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Morphological and anatomical structure of *Rubus saxatilis* leaf

Introduction to the practical use of local raw materials of medicinal plants has an important practical significance for pharmacy and cosmetic industry. Interest in raw materials of *Rubus saxatilis* is due to the biological activity of compounds, use in folk medicine, as well as significant amounts of raw materials growing in Central Kazakhstan. To introduce the raw material into practical application, the task of analyzing the anatomo-morphological structure and identifying diagnostic features of the raw material was set. The results showed that at the macroscopic level as diagnostic features of *Rubus saxatilis* raw material can be indicated the difference in the structure of the upper and lower side of leaves, type of veining, the presence of soft prickles, the structure of triple-complex leaves, almost complete absence of pubescence. At the microscopic level, the diagnostic features of this species are the shape of transverse sections, the shape of leaf epidermis cells and leaf petiole, the localization of receptacles in the mesophyll, the presence of rare simple unicellular trichomes and essential oil glands raised above the surface of the epidermis. The results obtained can serve as additional taxonomic characters in determining the species and authentication of raw materials of *Rubus saxatilis*.

Keywords: *Rubus saxatilis*, plant raw material, anatomo-morphological structure, diagnostic features, leaf, leaf petiole.

Introduction

The search for new sources of plant resources and biologically active substances in the natural flora is an important task for the food, pharmaceutical and parapharmaceutical industries of Kazakhstan [1, 2].

When making cosmeceutical preparations, the emphasis should be on local raw materials, which are widely distributed in nature, can be successfully grown in culture, contains a sufficient level of active components, that is, can become a substitute for expensive foreign analogs.

Sufficiently widespread object in the natural conditions of Central Kazakhstan [3], which is a medicinal plant used in folk medicine, is boneset (stone raspberry) — *Rubus saxatilis* L. (family Rosaceae).

In ancient times, people used *Rubus saxatilis* leaves and applied it to the skin, thus treating rubella and urticaria, and in case of biliary scabies the juice from the leaves was taken internally. In case of skin inflammations and acne, people prepared a decoction from its flowers and washed their faces with it. It has also been proven in scientific studies that leaf extracts are effective against platelet reactivity [4].

In folk medicine, *Rubus saxatilis* is known for its ability to support the health of the gastrointestinal tract, especially the intestines, helps not only to reduce the risk of constipation, but also to fight it. This is due to the presence of dietary fiber, namely pectin substances [5, 6].

Due to the presence of a large number of antioxidant substances, including ascorbic acid, vitamin E, carotenoids and polyphenols, the berries and leaves of *Rubus saxatilis* can resist Parkinson's and Alzheimer's diseases, as they promote brain activity and have neuroprotective properties, that is, they can protect nerve cells (neurons) from damage and death, and improve their functioning and survival [5].

The presence of ascorbic acid makes the berry an excellent remedy against scurvy, it also activates the function of cells, supporting the synthesis of collagen, which is important in the creation of cosmeceuticals, increases stress resistance and immunity, thus useful both for the prevention and treatment of avitaminosis, various colds, stimulates hematopoiesis, it is important for the prevention and treatment of cardiovascular diseases, increases the elasticity and density of capillary walls [5, 7]. In addition, ascorbic acid promotes the synthesis of cholesterol, a precursor of vitamin D [8]. A scientific study was conducted, which revealed that fruits of *Rubus* genera are able to inhibit the growth of pathogenic microorganisms, as well as *inhibit* the growth of some cancer cells *in vitro* [9].

Due to the presence of saponins in the leaves of *Rubus saxatilis*, the raw material has astringent, choleric, antiseptic, sedative, adsorbent and other actions. Due to its sedative properties, the raw material of

Rubus saxatilis can also be used to create medicinal preparations that are aimed at the treatment and prevention of insomnia. Saponins are also able to strengthen the human immune system and act against allergic skin reactions, and therefore they are widely used to create cosmeceuticals [10].

Based on the useful properties of *Rubus saxatilis* raw material and its potential use for the production of cosmeceutical preparations, we conducted an anatomico-morphological study of the leaves of this species to establish the features of the structure and diagnostic features of the raw material at the macro- and microscopic level.

Experimental

Collection of raw material *Rubus saxatilis* (leaves with petioles) was carried out in the stage of fruiting (1st decade of August 2023) in the tract Karaagash (Belodymovsky branch, State National Natural Park “Buiratau”).

In the analysis of morphological parameters, the appearance, surface structures, colors of leaves and petioles were investigated. Samples of raw material were examined with Digital Microscope Levenhuk DTX 30, using magnification 16*10, 16*4.

For microscopic study, the raw materials were soaked in a mixture of glycerol: alcohol: water (ratio 1:1:1), and microparameters were made manually with a razor [11]. Microphotographs were obtained using BiVisual Dio microscopy, photos were analyzed using Altamy Studio program, and processed in Paint 10.1 program. Classical summaries were used to describe plant morphology and anatomy [12–14].

Results and Discussion

Rubus saxatilis is a perennial herbaceous plant that grows widely under the canopy of small-leaved and mixed forests of Central Kazakhstan [3], dominating in the herbaceous layer.

Leaves of bonesetter are triple compound, raised above the surface on long petioles, rough, covered with stiff whitish hairs. The leaf length is 10–15 cm, width 12–16 cm. The terminal lobes are elliptical, with an acute apex, wedge-shaped base and coarsely serrated edge. The raw material is pieces of leaves and petioles, green and light green in color (Fig. 1). The upper side is dark green, matte, while the lower side is whitish, with well-developed veins (Fig. 2). The veins are weakly expressed on the upper side. The veining is pinnately setose. Rare white trichomes are noted along large veins.

Soft prickles are noted at the base of the leaves; the tip is pointed and darkly colored. The petiole is rounded, finely ribbed, light green with reddish spots. From other species of raspberries, the raw material of boneset is characterized by the structure of the leaf surface, soft prickles and long leaf petioles.

On the transverse section, the leaf of *Rubus saxatilis* is flat, dorsoventral type (Fig. 3). On both sides there is a single-layer epidermis, the cells of the upper epidermis are larger, round-rectangular in shape, the cells of the lower epidermis are small, rounded or prosenchyma-shaped. The epidermis is covered on the outer side by a layer of cuticle, which is thicker on the lower side. Stomata are few, located on both sides of the leaf plate. The leaf mesophyll is differentiated into columnar and spongy tissues. The columnar mesophyll is arranged in one layer and consists of elongated cells, while the spongy mesophyll is 2-3-layered. Small conductive bundles, collateral, of closed type, oval or rounded in shape, are noted in the mesophyll. Rounded receptacles are found in places.

The leaf epidermis and petioles bear rare essential oil glands (Fig. 4) and trichomes, simple, unicellular (Fig. 5). The glands are raised above the surface of the epidermis, multicellular, consisting of a rounded head and a 2-3-celled pedicel.

The leaf petiole on the transverse section is curved (Fig. 6). The internal structure is characterized by the presence of 3 conductive bundles: 2 bundles of rounded shape, located at the edges, 1 bundle in the middle, elongate-oval, slightly curved. The bundles are collateral, closed type, with a “cap” of a significant area of sclerenchyma. The inner part is filled with loose and thin-walled cells of spongy parenchyma.



Figure 1. Appearance of *Rubus saxatilis* raw material

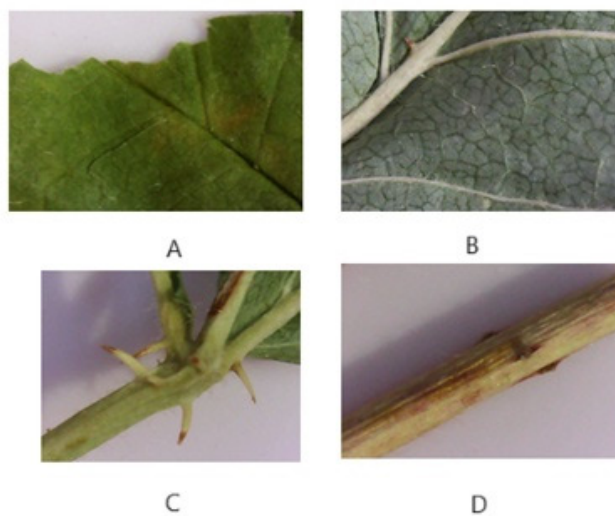
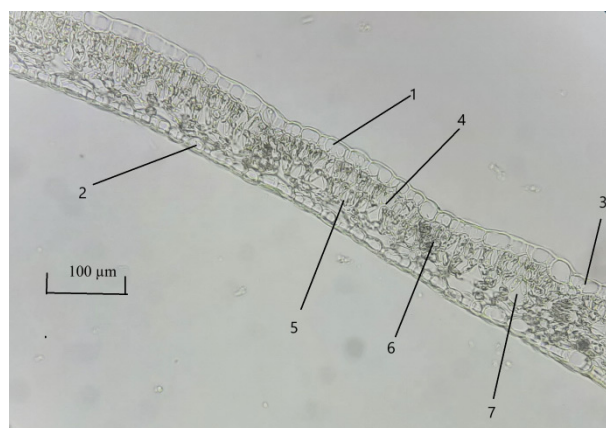


Figure 2: Appearance of leaf and petiole of *Rubus saxatilis*: A — upper side of leaf, B — lower side of leaf, C — prickles at the base of petiole, D — appearance of petiole



1 — upper epidermis, 2 — lower epidermis, 3 — stomata, 4 — columnar mesophyll, 5 — spongy mesophyll, 6 — conducting bundle, 7 — receptacle

Figure 3: Transverse section of a *Rubus saxatilis* leaf (fragment)



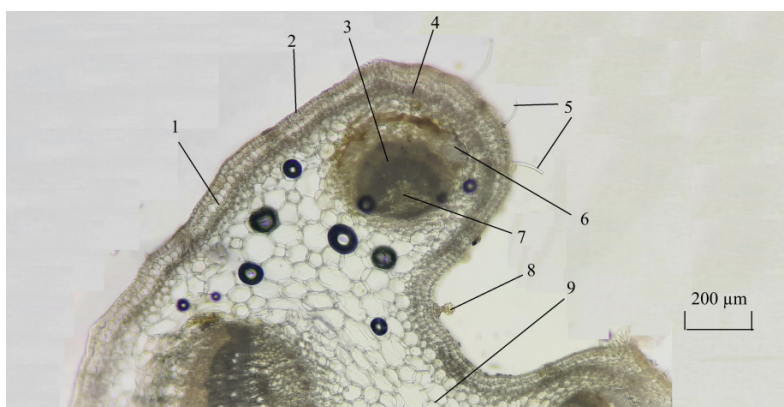
Figure 4: Appearance of the essential oil gland of *Rubus saxatilis*



Figure 5: Appearance of a simple trichome of *Rubus saxatilis*



A



Б

A — general view of leaf petiole (Eq. 16x4),
 B — fragment, 1 — bark parenchyma, 2 — epidermis, 3 — phloem, 4 — collenchyma,
 5 — simple trichomes, 6 — sclerenchyma, 7 — xylem, 8 — gland, 9 — spongy parenchyma

Figure 6. Transverse section of the leaf petiole of *Rubus saxatilis*

Single-layered epidermis consisting of rectangular cells with thickened outer walls and a layer of cuticle is noted along the perimeter. Rare simple trichomes, usually located in the corners, and single essential oil glands are noted on the epidermis. A thin layer of bark parenchyma and areas of angular collenchyma lie beneath the epidermis.

The investigated species differs from other species of the genus *Rubus* L. by smaller volume of sclerenchyma, more mesophytic structure of leaves, weak pubescence and absence of woodiness in the structure of leaf petiole.

Conclusion

As diagnostic signs of *Rubus saxatilis* raw material can be indicated the difference in the structure of the upper and lower sides of leaves, the type of veining, the presence of soft prickles and the shape of the leaf petiole. Analysis of anatomical slices allows us to determine the diagnostic features of this species, such as the shape of leaf epidermis cells and leaf petiole, the shape and localization of receptacles, the presence of simple trichomes and essential oil glands.

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***Rubus saxatilis* жапырағының морфологиялық және анатомиялық құрылымы**

Дәрілік өсімдіктердің шикізатын практикалық қолдануға енгізу фармация мен косметика өнеркәсібі үшін маңызды практикалық маңызы бар. *Rubus saxatilis* шикізатына деген қызығушылық қосылыстардың биологиялық белсенділігіне, халықтық медицинада пайдаланылуына, сондай-ақ Орталық Қазақстанда өсетін шикізаттың едәуір көлеміне байланысты. Шикізатты практикалық қолдануға енгізу үшін анатомиялық-морфологиялық құрылымды талдау және шикізаттың диагностикалық белгілерін анықтау міндеті қойылды. Нәтижелер макроскопиялық деңгейде *Rubus saxatilis* шикізатының диагностикалық белгілері ретінде жапырақтардың жоғарғы және төменгі жағының құрылымындағы айырмашылықты, венация түрін, жұмсақ тікенектердің болуын, үш қабатты жапырақтардың құрылымын, түктің толық болмауын көрсетті. Микроскопиялық деңгейде бұл түрдің диагностикалық белгілері көлденең кималардың пішіні, жапырақ пен жапырақ жапырақшасының эпидермис жасушаларының пішіні, мезофилдегі контейнерлердің локализациясы, сирек кездесетін қарапайым бір жасушалы трихомалар мен эпидермис бетінен жоғары көтерілген эфир майы бездерінің болуы. Алынған нәтижелер *Rubus saxatilis* шикізатының түрін анықтауда және түпнұсқалығын растауда қосымша таксономиялық белгілер бола алады.

Кілт сөздер: *Rubus saxatilis*, өсімдік шикізаты, анатомиялық-морфологиялық құрылымы, диагностикалық белгілері, жапырағы, жапырақшасы.

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Морфологическая и анатомическая структура листа *Rubus saxatilis*

Введение в практическое использование местного сырья лекарственных растений имеет важное практическое значение для фармации и косметической промышленности. Интерес к сырью *Rubus saxatilis* обусловлен биологической активностью соединений, использованием в народной медицине, а также значительными объемами сырья, произрастающего в Центральном Казахстане. Для введения сырья в практическое применение была поставлена задача анализа анатомо-морфологического строения и

выявления диагностических признаков сырья. Результаты показали, что на макроскопическом уровне в качестве диагностических признаков сырья *Rubus saxatilis* можно указать разницу в строении верхней и нижней сторон листьев, тип жилкования, наличие мягких колючек, строение тройчатосложных листьев, практически полное отсутствие опушения. На микроскопическом уровне диагностическими признаками данного вида являются форма поперечных срезов, форма клеток эпидермиса листа и листового черешка, локализация вместилищ в мезофилле, присутствие редких простых одноклеточных трихом и эфирномасличных железок, приподнимающихся над поверхностью эпидермиса. Полученные результаты могут служить дополнительными таксономическими признаками при определении вида и подтверждении подлинности сырья *Rubus saxatilis*.

Ключевые слова: *Rubus saxatilis*, растительное сырье, анатомо-морфологическое строение, диагностические признаки, лист, черешок листа.

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