

Morphological and anatomical study of promising herbal raw material - large-leaved gentian (*Gentiana macrophylla* Pall.)

V. A. Tsukurova¹, O. V. Evdokimova², V. I. Prokofieva¹, A. A. Matyushin¹

¹Sechenov First Moscow State Medical University, Trubetskaya Street, 8/2, Moscow, Russian Federation, 119991

²Federal State Budgetary Institution Scientific Centre for Expert Evaluation of Medicinal Products of the Ministry of Health of the Russian Federation, Petrovskiy Boulevard, 8/2, Moscow, Russian Federation, 127051

Abstract

The aim of the study was to examine dried wild-growing large-leaved gentian herb in order to establish its identification characteristics for the purpose of standardization and quality control. The examination of large-leaved gentian was performed visually (with the naked eye and using a microscope). Macro- and microscopic characteristics of large-leaved gentian aerial parts (leaves, flowers, and stems) were described. The results of the study can be used for standardization of large-leaved gentian herbal raw material.

Keywords: *Gentiana macrophylla* Pall., herbal raw material, large-leaved gentian, microscopic characteristics, traditional medicine.

INTRODUCTION

Currently, there is a strong need in studies aimed at search and evaluation of herbal raw materials as a source of biologically active substances with different pharmacological activities. One of the most perspective sources of such substances is the large-leaved gentian – a herb that is widely used in traditional medicine of Tibet for treatment of upper respiratory tract diseases, gastrointestinal disorders, and in neurasthenia. It is also used in Mongolian traditional medicine in epidemic fever and as an antipyretic and analgesic remedy [1-3].

Large-leaved gentian (*Gentiana macrophylla* Pall) belongs to the *Gentianaceae* family and grows in the Russian Federation in Altai region, in adjacent regions of Western Siberia, in all regions of Middle and Eastern Siberia, in the southern part of Amur Region and Khabarovsk Territory, in the Primorye Region, and in southern regions of Tuva Republic. *G. macrophylla* also grows in Mongolia, and in Western and Northern China. Its range includes meadows, grassland steppes, sparse larch, birch, pine, and mixed forests (along the outskirts and on clearings); cultivation is possible in European Russia, Southern Siberia, and Northwest China [2, 3].

To date, previous studies examined only anatomical and morphological features of large-leaved gentian leaves [4]. However, modern requirements for the quality of herbal drug products prescribe obligatory study of morphological and anatomical characteristics of all aerial parts of this type of herbal raw material: flowers, stems, fruits, etc [5: 272-279].

The aim of this study was to investigate morphological and anatomical characteristics of *G. macrophylla* aerial parts and propose an identification section (macroscopic and microscopic characteristics) for a pharmacopoeial monograph “Large-leaved gentian”.

MATERIALS AND METHODS

The study was performed using dried wild-grown large-leaved gentian herb gathered near regional center Mugur-Aksy (Mongun-tayginsk region, Tuva Republic, Russian Federation) as the object of the study.

The flowering herb was gathered in August 2016 and air-dried in the shade in a well-ventilated room. The herb was laid out in a thin layer and was mixed at regular intervals.

Macroscopic characteristics of the herb were assessed visually according to the general pharmacopoeial monograph “The Herb” of the State Pharmacopoeia, XIII ed. [5].

Microscopic characteristics were described according to the requirements of the general pharmacopoeial monograph “Microscopic and microchemical examination of herbal raw

materials and herbal medicinal products” of the State Pharmacopoeia, XIII ed. [5: 379-400]. The following method was used for preparation of samples for microscopic examination: about 0.1 g of raw material was placed into a 50 ml beaker, 5-10 ml of 5% sodium hydroxide solution were added, and the content was boiled for 10-20 minutes, followed by fractional washing of the material using 100-150 ml portions of purified water, which, in turn, was decanted after complete sedimentation of the particles. After last careful decantation, the material was transferred into a Petri dish, where it was separated into different parts. After that, the material was transferred into a drop of inclusive fluid (1:1 glycerol:water solution), covered with a cover glass, and examined using Olympus CX41 microscope (Olympus, Japan), equipped with 10× eyepiece and 4×, 20×, and 40× lenses, and a digital head PowerShot G1X (Canon, Japan). The photographs were processed using Adobe Photoshop 7.0 (Adobe, USA).

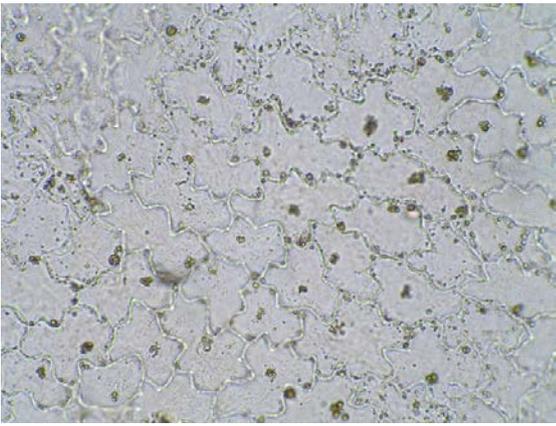
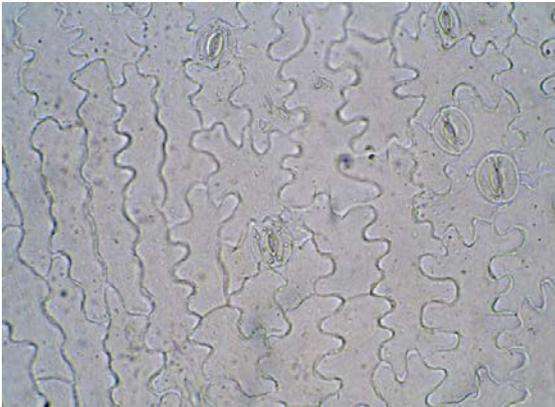
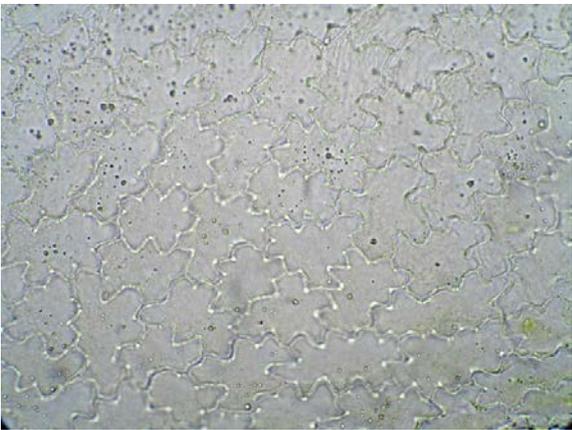
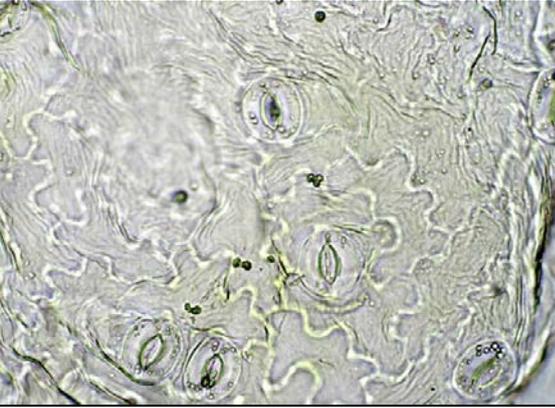
RESULTS

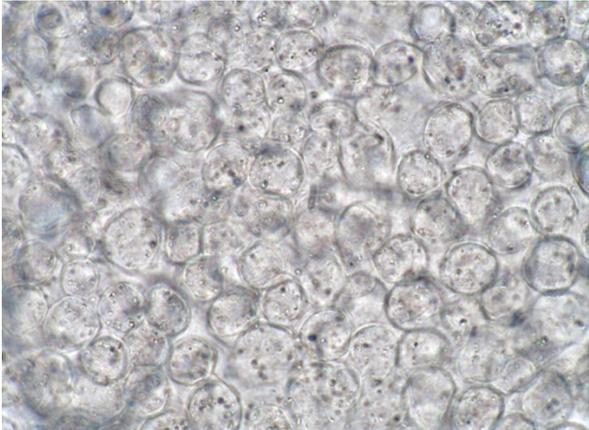
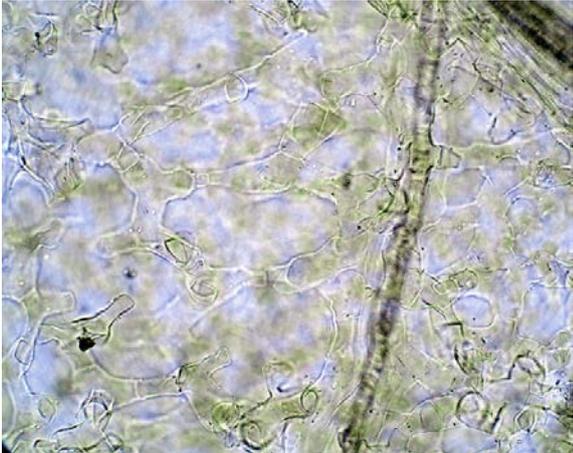
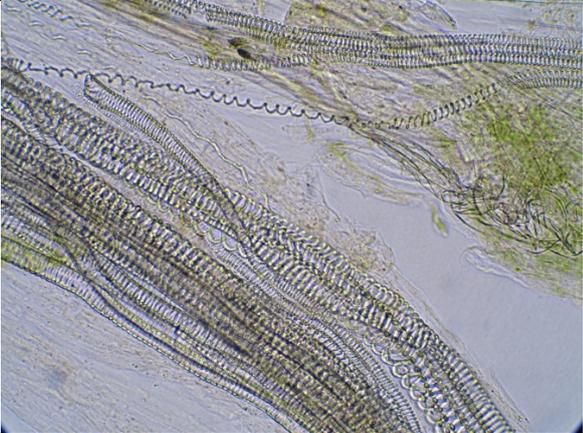
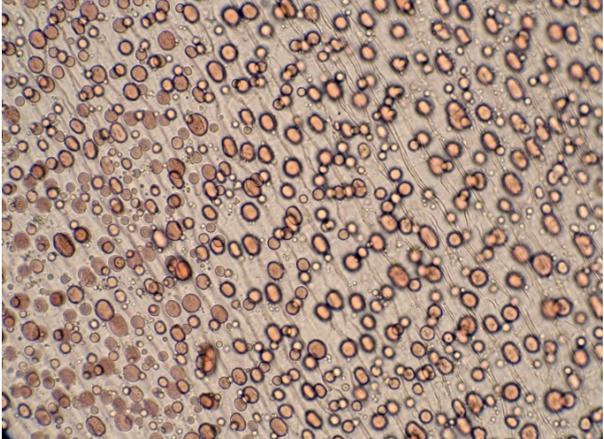
The visual examination has revealed the following macroscopic features of the material: a mixture of whole or partially fragmented stems with or without flowers, less frequently – with flower buds of various degree of development. The stems cut at the base are erect, cylindrical, slightly ribbed, 40-70 cm in length and 3-6 mm in diameter. Basal and stem leaves are opposite, sessile, simple or elliptic-lanceolate, narrowed at the base, apex acuminate, margins are smooth, venation is arcuate. The basal leaf blade is 20-40 cm long and 1.5-4.5 cm wide; stem leaf blade is 5-15 cm long and 0.5-2.0 cm wide. Inflorescences are pseudanthium; flowers and flower buds are gathered into multifloral whorls growing from leaf axils 1-2 cm in diameter. Perianth is double, actinomorphic. Calyx tube is membranous, white-greenish, about 6 mm in length, dentiform (dents are very short, triangulate, sharp). Corolla is sympetalous, five petals, bell-shaped, blue-purple, 15-20 mm long and 4-5 mm wide under fauces. Corolla lobes are triangular, sharp, about 2-4 mm long. Stamens are separate, filaments broadened. Ovaries are sessile [1, 3, 5-7].

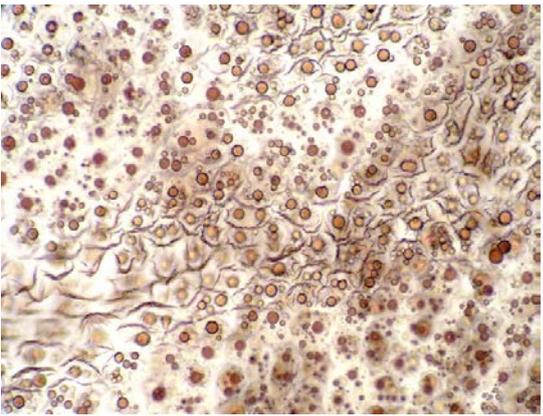
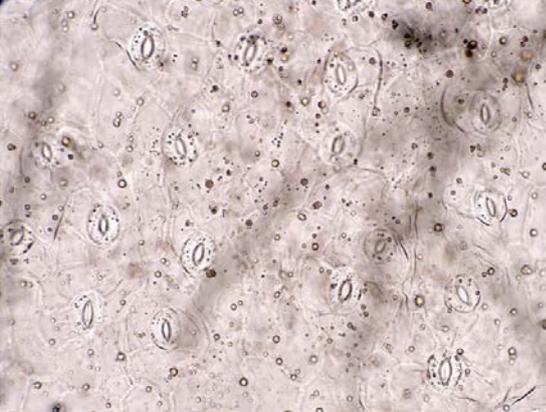
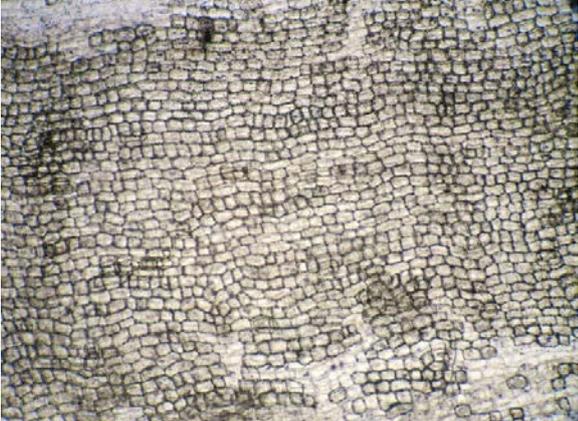
The stems are green, greenish-purple or brown, upper surface of leaves is green or dark-green, lower surface is grayish-green or light green. The corolla is purplish from the inside with blue inclusions and light green or light purple from the outside; calyx lobes are light green. The smell is faint, classless; water extract has bitter taste.

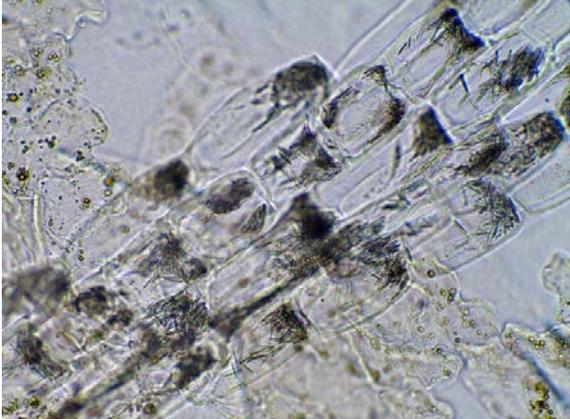
The most important anatomic and diagnostic features of large-leaved gentian aerial parts are presented in Table 1.

Table 1. The anatomic and diagnostic features of *Gentiana macrophylla* herba

Anatomic and diagnostic features	
Leaves	
<p>200× Zoom A fragment of stem leaf epidermis (upper side): cells with uniformly thickened walls and sinuous walls.</p>	
<p>200× Zoom A fragment of stem leaf epidermis (lower side): cells with with uniformly thickened walls and sinuous walls; large oval anomocytic stomata surrounded by bean-shaped guard cells.</p>	
<p>200× Zoom A fragment of basal leaf epidermis (upper side): cells with uniformly thickened walls and sinuous walls.</p>	
<p>200× Zoom A fragment of basal leaf epidermis (lower side): cells with uniformly thickened walls, sinuous walls, and striated cuticle; large oval anomocytic stomata surrounded by bean-shaped guard cells.</p>	

<p>200× Zoom Oval parenchymal cells.</p>	
<p>200× Zoom A fragment of basal leaf: spongy parenchyma cells with large intercellular spaces (aerenchyma).</p>	
<p>200× Zoom Helical vessels.</p>	
<p>Flowers</p>	
<p>200× Zoom A fragment of petal epidermis: axially elongated cells with drops of volatile oil (stained with Sudan III).</p>	

<p>200× Zoom A fragment of petal epidermis (outer side): papilla and drops of volatile oil. (stained with Sudan III)</p>	
<p>400× Zoom Trisulcate pollen. (stained with Sudan III)</p>	
<p>200× Zoom A fragment of sepal epidermis: cells with uniformly thickened walls and sinuous walls; large oval anomocytic stomata surrounded by bean-shaped guard cells. (stained with Sudan III)</p>	
<p>Stem</p>	
<p>40× Zoom A fragment of epidermis: square tightly closed cells.</p>	

<p>200× Zoom A fragment of epidermis: beaded thickening of cell walls and slot-like pores.</p>	
<p>200× Zoom A fragment of epidermis: square cells with large oval anomocytic stomata surrounded by bean-shaped guard cells.</p>	
<p>400× Zoom Parenchyma cells containing group of needle-like calcium oxalate crystals.</p>	
<p>200× Zoom Scalariform vessels.</p>	

CONCLUSIONS

Macroscopic characteristics, which allow identification of large-leaved gentian herbal raw material, are described in the article. The study of microscopic characteristics shows that only stem leaves have striated cuticle of the epidermis and aerenchyma. Also, anatomical and diagnostic characteristics of flowers and stems of *G. macrophylla* are described. It was noted, that the herbal raw material under test is aglandular and glabrous.

The results of the study can be used for standardization and quality control of large-leaved gentian herbal raw material.

REFERENCES

- [1] Makhov, A.A., *Green pharmacy: Medicinal plants of the Krasnoyarsk region*, Krasnoyarsk publishing office, Krasnoyarsk 1986, pp. 81-83.
- [2] Shapoval, T.P., Gentian - the most blue, *Floriculture* 2003, 5, 40-41.
- [3] Schreter, A.I., Valentinov, B.G., Naumova, E.M., *Natural raw materials of Chinese medicine*, Vol. 1, Tenerif, Moscow 2004.
- [4] Tikhonova, L.A., *Pharmacognostic study of gentian flora of Siberia and the Far East*, 1999, pp. 18-19.
- [5] Russian Federation State Pharmacopoeia, XIII ed., Vol. II, Moscow 2015.
- [6] Balandina, I.A., *Improvement of principles and methods of pharmacopoeial analysis in the system of standardization medicinal plant raw materials and medicinal products on its basis*, Moscow 2004, pp. 134-143.
- [7] Evdokimova, O.V., Matyushin, A.A., Prokofieva, V.I., Pheasant's eye: a pharmacopoeial study of medicinal plant, *J. Pharm. Sci. & Res.* 2018, 10(2), 340-343.