PHARMACOGNOSTIC INVESTIGATION OF GALANTHUS WORONOWII LOSINSK. AND GALANTHUS NIVALIS L. HERBAL PHARMACEUTICAL SUBSTANCES (MICROSCOPIC AND MACROSCOPIC ANALYSIS)

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ABSTRACT

Objective: Today drug produced from snowdrop species (Galanthus woronowii Losinsk. and Galanthus nivalis L.) used in Russian traditional medicine for nervous and cardiovascular systems disorders treatment. Pharmacognostic study of fresh snowdrop plants including macroscopic and microscopic (morpho-anatomical diagnostic features) evaluation for identification of herbal pharmaceutical substances (HPS).

Methods: Macro- and microscopic evaluation was carried out according to general pharmacopeial monographs of State Pharmacopoeia of Russian Federation XIII ed.; Photographs were obtained by the microscope "Altami 139T" (10× eyepiece and lenses: 4×, 10×, 40×, 100×) with a digital camera eyepiece UCMS05100KPA; images were processed using Altami Studio program.

Results: In a pharmacognostic study of G. nivalis and G. woronowii HPS linear dimensions were determined. Several microscopic diagnostics and anatomical signs of snowdrops were investigated: Adaxial and abaxial leaf epidermis; epidermis of corolla, peduncle; internal and external outer scale epidermis, internal and external storage scale epidermis, and sizes of cells and cellular inclusions (starch grains and calcium oxalate raphides). G. woronowii and G. nivalis HPS possess differences both in the micro and macro levels in the linear dimensions. In general, dimensions of G. nivalis organs are much smaller than G. woronowii ones, this aspect is also expressed in the cell structures linear dimensions. The complex of macro- and micro-diagnostic signs allow to identify the snowdrop species.

Conclusion: The results of the investigation can be used in routine quality control and for inclusion of pharmacopeial monographs for snowdrop HPSs.

Keywords: Galanthus woronowii, Galanthus nivalis, Herbal pharmaceutical substances.

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 Checklist of Selected Plant Families) [2]. Galanthus woronowii Losinsk. (Woronowii snowdrop) and 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. Herbal pharmaceutical substances (HPS), prepared from plants of the genus Galanthus L., contain several biologically active compounds: Amaryllidaceae alkaloids [3-5], flavonoids, organic, and hydroxyacetic acids [6]. Alkaloids are biologically active substances that pose strong pharmacological activities of medicinal plants [7,8]. Mother tinctures produced from both Galanthus species are used in the preparation of homeopathic drugs [9]. Macro- and micro-scopical determinations of diagnostic features in HPS are very important stage of pharmacognostic analysis (herbs, fruits, leaves, etc.) [10-15].

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

METHODS

The plants were collected at blooming period at the Botanical Garden in Sechenov University in April 2017. The whole plants (HPS) - aerial parts (flowers and leaves) and the bulbs with roots - of G. woronowii and G. nivalis were used for pharmacognostic analysis.

Macro- and micro-scopical evaluations were carried out according to general pharmacopeial monograph of State Pharmacopoeia of Russian Federation XIII ed., Vol. 2 “The root, rhizome, bulb, tuber, corn;” “Herbs;” “Method of microscopic and microchemical studies of medicinal plants and medicinal plant preparations” [16]. Photographs were obtained by the microscope “Altami 139T” (10× eyepiece and lenses: 4×, 10×, 40×, 100×) with a digital camera eyepiece UCMS05100KPA; images were processed using Altami Studio program.

RESULTS AND DISCUSSION

Macroscopic evaluation of Galanthus HPS

Macroscopic evaluation of G. woronowii

The G. woronowii HPS is presented at Fig. 1a. The bulb is pyriform, 3.0 cm in length, 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 color after covering scales removal is white. The roots are cylindrical, threadlike, 25 cm in length, and 2 mm in diameter, white. Leaves are simple, broadly, pointed, bright green with a yellowish tinge and 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 in length, leaf is 1.5–2.5 cm in wide, and 15–23 cm in length. In a bud, one leaf covers another. Wax coating of the leaves is absent. The edge of leaf is entire; venation is...
Macrosopic evaluation of Galanthus nivalis

The G. nivalis HPS is presented at Fig. 1b. The bulb is pyriform or conical, 2.0 cm in length, and 1.5 cm in diameter. The outer surface is slightly wrinkled, covered with light-brown leathery scales. The outer bulb scales are arranged at the same level. Bulb's color after the covering scales removal is white. The roots are cylindrical, thin, filamentous 15 cm in length, 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, and in the bud are flatly adjacent to each other. The edge of the leaf is entire; venation is parallel. The leaf sheath is 2.0–4.0 cm in length, leaf is 6–12 cm in length, 0.4–0.5 cm in wide. Peduncle is slightly ribbed, cylindrical, glabrous; 7–10 cm in length, 1.5 mm thick. Green. Flower is solitary, dialypetalous, hermaphrodite, has a white corolla perianth. Pedicel is 1.0 mm in length, has three locules. Stile is thread-like, has acute stigma. The HPS smell is specific, weak; the taste is not determined (toxic HPS).

Microscopic evaluation of Galanthus HPS

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 and 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 (Figs. 3 and 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 also 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 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 include netted and ladder-shaped vessels and spiral tracheids.

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, monocotilate, and heteropolar with a glabrous surface (Fig. 7).

The peduncle epidermis cells are rectangular; the walls are straight, the cuticle is smooth (Fig. 8). Stoma type is tetracytic, size is similar to leaf stoma. A. 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 (Figs. 9 and 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 (Figs. 11 and 12).

Ground tissue consists of 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.

Nevertheless, plants of *G. woronowii* and *G. nivalis* are not pharmacopeial HPS, and as a consequence, there are no standardization approaches to its quality control. Although several research papers for botanical evaluation of *Galanthus* species were published, there are no systematic data for standardization of HPS [17-21]. We have conducted the first study in pharmacognostic aspect. Obtained data will be used in creating documentation regulating the quality of *Galanthus* HPS.

**CONCLUSION**

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

As the result of this study *Galanthus* herbal pharmaceutical substances (HPS), linear dimensions were determined. Several microscopic diagnostics and anatomical signs of snowdrops were investigated: adaxial and abaxial leaf epidermis; epidermis of corolla, peduncle; internal and external outer scale epidermis, internal and external storage scale epidermis, size of cells and cellular inclusions (starch grains and calcium oxalate raphides). It has been established that *G. woronowii*, *G. nivalis* HPS possess differences both in the micro and in the macro levels in the linear dimensions. In general, dimensions of *G. nivalis* organs are much smaller than *G. woronowii* ones, this aspect is also expressed in the cell structures linear dimensions. Thus, complex of macro- and micro-diagnostic signs allows to identify the snowdrop species.

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**AUTHOR’S CONTRIBUTIONS**

I declare that this work was done by the author named in this article.
Table 1: Comparison of microscopic Galanthus woronowii, Galanthus nivalis features

| Feature                          | Galanthus woronowii          | Galanthus nivalis          |
|---------------------------------|------------------------------|----------------------------|
| Leaf                            | Rectangular, 200–480 µm long, 25–35 µm wide | Rectangular, 185–395 µm long, 23–31 µm wide |
| Epidermis cells                 |                              |                            |
| Stomata                         | Tetracytic (rarely pentaacytic), rounded 45–55 µm in diameter | 40–50 µm in diameter |
| Stomata density of adaxial surface | Up to 10–15 per 1 mm²         | Up to 15 per 1 mm²         |
| Stomata density of abaxial surface | 20–25 per 1 mm²              | 25–30 per 1 mm²            |
| 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 mm²              | 1.5–3 per 1 mm2            |
| Flower                          |                              |                            |
| Corolla epidermis cells         | Iso-diametric, wing cells with papillate projections | 50–70 µm in diameter |
| 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                         | Tetracytic, rounded, 45–55 µm in diameter | Tetracytic, rounded, 40–50 µm in diameter |
| Stomata density                 | 19–22 per 1 mm²              | 23–28 per 1 mm²            |
| Calcium oxalate raphides        | 75–135 µm                    | 50–65 µm                   |
| Density of cells containing raphides | 1–2.5 per 1 mm²              | 1.5–3 per 1 mm²            |
| Bulb                            |                              |                            |
| Outer scale external epidermis of outer scale | 120–230 µm long, 30–40 µm wide | 100–210 µm long, 20–30 µm wide |
| Outer scale internal epidermis of outer scale | 110–225 µm long, 25–35 µm wide | 90–200 µm long, 18–25 µm wide |
| Storage scale external epidermis | 130–245 µm long, 55–70 µm wide | 110–220 µm long, 50–65 µm wide |
| Storage scale internal epidermis | 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 mm²              | 2–3.5 per 1 mm²            |
| Storage granules                | Rounded-ovate, 5–45 µm in diameter | Rounded-ovate, 2.2–26 µm in diameter |
| Roots                           |                              |                            |
| Structure                       | Primary                      | Primary                    |
| The structure of the central axial cylinder, its diameter | Tetrarch, 55–60 µm | Triarchic, 45–50 µm |
| Ring of deformed parenchyma     | Absent                       |                            |

Fig. 14: Ground tissue cells of Galanthus woronowii storage scale (a): Galanthus nivalis (b) storage scale with starch grains (SG) (>400)

CONFLICTS OF INTEREST

The author had no conflicts of interest.

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