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Research Article

Macroscopic and Microscopic Evaluation of *Galanthus woronowii* Losinsk. and *Galanthus nivalis* L. Homeopathic Crude Herbal Drugs

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ABSTRACT

Galanthus woronowii Losinsk. and *Galanthus nivalis* L. are two snowdrop species that are used in traditional medicine and homeopathy for nervous system disorders treatment. During pharmacognostic research linear dimensions of *G. woronowii* and *G. nivalis* homeopathic crude herbal drugs (HomCHD) were determined. The following microscopic anatomical and diagnostic features of snowdrop species were investigated: the structure of the adaxial and abaxial leaf epidermis, peduncle epidermis, the corolla epidermis, external and internal epidermis of outer scale, external and internal epidermis of storage scale, cell size and cellular inclusions (calcium oxalate raphides, starch grains). There are differences in the linear dimensions both in the macro and in the micro level. Overall dimensions of *G. woronowii* organs are significantly greater than *G. nivalis* organs, this fact is also reflected in the linear dimensions of the cell structures. The complex of diagnostic features allows the identification of the snowdrop species.

Keywords: *Galanthus woronowii*, *Galanthus nivalis*, macroscopy, microscopy, homeopathic crude herbal drugs.

INTRODUCTION

The *Amaryllidaceae* family consists of about 85 genera and 1100 species. These plants are distributed throughout the warm temperate and tropical regions of the world[1]. It's well aware that the genus *Galanthus* numbers 19 species, six varieties and two natural interspecies hybrids (World Cheklist of Selected Plant Families)[2]. *Galanthus woronowii* Losinsk. (Woronow's snowdrop), *Galanthus nivalis* L. (common snowdrop) are an early-spring flowering bulbous plant species cultivated for its ornamental qualities in gardens and found application in medicine. Homeopathic crude herbal drugs (HomCHD), prepared from plants of the genus *Galanthus* L., contain biologically active compounds: Amaryllidaceae alkaloids, flavonoids, organic and hydroxycinnamic acids[3]. Mother tinctures produced from both *Galanthus* species are used in preparation of homeopathic drugs[4]. Nevertheless, *planta tota* of *G. woronowii* and *G. nivalis* is not pharmacopoeial HomCHD and as a consequence there are no standardization approaches to its quality control[5]. Although several research papers for botanical evaluation of *Galanthus* species were published, there are no systematic data for standardization of HomCHD[6-10].

The aim of this research is pharmacognostic study of fresh snowdrop planta tota including macroscopic and microscopic (morpho-anatomical diagnostic features) evaluation for identification of HomCHD.

MATERIALS AND METHODS

The plant material was collected at blooming period from the Botanical Garden of I.M. Sechenov First Moscow State Medical University in April 2016. The whole plants (HomCHD) – aerial parts (flowers, leaves) and the bulbs with roots – of *G. woronowii* and *G. nivalis* were used for pharmacognostic analysis.

Macroscopic and microscopic evaluation was carried out according to general pharmacopoeial monograph of State Pharmacopoeia of Russian Federation XIII ed., Vol. 2, p. 304 "The roots, rhizomes, bulbs, tubers, corms" (GPM.1.5.1.0006.15); p. 272. "Grass" (GPM.1.5.1.0002.15); p. 379 "Method of microscopic and microchemical studies of medicinal plants and medicinal plant preparations" (GPM.1.5.3.0003.15). [11] Photographs were obtained by the microscope "Altami 139T" (10× eyepiece and lenses: 4×, 10×, 40×, 100×) with a digital camera eyepiece UCMOS05100KPA; images were processed using Altami Studio program.

RESULTS AND DISCUSSION

Macroscopic evaluation of Galanthus HomCHD.

Macroscopic evaluation of G. woronowii.

The *G. woronowii* HomCHD is presented at Fig. 1A. The bulb is pyriform, 3.0 cm long, 2.5 cm in diameter. The outer surface is slightly wrinkled, covered with yellow-brown leathery scales. The outer bulb scales are arranged in step order. Bulb's colour after covering scales removal

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Figure 1: Appearance of *G. woronowii* (A) and *G. nivalis* (B) HomCHD.

Table 1: Comparison of microscopic *G. woronowii* and *G. nivalis* features.

Feature	*G. woronowii*	*G. nivalis*
	Leaf	
Epidermis cells	rectangular, 200-480 µm long, 25-35 µm wide	rectangular, 185-395 µm long, 23-31 µm wide
Stomata	Tetracytic type (rarely pentacytic), rounded	
	45-55 µm in diameter	40-50 µm in diameter
Stomata density of adaxial surface	up to 10-15 per 1 mm^2	up to 15 per 1 mm^2
Stomata density of abaxial surface	20-25 per 1 mm^2	25-30 per 1 mm^2
Mesophyll cells	rounded, 35-60 µm	rounded, 30-50 µm
Calcium oxalate raphides	70-120 µm	45-50 µm
Density of cells containing raphides	1-2,5 per 1 mm^2	1,5-3 per 1 mm^2
	Flower	
Corolla epidermis cells	isodiametric, wing cells with papillate projections	
	60-80 µm in diameter	50-70 µm in diameter
Pollen grains	oval, monocolpate, heteropolar with a glabrous surface	
	21-26 µm long, 16-19 µm wide	18-20 µm long, 12-15 µm wide
	Peduncle	
Peduncle epidermis cells	rectangular, the walls are straight	
	190-470 µm long, 23-34 µm wide	180-390 µm long, 22-30 µm wide
Stomata type	tetracytic, rounded, 45-55 µm in diameter.	tetracytic, rounded, 40-50 µm in diameter.
Stomata density	19-22 per 1 mm^2	23-28 per 1 mm^2
Calcium oxalate raphides	75-135 µm	50-65 µm
Density of cells containing raphides	1-2,5 per 1 mm^2	1,5-3 per 1 mm^2
	Bulb	
External epidermis of outer scale	120-230 µm long, 30-40 µm wide	100-210 µm long, 20-30 µm wide
Internal epidermis of outer scale	110-225 µm long, 25-35 µm wide	90-200 µm long, 18-25 µm wide
External epidermis of storage scale	130-245 µm long, 55-70 µm wide	110-220 µm long, 50-65 µm wide
Internal epidermis of storage scale	120-240 µm long, 45-65 µm wide	100-215 µm long, 30-55 µm wide
Ground tissue cells	rounded, 80-105 µm in diameter	rounded, 70-95 µm in diameter
Calcium oxalate raphides	85-145 µm	60-75 µm
Density of cells containing raphides	1,5-3 per 1 mm^2	2-3,5 per 1 mm^2
Starch grains	Rounded-ovate, 5-45 µm in diameter	Rounded-ovate, 2,2-26 µm in diameter

is white. The roots are cylindrical, threadlike, 25 cm long, and 2 mm in diameter, white. Leaves are simple, broadly, pointed, bright green with a yellowish tinge, have glabrous shiny surface, characteristic lenticels and two undeveloped longitudinal folds. Leaves without stem have the keel on the lower surface, tapering at the base and gradually turning into a long sheath. The leaf sheath is 3.5-6.0 cm long, leaf is 1.5-2.5 cm wide, 15-23 cm long. In a bud one leaf covers another. Wax coating of the leaves is absent. The edge of leaf is entire, venation is parallel. Peduncle is cylindrical, ribbed, glabrous, 9-15 cm long, 2.5 mm thick, green. Flower is solitary, dialypetalous, hermaphrodite, has a white corolla perianth. Bract is linear, membranous, up to 4 cm long, pedicels are 4.5 cm long, 3 outer tepals are obovate, slightly curved 2.3 cm long, 1.3 cm wide; 3 inner tepals are 0.8 cm wide, 1.2 cm long, tapering

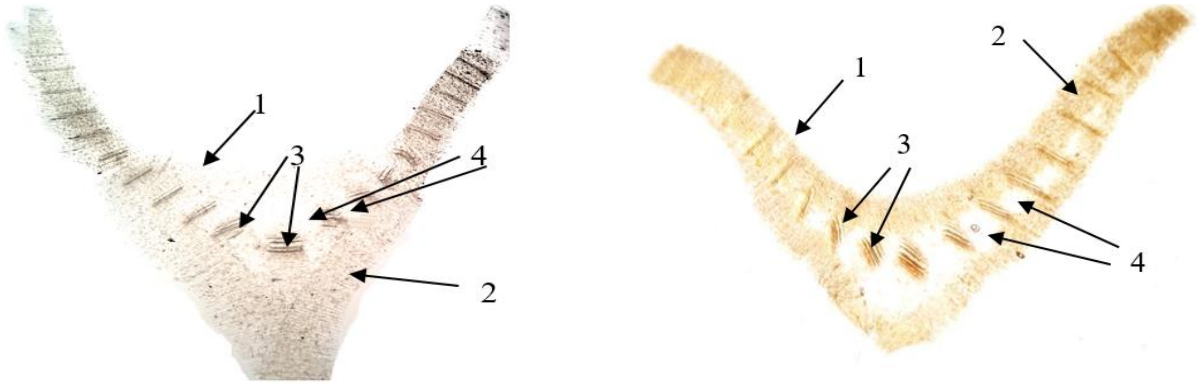

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Figure 2: Cross section of *G. woronowii* (A) and *G. nivalis* (B) leaf, 40×.1 – epidermis, 2 – mesophyll, 3 – conducting bundles, 4 – air cells.

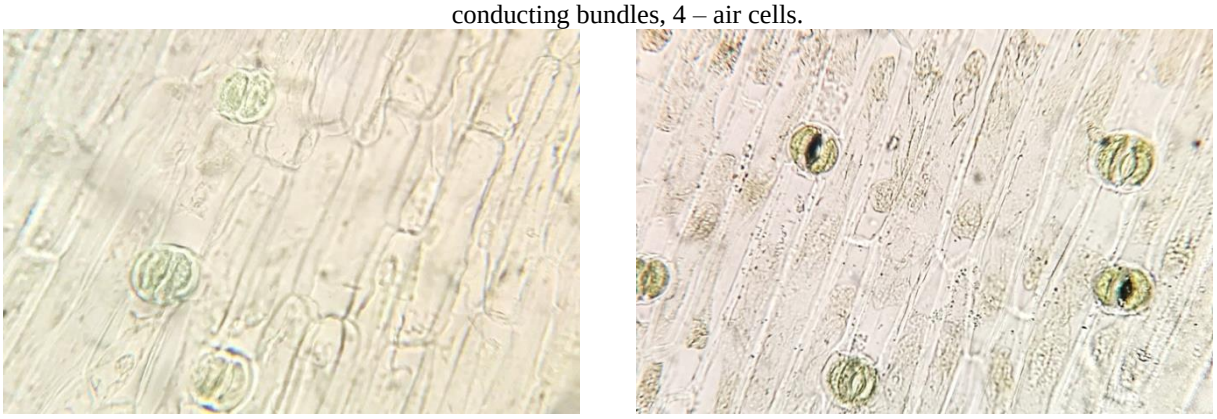

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Figure 3: Adaxial epidermis of *G. woronowii* (A) and *G. nivalis* (B) leaf, 400×.

A B

Figure 4: Abaxial epidermis of *G. woronowii* (A) and *G. nivalis* (b) leaf, 400×.

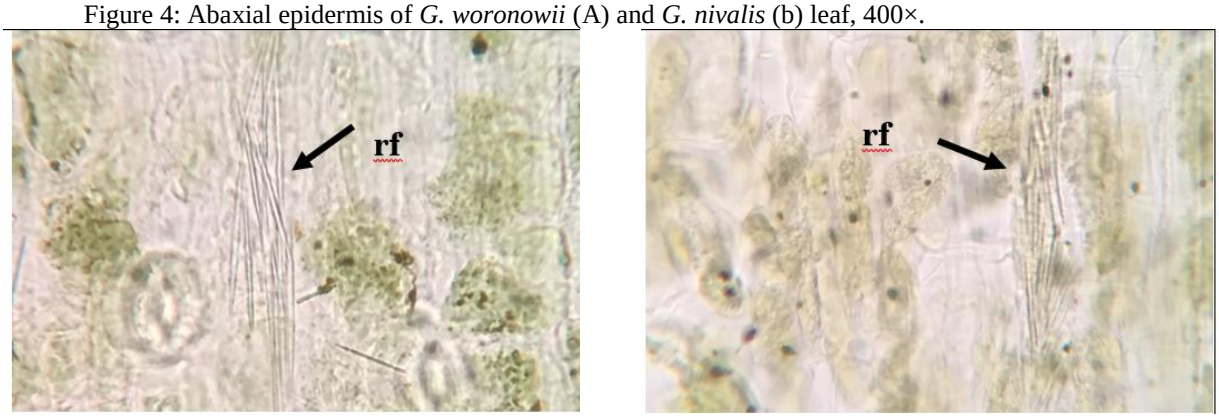

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Figure 5: Calcium oxalate raphides (rf) in mesophyll cells of *G. woronowii* (A) and *G. nivalis* (B) leaf, 400×.

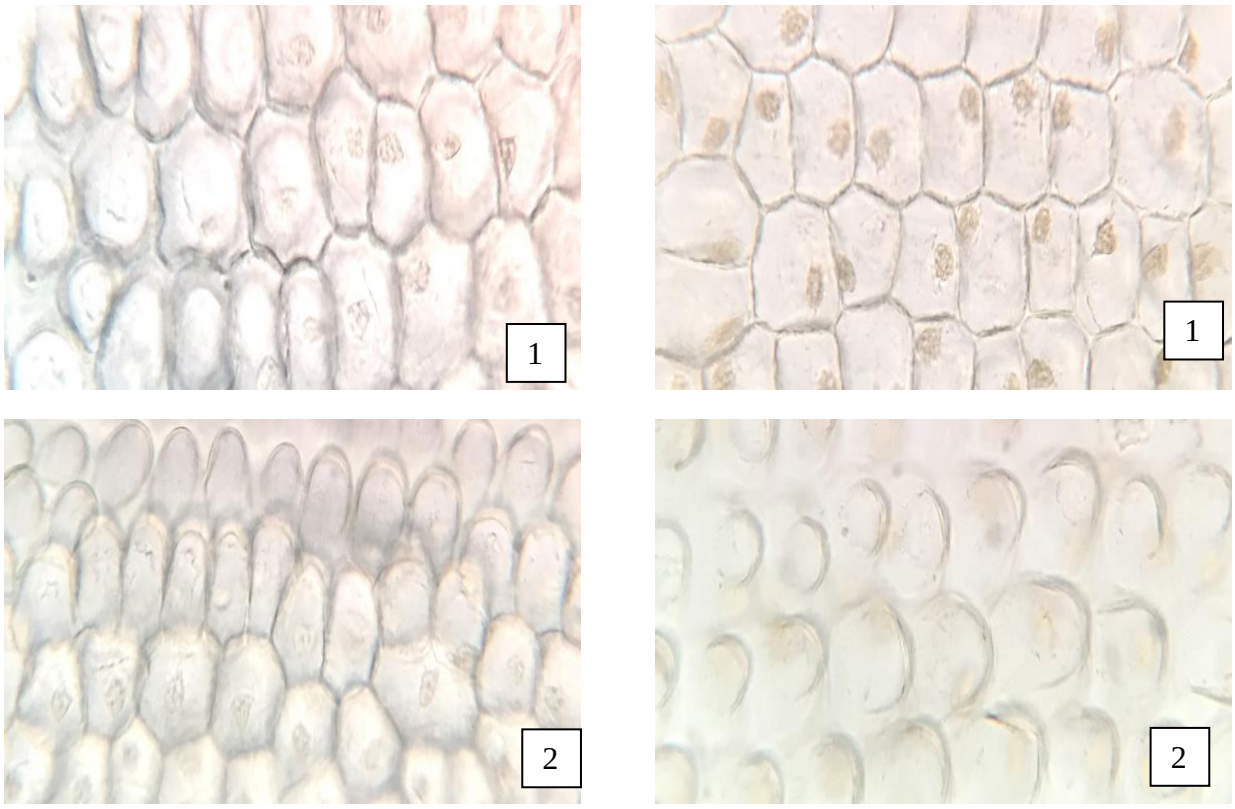

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Figure 6: Epidermis cells of *G. woronowii* (A) and *G. nivalis* (B) corolla. 1 – isodiametric, wing cells; 2 – papillate projections, 400×.

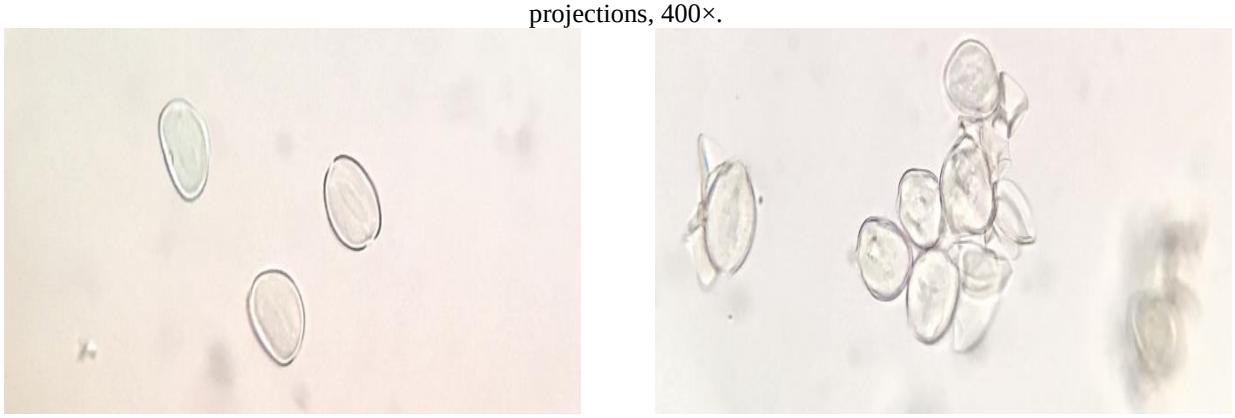

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Figure 7: Pollen grains of *G. woronowii* (A) and *G. nivalis* (B), 400×.

downward wedge, flat, erect, with a notch and large 1.6 mm horseshoe-shaped green spot at the top. 6 stamens are 0.6 cm long, have short filaments that are attached to the base of the perianth; anthers have a cusps; ovary is lower, oblong, 0.3-0.4 cm long, has three locules. Stile is thread-like, has acute stigma. The HomCHD smell is specific, weak, the taste is not determined (toxic HomCHD).

Macroscopic evaluation of G. nivalis

The *G. nivalis* HomCHD is presented at Fig. 1B. The bulb is pyriform or conical, 2.0 cm long, 1.5 cm in diameter. The outer surface is slightly wrinkled, covered with a light-brown leathery scales. The outer bulb scales are arranged at the same level. Bulb's colour after the covering scales removal is white. The roots are cylindrical, thin, filamentous 15 cm long, 1 mm in diameter, white. Leaves are simple, linear, dark green or gray, glabrous, with a wax coating; on the tip are obtuse, at the base are slightly tapered and gradually turning into a long sheath, in the bud are flatly adjacent to each other. The edge of leaf is entire, venation is parallel. The leaf sheath is 2.0-4.0 cm long, leaf is 8-12 cm long, 0.4-0.5 cm wide. Peduncle is slightly ribbed, cylindrical, glabrous, 7-10 cm long, 1.5 mm thick, green. Flower is solitary, dialypetalous, hermaphrodite, has a white corolla perianth. Bract is linear, membranous, up to 2 cm long, pedicels are 2.5 cm long, 3 outer tepals are oblong-obovate, 1.8 cm long, 0.6 cm wide; 3 internal tepals are 1.0 cm long, 0.4 cm wide, wedge-shaped, flat, with notch and large 1 mm horseshoe-shaped green spot at the top. 6 stamens are 0.5 cm long, have short filaments that are attached to the base of the perianth; anthers have a

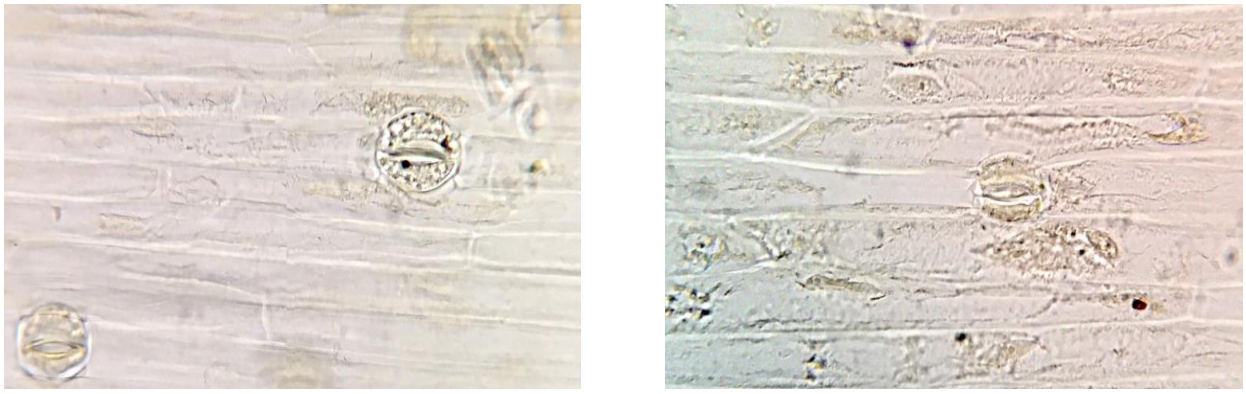

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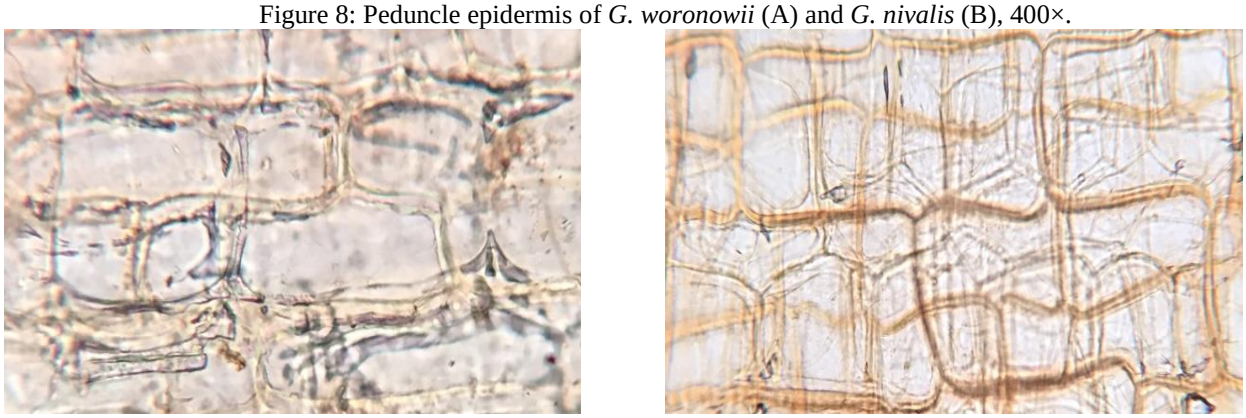
Figure 8: Peduncle epidermis of *G. woronowii* (A) and *G. nivalis* (B), 400×.



A B

Figure 9: External epidermis of *G. woronowii* (A) and *G. nivalis* (B) outer scale, 400×.

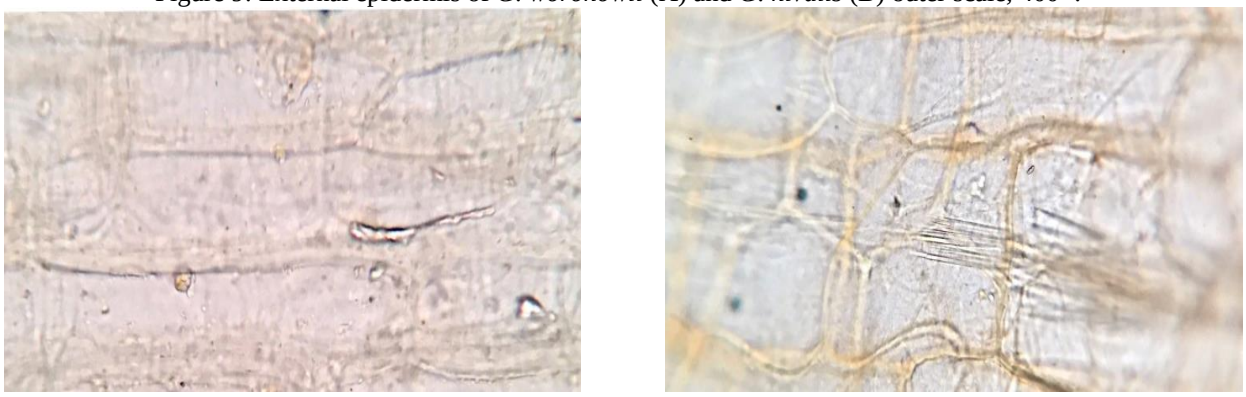


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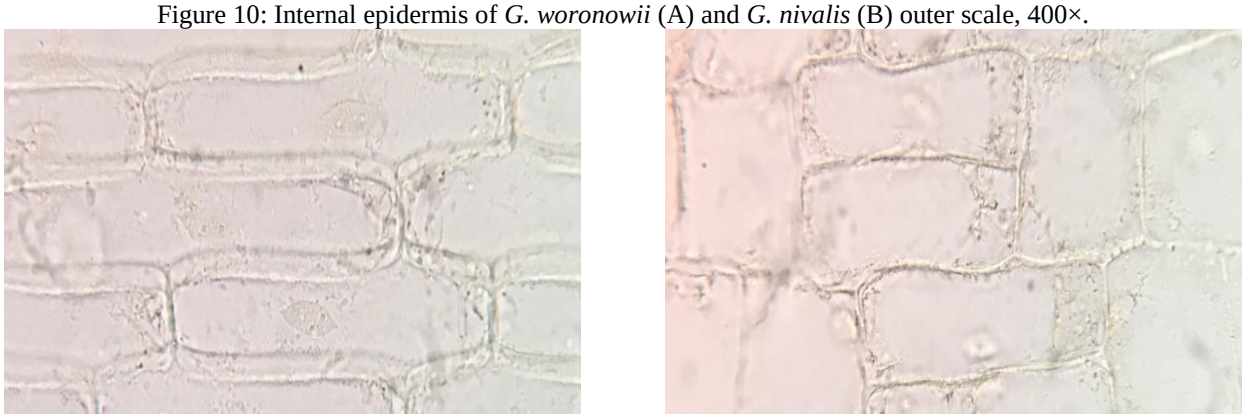
Figure 10: Internal epidermis of *G. woronowii* (A) and *G. nivalis* (B) outer scale, 400×.



A B

Figure 11: External epidermis of *G. woronowii* (A) and *G. nivalis* (B) storage scale, 400×.

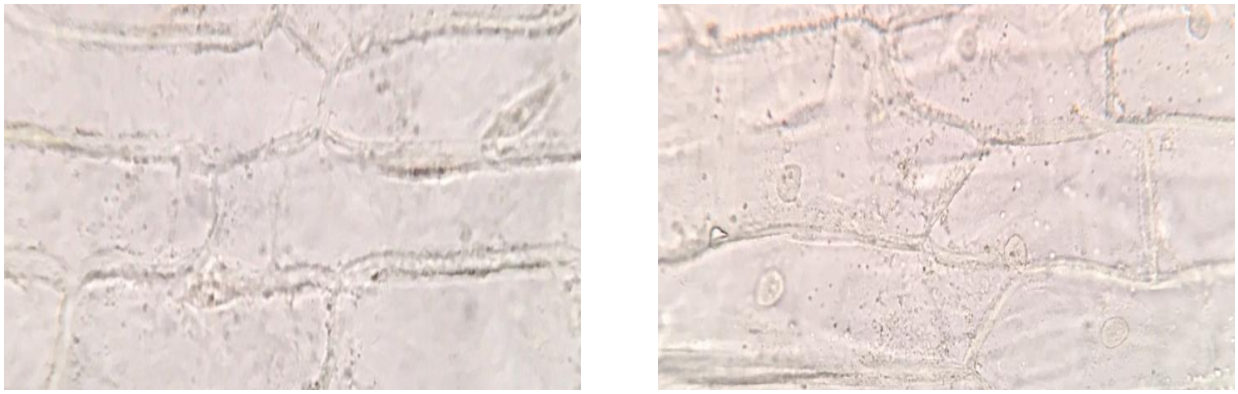
cusps; ovary is lower, oblong, 0.1-0.2 cm long, has three locules. Stile is thread-like, has acute stigma. The HomCHD smell is specific, weak, the taste is not determined (toxic HomCHD).

Microscopic evaluation of Galanthus HomCHD.

Microscopy of Galanthus leaf.

G. woronowii lamina is dorsoventral, triangular at the base, has long edges, which are bent inward, leaf width is 3.6-4.2 mm, the number of conducting bundles is 28-31 (Fig. 2A). *G. nivalis* lamina is dorsoventral, has the shape of a concave triangle with short edges at the base. Leaf width is 2,4-3,1 mm, the number of conducting bundles is 12-20 (Fig. 2B).

At both sides of *Galanthus* leaf (Fig. 3, 4) the epidermis cells are elongated, rectangular with straight walls. The epidermis cell walls have beaded thickening. The stomata are round, surrounded by 4 (rarely 5) epidermal cells (tetra- and penta- cytic types). Cuticle is smooth, sometimes wrinkled longitudinally. Leaf mesophyll is not clearly differentiated into palisade and spongy tissue, consisting of round cells. Several mesophyll cells rows, adjacent to the upper epidermis, consist of slightly radially elongated cells. In the central part mesophyll has loose structure, thereby forming a large air cells which are disposed between the conducting bundles. In the peripheral part of mesophyll there are cells containing calcium oxalate raphides bundles (Fig. 5). Conducting bundles type of side and central ribs is collateral. Fiber vascular bundles includes netted and ladder-shaped vessels and spiral tracheids.



A B

Figure 12: Internal epidermis of *G. woronowii* (A) and *G. nivalis* (B) storage scale, 400×.

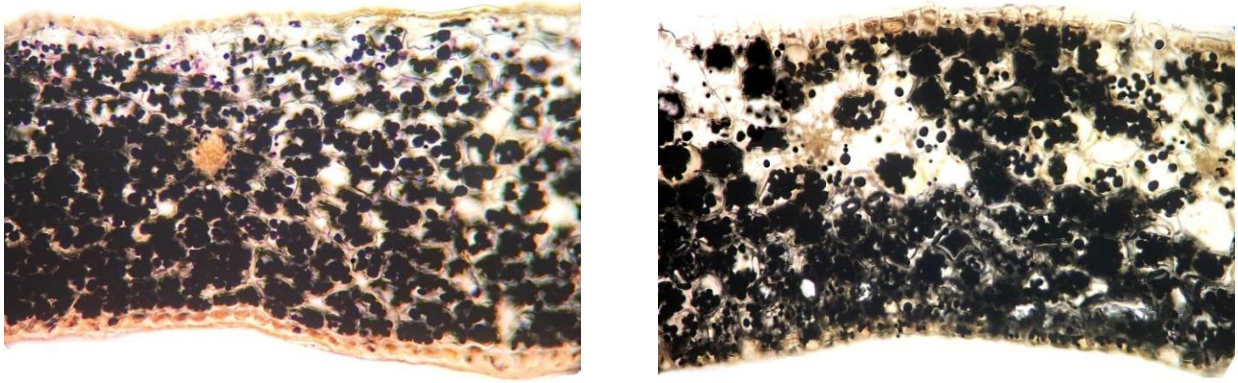


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Figure 13: Cross section of *G. woronowii* (A) and *G. nivalis* (B) storage scale, Lugol solution staining, 100×.

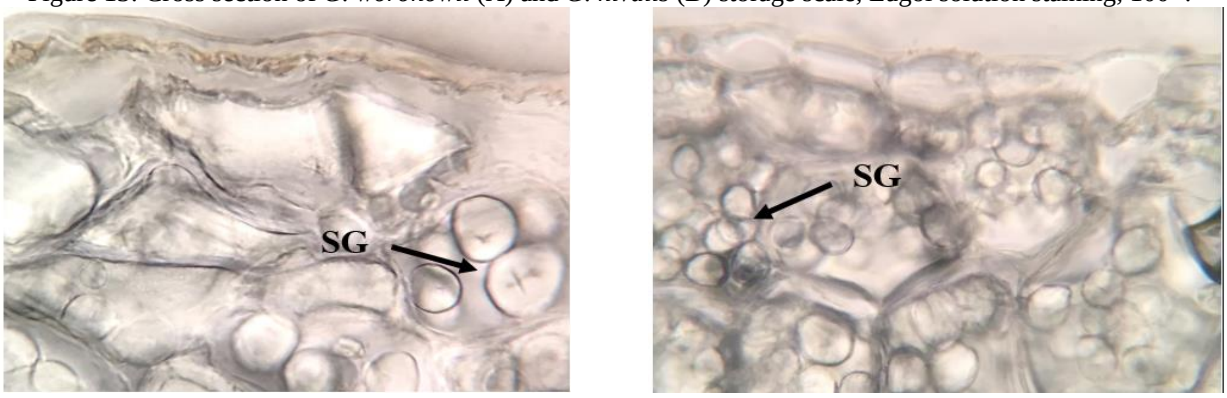

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Figure 14: Ground tissue cells of *G. woronowii* (A) and *G. nivalis* (B) storage scale with starch grains (SG), 400×.

Microscopy of Galanthus flower.

At both sides the corolla epidermis consists of isodiametric wing cells with papillate projections (Fig. 6). Papillate projections of abaxial epidermis are developed better. Cuticle is longitudinally wrinkled. Stomata are absent. Pollen grains are oval, monocolpate, heteropolar with a glabrous surface (Fig. 7).

The peduncle epidermis cells are rectangular, the walls are straight, the cuticle is smooth (Fig. 8). Stomata type is tetracytic, size is similar to leaf stomata. In the peripheral part of the peduncle mesophyll there are cells containing calcium oxalate raphides.

Microscopy of Galanthus bulb.

The outer scales are dead shell with completely deformed parenchyma cells. External and internal outer scale epidermis is composed of prosenchymatous cells with rounded corners and beaded thickening (Fig. 9, 10).

Storage scales structure is similar to the leaf. External and internal epidermis of these scales consists of oval cells that are slightly elongated in the tangential direction or isodiametric (Fig. 11, 12).

Ground tissue consists of a thin-walled round-shaped cells filled with rounded-ovate starch grains (Fig. 13). Large starch grains have 2-3, rarely 4 radial cracks (Fig. 14). Raphides are contained in the outer part of scales ground tissue, and are arranged in bundles, parallel to the longitudinal axis of the bulb. Conducting bundles type is closed collateral, they are located closer to the inner side of the scales, and they have parenchymal lining.

Comparison of microscopic *G. woronowii* and *G. nivalis* features is presented in table 1.

During pharmacognostic research macroscopic and microscopic (morpho-anatomical diagnostic features) evaluation for identification of *G. woronowii* and *G. nivalis* HomCHD were carried out, linear dimensions of plant organs were determined.

As the result of this study microscopic anatomical and diagnostic features of HomCHD Voronov's and common snowdrop were investigated: the structure of the adaxial and abaxial leaf epidermis, peduncle epidermis, the corolla epidermis, external and internal epidermis of outer scale, external and internal epidermis of storage scale, cell size and cellular inclusions (calcium oxalate raphides, starch grains).

It has been established that there are differences in the linear dimensions of both of the macro and micro levels. Overall dimensions of *G. woronowii* organs are significantly greater than *G. nivalis* organs, this fact is also reflected in the linear dimensions of the cell structures. Thus, the complex of diagnostic features allows the identification of the snowdrop species.

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