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STUDY OF MORPHOLOGICAL AND ANATOMICAL FEATURES OF THE PLANT RAW MATERIAL CROCUS ALATAVICUS

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Abstract

Introduction. Plants of the genus *Crocus* L. have a wide spectrum of pharmacological action. To date, many of their species have not been fully studied. Morphological and anatomical features of *Crocus alatavicus* Regel & Semenow have not been studied and there is no regulatory documentation. The literature review indicates the absence of a specific, complete description of these features that make it possible to identify and standardize *Crocus alatavicus* raw materials.

The aim of this study is to research the features of morphological and anatomical diagnostic features of the plant *C. alatavicus*.

Materials and methods. Morphology of raw materials were studied using a binocular magnifier. Anatomical and diagnostic signs were determined by preparing temporary preparations according to the pharmacopoeia technique using an MS-300 microscope with a camera. Morphological and anatomical features of the reduced stem, leaves, and flower were studied.

Results. It has been established that the leaves of *C. alatavicus* have a unique and peculiar shape in cross-section, like most species of *Crocus* L. It consists of a central quadrangular "keel" and two side "arms". *C. alatavicus* differs from other species in the triangular shape of the "keel" and the tips of the "arms" are strongly curved to the "keel". There is a pale stripe in the axial direction along the center of the "keel". The leaves of *C. alatavicus* are amphistomatic in the arrangement of stomata and belong to the anomocytic type.

Conclusions. These signs of plant are used to identify medicinal plant raw materials. It is important to emphasize that the standardization of *C. alatavicus* domestic raw materials creates new opportunities for its further use in medicine.

Keywords: *Crocus* L., *Crocus alatavicus*, anatomical and diagnostic signs, morphological features of *C. alatavicus*, anatomical features of *C. alatavicus*, plant raw materials.

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Introduction. The genus *Crocus* L. from the *Iridaceae Juss* family includes more than 80 species distributed from south-western Europe through Central Europe to the south-western regions of Asia and Central Asia, up to Western China [1]. A literary search by the keywords "Crocus" and "Saffron" shows that a wellstudied species is *Crocus sativus* L., commonly known as saffron. It is used in cooking, producing alcoholic beverages, cosmetology and pharmacy. The results of research done by foreign scientists show a rich phytochemical composition of plants of the genus *Crocus* L. and good antitumor, anti-inflammatory, antioxidant, antidepressant, immunomodulatory, antihypertensive and hypolipidemic activity [2-4].

Two species grow on the territory of Kazakhstan: *Crocus alatavicus* Regel et Semenow and *Crocus korolkowii* Regel et Maw. This article presents the results of a study of the morphological and anatomical features of *C. alatavicus*.

The results of the study of morphological and anatomical features of *C. alatavicus* plant raw materials are necessary for the development of parts of the regulatory documentation 1) definition and 2) microscopy.

When drawing up a specification and a regulatory document on the quality of medicinal products, pharmacognostic analysis of raw materials is necessary. This analysis includes phytochemical, macroscopic and microscopic studies of plant materials.

Our phytochemical analysis [5] of *C. alatavicus* showed that kaempferol derivatives account for 96.5% of all identified flavonoid compounds (22 compounds in total). The rich flavonoid composition determines its antibacterial, antifungal activity for hands, a noticeable antiviral effect against HHV-1 and significant antitumor activity against metastatic prostate cancer. Morphological and anatomical features of *C. alatavicus* are the leading indicators for the standardization of medicinal plant raw materials. To date, the macro and micro diagnostic features of this species have not been studied enough. The available literature contains information about the morphological and anatomical features of the transverse section of the leaf of the introduced *C. alatavicus* on the territory of Uzbekistan. However, the distinguishing features of raw materials are given in the form of diagrams and there are no photographs of microscopy of the above-ground parts of the raw materials [6].

The leaves of plants of the genus *Crocus* L. in cross section have a unique distinctive shape, consisting of a central square or rectangular "keel" and two lateral edge "arms", which are usually curved towards the "keel". There is a characteristic pale streak running axially along the centre of the leaf, caused by the presence of parenchymal cells in the keel, which lack chloroplasts. This shape of the cross section is one of the main features in the identification of a particular species of plants of the genus *Crocus* L. The authors [7-10] of previously published systematic studies of the anatomy of the leaves of the genus *Crocus* L. plants concluded that the shape of the transverse section of the leaves has a certain taxonometric value.

Ukrainian researchers studied the biomorphological features of 6 species of the genus *Crocus* L. growing on the territory of Ukraine: *C. banaticus, C. heuffelianus, C. reticulatus, C. angustifolius, C. tauricus, C. pallasii, C. speciosus.* The results showed that the shape and color of the inner and outer segments of the perianth, the form and degree of branching of the

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stigma, as well as the structure of the tunics of saffron corms have individual characteristic features. The perianths of C. angustifolius are yellow, the other species have different shades of purple [11, 12].

The genus *Crocus* L. in Turkey is represented by 32 species. Morphological and anatomical characteristics of four species: *C. sativus, C. ancyrensis., C. antalyensis, C. chyrsanthus* were studied by Kyuchuk S. and Soarer M. The key morphological differences between the species are the color of the perianth segments and the shape of the cross section of the leaf blade [13]. Akyul Y. and his colleagues [14] presents the morphological and anatomical signs of *C. asumaniae* and *C. mathewii*, where the characteristic feature is the presence of sclerenchymal tissues around the leaf vascular bundles. Ozdemir K. and his colleagues [15] studied the *C. danfordiae, C. fleischeri*, where for the first species the distinguishing characteristic is anthers with black basal lobes at the base, and for *C. fleischer* flowers with purple spots at the base and on the tube. Turkish endemics *C. flavus* Weston and *C. flavus* subsp. *Dissectus* [16], two closely related taxa, are outwardly similar, and have yellow flowers, but differ significantly from each other in the shape of the transverse section of the leaves. Erol O. and Kuchuker O. [17] studied the morphological and anatomical features of six endemic crocus taxa: *C. fleischeri, C. gargaricus, C. wattiorum, C. antalyensis, C. olivieri* and *C. candidus.* The authors [18-22] revealed differences in morphological characters between taxa, but they recommend using a leaf crosssection scheme as a diagnostic key for species identification. They describe anatomical features of the cross section and the vascular bundles size of *C. reticulatus, C. danubensis, C. variegatus, C. heuffelianus, C. leichtlinii* and *C. pestalozzae* leaves. In order to determine the phylogenetic relationship of *Crocus* L. taxa from the flora of Bulgaria, the morphological and anatomical characters of *C. adamioides* and *C. pallidus* were studied and the cross sections of the leaves were presented [23, 24]. The authors consider the shape of the transverse section of the leaf, the number of large and smaller vascular bundles, the colour of the perianth, and the degree of stigma branching as characteristic features.

The aim of this study is a full-scale study of the features of morphological and anatomical diagnostic signs of the aerial part of *Crocus alatavicus*.

Materials and methods

The object of the study was the dried aerial part of *Crocus alatavicus* cultivated in the plantation of Fitoleum LLP. The appearance, anatomical and diagnostic features of plants were determined in accordance with pharmacopoeial methods. Temporary micropreparations for the detection of anatomical and diagnostic features were prepared according to the article of the State Pharmacopoeia of the Republic of Kazakhstan - Technique for microscopic and microchemical examination of medicinal plant raw materials and medicinal plant preparations [25]. The surface and transverse sections of leaves were prepared using a microtome with a TOC-2 freezing device. Micro preparations were studied using an MS-300 microscope with a camera. Clarification of the preparations was carried out in a solution of chloral hydrate-water (1:1). The measurements were carried out in ten repetitions; MS Excel was used to analyze the static data.

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Results and discussions

Botanical description. *C.alatavicus* is an herbaceous perennial with a height of 10-20 cm. Bulbous ephemeroid with a round-spherical corm, 1.5-2 cm in diameter. It blooms in early spring, from late February to mid-March. The fruiting period is April-May. The fruits are three-celled capsules, with 8-15 small blackbrown seeds. After the formation of fruits, the aerial part dies off completely, while the corm remains. The vegetation resumes in a more favorable period for the plant.

Corms outwardly resemble bulbs, but their scaly leaves do not serve as storage; they are dry and membranous. The actual storage organ is the stem part of the corm, it is thickened and parenchymatized. Thus, a corm is a leafy tuber, not a bulb. On the axis of the corm, nodes and internodes, axillary buds are usually clearly visible. Flattened-spherical corms are 1.0-1.5 cm in height and 2.0-2.5 cm in thickness; their shells are membranous, and soft, with scales of parallel fibres connected by a thin membrane. The appearance of the plant is shown in Figure 1.

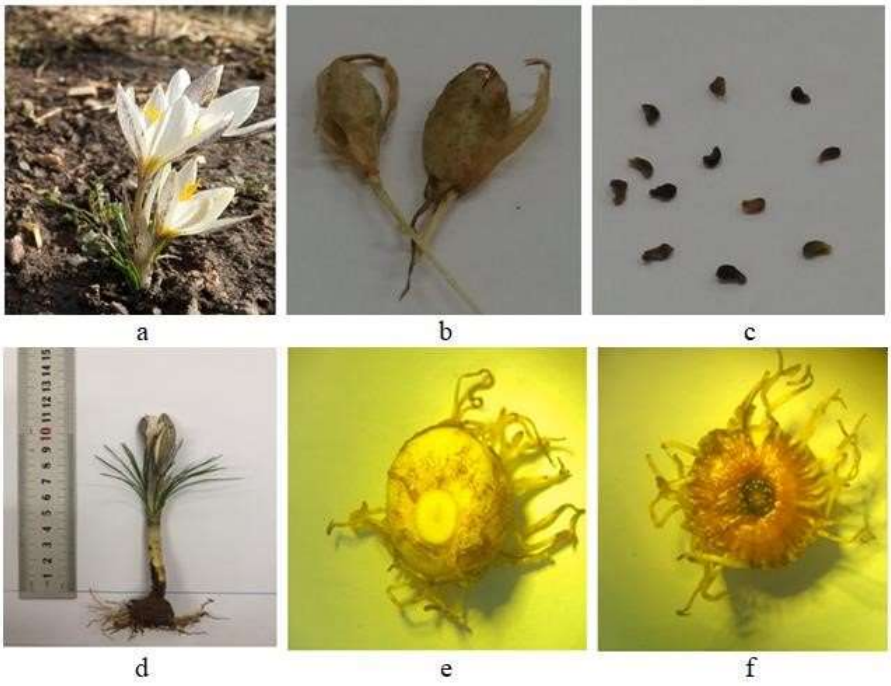


Figure 1 - Appearance or habitus of the plant Crocus alatavicus
a) above-ground part; b) fetus; c) seeds; d) whole plant;
e) corm; f) the stem part of the corm

Morphological study. *C. alatavicus* plant material is a partially crushed grass, including fragments of leaves, flowers and a reduced stem or whole grass up to 10-12 cm long.

Partially crushed raw material. The raw material is an herb consisting of plant parts: needle-shaped leaves, up to 5-6.5 cm long, up to 1 mm wide; funnelshaped flowers with petals up to 2-2.5 cm, white inside, grey-violet on the back; stamens three in number, with

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long orange anthers; ovary columns long, filiform, with wedge-shaped dentate stigmas. The raw material is grey-violet (Figure 2).

Organoleptic indicators of whole or partially crushed raw materials: spicy smell, specific to saffron; the taste is spicy-bitter, slightly tart.



Figure 2 – Crushed raw material *C. alatavicus*

Whole raw material. Whole plant material (grass) consists of a reduced stem, leaves and flowers (Figure 3a).

The stem is underdeveloped, so the tube carries the flower above the soil surface (Figure 3b).

The leaf blades are narrow with whitish veins in the centre, almost needlelike in shape, the leaf apex is pointed-subulate, the venation is linear, and the leaf margin is entire (Figure 3 c); in the amount of 8-16 leaves (the number depends on the age condition) are collected in a surface bunch, usually 8-10 cm long (up to 15 cm during fruiting), surrounded by long membranous sheaths (Figure 3d). The leaves grow directly from the corm.

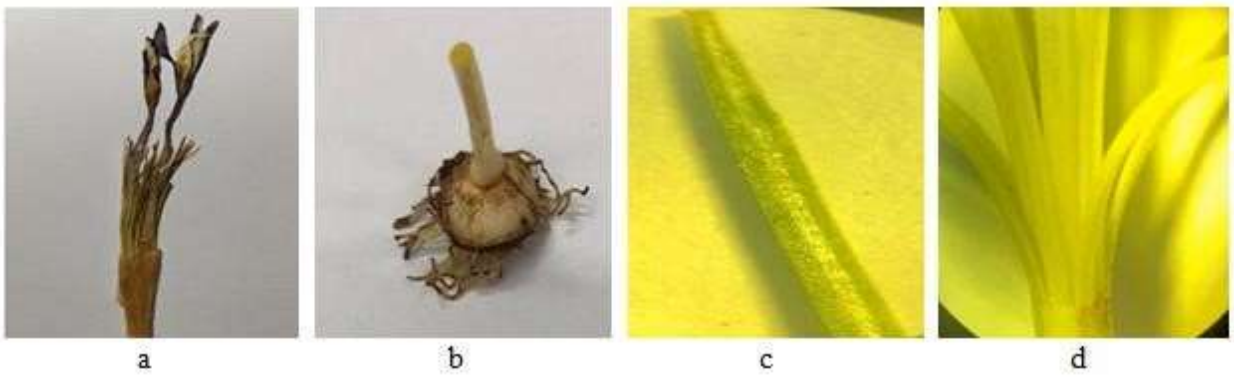


Figure 3 - Morphological features of whole raw materials, reduced stem and leaf a) whole raw materials; b) reduced stem; c) sheet; d) membranous leaf wrapper.

The flowers are funnel-shaped, and grow from 1 to 5 bulbs. The tepals are snow-white, painted with grey-violet strokes on the outside, up to 3-5 cm and the fused segments of the perianth form a long tube that takes on the role of a stem (Figure 4 a, b). The results of the

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analysis of taxa photographs on the site "Plantarium" show that most species of the genus *Crocus* L. have a colour from deep purple to bright yellow. Only about 24% of the species are pure white or white on the inside of the perianths [26]. The color of the *Crocus alatavicus* tepals can be used as an identification feature.

The number of stamens is 3; they are linear, about 1.5-1.8 cm. The stamen consists of an anther of orange and a short yellow filament. The pistil consists of an ovary, a column and an orange stigma. The stigma is slightly branched on 1/3 of the upper part (Figure 4 c, d). The ovary is three-celled, remains underground, and rises to the soil surface only when the seeds ripen. According to Gilbert's classification, the *Crocus alatavicus* belongs to the second form. J. Gilbert Baker [27] used the degree of branching of the stigma (styloid) as a taxonomic feature of the genus *Crocus* L. and divided the plants of the genus into three forms according to the degree of separation of the stigma: Holostigma - with a complete stigma, Odontostigma - with serrated or slightly divided stigmas and Schizostigma - with deeply divided stigmas.

Therefore, the petals colour and branching form of the stigma are characteristic features of the *C. alatavicus*.

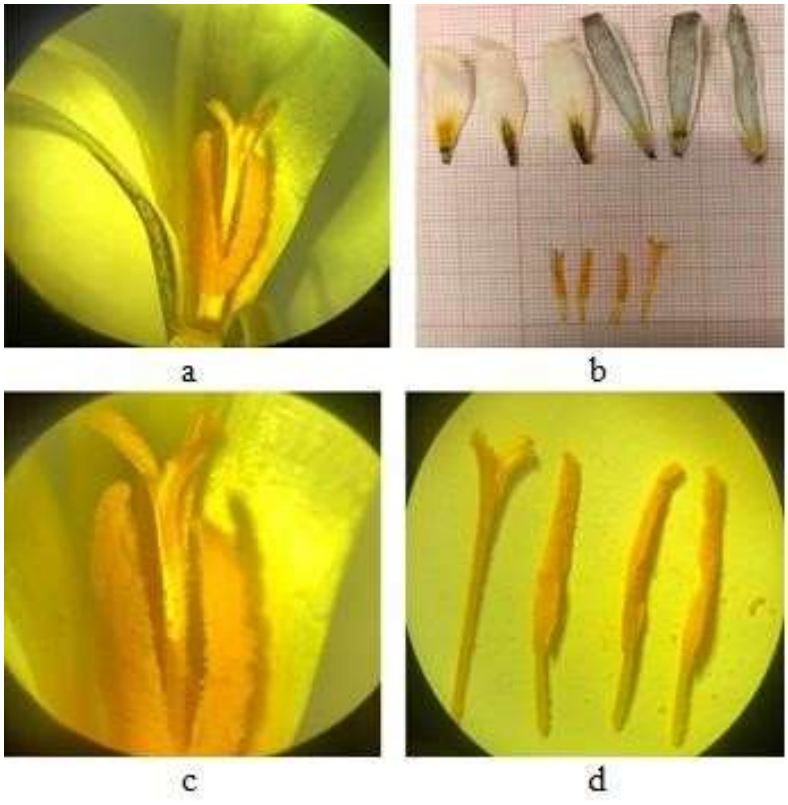


Figure 4 - The structure of: a) flower, b) petals, stamens, pistil; c, d) pistil and stamens.

Crocus flowers are unisexual, and pollinated by insects. Crocus flowers close at night and open in the sun.

Anatomical study. Microscopic analysis of a cross section of a *C. alatavicus* leaf showed a unique shape characteristic of *Crocus* L. species, consisting of a central «keel» and

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two lateral «arms» curved towards the «keel» (Figure 5). Sedelnikova L. L. [28, 29] grouped the cross sections of the leaf blade of introduced species of the genus *Crocus* L. into 4 morphological forms: pterygoid, grooved pterygoid, reverse trapezoid, and trapezoid. According to this classification, the *C.*
alatavicus belongs to the pterygoid. Unlike other species, the ―arms‖ of the *C. alatavicus* differ from other species in mesomorphic features and a strong curvature towards the ―keel‖, as well as the triangular shape of the ―keel‖. There is a characteristic pale stripe running axially along the centre of the leaf, due to parenchymal cells lacking chloroplasts.

The thickness of the leaf blade is 70.51±1.85 μm. The leaf blade is covered on the outside by the single-layered epidermis. The square-shaped epidermal cells are arranged in two rows on the adaxial and in one row on the abaxial side. Epidermal cells are large, thick-walled, and cell walls are straight. The height and width of the cells of the upper epidermis are 6.12±0.06 μm and 7.28±0.04 μm; for the lower epidermis, these figures are 5.91±0.63 μm and 6.34±0.58 μm, respectively.

Under the epidermis, there is a mesophyll, which consists of 2 rows of cells of the palisade parenchyma, which are oriented perpendicular to the surface of the leaf blade, and 4-6 rows of spongy cells of various shapes.

The cells of the palisade parenchyma have an elongated rectangular shape and are well-defined, and densely spaced. The cell walls of the palisade parenchyma are slightly torturous. The height and width of the cells of the palisade parenchyma are 12.18±0.18 μm and 9.11±1.73 μm, respectively. The spongy parenchyma is the main tissue of the leaf blade with thickness of 9.67 ± 0.29 μm. The anatomical and diagnostic parameters of a *C. alatavicus* leaf are shown in Table 1.

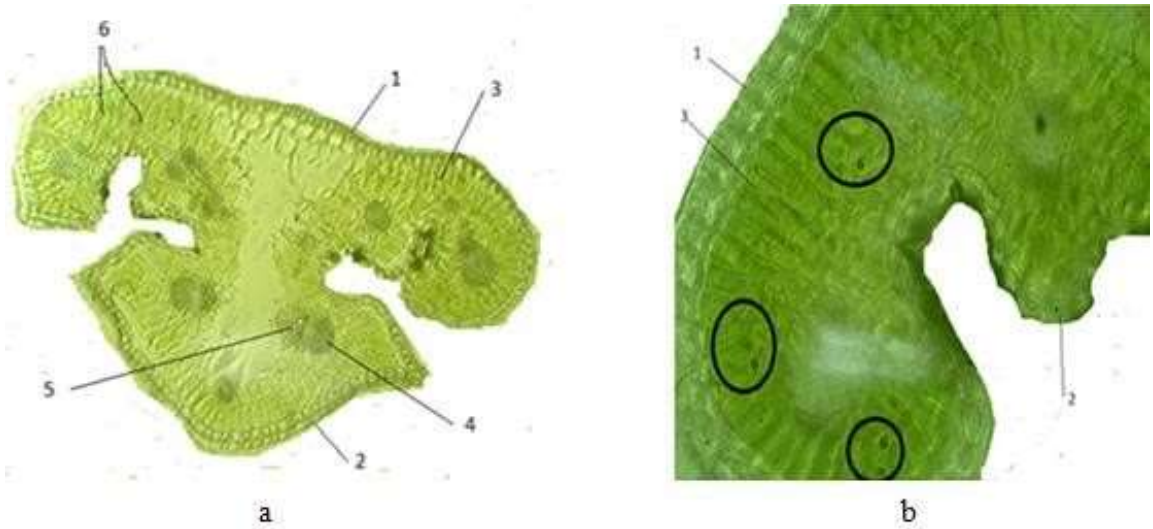


Figure 5 - Cross section of a *C. alatavicus* leaf at microscope magnification
a) ×180 and b) ×720
1 - upper epidermis; 2 - lower epidermis; 3- mesophyll; 4 - xylem; 5-phloem; 6-idioblasts.

Conductive bundles of crocus leaves are very specific. The authors [29] found four main vascular bundles, when studying transverse sections of the leaves of some types of

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crocuses. Two of them are located at the corners of the keel; the other two are at the ends of the "arms". On the "arms", there are also medium and small vascular bundles. The authors concluded that the number of such vascular bundles depends on the leaf width. The vascular bundles of *C. alatavicus* are nearly symmetrical. Four main vascular bundles were found in *C. alatavicus*. Two of them are located at the corners of the "keel", and the other two are at the tips of the "arms". These are the largest vascular bundles, which consist of the phloem and xylem. There are smaller or medium-sized vascular bundles that are located in the folds of the shoulder. *C. alatavicus* differs from other species by the presence of a medium-sized conductive bundle at the tip of the ―keel‖, located in the middle of two large bundles. And also, there are small conductive bundles distributed along the perimeter of the "arms" and in the bends. The total number of vascular bundles ranges from 15 to 17. Small dark idioblasts are also found in the mesophyll. Perhaps they accumulate essential oils.

C. alatavicus is characterized by well-developed chlorophyll-bearing parenchyma in the lower part of the leaf. The middle and upper parts of the leaf differ from the lower part by a strongly developed palisade parenchyma, the cells of which are smaller and have a more sinuous wall.

Table 1 - Anatomical and diagnostic features of the *C. alatavicus* leaf

Anatomical and diagnostic indicators, microns	**Size**
Sheet plate thickness, µm	70.51±1.85
Height of cells of the upper epidermis, µm	6.12±0.06
Width of cells of the upper epidermis, µm	7.28±0.04
Height of cells of the lower epidermis, microns	5.91±0.63
Cell width of the lower epidermis, µm	6.34±0.58
Height of palisade parenchyma cells, µm	12.18±0.18
Width of palisade parenchyma cells, µm	9.11±1.73
Spongy parenchyma thickness, µm	9.67±0.29
Number of conductive bundles, pcs	15-17
Stomata size, µm	0.02±0.03
Stomatal index (adaxial side) per 1 mm2	14.2
Stomatal index (abaxial side) per 1 mm2	21.3

In *Crocus alatavicus* the adaxial and abaxial sides of the leaf are clearly expressed morphologically and anatomically, despite the insignificant surface of the plate. According to the location of the stomata, the leaves are of the amphistomatic type. The stomata are 0.02±0.03 µm in size; the stomatal index for the upper side is -14.2 and for the lower side - 21.3. The stoma has an anomocytic type of structure; guard cells have an elongated shape (Figure 6 a, b).

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Figure 6 - Type and location of the stomatal apparatus of the *C. alatavicus* leaf: a) the top surface of the sheet; b) the lower surface of the sheet 1- stomata; 2 - epidermal cells

The reduced stem of C. *alatavicus* on a cross section has a round shape (Figure 7 a, b). Outside it is covered with cells of the epidermis and 1–3 layers of also corky cells of the primary cortex. Next is the storage parenchyma of the primary cortex. The inner layer of the primary cortex is the endoderm, which is similar to the root.

In the central cylinder, many conducting beams are located diffusely; at the same time, most of them are concentric, especially in the central part, and on the periphery, they are collateral, while all of them are of a closed type. In contrast to the typically reduced stem, the

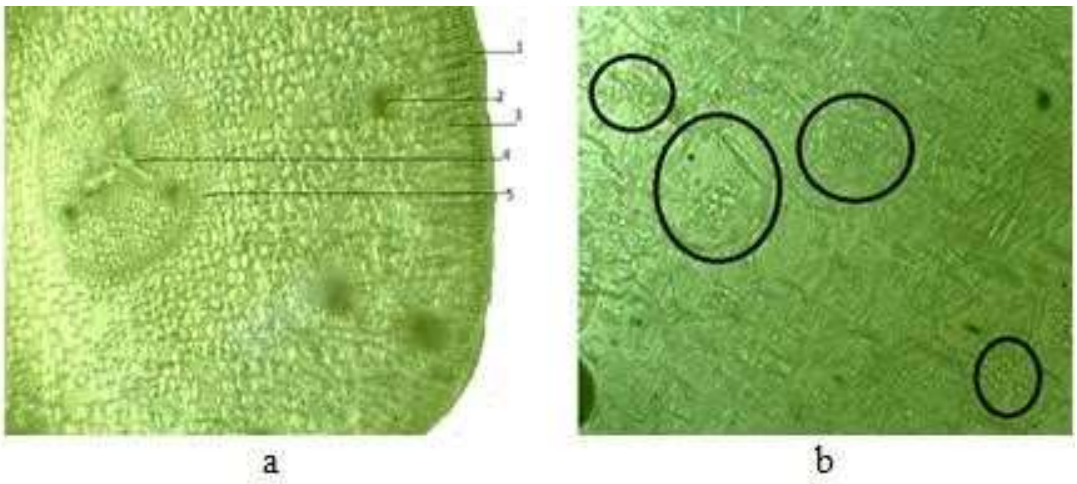


Figure 7 - Microscopic structure in a transverse section of *C. alatavicus* areduced stem

a) 1-epiderm; 2-storage parenchyma of the cortex; 3- conductive bundles; 4concentric centrophloem bundle; 4-phloem; 5-endoderm; b) starch grains.

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central cylinder of the reduced stem of *C. alatavicus* does not form pericyclic sclerenchyma, and the parenchyma of the central cylinder, like the parenchyma of the primary cortex, is storage.

The thickness of the epidermis is 10.58±1.04 μm. The thickness of the parenchyma is 23.47±1.08 μm. Endoderm consists of 1-2 rows of parenchymal cells. Conductive bundles are arranged in a circle. The phloem of vascular bundles is single-row; the xylem part is more developed and separated by intermediate sclerenchyma. The area of the conducting beam is 48.45±2.16×10-3. The area of xylem vessels is 1.67±0.28×10-3. Anatomical and diagnostic parameters of the reduced leaf cross section are shown in Table 2.

Table 2 - Features of the microscopic structure of the reduced *C. alatavicus* stem

Thickness of the epidermis, μm	Thickness of the parenchima, μm	Area of the conductive bundle, ×10-3	Area of xylem vessels, $\times 10^{-3}$
10.58±1.04	23.47±1.08	48.45±2.16	1.67±0.28

Conclusion

Thus, as a result of the study, the following morphological and anatomical features of *Crocus alatavicus* were established:

1) the colour of the petals and branching form of the stigma are characteristic features of this species: the perianth is white on the inside, the outer part is coloured with grey-violet strokes and the stigma is slightly branched on 1/3 of the upper part;
2) the stem is underdeveloped and has a reduced form.
3) the leaves of *C. alatavicus* in cross-section have a unique and peculiar shape, consisting of a central triangular “keel” and two lateral “arms” with strongly curved edges to the keel;
4) the leaf blade has 4 large, 3 medium and many small vascular tissues. Unlike other species, there is a medium-sized bundle at the tip of the "keel" between two large conducting vessels.
5) stomata are present on both sides of the leaf blade and belong to the anomocytic type.

The results of our study are of practical value - they supplement the available information about plants of the genus *Crocus L.*, allow the identification and standardization of raw materials of these plants and expand the possibilities of their use as domestic ones.

Authors' Contributions.
Conceptualization: Z. B. Allambergenova, Z. B. Sakipova
Investigation: Z. B. Allambergenova, A. T. Mamurova
Processing of results: G. M. Kadyrbaeva, K. K. Kozhanova
Scientific interpretation: E. A. Severova, N. U. Aliyev
Article Writing: Z. B. Allambergenova, G. T. Zhumashova

No conflicts of interest have been declared.

This material has not been previously submitted for publication in other publications and is not under consideration by other publishers.

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CROCUS ALATAVICUS ӨСІМДІК ШИКІЗАТЫНЫҢ МОРФОЛОГИЯЛЫҚ ЖӘНЕ АНАТОМИЯЛЫҚ ЕРЕКШЕЛІКТЕРІН ЗЕРТТЕУ

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Түйіндеме

Кіріспе. *Crocus* L. тұқымдасының өсімдіктері фармакологиялық әсердің кең спектріне ие, олардың көптеген түрлері бүгінгі күнге дейін аз зерттелген. Олардың ішінде шикізатқа нормативтік құжаттама жетіспейтін *Crocus alatavicus* Regel & Semenow бар, өйткені бұл өсімдіктің морфологиялықанатомиялық белгілері зерттелмеген. Әдебиеттерге шолу *Crocus alatavicus* шикізатын анықтауға және стандарттауға мүмкіндік беретін осы белгілердің нақты, толық сипаттамасының жоқтығын көрсетті.

Зерттеудің мақсаты *C. alatavicus* шикізатының морфологиялық және анатомиялық-диагностикалық белгілерін зерттеу.

Материалдар мен әдістер. Шикізаттың морфологиялық белгілері бинокулярлық лупаның көмегімен зерттелді, анатомиялық-диагностикалық белгілер фармакопеялық әдіске сәйкес уақытша препараттарды дайындау арқылы фотокамералы MC300 микроскопын қолдана отырып анықталды.

Нәтижелер. Қысқартылған сабақтың, жапырақтың, гүлдің морфологиялық және анатомиялық-диагностикалық белгілері зерттелді. *C. alatavicus* жапырағының көлденең қимасы *Crocus* L. тусының басқа түрлері сияқты ерекше пішінге ие екені анықталды. Ол Орталық төртбұрышты "кильден"және екі бүйірлік "қолдардан" тұрады. Алатау шафранының зерттелген басқа *Crocus* L. түрлерінен айырмашылығы, "киль" үшбұрыш пішінді және "қолдарының" ұштары "кильге" өте иілген болып келеді. Осьтік бағытта "киль" орталығының бойында бозғылт жолақ бар. Алатау шафранында лептесіктердің орналасуы бойынша жапырақтары амфистоматикалық, лептесіктері – аномоциттік типті болып келеді.

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Қорытынды. Өсімдік шикізатының осы белгілері шикізатты сәйкестендіру үшін қолданылады және дәрілік өсімдік шикізатын стандарттаудың маңызды аспектілерінің бірі болып табылады.

Түйін сөздер: *Crocus* L., *Crocus alatavicus*, анатомиялық диагностикалық белгілер, *C. alatavicus* морфологиялық ерекшеліктері, *C. alatavicus* анатомиялық ерекшеліктері, өсімдік шикізаты.

ИЗУЧЕНИЕ МОРФОЛОГИЧЕСКИХ И АНАТОМИЧЕСКИХ ОСОБЕННОСТЕЙ РАСТИТЕЛЬНОГО СЫРЬЯ CROCUS ALATAVICUS

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Аннотация.

Введение. Растения рода *Crocus* L. обладают широким спектром фармакологического действия, многие их виды на сегодняшний день мало изучены. В их числе *Crocus alatavicus* Regel & Semenow, на сырье которого отсутствует нормативная документация, так как не изучены морфологоанатомические признаки этого растения. Обзор литературы свидетельствует об отсутствии конкретного, полного описания этих признаков, которые делают возможным идентификацию и стандартизацию сырья *Crocus alatavicus*.

Целью настоящего исследования является изучение морфологических и анатомо-диагностических признаков растение *C. alatavicus*.

Материалы и методы. Морфологические признаки сырья изучали с помощью бинокулярной лупы, анатомо-диагностические признаки определяли путем приготовления временных препаратов согласно фармакопейной методике, используя микроскоп МС-300 с фотокамерой.

Результаты. Изучены морфологические и анатомо-диагностические признаки редуцированного стебля, листьев, цветка. Установлено, что листья *C. alatavicus*, как и большинство видов *Crocus* L. в поперечном сечении имеет уникальную и своеобразную форму. Состоит из центрального четырехугольного «киля» и двух боковых «рук». В отличие от других изученных видов *Crocus* L., у *C. alatavicus* «киль» имеет трехугольную форму и кончики «рук» очень изогнуты к «килю». В осевом направлении вдоль центра «киля» имеется бледная полоса. По расположению устьиц листья *C. alatavicus* называются амфистоматическими, по структуре относиться аномоцитному типу.

Выводы. Данные признаки растительного сырья применяется для идентификации сырья, и являются одним из важных аспектов стандартизации лекарственного растительного сырья.

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Ключевые слова: *Crocus* L., *Crocus alatavicus*, анатомо-диагностические признаки, морфологические особенности *C. alatavicus*, анатомические особенности *C. alatavicus*, растительное сырье.