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THE MICROSCOPIC ANALYSIS HERB OF CARDAMINE QUINQUEFOLIA (M.BIEB.) SCHMALH

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Abstract

Objective. Implement microscopic analysis herb of *Cardamine quinquefolia (M.Bieb.) Schmalh.* The genus *Cardamine* has only 4 species in the flora of the Caucasus: *C. quinquefolia, C. microphylla, C. bipinnata* and *C. bulbifera*. Starting the study of the *Cardamine* genus in the North Caucasus (Russia), we selected as the first object *Cardamine quinquefolia (M.Bieb.) Schmalh.* (syn. *Dentaria quinquefolia M. Bieb*.) since its anatomical description has already begun and some data are available on its chemical composition, it includes alkaloids, organic acids, ascorbic acid.

Methods. The herb of *C. quinquefolia* was harvested during flowering in Pyatigorsk, Stavropol region. As research methods, macro- and microscopic analysis were used.

Results. The whorled arrangement of leaves under the inflorescence is the main distinguishing macroscopic sing of the herb of *C. quinquefolia*. The main microscopic signs of *C. quinquefolia* are: heavily sinuous walls of cells of the upper and lower epidermis, stomata anisocytic type, unicellular trichomes with thick walls. On the cross section of the leaf are visible epidermis, mesophyll palisade and spongy, collenchyma, parenchyma, collateral vascular bundle. The cross-section of the stem is characterized by collateral, open vascular bundles arranged in a circle. The lignified parenchyma takes place between the vascular bundles. The sclerenchyma is located near the phloem. The diagnostic signs of a flower are polygonal cells of the sepal and petal epidermis, stomata of anisocytic type of sepal outgrowths on the edge of the petal.

Conclusions. Introduction to the pharmaceutical practice of new plants is a promising direction in expanding the methods of herbal medicine.

Keywords: *Cardamine quinquefolia*, collenchyma, cross-section, mesophyll, stomatal anisocytic type, trichomes, vascular bundle

МИКРОСКОПИЧЕСКИЙ АНАЛИЗ ТРАВЫ CARDAMINE QUINQUEFOLIA (M.BIEB.) SCHMALH

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Резюме

Цель. Провести микроскопический анализ травы *Cardamine quinquefolia (M.Bieb.) Schmalh.* Род *Cardamine* во флоре Кавказа насчитывает всего 4 вида: *C. quinquefolia, C. microphylla, C. bipinnata* и *C. bulbifera*. Начиная изучение рода *Cardamine* на Северном Кавказе (Россия), мы выбрали в качестве первого объекта *Cardamine quinquefolia (M.Bieb.) Schmalh. (син. Dentaria quinquefolia M. Bieb.)*, поскольку его анатомическое описание уже начато и имеются некоторые данные о его химическом составе, он включает алкалоиды, органические кислоты, аскорбиновую кислоту.

Методика. Траву *C. quinquefolia* собирали в период цветения в г. Пятигорске Ставропольского края. В качестве методов исследования использовали макро- и микроскопический анализ.

Результаты. Основным макроскопическим признаком травы *C. quinquefolia* является мутовчатое расположение листьев под соцветием. Принципиальные микроскопические признаки *C. quinquefolia* представлены сильно извилистыми стенками клеток верхнего и нижнего эпидермиса, устьицами анизоцитного типа, одноклеточными волосками с толстыми стенками. На поперечном срезе листа видны эпидермис, мезофилл палисадный и губчатый, колленхима, паренхима,

коллатеральный проводящий пучок. На поперечном срезе стебель характеризуется коллатеральными открытыми проводящими пучками, расположенными по кругу. Между проводящими пучками располагается одревесневшая паренхима. Склеренхима располагается вблизи флоэмы. Диагностическими признаками цветка являются полигональные клетки эпидермиса чашелистиков и лепестков, устьица анизоцитного типа, выросты по краю лепестка.

Заключение. Внедрение в фармацевтическую практику новых растений является перспективным направлением расширения методов фитотерапии.

Ключевые слова: ***Cardamine quinquefolia***, колленхима, поперечный срез, мезофилл, устьица анизоцитного типа, волоски, проводящий пучок

Introduction

Cardamine L. (family ***Brassicaceae***) - a European sub-oceanic genus distributed in deciduous forests of the European part of Russia [4, 6], the Mediterranean countries [7, 10, 11], Caucasus, Turkey, Iran [8]. A large number of species are represented by nemoral plants, some of which play a prominent role in the grassy layer of deciduous forests. The northern boundary of the distribution of the genus is limited by the northern limits of the natural growth of broad-leaved species such as ***Tilia cordata, Ulmus glabra, Quercus robur*** and ***Acer platanoides*** [1, 3].

The genus ***Cardamine*** has only 4 species in the flora of the Caucasus: ***C. quinquefolia, C. microphylla, C. bipinnata*** and ***C. bulbifera***. All presented species are found both north of the Greater Caucasus Mountain Range and south. ***C. bipinnata*** is endemic to the Greater Caucasus. According to a number of morphological characters, ***Cardamine*** is well divided into three sections: ***Verticillatae V.I. Dorof. sect. nov. (C. quinquefolia), Cardamine sect. nov. (C. microphylla, C. bipinnata)*** and monotypic ***Bulbiferae sect. nov. (C. bulbifera)*** [3].

Starting the study of the ***Cardamine*** genus in the North Caucasus, we selected as the first object ***Cardamine quinquefolia (M.Bieb.) Schmalh.*** (syn. ***Dentaria quinquefolia M. Bieb***.) Since its anatomical description has already begun and some data are available on its chemical composition, it includes alkaloids, organic acids (4.09 %), ascorbic acid (0,05 %) [5].

The aim of the work was to search for the main diagnostic signs characterizing ***C. quinquefolia*** as a raw material for establishing its authenticity.

Methods

For analysis, ***C. quinquefolia*** herb was used (fig. 1). It was harvested during flowering (April, 2020); herb harvesting place: Russia, Stavropol region, Pyatigorsk.



Fig. 1. ***Cardamine quinquefolia (M.Bieb.) Schmalh.***

Two types of micropreparations were used: from fresh and dried herbs of *C. quinquefolia.* Dry raw materials were fixed before use in the system ethyl alcohol 95%-glycerol-water in the ratio 1:1:1. The micropreparations were stained with phloroglucin and concentrated sulfuric acid. The microscope "Biomed", lenses ×4, ×10, eyepiece 10× was used in the work. Microphotographs were made on a digital camera «3.0mp cmos microscope eyepiece new». Microscopic signs of *C. quinquefolia* have been studied according to the requirements of the State Pharmacopoeia XIV [2].

Results

Morphological study. Stem of *C. quinquefolia* 20-35 cm, no branching, no pubescence. There is no leaf below the stem, 3 leaves at the top. The main diagnostic feature that distinguishes *C. quinquefolia* from other representatives of the Caucasian flora is a whorled arrangement of leaves under an inflorescence.

The leaves are simple, sect, there are petioles, lanceolate leaflets. The margin of the leaf is dentate. Venation is pinnate. The upper side of the leaves is green, the lower one is lighter. Inflorescence raceme, consists of 6-15 flowers. 4 sepals, oblong shape, length up to 5 mm. 4 petals, color violet, rarely rose, length up to 15 mm. The odor is specific.

Microscopic study of the leaf. Upper epidermis of the leaf, in frontal view has polygonal cells, walls of cells heavily sinuous. Stomata are rare, anisocytic type (cells around stomata 3, one of them is small). The lower epidermis differs from the upper epidermis by a large number of stomata and more sinuous cell walls (fig. 2A). Unicellular trichomes with thick walls are located on the entire surface of the leaf and along the edge of the leaf (fig. 2B).

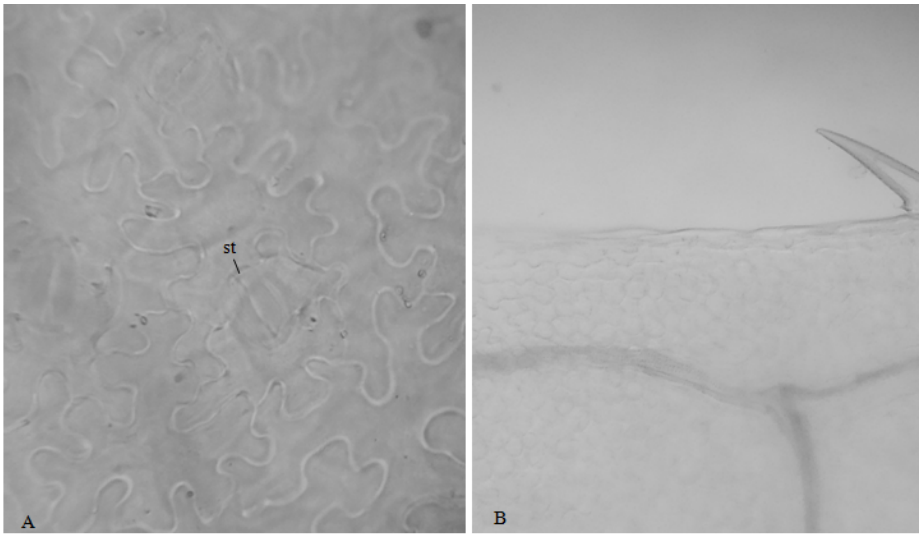


Fig. 2. Leaf of *C. quinquefolia:* A – lower epidermis (400×); B – edge of the leaf (100×): st – stomata

Cross-sections of the leaf (fig. 3A). The cells of the upper and lower epidermis near the vein are round and rectangular, in the rest of the leaf are rectangular, the cells are densely arranged in one layer (fig. 3D). On the epidermis there are stomata and trichomes.

Under the upper epidermis are 2 layers of rectangular cells with thin walls and a large number of chloroplasts. This is the mesophyll palisade. Between the mesophyll palisade and the lower epidermis there are round or oval-shaped cells with the same number of chloroplasts as in the cells of the palisade mesophyll. This is the mesophyll spongy (fig. 3C).

On the cross section of the leaf is visible collenchyma. Its first small area is located under the upper epidermis and several layers of cells are located under the lower epidermis. Parenchyma cells are rounded or multifaceted.

The central part of the vein is a large vascular bundle. Vascular bundle of ovoid shape, collateral type, there is cambium (fig. 3B).

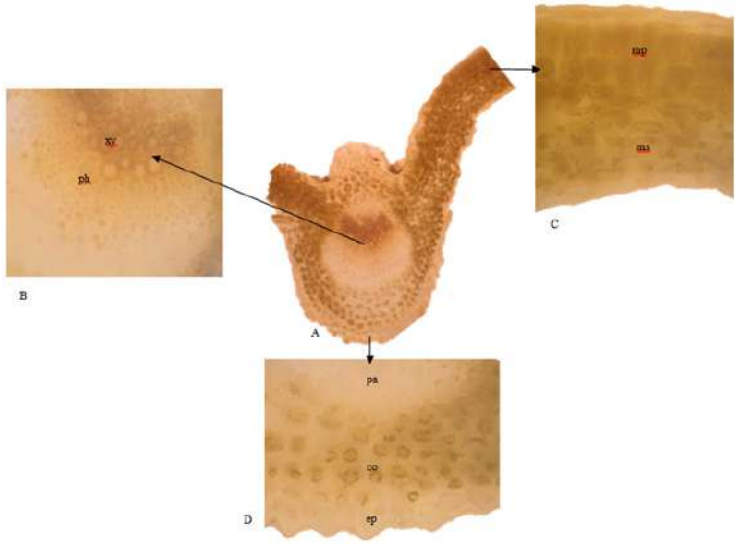


Fig. 3. Cross-sections of the leaf of *C. quinquefolia* (A – ×40; B, C, D – ×400)*:* ep – epidermis, co – collenchyma, xy – xylem, ph – phloem, pa – parenchyma, mp - mesophyll palisade, ms - mesophyll spongy

Cross-sections of the petiole (fig. 4A). On the cross section of the petiole is visible epidermis, its cells are square in shape, are located in one layer, there is the cuticle (fig. 4D). On the epidermis, trichomes are rarely found. Under the epidermis is the collenchyma, consisting of 2-3 rows of oval cells with chloroplasts (fig. 4B). Vascular bundles open, collateral. The phloem is represented by small sieve elements. Vessels of various diameters form xylem (fig. 4C).

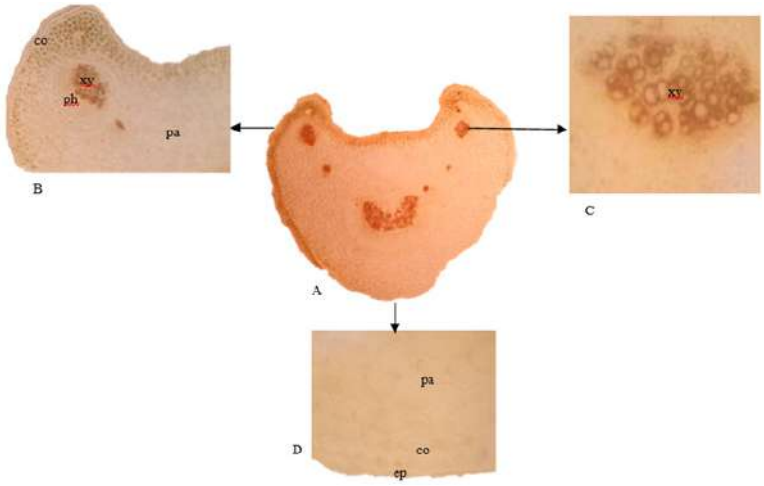


Fig. 4. Cross-sections of the petiole of *C. quinquefolia* (A – ×100; B, C, D – ×400)*:* ep – epidermis, co – collenchyma, xy – xylem, ph – phloem, pa – parenchyma

Cross-sections of the stem (fig. 5A). The integumentary tissue is the epidermis. Epidermal cells are located in one layer close to each other. The shape of the cells is square, covered with cuticles (fig. 5B).

After the epidermis, the collenchyma is located. It consists of 2-3 layers, many cells of the collenchyma contain chloroplasts (fig. 5B).

Vascular bundles 24-25, they are arranged in a circle (fig. 5A), the shape is ovoid, the vascular bundles are collateral, there is a cambium (fig. 5C). Near the phloem is sclerenchyma. The lignified parenchyma takes place between the vascular bundles (fig. 5D). Cells of different sizes oval and round in shape, which are located in the center, this is the parenchyma.

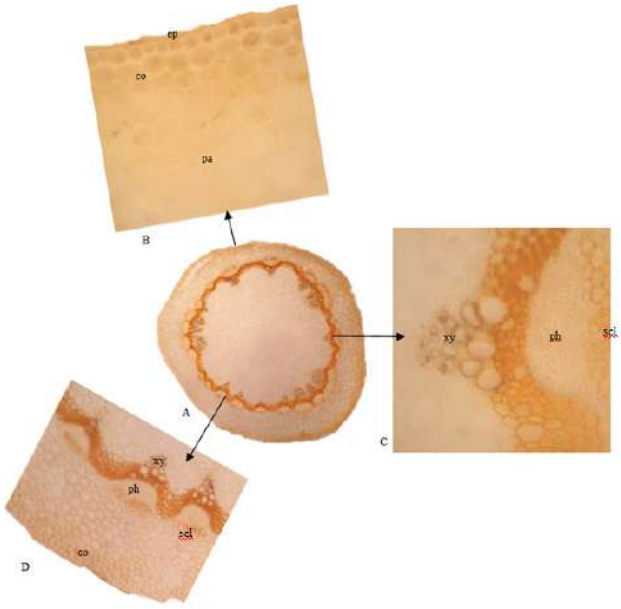


Fig. 5. Cross-sections of the stem of *C. quinquefolia* (A – ×40; B, C, D – ×400)*:* ep – epidermis, co – collenchyma, xy – xylem, ph – phloem, pa – parenchyma, scl – sclerenchyma

Anatomical structure of the flower. Sepal epidermal cells are polygonal, more elongated at the periphery (fig. 4A), there are stomata of anisocytic type (fig. 4B). Petal epidermal cells are also polygonal, there are outgrowths on the edge of the petal (fig. 4C). Pollen round (fig. 4D).

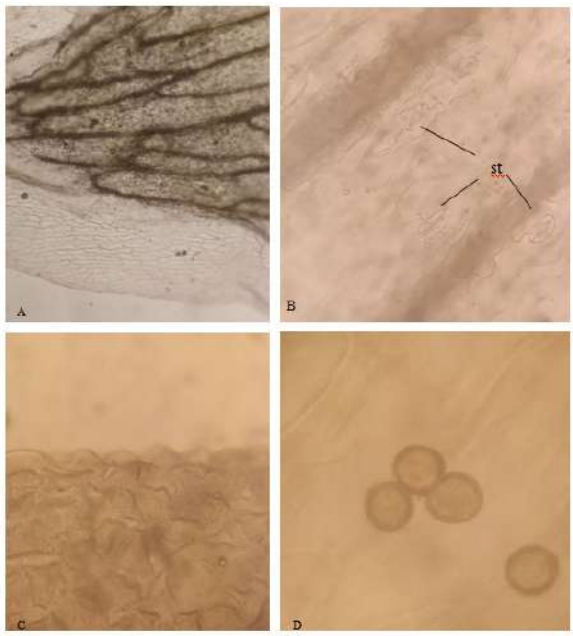


Fig. 4. Flower of *C. quinquefolia*: A – epidermis of sepal (A – 100×; B – 400×); C – epidermis of petal (×400) D – pollen grains (×400): st – stomata

Discussion

The main diagnostic sign that distinguishes *C. quinquefolia* from other representatives of the Caucasian flora is a whorled arrangement of leaves under an inflorescence. Microscopic examination of the stem revealed that the integumentary tissue is the epidermis covered with cuticles. Next is the collenchyma,

many cells of the collenchyma contain chloroplasts Vascular bundles are ovoid, collateral, open. Near the phloem is sclerenchyma. The lignified parenchyma takes place between the vascular bundles. There are heavily sinuous walls of cells of the upper and lower epidermis of the leaf, stomata anisocytic type, unicellular trichomes with thick walls. On the cross section of the leaf are visible epidermis (near the vein are round and rectangular, in the rest of the leaf are rectangular), mesophyll palisade and spongy, collenchyma (its first small area is located under the upper epidermis and several layers of cells are located under the lower epidermis), parenchyma, collateral vascular bundle. The cross section of the petiole consists of epidermis, collenchyma, parenchyma and vascular bundles. The diagnostic signs of a flower are polygonal cells of the sepal and petal epidermis, stomata of anisocytic type of sepal outgrowths on the edge of the petal.

Conclusion

Modern medicine cannot be imagined without pharmacotherapy using synthetic medicament, but one cannot do without phytotherapy. Plant raw materials in its composition contain a complex of active substances, which simultaneously affects several organs and systems of the body. Phytotherapy not only retains its relevance, but also expands opportunities through the active introduction of new medicinal plants into pharmaceutical practice and the relationship with pharmacotherapy. ***Cardamine quinquefolia (M.Bieb.) Schmalh***. is a promising plant in this area, to which the interest of researchers is currently increasing. Species of this genus grow in many regions of the world, including Russia. To standardize and establish the authenticity of raw materials, it is necessary to study the basic macro and microscopic signs. This will allow the identification of raw materials for further research with the aim of introducing into the pharmacy.

References

1. Берхманн А.А. Оценка состояния ценопопуляции зубянки сибирской (*Dentaria sibirica*) в окрестностях города Абаза // Экология Южной Сибири и сопредельных территорий / Под. ред. В.В. Анюшина. – Абакан, 2015. – С. 109-110. [Berhmann A.A J*ekologija Juzhnoj Sibiri i sopredel'nyh territorij / Pod. red. V.V. Anjushina*. Ecology of Southern Siberia and adjacent territories / Ed. V.V. Anjushin – Abakan, 2015. – P. 109-110. (in Russian)]
2. Государственная Фармакопея Российской Федерации. 14 изд. – Министерство Здравоохранения РФ, 2018. [*Gosudarstvennaja Farmakopeja Rossijskoj Federacii. 14 izd.* State Pharmacopoeia of the Russian Federation 14th ed. – Ministry of Health RF, 2018. (in Russian)]
3. Дорофеев В.И. Род *Dentaria (Brassicaceae)* во флоре Кавказа // Turczaninowia. – 2000. – Т.3, № 3. – С. 14-17. [Dorofeev V.I. Turczaninowia. – 2000. – V.3, N 3. – P. 14-17. (in Russian)]
4. Семенищенков Ю.А., Шапурко А.В. Особенности биологии и фитоценотические связи редкого вида *Dentaria bulbifera L. (Cruciferae)* у северной границы ареала в Брянской и Смоленской областях // Бюллетень Брянского отделения Русского ботанического общества. – 2017. – № 2 (10). – С. 35-44. [Semenishhenkov Ju.A., Shapurko A.V. *Bjulleten' Brjanskogo otdelenija Russkogo botanicheskogo obshhestva*. Bulletin of the Bryansk branch of the Russian Botanical Society. – 2017. – N 2(10). – P. 35-44. (in Russian)]
5. Серебряная Ф.К., Хартюнова Е.И. Морфолого-анатомическое и фитохимическое исследование надземных органов зубянки пятилистной (*Dentaria quinquefolia Bieb*.) семейства *Brassicaceae* // Современные проблемы науки и образования. – 2015. – № 2. – С. 528. [Serebrjanaja F.K., Hartjunova E.I *Sovremennye problemy nauki i obrazovanija.* Modern problems of science and education. – 2015. – N.2. – P. 528. (in Russian)]
6. Чистякова А.А. Распространение и популяционная организация зубянки пятилистной *(Dentaria quinquefolia Bieb.)* в Пензенской области // Известия Пензенского государственного педагогического университета им. В.Г. Белинского. – 2007. – №9. – С. 29-32. [Chistjakova A.A *Izvestija Penzenskogo gosudarstvennogo pedagogicheskogo universiteta im. V.G. Belinskogo*. News of Penza State Pedagogical University named after V.G. Belinsky. – 2007. – N9. – P. 29-32. (in Russian)]
7. Gustafsson C.D. Plant population dynamics and biotic interactions in two forest herbs (*Sanicula europaea, Dentaria bulbifera*): Candidate Thesis. – Sweden, 2003.
8. Karaismailoğlu M.C. New Chromosome numbers in five *Cardamine* taxa from Turkey // Cytologia. – 2021. – V.86, N1. – P. 11-13. Doi:10.1508/cytologia.86.11
9. Marhold K. Lectotypification of names of the European representatives of *Cardamine subgen. Dentaria (Cruciferae)* // Willdenowia. – 2001. – N.31. – P.43-49. Doi:10.3372/wi.31.31103

10. Marhold K., Lihova J., Perny´ M., Grupe R., Neuffer B. Natural hybridization in *Cardamine (Brassicaceae)* in the Pyrenees: evidence from morphological and molecular data // Botanical Journal of Linnean Society. – 2002. – N139. – P.275-294.
11. Lihova J., Aguilar J.F., Marhold K., Feliner G.N. Origin of the disjunct tetraploid *Cardamine amporitana (Brassicaceae)* assessed with nuclearand chloroplastdnasequence data // American Journal of Botany. – 2004. – V.91,N8. – P. 1231-1242.

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