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Research Article

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 micro-scopic 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 UCMOS05100KPA; 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 allows 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.

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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. (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 hydroxycinnamic acids [6]. Alkaloids are biologically active substances that posse 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-scopic determinations of diagnostic features in HPS are very important stage of pharmacognostic analysis (herbs, fruits, leafs, 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-scopic evaluations were carried out according to general pharmacopeial monograph of State Pharmacopoeia of Russian Federation XIII ed., Vol. 2 "The root, rhizome, bulb, tuber, corm;" "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 UCMOS05100KPA; 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

parallel. Peduncle is cylindrical, ribbed, glabrous, 9–15 cm in length, 2.5 mm thick, and green. Flower is solitary, dialypetalous, hermaphrodite, has a white corolla perianth. Bract is linear, membranous, up to 4 cm in length, pedicels are 4.5 cm in length, 3 outer tepals are obovate, slightly curved 2.3 cm in length, 1.3 cm in wide; 3 inner tepals are 0.8 cm in wide, 1.2 cm in length, tapering 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 in length, 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 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).

Macroscopic 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 8–12 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. Bract is linear, membranous, up to 2 cm in length, pedicels are 2.5 cm in length, 3 outer tepals are oblong-obovate, 1.8 cm in length, 0.6 cm in wide; 3 internal tepals are 1.0 cm in length, 0.4 cm in wide, wedge-shaped, flat, with notch and large 1 mm horseshoe-shaped green spot at the top. 6 stamens are 0.5 cm in length, have short filaments that are attached to the base of the perianth; anthers have cusps; ovary is lower, oblong, 0.1–0.2 cm 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, monocolpate, and 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.

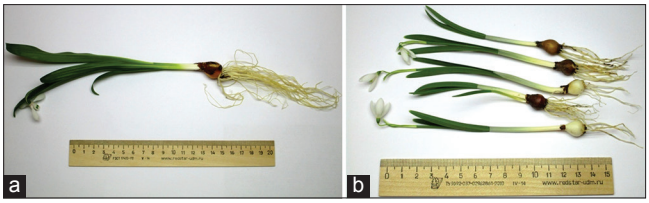

Fig. 1: Appearance of *Galanthus woronowii* herbal pharmaceutical substances (a); *Galanthus nivalis* herbal pharmaceutical substances (b)

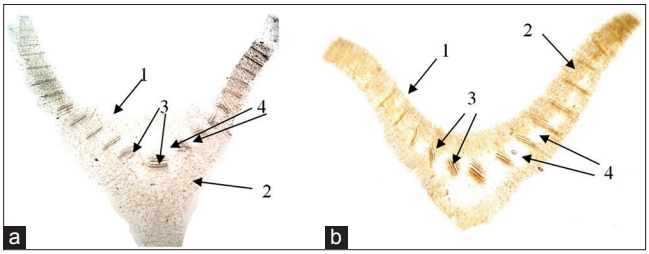

Fig. 2: Cross section of *Galanthus woronowii* leaf (a); *Galanthus nivalis* leaf (b) (×40). (1) epidermis, (2) mesophyll, (3) conducting bundles, (4) air cells

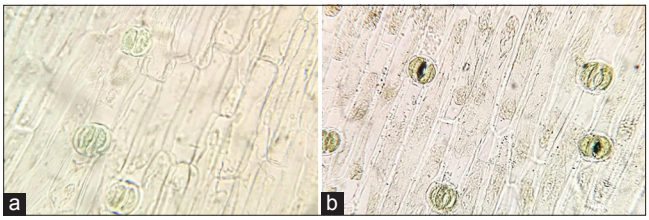

Fig. 3: Adaxial epidermis of *Galanthus woronowii* leaf (a); *Galanthus nivalis* leaf (b) (×400)

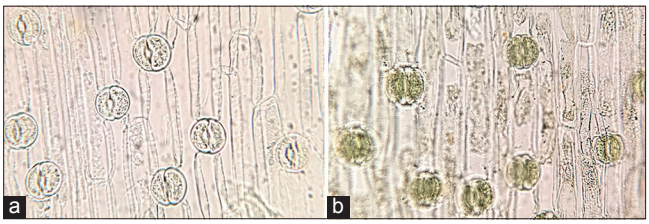

Fig. 4: Abaxial epidermis of *Galanthus woronowii* leaf (a); *Galanthus nivalis* leaf (b) (×400)

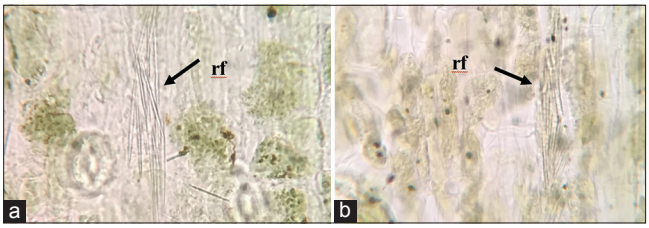

Fig. 5: Calcium oxalate raphides (rf) in mesophyll cells of *Galanthus woronowii* leaf (a); *Galanthus nivalis* leaf (b) (×400)

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).

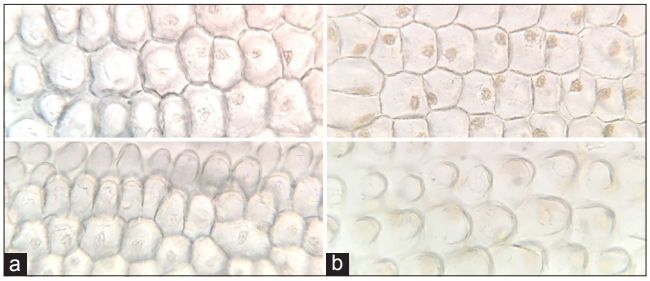

Fig. 6: Epidermis cells of *Galanthus woronowii* corolla (a); *Galanthus nivalis* (b) corolla. (1) Isodiametric, wing cells; (2) papillate projections (×400)

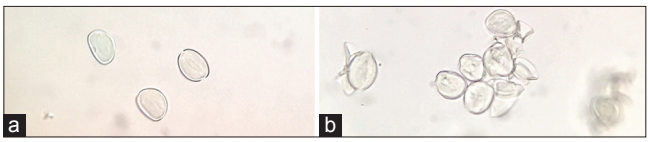

Fig. 7: Pollen grains of *Galanthus woronowii* (a); *Galanthus nivalis* (b) (×400)

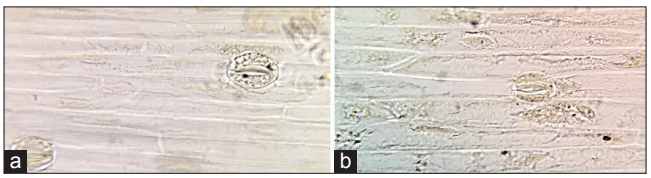

Fig. 8: Peduncle epidermis of *Galanthus woronowii* (a); *Galanthus nivalis* (b) (×400)

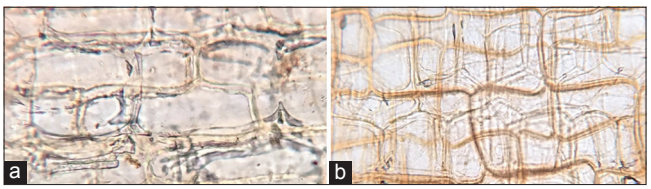

Fig. 9: External epidermis of *Galanthus woronowii* (a) and *Galanthus nivalis* (b) outer scale (×400)

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.

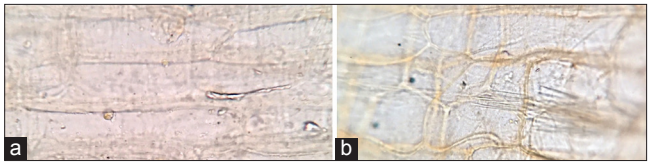

Fig. 10: Internal epidermis of *Galanthus woronowii* (a) and *Galanthus nivalis* (b) outer scale (×400)

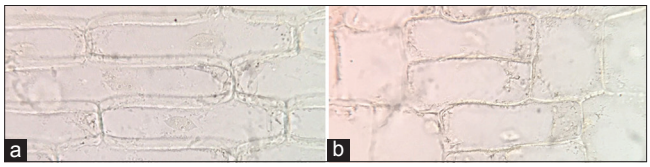

Fig. 11: External epidermis of *Galanthus woronowii* storage scale (a); *Galanthus nivalis* (b) storage scale (×400)

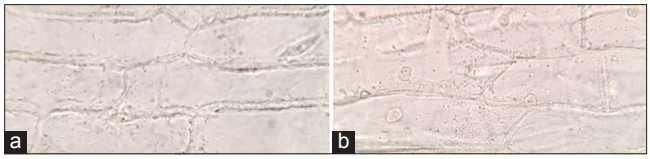

Fig. 12: Internal epidermis of *Galanthus woronowii* storage scale (a); *Galanthus nivalis* (b) storage scale (×400)

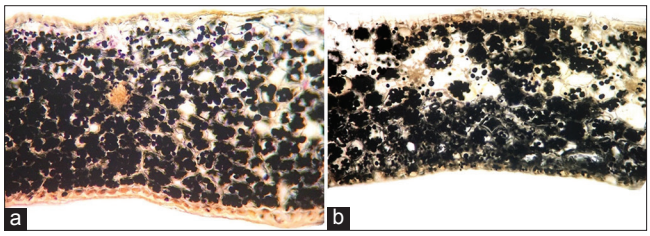

Fig. 13: Cross-section of *Galanthus woronowii* storage scale (a); *Galanthus nivalis* (b) storage scale, Lugol solution staining (×400)

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.

ACKNOWLEDGMENT

I would like to thank professor, corresponding member of Russian Academy of Sciences Irina Aleksandrovna Samylina, for her useful communications and constant help.

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		
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 mm2
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		
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 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
Roots		
Structure	Primary	Primary
The structure of the central axial cylinder, its diameter	Tetrarch, 55–60 µm	Triarchic, 45–50 µm
		Occurred in the outer part of the crust
Ring of deformed parenchyma	Absent	

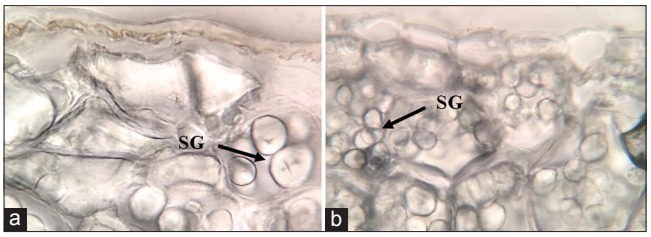

Fig. 14: Ground tissue cells of *Galanthus woronowii* storage scale (a); *Galanthus nivalis* (b) storage scale with starch grains (SG) (×400)

CONFLICTS OF INTEREST

The author had no conflicts of interest.

REFERENCES

1. Willis JC. *Amaryllidaceae*. A Dictionary of the Flowering Plants and Ferns. 8th ed. Cambridge: Cambridge University Press; 1988.
2. World Checklist of Selected Plant Families (WCSP). Royal Botanic Gardens, Kew. Available from: http://apps.kew.org/wcsp/home.do. [Last cited on 2018 April 25].
3. Bokov DO, Samylina IA, Malinkin AD, Nikolov S. Application of HILIC-UV method in analysis of medicines containing *Amaryllidaceae* alkaloids. Russ J Biopharm 2017;9:52-8.
4. Sarikaya BB, Kaya GI, Onur MA, Bastida J, Somer NU. Phytochemical investigation of *Galanthus woronowii*. Biochem Syst Ecol 2013;51:276-9.
5. Berkov S, Codina C, Viladomat F, Bastida J. Alkaloids from *Galanthus nivalis*. Phytochemistry 2007;68:1791-8.
6. Bokov DO, Malinkin AD, Samylina IA, Bessonov VV. Hydroxycinnamic and organic acids of snowdrops (*Galanthus* L.). J Appl Pharm Sci 2017;7:36-40.
7. Prakasia PP, Nair AS. Evaluation of *in vitro* antioxidant potential of the total crude alkaloid extract of *Glycosmis pentaphylla* (Retz.) Correa leaves. Int J Pharm Pharm Sci 2016;8:85-91.
8. Debnath B., Uddin MD J, Patari P, Das M, Maiti D, Manna K. Estimation of alkaloids and phenolics of five edible cucurbitaceous plants and their antibacterial activity. Int J Pharm Pharm Sci 2015;7:223-7.
9. Boericke W. Homeophatic Materia Medica. New York: Kessinger Publishing; 2004.
10. Yanala SR, Sathyanarayana D. Powder microscopic studies of the fruits of tribulus terrestris linn collected from different geographical locations of South India–a comparative stdy. Int J Pharm Pharm Sci 2017;9:158-64.
11. Saini S, Dhiman A, Nanda S. Pharmacognostical and phytochemical studies of *Piper betle* Linn. Leaf. Int J Pharm Pharm Sci 2016;8:222-6.
12. Sharmila S, Kalaichelvi K, Dhivya SM. Pharmacognostic standardisation of *Cayratia pedata* (Lam.) *Gagnep var glabra* gamble–an endemic and endangered medicinal climber in Thiashola, Nilgiris. Int J Pharm Pharm Sci 2017;9:57-63.
13. Ali I, Rizwani GH, Shareef H, Khan S. Pharmacognostic studies of *Dalbergia sisso* Roxb. Int J Pharm Pharm Sci 2016;8:48-53.
14. Ramamoorthy SK, Manickam D, Subramaniam S, Subramaniam S. Standardisation and phytochemical screening of traditional formulation. Int J Curr Pharm Res 2017;9:70-4.
15. Kaur M, Yadav R. Pharmacognostic, ethnopharmacological,

phytochemical and pharmacological profile of wild guava I.E. *Careya arborearoxb*. Int J Curr Pharm Res 2017;9:1-7.

16. State Pharmacopoeia of the Russian Federation. 12th ed, Vol. 2. Available from: http://www.193.232.7.107/feml. [Last cited on 2018 April 25].
17. Davis AP, Barnett JR. The leaf anatomy of the genus *Galanthus* L. (*Amaryllidaceae* J. St.-Hil.). Bot J Linn Soc 1997;123:333-52.
18. Sahin NF. Morphological anatomical and physiological studies on *Galanthus ikariae* Baker and *G. rizehensis* Stern (*Amaryllidaceae*) grown around NE Turkey. Pak J Bot 1998;30:117-31.
19. Chudzik B, Snieżko R, Szaub J. Biology of flowering of *Galanthus nivalis* L. (*Amaryllidaceae*). Ann Univ Mariae Curie-Skłodowska EEE Hortic 2002;10:1-10.
20. Budnikov G. Morphological variation of specimens and populations of *Galanthus nivalis* L. in Western regions of Ukraine. Thaiszia J Bot Košice 2011;21:95-109.
21. Dönmez EO, IşIK S. Pollen morphology of Turkish *Amaryllidaceae*, *Ixioliriaceae* and *Iridaceae*. Grana 2008;47:15-38.