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MACROSCOPIC AND MICROSCOPIC ANALYSIS OF *ALFREDIA NIVEA* KAR. & KIR HERB

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Kazakhstan flora is rich in promising little-studied plants that are used in folk medicine, but additional deep research is required for their introduction into medical practice with the help of modern scientific techniques. An interesting object for introduction into the official medical and pharmaceutical practice is the Alfredia Nivea Kar. & Kir, family Asteraceae, whose extracts have a neurotropic effect.

The aim. *Regarding mentioned above, in order to create new medicines based on the grass of the Alfredia nivea, it is necessary to develop methods of its macro and microscopic identification.*

Materials and methods. *Macro- and microscopic studies of the grass of Alfredia nivea were carried out in accordance with the methodology of SPhU 2.8.23 "Microscopic examination of medicinal vegetable raw materials". Macroscopic studies were performed using magnifying glass and binocular microscope MBS-9, microscopic-using MS Microscopes 10 (eyepiece X5, X10, X15, lenses x10, x40), micromed XS-4130 (WF15X, Opera, lens X40/0, 65, x10/0,25) with microphoto addon (China).*

Results. *The morphological and anatomical features of the Alfredia nivea grass. Diagnostic features of the structure of stems, leaves and flowers of this species were revealed.*

Conclusions. *The macroscopic and anatomical identification parameters of the grass of the Alfredia of Snow. The study of the morphological and anatomical features of the stems, leaves and flowers of Alfredia nivea, considering the requirements of European pharmacopoeia. Diagnostic features of the structure of stems, leaves and flowers of the species were found, which were used as key quality indicators for standardization and identification of raw materials*

Keywords: *Alfredia nivea, medicinal plant raw material, morphological and anatomical signs, standardization*

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1. Introduction

In the structure of the modern pharmaceutical market, the proportion of medicines based on medicinal plant raw materials is steadily growing. According to the World Health Organization, about 80 % of the population use herbal preparations to treat and prevent various diseases. The wide range of their actions is explained by the multicomponent of the chemical composition and a soft comprehensive effect on the body.

The flora of Kazakhstan is rich in promising little-studied plants that are used in folk medicine, but they require additional deep studies to implement them in medical practice, using modern scientific methods [1, 2]. An interesting object for implementing in official medical and pharmaceutical practice is *Alfredia nivea Kar. & Kir*, family *Asteraceae* [3, 4].

A. nivea Kar. & Kir (Fig. 1) has several synonyms: *Alfredia suavelens* Ruprecht; *Arctium niveum* (Karelin & Kirilov) Kuntze, *Carduus karelinii* B. FedtSchenko, *Cousinia trautvetteri*. Regel [4, 5].

Alfredia nivea – perennial plant 25–70 cm high; The leaves are leathery, oblong-lanceolate, pinnati-lobed, less often toothed, with blades and teeth, turning into strong, yellowish thorns, greenish, naked or absent-minded-cauty, with yellow, slightly outstanding veins, turning into thorns, below white-tomentose, sometimes grayish, with protruding veins, blooms in July, fruit in August - September. It grows in the subalpine and alpine belts of the mountains, along the stepped rocky slopes, in spruce-fir forests, is found in Tarbagata, Dzungarsky, Zailiysky and Kungay Alatau [5, 6].

Plants of the genus Alfredia are not examined enough. So, in the international base Scopus currently there are only 19 links containing information on the keyword alfredia, of which only two have studies on *A. Nivea* [7, 8].

Most other studies are devoted to the study of *A. cernua*. So, it was shown that extracts of this type affect the operation of the central unequal system, and improve memory [9, 10]. It was blished that *A. cernua* extracts have nootropic properties, help improve indicators of indicative and research behaviour, preserve the reflex of passive avoidance in hypoxic shock, and increase physical performance in mice [11, 12]. The most pronounced effect was

observed with the introduction of the *A. cernua* extract obtained by 95 % ethanol [12, 13]. It was also shown that the extract of the aboveground part *A. nivea* by 95 % ethanol at a dose of 100 mg/kg restores exploitabehaviour and safety of the reflex after a hypoxic trauma [14]. Thus, the grass of *A. nivea* is a promising raw material to create new medicines with neurotropic activity.

Previously, the standardization of the grass of *A. cernua* [15] was carried out, the data of which were considered when determining macro- and microscopic diagnostic signs of grass *A. nivea*, which would be based on the instructions for the cultivation of the plant and the harvesting of this raw materials, as well as the methods of monitoring its quality.

Therefore, **the aim of the research** was to conduct macro- and microscopic analysis of grass *Alfredia nivea* Kar. & Kir. to establish diagnostic features that will be the basis of methods for identifying raw materials during standardization.

Fig. 1. *Alfredia nivea* KAR. & KIR, family *Asteraceae*

2. Planning (methodology) of the research

In Fig. 2 a graphical representation of the research planning process is shown.

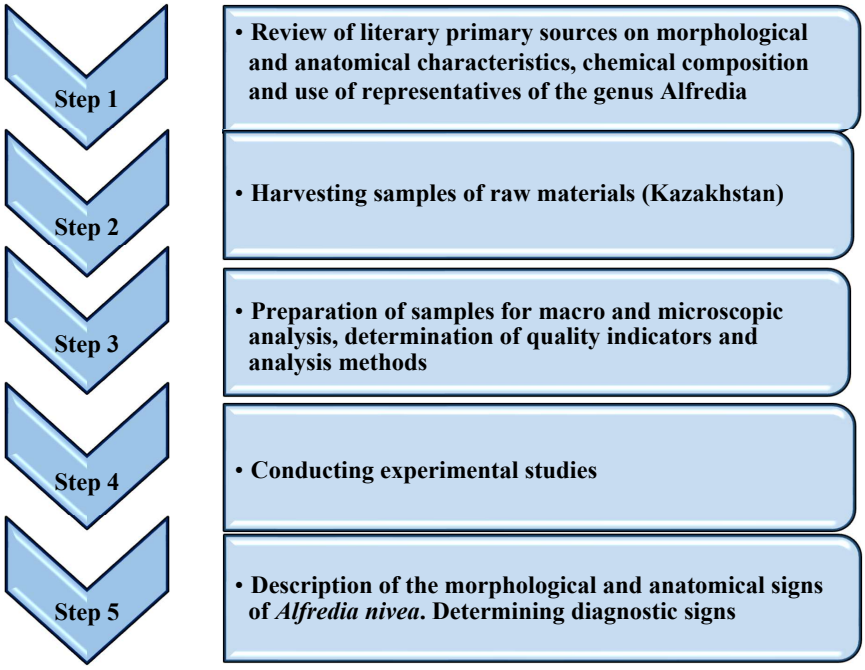

Fig. 2. Planning of the research

3. Materials and methods

The objects of the study were samples of grass of *A. nivea*, which were prepared by Kungay Alatau, 4.3 km southeast of the Karabulak village, gorge East Karabulak, Almaty Region, Kazakhstan (1762 m above the city, N 43°02'30.2", E 078°34'16.0") 06.07.2021. The identity of the plant was established by professor Tetiana Gontova, D. Sc. [6, 16]. Voucher specimens were deposited at the School of Pharmacy, Asfendiyarova Kazakh National Medical University (Almaty, Kazakhstan, No. 432–435). The raw material was dried at room temperature [17–19] in a well-ventilated area for ten days and stored in paper bags [20–22]. Raw materials studies were conducted in January–February 2022.

Macro- and microscopic studies of raw materials were carried out according to the methodology of SPhU 2. 8. 23 "Microscopic examination of drug plant materials" [20, 23]. Macroscopic studies were carried out using a magnifying glass and binocular microscope MBS-9. The study of the anatomical structure of the *A. nivea* was carried out on samples of whole and cut raw materials in accordance with the requirements of SPhU. The shoots were fixed in a mixture of 96 % ethanol P – glycerin P – purified water P (1:1:1). The structure of the stems and leaves was studied on transverse sections. The epidermal of organs was considered from the surface according to generally accepted methods [20, 24, 25]. The raw materials were crushed according to the requirements of the SPhU monograph 2. 9. 12 "Sit analysis" and enlightened with the help of chloralhydrate P [20, 23, 26]. Studies of transverse and longitudinal sections, epiderma and preparations from the surface were carried out using microscopes of MBS 9, MS 10 (oculars X5, X10, 15, lenses X10, X40), Micromeed XS-4130 (oculars WF15X, lenses x40/0.65, x10 /0.25) with microphoto add-on (China). The results of the study were recorded using the Canon IXUS 220 HS camera.

4. Research results

The raw materials are dried whole or broken leaves, flowers and remains of *Alfredia nivea* KAR. & KIR.

According to the results of a macroscopic analysis of whole raw materials, *Alfredia nivea* (Fig. 3), such macroscopic features are established.

The stem is rough, unbranched, ribbed, tomentose (Fig. 3.1). The top of the flowering stem is unbranched or light branched. Leaves are leathery, lanceolate, pinnatipartite, rosette – petiolar, stem – sessile

(Fig. 3.2, a, b). Leaf blade from the adaxial side green, naked; with abaxial – grayish-silver and densely tomentose; pinnate venation, lateral veins hard, lignified, straw color, passing into sharp teeth along the edge of 5.0–10 mm long (Fig. 3.2, a, b, 3.3).

Basal and lower stem leaves are from 15 to 36 cm long and 2.5–6.0 cm wide. The middle stem leaves are short-petiolar, the upper ones are sessile, from lanceolate to linear-lanceolate, up to 5.0–7.0 cm in length, 1.5–2.5 cm in width.

Inflorescence (Fig. 3.1, 3.4, 3.5) is spherical drooping head 3.0–6.0 cm in diameter. Involucre (Fig. 3.5, a, b) 3–5-row, tiled. Leaflets (Fig. 3.5, d) are rough, pointed, straw colour. The outer and middle leaflets are 2.5–3.0 cm in length, laterally expanded in the middle part, with membranous fringed-torn edges at the base or middle part. The inner leaflets of the involucres are oblong-lanceolate, up to 3.0 cm in length; rigid, filmy, entire margin, pointed apex.

The flowers are tubular (Fig. 3.5, b, 3.6, a, b), thin, 2.0–3.0 cm in length, pink corolla, 5-toothed. Numerous sepals modified to thin long (up to 2.0 cm) hairs. The stamens and pestle protrude from the corolla tube. There are 5 stamens, coenocarpous gynoecium of 2 fused carpels. The stigma of the pistil (Fig. 3.6, c) with an elongated oval brown top. Cypsela up to 6.0 mm, pale yellowish with variegated brown spots and stripes on the surface.

Microscopic features of *Alfredia nivea* were studied on samples of whole and cut raw materials in accordance with the requirements of SPhU.

On the epidermal of the stems and leaves of *Alfredia nivea* there are covering and glandular trichomes (Fig. 4). The tomentose pubescence of the stem and the lower side of the leaves provide long, filamentous hairs with a 2-cell base (Fig. 4.1, 4.4), multicellular hairs with collapsed cells (Fig. 4.2) and multicellular narrow hairs with collapsed cells are also often found (Fig. 4.3). Less commonly found hairs with a 1-cell head and a stalk (Fig. 4.5) and glandular hairs with a multicellular stalk and a multicellular head with collapsed cells (Fig. 4.6).

The stem on the cross section is rounded with slightly protruding rounded ribs. The pubescence is scattered, but more often the cluster of hairs can be observed at the base of the stem ribs. The epidermis of the stem is covered by a thin layer of a folded cuticle. Epidermal cells are prosenchymatous, straight-walled, with slightly thickened walls pierced by straight simple pores. (Fig. 5.1). Stomata are rare, large, oval (Fig. 5.1, a). Stomata apparatus type is anomocytic.

The primary cortex is well developed, represented by collenchyma, cortex parenchyma with collenchyma walls and endoderm (Fig. 5.2–5.4). Collenchyma is of two types, angular and lacunar (Fig. 5.3, d, f). Collenchyma lies unevenly, from 2 to 6–8 (in the ribs) layers.

Fig. 3. Macroscopic features of the *Alfredia nivea* herb:
1 – general view of the plant;
2 – lower (a) and stem (b) leaves;
3 – leaf fragment with veins modified into spines;
4 – shoot tip with inflorescences;
5 – a flower head with multi-row involucre on the lower (a) and on the upper (b) side, c – a fragment of the top of the inflorescence, d – involucral leaves;
6 – flowers with a modified calyx (a), without a calyx (b), the top of the corolla tube with stamens and pistil (c)

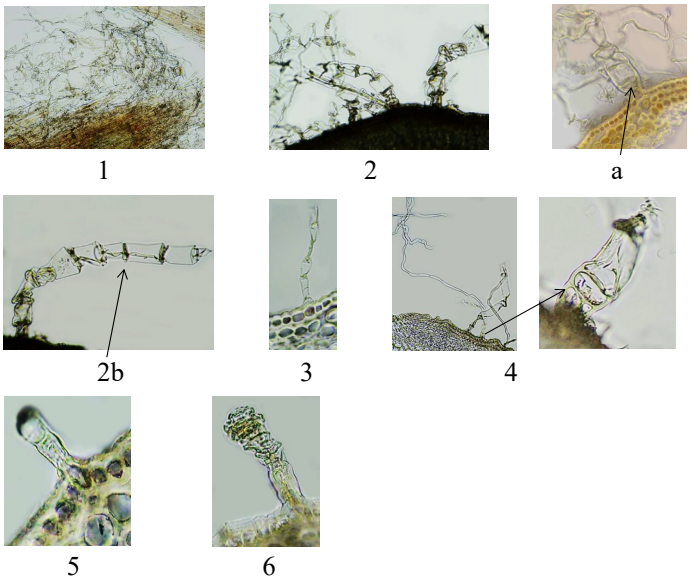

Fig. 4. Types of trichomes: 1 – pubescence (top view);
2 – multicellular wide hairs with collapsed cells,
a – hair base, b – hair body; 3 – multicellular narrow hair with collapsed cells; 4 – filamentous hair with a 2-cell base;
5 – 2-cellular capitate hair; 6 – glandular hair with a multicellular stalk and a multicellular head with collapsed cells

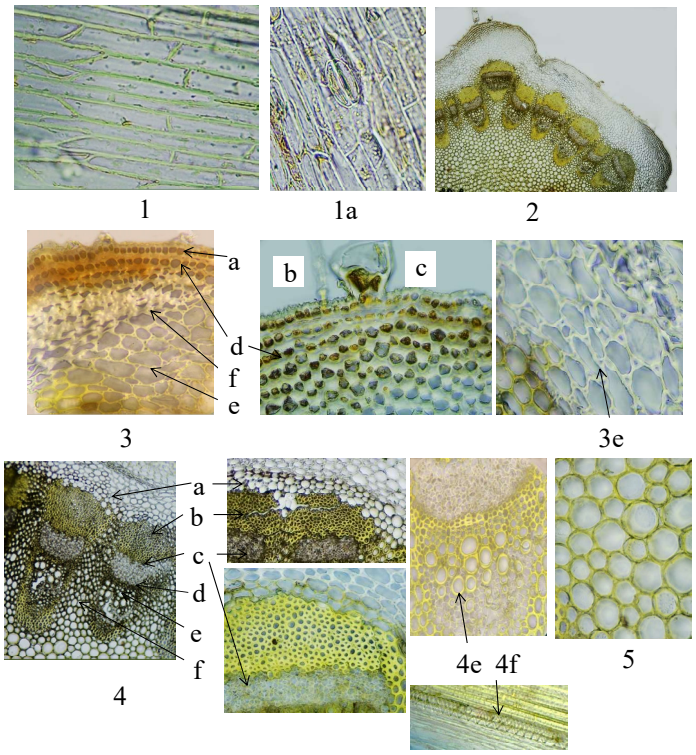

Fig. 5. Microscopic features of the stem: 1 – epidermis (top view), a – stomata; 2 – a fragment of a cross section; 3 – a fragment of the primary cortex: a – epidermis, b – base of a narrow-lumen multicellular hair, c – base of a wide-lumen multicellular hair, d – angular collenchyma, e – cortex parenchyma with collenchymal membranes, f – lacunar collenchyma; 4 – a fragment of the primary cortex and the central cylinder: a – endoderm, b – sclerenchyma, c – phloem, d – cambium, e – xylem vessels in a transverse section, f – vessels in a longitudinal section, f – cove ray; 5 – core

The epidermis can be underlain by angular and lacunar collenchyma (Fig. 5.3). Cells of collenchyma are of different diameters; as a rule, 2–4 rows of collenchyma, lying under the epidermis, are small, and layers of larger cells are located below. The cortex parenchyma is well developed, 6–12-row, lacunar, the cells are large, less often small, rounded, oval, collenchymatous walls (Fig. 5.3, e). 2–3 layers of cortex cells adjacent to the endoderm (Fig. 5.4, *a*) are smaller in size and dense location. Endoderm (Fig. 5.4, *a*) is a single-row, cells are rounded and wide. The central axial cylinder of the transitional stem. Vascular-fibrous bundles (Fig. 5.2, 5.4) are located tightly to each other. The bundles are open collateral, wide or narrow, oval, with well-developed sclerenchyma (Fig. 5.4, *b*) above the xylem and under phloem. Sclerenchyma cells with thickened, lignified membranes pierced by chinked. Phloem (Fig. 5.4, *c*) is well expressed, represented by sieve tubes with companion cells and bast parenchyma. Vessels of the xylem (Fig. 5.4, *e*) are porous, spiral, narrow- and wide-lumen, with lignified membranes, often have a radial location, less often scattered. The core rays (Fig. 5.4, *f*) have different degree of lignification of cell membranes. The core walls (Fig. 5.5) are parenchymal, with slightly thickened walls and numerous straight pores.

The leaves of the rosette of *Alfredia nivea* have petioles of different lengths. When studying the anatomical structure of the transverse sections of the petiole in the basal, middle and upper part (Fig. 6–9), a change in the shape of the petiole, its thickness, the shape of the "wings", the amount and size of the bundles is observed.

In the basal part, the petiole (Fig. 6.1, 7) has the shape of an elongated triangle in outline. The "wings" are elongated and directed upwards at 45 degrees, at the ends are narrow. The adaxial side of the petiole is deeply concave, and the abaxial side in the central part of the petiole is rounded and wavy at the transition from the base to the "wings". In the middle part, the petiole (Fig. 6.2, 8) thickens, the "wings" are shorter and wider at the ends, directed upwards at an angle of 25–35 degrees. The adaxial side of the petiole is poorly concave, and abaxial in the central part of the petiole in outlines and wavy when moving from the base to the "wings". In the upper part, the petiole (Fig. 6.3, 9) in the outlines has a half-moon shape. The adaxial side of the petiole is deeply concave, and the abaxial rounded. The "wings" at the ends are well developed and bent down.

The topography of tissues and bundles in the basal, middle and upper parts of the petioles is the same. The adaxial side of the petioles is evenly pubescent with short covering hairs and rarely glandular, and the abaxial side is densely pubescent with glandular hairs and short and long covering hairs characteristic of the stem epidermis (Fig. 4, 7.2, a, 8.3, 9.2, b). Long hairs numerically prevail on the abaxial side of the petiole. The epidermis is covered with a thin layer of cuticle. The epidermis on the adaxial side by 2–3 layers of the angular collenchyma (Fig. 7.2, b, 8.2, a, 9.2, d), and from the abaxial side at the base of the petiole up to 8 layers and up to 4-6-between the base and "wings". The main parenchyma is represented by rounded cells different in size – from large to small.

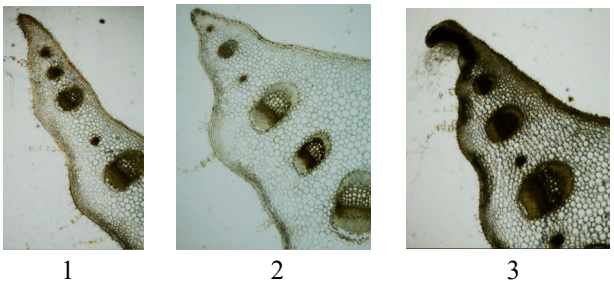

Fig. 6. Schemes of the edge of the petiole of the rosette leaf: 1 – in the basal part, 2 – in the middle part, 3 – in the upper part

In the central part of the petiole in the basal, middle and upper parts there is one vascular bundle (Fig. 7.1, 2, 8.1, 9.1, 3). To the right and left of the main bundles are single bundles of different sizes from medium to very small, oval. Small bundles that are located in the alar outgrowths have a rounded. The total number of

bundles ranges from 10–12. Conductive tissues are well developed in the bundles, to which areas of sclerenchyma are adjacent (Fig. 7.4, b, 8.1, 9.3). Phloem is represented by a sieve tube with companion cells, the vessels of the xylem of the same diameter have a radial location.

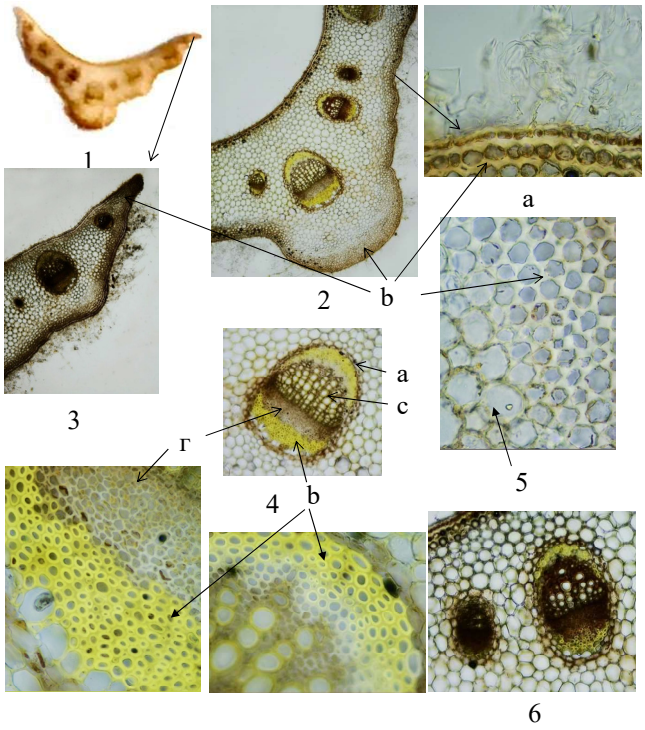

Fig. 7. Microscopic signs of the basal part of the petiole of the rosette leaf: 1 – cross section (general view); 2 – central part: a – epidermis with trichomes, b – angular collenchyma; 3 – "wing"; 4 – main bundle: a – parenchyma sheath, b – sclerenchyma, c – xylem; d – phloem; 5 – main parenchyma; 6 – additional bundles

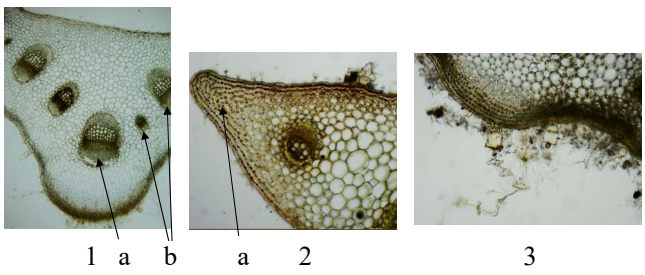

Fig. 8. Microscopic signs of the middle part of the petiole of the rosette leaf: 1 – central part: a – main bundle, b – additional bundles; 2 – "wing" (fragment): a – angular collenchyma; 3 – trichomes

The sclerenchyma on both sides of large bundles is well developed, multi-layered, less pronounced in small bundles, 1–2 layers. Sclerenchyma cells in the transverse section are oval or rounded, of different diameters with woody membranes.

On the cross section of the rosette and stem leaves *Alfredia nivea*, the main vein on the adaxial side is from slightly concave to flat or slightly convex (Fig. 10.1, 2), and on the abaxial side it is rounded at the base and slightly wavy on the sides. Depending on the degree of development of the leaf blade, the lateval "wings" are short, bent down or long with additional bundles of single-bundle lateral veins (Fig. 10.2, *a*, *b*), strongly protruding from the underside. The main vein is densely pubescent on the lower side (Fig. 10.2, 3), with trichomes characteristic of other parts of the plant (Fig. 4) and rarely on the upper side with capitate hairs and narrow colourless hairs with collapsed

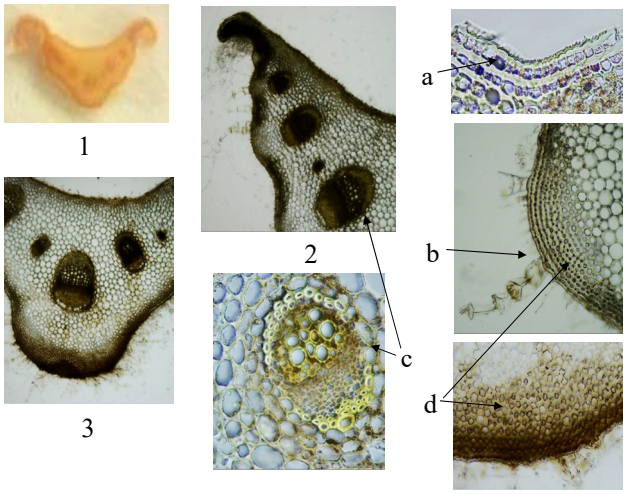

Fig. 9. Microscopic signs of the upper part of the petiole of the rosette leaf: 1 – cross section (general view); 2 – "wing" (fragment): a – epidermis with cuticle, b – trichomes, c – bundle, d – angular collenchyma; 3 – central part

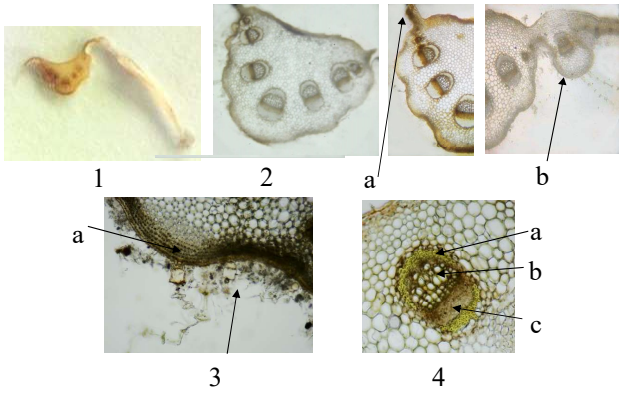

Fig. 10. Microscopic signs of the leaf: 1 – cross section (general view); 2 – main vein, a – "wing" (fragment), b – wing (fragment with lateral vein); 3 – pubescence along the epidermis, a – collenchyma; 4 – vascular bundle: a – sclerenchyma, b – xylem, c – phloem

cells and broken top.

The subepidermal collenchyma is located unevenly: 5–6-row at the base of the main vein in protruding places and on the adaxial side at the points of transition of the vein into the leaf blade, 2–4 rows in other areas. The main vein includes 7 conductive bundles (Fig. 10.2, 4). 5 large, oval bundles are located in the lower part of the petiole evenly semi-lunar, and 2 small ones – at the point of transition of the main vein to the leaf blade, adjoin the main bundles. Conductive tissues are well developed; small areas of sclerenchyma adjoin the phloem and xylem. The main parenchyma is represented by parenchymal rounded thin-walled cells.

The top of the leaf and the edges have sharp thorns (Fig. 3.2, *a*, *b*, 3.3). On the transverse section of the leaf apex in the central part, there is a rounded main vein, strongly protruding from the abaxial and adaxial sides. Moreover, the adaxial side is wider than abaxial one. The central position is occupied by a bundle (Fig. 11.1, *a*) with poorly developed conductive tissues, surrounded by sclerenchyma.

The main parenchyma is small celled (Fig. 11.1, *b*). The leaf blade is developed the same on both sides. The upper epidermis is not pubescent with hairs, and the lower epidermis is densely pubescent with thin, filamentous, tangled hairs (Fig. 11.2). Leaf blade is dorsiventral type. The palisade mesophyll is 2-rod (Fig. 11.3, *a*), the cells are narrow, tightly closed, located perpendicular to the upper epidermis. Spongy mesophyll (Fig. 11.3, *b*) has 4–5 rows, cells are small, oval, rounded, evenly spaced, oriented horizontally to the lower epidermis. In the mesophyll there are single small additional bundles.

The cells of the upper epidermis of the leaf along the veins are prosenchymatous, straight-walled, with slightly thickened walls and straight pores (Fig. 12.1, *a*). Cells adjacent to veins from elongated to parenchymal, 5–6-angular. The cells of the epidermis between the veins (Fig. 12.1, *b*) are also 5–6-angled, isodiametric in shape, often slightly elongated with straight-out poorly thickened membranes and frequent simple pores. Stomata are rare, large. The type of stomatal apparatus is anomocytic and anisocytic (Fig. 12.1, *b*, 2, *a*, *b*).

The lower epidermis along the veins is formed by narrow prosenchymatous cells with straight-walled, slightly thickened walls (Fig. 12.3, *a*). Between the veins of the cells are parenchymal, with sinuous-walled slightly thickened membranes (Fig. 12.3, *b*). Stomata are frequent, stomata apparatus is anomocytic. The upper epidermis is rarely pubescent with thin long hairs, and the lower is densely pubescent (Fig. 12.4, *a*, *b*) with filamentous hairs with a 2-cell base that dominate (Fig. 12.5) and multicellular wide-lumen hairs with collapsed cells (Fig. 12.6).

Involucral leaflets of *Alfredia nivea* are unevenly roughly bordered at the base or middle part (Fig. 3.5, g) and rarely coarsely serrated at the apex (Fig. 13.1).

The leaflets are dense, and along the edges are filmy. The cells of the outer epidermis along the edge of the leaflets are elongated in the direction of the top or teeth (Fig. 13.2), straight-walled, with slightly thickened walls. The contents of the cells are colored light brown. In the upper part of the leaflets above the mesophyll the epidermal cells are narrow, strongly elongated, with sharp or flat tops and an even thick-walled edge with simple pores (Fig. 13.3), and less elongated at the base (Fig. 13.4). Stomata and pubescence are absent. The inner epidermis of the leaflets at the apex and base (Fig. 13.5, 7) also consists of prosenchymatous cells with straight-walled, poorly thickened walls, flat or slanting tops, and in the middle part of the epidermal cell are less elongated, there are stomata and rare filamentous trichomes (Fig. 13.6). Stomata are of anomocytic type.

The flowers of the *A. nivea* are tubular. The top of the petal is rounded (Fig. 14.1).

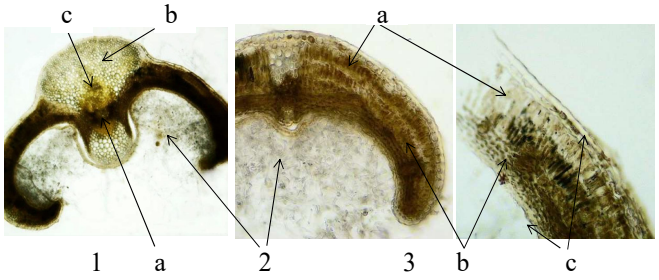

Fig. 11. Microscopic signs of the tip (apex) of the rosette leaf:
1 – transverse section: a – vascular bundle,
b – main parenchyma, c – sclerenchyma; 2 – hairs;
3 – mesophyll: a – palisade, b – spongy, c – epidermis

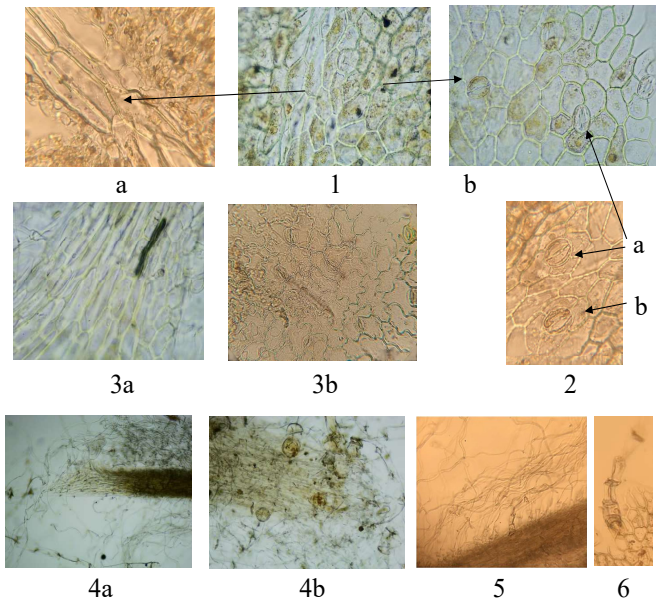

Fig. 12. Microscopic signs of leaf epidermis:
1 – upper epidermis along the vein (a), between the veins (b); 2 – stomata apparatus: a – anomocytic, b – anisocytic;
3 – lower epidermis along the vein (a), between the veins (b);
4 – pubescence along the vein (a, b);
5 – filamentous hairs with a 2-cell base; 6 – multicellular wide-lumen hair with collapsed cells

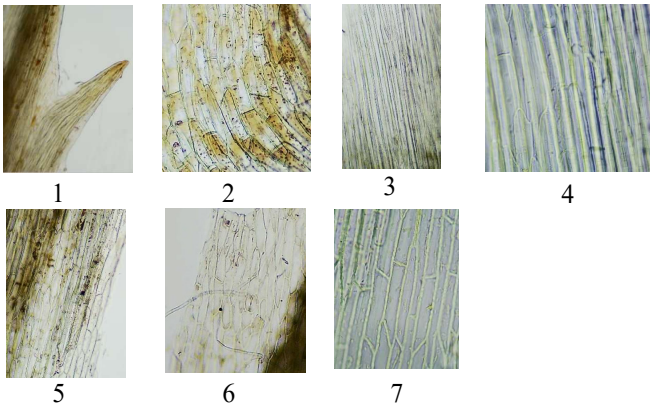

Fig. 13. Microscopic features of the involucral leaflet:
1 – tothed edge, *outer epidermis*; 2 – along the edge;
3 – at the top; 4 – at the base; *inner epidermis*; 5 – at the apex;
6 – in the middle part with stomata and filamentous hairs;
7 – at the base

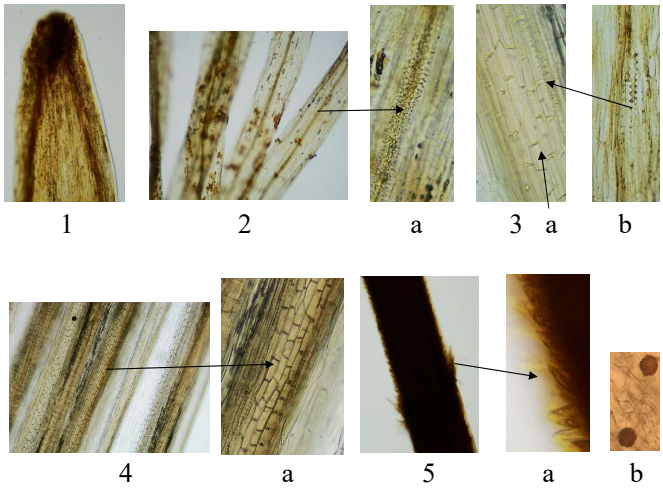

Fig. 14. Microscopic signs of a flower:1 – top of the petal; 2 – fragments of corolla petals (l/m), a – spiral vessels of the vein; 3 – epidermis of the corolla petal: a – thin-walled cells, b – thick-walled cells; 4 – epidermis of the corolla tube, a – on l/m; 5 – fragment of the pistil style, a – conical hairs; 6 – pollen

Occasionally there are cells of the epidermis with brown contents (Fig. 14.1, 2). The epidermal cells of the petal are prosenchymatous, thin-walled, straight-walled with a weakly pronounced folded cuticle (Fig. 14.3, *a*). Occasionally there are groups of prosenchymatous cells with heavily thickened membranes (Fig. 14.3, *b*). The vessels are thin and spiral (Fig. 14.2, *a*). The cells of the corolla tube are prosenchymatous, rarely parenchymal, straight-walled, with weakly thickened walls (Fig. 14.4, *a*). The surface of the style of the pistil is densely covered with short conical hairs (Fig. 14.5, *a*). Pollen grains are rounded, yellow-brown, the structure of ectine is slightly spiny(Fig. 14.6).

5. Discussion of the results

Thus, the studies of the morphological and anatomical structure of the *Alfredia nivea* grass allowed to determine the diagnostic signs of the structure of the stem, leaves, leaflets of the involucre and flowers of this species.

The diagnostic morphological signs of *Alfredia nivea* can be attributed:

– rough, unbranched, ribbed and tomentose stem;

– sessile and petiolar rosette leaves leathery, linear-lanceolate and lanceolate, pinnatipavtite, with lignified veins of sharp ones at the ends, non-tomentose on the upper side and tomentose from the lower;

– inflorescence-a spherical drooping head up to 6.0 cm in diameter with a 3–5-row, tiled involucre made of hard lanceolate, pointed leaflets with filmy fringrowed outgrowths on the sides;

– the flowers are tubular, 5-tooth, thin, up to 3.0 cm in length, the corolla is pink, numerous sepals are modified to thin long hairs; stamens and pistil protrude from the corolla's tube;

– cypsela up to 6.0 mm, pale yellowish with variegated brown spots and stripes on the surface.

The diagnostic anatomical signs of *Alfredia nivea* can be attributed:

– pubescence of stems and leaves is represented by covering and rarely glandular trichomes; covering trichomes of three types – long filamentous hairs with a 2-cell base, large multicellular wide lumen hairs with collapsed cells; and multicellular narrow lumen hairs with collapsed cells; glandular hairs of 2 types – 1-headed with a 1-celled stalk, and headed with a multicellular stalk and a multicellular head with collapsed cells;

– the stem is rounded with weakly protruding ribs, the transitional type, the epidermis is covered with a layer of a folded cuticle, unevenly pubescent, prosenchymatous cells are straight-walled with slightly thickened walls and simple straight pores, stomata rare, type of stomatal apparatus anomocytic; angular and lacunar collenchyma, cortical parenchyma cells with collenchymatous walls; expressed endoderm; open collateral bundles with well-developed sclerenchyma, xylem vessels porous and spiral;

– tissue and bundles topography in the basal, middle and upper parts of the petioles of the rosette leaves are the same, the petioles differ in the shape, thickness, shape of the "wings", the number and size of the bundles;

– the leaf blade has dorsiventral type; the cells of the upper and lower epiderma above the veins are prosenchymatous, straight-walled, with slightly thickened membranes and straight pores; between the veins of the the cells of the upper epidermis are 5–6-angle, isodiametric and on the lower – parenchymal, sinuous-walled, with slightly thickened membranes; stomata are frequent only on the lower epidermis, the type of stomatal apparatus is anomocytic;

– the upper epidermis of the leaves is rarely pubescent with thin long hairs, and the lower densely pubescent with filamentous hairs with a 2-cell base and less often multicellular hairs with collapsed cells;

– the cells of the outer and inner epidermis of the leaflets of the involucre of the inflorescence are elongated, straight-walled, with slightly thickened membranes, the contents of the cells are often colored light brown; stomata and single filamentous trichomes are rare on the inner epidermis; the type of stomatal apparatus is anomocytic;

– cells of the epidermis of the petals and corolla tube are prosenchymatous, thin-walled, straight-walled with colourless, rarely brown contents, less often there are groups of prosenchymatous cells with heavily thickened membranes; the epidermis is covered with a weakly expressed folding cuticle; vessels are thin spiral; style of pistil densely covered with short conical hairs; pollen grains are rounded, yellow-brown, the structure of ectine is slightly spiny [27].

The data obtained were used in the development of quality control methods and are included in the description of macroscopic and anatomical signs of raw materials in identification A and B.

Study limitations. The study used raw materials prepared in one region. The expansion of the geography of the harvesting of raw materials may allow determining new patterns of dependence of a change in morphological and anatomical signs on the conditions of growth.

The prospects for the further research. The results will be used in the development of instructions for the harvesting of grass *Alfredia nivea* and methods of quality control of this raw material. Further phytochemical and pharmacological studies of raw materials will show the prospect of creating new medicines with neurotropic activity.

6. Conclusions

The parameters of the macroscopic and anatomical identification of the *Alfredia nivea* are determined. Studies have been conducted morphological and anatomical signs of stems, leaves and flowers of *Alfredia nivea*, considering the requirements of European pharmacopeia. Diagnostic features of the structure of stems, leaves and flowers of the species were identified, which are used as key quality indicators in standardization and identification of raw materials.

Conflicts of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

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Data availability

The manuscript does not have related data.

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