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Macro- and microscopy of upper parts from *Limonium gmelinii* genus plants

Abstract

This article presents data on macroscopy and microscopy of upper parts of plants from *L. gmelinii* genus in accordance with the regulatory requirements for medicinal plants introduced into medicine, by leading pharmacopoeias of the world, in particular by the European Pharmacopoeia, and harmonized with the State Pharmacopoeia of the Republic of Kazakhstan.

Key words: macroscopy, microscopy, plant, upper parts, *Limonium gmelinii*, pharmacopoeia.

Introduction

For the development of pharmaceutical production in Kazakhstan an important source is its rich and diverse wild flora, with over 6000 species of the plants, of which over 100 are medicinal. Of the available medicinal plants only 5% are of commercial importance.

In recent decades there has been a clear tendency for increase in the total share of issued medicines and herbal preparations. To date, the figure is over 50%, which is due to the softness of their action, low toxicity and rare induction of allergic reactions, the latter is particularly important in the case of diseases requiring long-term treatment. Among the medicinal plants of the native flora, related to halophytes and takyr, promising are those of the *Plumbagenaceae* family *Limonium* genus, consisting of 18 species, growing in all regions of Kazakhstan. These plants can be reproduced vegetatively and by seed, differ by rapid growth and high yield. The most known and studied is *L. gmelinii*, harvesting is possible in areas of wildgrowing bushes of Zhambyl and Enbekshikazhskii district of Almaty region.

Productive supply of dry roots of two commercially important species *L. gmelinii* and *L. myrianthum* in Almaty, Semipalatinsk, Zhambyl, Atyrau, West and East Kazakhstan regions exceeds 54.4 thousand tons for the area of 160 thousand hectares [1-3].

Roots of *L. gmelinii* were introduced into medicine and State Pharmacopoeia of the Republic of Kazakhstan as a source for effective medicines on their basis, such as tincture "Limonidin" and substance "Limonidin", registered and recommended by the Ministry of Health care to the industrial production and use in medicine as anti-inflammatory and antiviral remedy. Syrup and ointment under a unified name "Limonidin" were obtained on the basis of the substance "Limonidin" [4].

However, high antioxidant activity of substance extracted from the upper parts of *L.gmelinii*, commensurate with that from the roots of *L.gmelinii*, leading to conclusions about the prospects for its implementation into practice, which will allow to use the whole plant, increase their resource base and promoting creation of new, original, native herbal medicines [5]. One of the most important indicators for the identification and standardization of studied medicinal plant objects at their introduction to the medicine is their macroscopic and microscopic analysis.

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Materials and methods

For structural analysis upper vegetative parts of *L.gmelinii* plants were fixed. Fixation was performed in 70% alcohol by the method of Flemming and Strasburger (alcohol/ glycerol/ water, ratio = 1:1:1) [6]. Anatomic specimens were prepared manually and using a microtome with freezing unit TOC-2. Glycerin and balsam embedded sections were processed in accordance with conventional techniques [7-8]. Thickness of anatomic sections was 10-15 microns.

More than 500 temporary slides were obtained. Microphotographs have been taken on microscope MC-300 (magnification x100, x400; Micros, Austria).

Optical microscopy facilitated study. Inspecting light – artificial, reflected. Magnification – from 18 to 40 times. Plant anatomic features are usually clearly identified in such conditions.

Results and their discussion

Macroscopy. *L. gmelinii* is a wild 30-80 cm tall perennial herb. Stem – straight, short at the top, with two or few, usually paired branches with wide somewhat corymbose apical whisk. Rosettes of leaves are numerous, green or blue-green, reddish at fissures, oblong-obovate to wide elliptical. One or more peduncles are terminal or axillary. Flowers are in small, 1-3-4-flowered semi curled spikes, forming almost corymbose or pyramidal inflorescences. Spikelets are 4-5-6 mm long, calyx is 4-4.5 mm long, at the base and up to half of the veins, sometimes on two internal veins densely and rather long pubescent. Limb is whitish or pale purple, 5 less 10 notched. Petals are blue-purple, rarely white. Seeds are oblong-ovate, 2 mm long, 0.6 mm wide, dark purple-brown.

Microscopy. Lamina is covered with epidermis from the outside. Epidermal cells are tightly packed and have no intercellular spaces. The cells of the upper and lower epidermis are covered with finely granulate cuticle. Leaf is controversial, with two-rowed tightly packed, well-developed palisade tissue located on the upper, adaxial side. Spongy tissue is loose, composed of variously shaped cells, elongated along the width of the leaf and lying in a plane parallel to the surface of the leaf (Fig. 1).

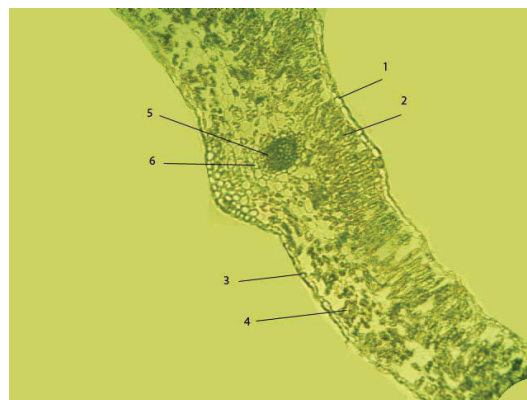


Figure 1 – Anatomy of the leaf. Note: 1 – upper epidermis, 2 – column-shaped mesophyll cells, 3 – lower epidermis, 4 – spongy mesophyll, 5 – conductive bundle, 6 – bundle lined with parenchyma

Fibrovascular bundles penetrate the leaf mesophyll. Type of vascular bundle is collateral. Subepidermal angled collenchyma has three rows above the rib and four rows below it. Epidermis on both sides has almost the same structure and is composed of small cells, polygonal in outline. Stomata are numerous on both sides. They are surrounded by 2-3, sometimes 4 cells of the epidermis (Fig. 2).

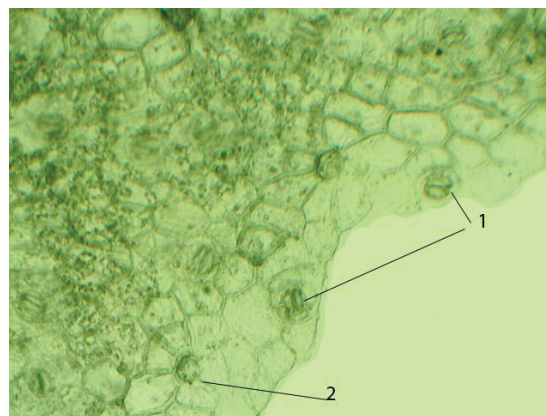


Figure 2 – Upper epidermis of the leaf. Note: 1 – stomata, 2 – trichome bases

Hairs in large numbers along the surface of the leaf are unicellular, slightly curved with pointed tip and roughly warty surface. They often fall apart.

Then at the point of their attachment remains a small round bolster surrounded by rosette of epidermal cells (Fig. 3).

All veins on the leaf are lined out with parenchyma.

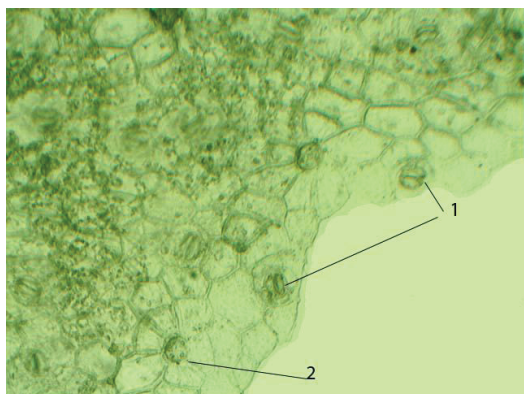


Figure 3 – Lower epidermis of the leaf.
Note: 1 – stomata, 2 – trichome bases

Stem is covered with epidermis, consisting of tubular cells; the outer walls of the epidermis are covered with finely papillose cuticle (Fig. 4).

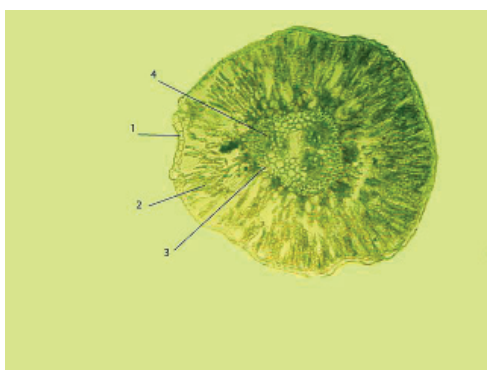


Figure 4 – Anatomy of the young stem.
Note: 1 – epidermis, 2 – primary cortex, 3 – sclerenchyma, 4 – parenchyma of conducting bundle

Below is a layer of the parenchyma of the primary cortex, separated from central cylinder by groups of sclerenchyma cells (Fig. 5).



Figure 5 – Anatomy of the stem during generative phase.
Note: 1 – epidermis, 2 – primary cortex, 3 – sclerenchyma, 4 – pith, 5 – xylem, 6 – phloem

Primary cortex consists of two layers.
The first layer of the stem consists of assimilating tissue containing chlorophyll.

Cells are more elongated and located perpendicularly to the epidermis.

Young plants possess more developed chlorophyll-containing parenchyma.

The second layer contains parenchymal cells of different shapes.

Sclerenchymatic cells are thick-walled, circular in cross section, with a pointed cavity.

In this area, some adjacent cortical cells contain single crystals – drusen (Fig.6).

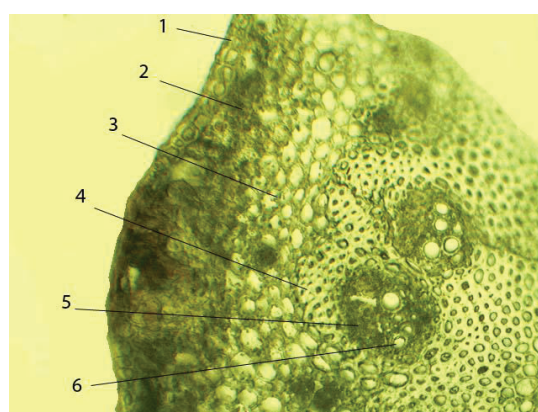


Figure 6 – Anatomy of the stem at postgenerative phase.
Note: 1 – epidermis, 2 – assimilation tissue, 3 – parenchymal cells of the primary cortex, 4 – sclerenchyma, 5 – phloem, 6 – xylem

In central cylinder conductive tissue is presented by bundles, in which cambium is functioning. Vessels are located in groups and have thick walls (Fig. 7).

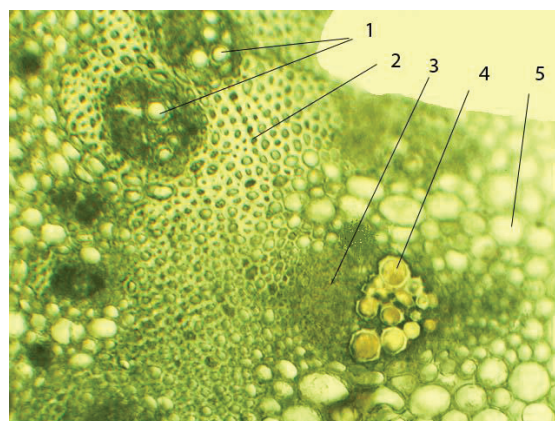


Figure 7 – Anatomy of the core portion of the stem.
Note: 1 – xylem, 2 – sclerenchyma, 3 – phloem, 4 – extractives in xylem vessels, 5 – pith

There is a group of pericyclic fibers around each bundle. Pith is scattered. The core is round in cross section, composed of large thin-walled cells. In parenchyma cells containers with biologically active compounds could be found (Fig. 8).



Figure 8 – The internal structure of the stem. Note: 1-cell contents, 2 – sclerenchyma, 3-phloem

As a result of the study the following was discovered:

- Basic anatomic-morphological features of *Limonium gmelinii* are:
 - particles of the upper and lower epidermis of the leaves;
 - unicellular trichomes, slightly curved with pointed tip and roughly warty surface;
 - presence in the pith of the stem receptacles with biologically active substances.

Assimilating tissue in the stem of the plant maintains even during the post-generative stage.

Moreover, its presence is a consistent feature of the *L. gmelinii* plants.

The combination of such features testifies that studied substance is a mixture of various upper parts of the *L. gmelinii* plants.

Detailed macro- and microscopic analysis of upper parts of the *L. gmelinii* species plants in accor-

dance with the requirements of the State Pharmacopoeia of the Republic of Kazakhstan may facilitate identification of such type of plant material during its introduction into the medicine.

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