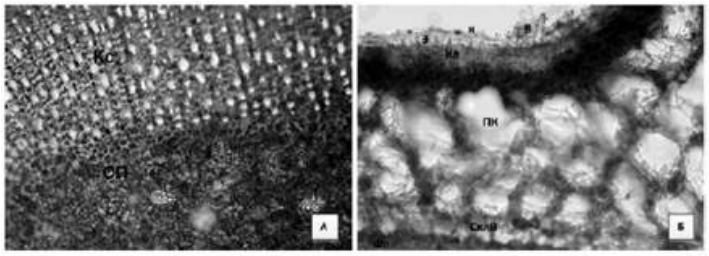
The layer following collenchyma is represented by parenchymal cells of the primary cortex with abundant chloroplasts. Further, towards the center, there is a layer of cells of the main parenchyma of the cortex with extensive intercellular spaces. In this part of the stem, there are inclusions in the form of large prismatic crystals of calcium oxalate. The mechanical elements of the primary cortex are represented by fibers folded in 2–4 layers, which surround the phloem part of the stem with an almost continuous ring. Phloem borders on cambium. Further to the center, there is a xylem of the scattered vascular type with single-row chains of medullary rays. The perimedullary zone with small cells of the primary xylem is clearly visible. The core is represented by large

parenchymal cells with lignified strongly thickened walls with pores. The pith cells contain a large amount of starch grains at all three levels of the stem.

When examining a series of sections in the direction from the top to the bottom of the stem, it can be seen that its anatomical architecture is the same at all three levels of growth. Despite the fact that the location of tissues in the anatomical structure of a one-year-old stem of V. myrtillus is typical for shrub forms, there are a number of microscopic features that have a certain diagnostic value in terms of diagnostics. Diagnostically significant microscopic features of V. myrtillus shoots recommended for harvesting are: the characteristic sharp-ribbed shape of the stem on the cross section, the absence of peridermis, acicular hairs on the stem epidermis, a network of intercellular spaces in the primary cortex, and a well-developed continuous ring of mechanical tissue in the phloem part of the stem (Fig. 2).

When studying the anatomical and diagnostic signs of leaves, we took into account the information available in the literature [3, 5]. The table shows the generalized literature data and the results of our own studies of the anatomical structure of the epidermis of the leaves, which are proposed to be used in the diagnosis of raw materials of this species. Photographs of microscopic features are shown in Fig. 3.

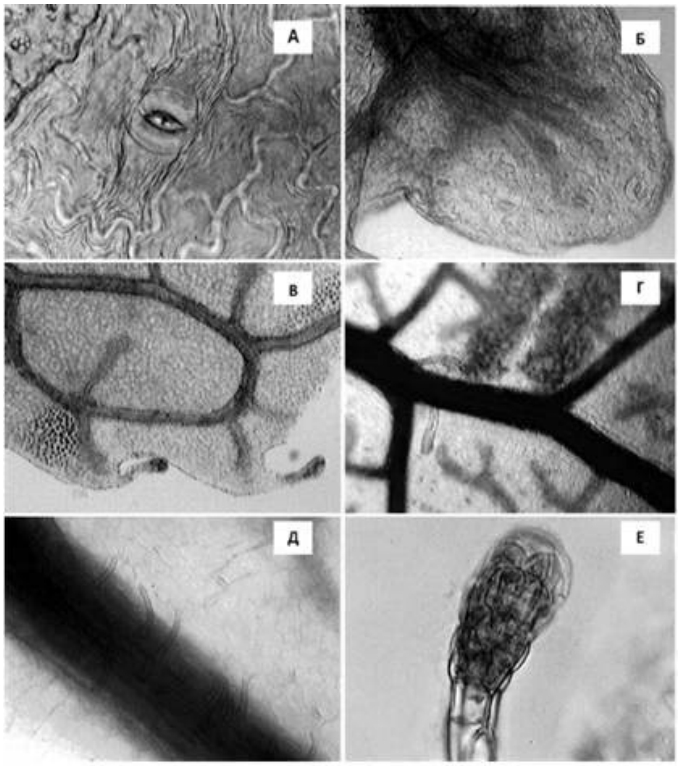
Our studies of the anatomical structure of leaves were mainly limited to determining the biometric parameters of characters that were absent in the literature.



Rice. 2 Fragments of a cross-section of the stemV. myrtillus.

A - a fragment of the xylem and core, taken away. 400× - X-xylem, SP-parenchyma of the core with grains starch;

B - a fragment of the integumentary and crustal parts, taken away. 400× - K-cuticle, E-epidermis, B-hairs, C-collenchyma, PC-parenchyma of the cortex with large intercellular spaces, SklB-sclerenchymal fibers, Fl-phloem.



Rice. 3. Microscopy of the leaf epidermisV. myrtillus.

A - a fragment of the lower epidermis of a leaf with an anamocytic stomatal complex and folding cuticles, took away. 800×

B - a fragment of the leaf epidermis with a hydatode, taken away. 100×

B - a fragment of the edge of a leaf with clavate glands on the denticles, taken away. 100×D - a fragment of the upper epidermis of a leaf with clavate glands on the vein, taken away. 100×

D - a fragment of the upper epidermis of a leaf with hairs on a vein, taken away. 100×

E - the head of the clavate gland, led away. 400×

table

Anatomical and diagnostic features of V. myrtillus leaves

Анатомо-диагностические признаки	Характеристика анатомо-диагностического признака	Встречаемость анатомо-диагностического признака
Клетки верхней и нижней эпидермы листа	В очертании клетки тонкостенные сильно извилистые, длиной 44–89 мкм, шириной 22–57 мкм	
Устьица	Устьица мелкие, с 4–6 околоустьичными клетками (аномоцитный тип устьичного аппарата), длиной 24–34 мкм, шириной 13–25 мкм	Устьица располагаются на обеих сторонах листовой пластинки, с преобладанием на нижней стороне
Железки	Булавовидные железки. Железка состоит из овальной многоклеточной головки с коричневым содержимым и многоклеточной двурядной ножки, длина железки 94–161 мкм	Располагаются на обеих сторонах листовой пластинки, а также встречаются по краю листовой пластинки на верхушках зубцов
Трихомы (волоски)	Грубобородавчатые толстостенные волоски, одноклеточные прямые или изогнутые, длиной 36–110 мкм	Располагаются на верхней стороне листовой пластинки, по жилкам
Гидатода	Сопровождается ответвлениями проводящих пучков и несколькими водяными устьицами	Располагается на верхушке листовой пластинки
Кутикула	Кутикула складчатая	Складчатость кутикулы наблюдается с обеих сторон листа.
Включения	Крупные кристаллы оксалата кальция	Встречаются с нижней стороны листовой пластинки вдоль жилок

For biometric studies of microdiagnostic elements of V. myrtillus leaves, the following parameters were chosen: length and width of epidermal cells on the lower and upper sides of the leaf, length and width of stomata, length of clavate gland, length of coarse hair.

The representativeness of the sample for carrying out biometric studies was ensured by a sufficient number of samples (5 batches of raw materials, covering a significant range of the species) and the amount of data obtained. In total, 125 measurements of each parameter of the microfeature were carried out, both from the lower and upper sides of the leaf blade. The exception was the length of coarsely warty hairs, since they are found only on the upper side of the leaf blade. The research results are presented in the table.

When comparing the sizes of anatomical and diagnostic signs, we did not find significant differences between the length and width of epidermal cells, length and width of stomata, length and width of the head of the clavate gland located on the upper side of the leaf blade with those located on the lower side of the leaf blade (tcr> texp). In most cases, biometric parameters are characterized by moderately pronounced variation (CV = 11–16%). A relatively small variation in size was found for the stomatal apparatus (CV = 7–10%). The variability of the length of the coarsely warty hair is noted as high (CV = 28%).

Thus, the experiment made it possible to establish the sizes of the anatomical and diagnostic features of V. myrtillus leaves, which are included in the draft of the monograph.

Shredded raw materials. Crushed blueberry shoots are a mixture of pieces of raw materials (leaves, stems, occasionally buds, flowers and fruits) of various shapes, passing through a sieve with 5 mm holes. Studies of crushed raw materials have shown that the sizes of stems and leaves are quite acceptable for the preparation of microslides, similar to micropreparations of whole raw materials: leaf from the surface, stem - cross section. In this case, all diagnostically significant traits for V. myrtillus raw material appear in full. Therefore, we consider it appropriate not to distinguish between the description of microdiagnostic signs of whole and crushed shoots in the draft pharmacopoeial monograph.

Powder. When considering micropreparations of the coarse fraction (particle size 2–0.25 mm) and fine fraction (sifting through a sieve with holes with a diameter of 0.25 mm) of the powder, we established

fragments of leaves with their inherent anatomical and diagnostic features. Scraps of leaf epidermis with winding thin cell walls 44–89 μm long, 22–57 μm wide and anomocytic stomata of 24–34 μm long and 13–25 μm wide are visible. On scraps of leaf epidermis (mainly along the veins), as well as on scraps of the leaf edge, on the denticles, clavate glands 94–161 μm long with a multicellular two-row stem and an oval multicellular head with brown content are often found. On scraps of the upper epidermis of the leaf along the veins, coarsely warty, thick-walled, unicellular straight or curved hairs 36–110 μm long are visible. Sometimes on the scraps of the epidermis of the leaf, folds of the cuticle are observed. Scraps of veins with a crystalline sheath are often seen.

The anatomical picture of the stem parts of the raw material is somewhat different from that in the crushed raw material. Stem elements in coarse and fine powder fractions are presented both in transverse (scraps) and in longitudinal section.

CONCLUSIONS

1. Diagnostically significant microscopic features of V. myrtillus stem were established.
2. Biometric parameters of micro-traits of V. myrtillus leaves were determined for the first time.
3. For the first time, the anatomical signs of crushed raw materials and powder of shoots of V. myrtillus.
4. The research results are included in the "Microscopy" section of the draft pharmacopoeial monograph for GF 12th ed., Regulating the quality of shoots of V. myrtillus.

LITERATURE

1. Rules for the collection and drying of medicinal plants (collection of instructions). - M .: Medicine, 1985. - 328 p.
2. State Pharmacopoeia of the USSR: Vol. 1. General methods of analysis / Ministry of Health of the USSR. 11th ed., Add. - M .: Medicine, 1987 .-- 336 p.
3. Samylina I.A., Ermakova V.A., Bobkova I.V., Anosova O.G. Pharmacognosy. Atlas: educational manual: in 3 volumes. - M .: GEOTAR-Media, 2009. - Vol. 3. - 488 p.
4. Eremin V.M., Boyko V.I. Anatomical structure of the stem bark of some species of the family Ericaceae // Botanical Journal. - T. 83. - No. 8. - P.1-15.
5. Nikitin A.A., Pankova I.A. Anatomical atlas of useful and some poisonous plants. - L., Nauka, 1982.768 p.

Author's address

Ph.D. Mechikova G.Ya., Associate Professor of the Department of Pharmacognosy and Botany, State Budgetary Educational Institution of Higher Professional Education, Far Eastern State Medical University, Ministry of Health of Russia (Khabarovsk)

galina.m.ya@mail.ru

Mechikova, G. Ya. Study of morphological and anatomical features of Vaccinium myrtillus L. raw materials / G.Ya. Mechikova, T.A. Kopoteva, O. V. Smirnova // Traditional medicine. - 2013. - No. 3 (34). - S.43-46.