

New approaches to diagnostics of medicinal plant raw materials *Echinacea purpurea*

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New approaches to the diagnostics of medicinal plant drugs of *Echinacea purpurea*

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RESUME

Petiole anatomy is a new and promising method for diagnostics of medicinal plants, both fresh and air-dried. With the help of digital microscopy there were studied the anatomical and morphological features of the structure petiole of fresh leaf of *Echinacea purpurea* (L.) Moench. (Asteraceae), and also identified the main diagnostic features of the object in order to further the recommendations of the data to be included in the section "Microscopy" project pharmacopoeial monograph, which regulates the quality of fresh raw material of *Echinacea purpurea*.

Keywords: *Echinacea purpurea*, petiole anatomy, petiole, microscopy, diagnostic characteristics.

SUMMARY

Petiolar anatomy is a new and promising method for diagnosing medicinal plants, both fresh and air-dry. Using digital microscopy, the anatomical and morphological features of the structure of the leaf petiole of fresh *Echinacea purpurea* [*Echinacea purpurea* (L.) Moench., Family. Compositae - Asteraceae], the main diagnostic signs of the object under study were identified with the aim of further recommending the obtained data for inclusion in the "Microscopy" section of the draft pharmacopoeial monograph regulating the quality of fresh raw materials of *Echinacea purpurea*.

Key words: *Echinacea purpurea*, petiolar anatomy, leaf petiole, microscopy, diagnostic signs.

Introduction

Currently, herbal medicines used for the prevention and treatment of immunodeficiency conditions are of particular importance. The advantage of herbal medicines over drugs of synthetic origin lies in the mildness of their therapeutic action, along with the absence of pronounced side effects. *Echinacea purpurea* [*Echinacea purpurea* (L.) Moench., Family. Asteraceae] is one of the most promising plants as a source of plant immunomodulators, since it combines pronounced immunomodulatory, anti-inflammatory, antiviral and tonic properties [1, 2, 3].

Considering the above, the search for optimal methods of analysis and the development of quality control methods for raw materials (both fresh and air-dry), which is the starting point for obtaining immunomodulatory drugs based on *Echinacea purpurea*, also becomes relevant.

It is known that the anatomical structure of such main organs as the root, stem and leaf is characterized by a certain constancy, typical for dicotyledonous plants, while the petiolar anatomy (the structure of the leaf petiole) is characterized by a wide variety of diagnostic features [4, 5]. The most important of them should be considered such signs as the shape of the cross-section of the petiole, the location of the conductive bundles, lignification or destruction of the central parenchyma, the presence of sclerenchyma, collenchyma, secretory tubules, trichomes, etc. Thus, the study and identification of diagnostic features of the structure

The leaf petiole of a plant can be used to determine the authenticity and analyze the quality of medicinal plant materials.

Purpose of the study: anatomical and histological study of the petiolar signs of echinaceapurple, which can be further used in the diagnosis of medicinal plant raw materials of this plant.

Materials and methods

The material for the study was a freshly harvested aerial part of *Echinacea purpurea*, harvested in the village of Alekseevka, Kinelsky District, Samara Region, from June to July 2011.

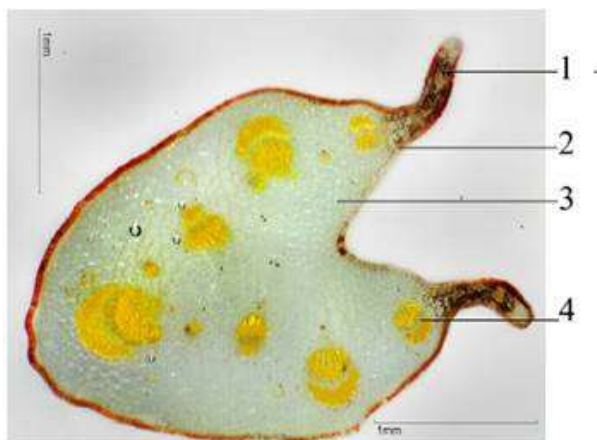
The freshly collected aerial part of *Echinacea purpurea* was fixed in a mixture of ethyl alcohol 96%, rectified glycerin and purified water in a ratio of 1: 1: 1. The object was infused for 24 hours, after which it was subjected to anatomical and histological analysis.

The preparation of micropreparations was carried out according to the pharmacopoeial method of the State Pharmacopoeia of the USSR, XI edition [6].

The study was carried out using digital microscopes "Motic" (Korea): DM-111 and DM-39C-N9GO-A. For a more complete characterization of the studied objects, we used histochemical reactions to lignified shells with aniline sulfate solution, prepared according to the appropriate methods [5, 7].

results

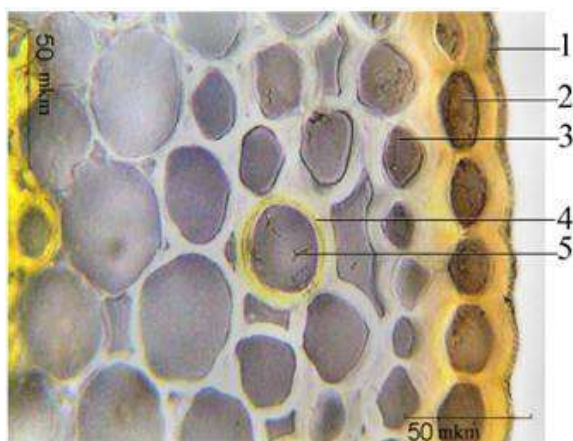
At the initial stage, we considered a cross section of the petiole (Fig. 1). The cross-sectional shape is a rhombus with a V-shaped notch on the adaxial side (facing the stem). The edges of the cut are represented by the remnants of the descending base of the sheet plate. They are usually turned towards the underside of the sheet (Fig. 1).



Rice. 1. Cross section of leaf petiole (x40).

Designations: 1 - a fragment of a leaf blade; 2 - a cell of the upper epidermis; 3 - cage parenchyma; 4 - conducting bundle.

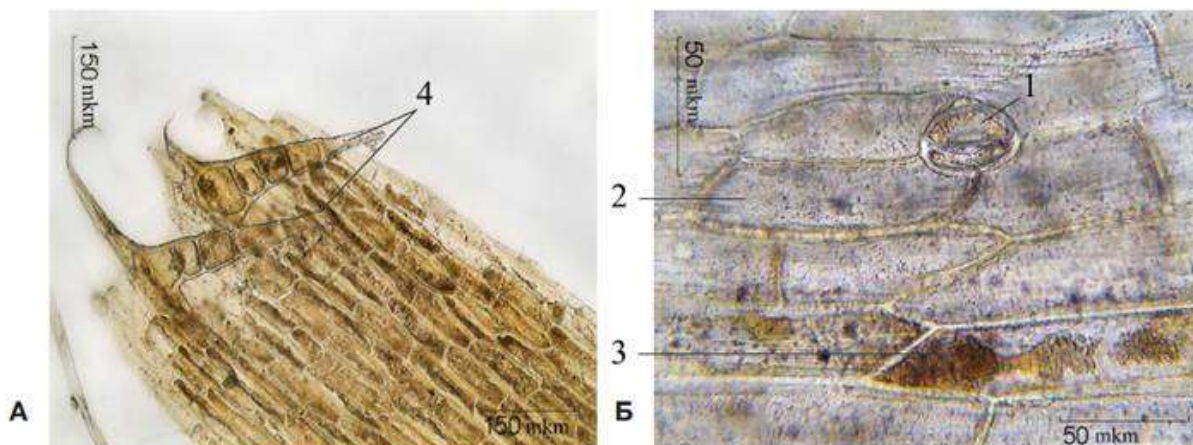
The leaf petiole is made by the main parenchyma, the cells of which have an isodiametric rounded shape in cross section. The size of parenchymal cells varies from 40 to 100 μm in diameter, with the largest cells located in the center, and the smallest ones along the periphery. When examining the longitudinal section of the petiole, it was revealed that the cells of the main parenchyma have an elongated rectangular shape. The cell walls are slightly thickened and have clearly visible simple pores. In the main parenchyma, more often to the periphery, cells with lignified membranes are found (Fig. 2).



Rice. 2. Cross section of the leaf petiole. Staining with aniline sulfate solution (x400). Designations: 1 - cuticle cell; 2 - epidermis cell; 3 - collenchyma cell; 4 - lignified parenchymal cell; 5 - cell cavity.

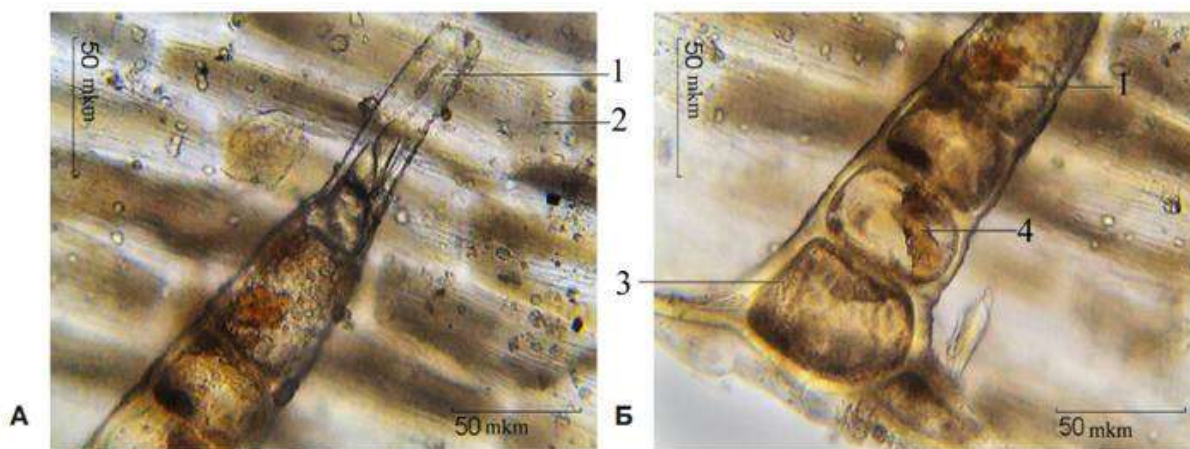
The epidermal cells of the leaf petiole from the surface are elongated, polygonal, of different sizes, reaching 130–150 μm in length and 50 μm in width (Fig. 3). In cross-section, epidermal cells have a rounded shape with highly thickened cell walls. The cell walls of the epidermis are initially colored in a slightly orange color, which is enhanced by treatment with aniline sulfate solution.

On the surface of the petiole, stomatal apparatuses are occasionally found, surrounded by four to five peri-stomatal cells, which do not differ from the cells of the main epidermis (Fig. 3A).



Rice. 3. Epidermis of leaf petiole: A - trichomes (x100); B - stomata (x400). Designations: 1 - stomata; 2 - peri-stomatal cell; 3 - epidermal cell; 4 - trichomes.

The epidermis of the petiole is pubescent. Trichomes are represented by multicellular hairs reaching a length of 300 microns. Hair cells contain a protoplast. The shape of the cells at the base is rounded, slightly flattened. The terminal cell of the hair is longer than the others and strongly narrowed (Fig. 4), and it often breaks off.

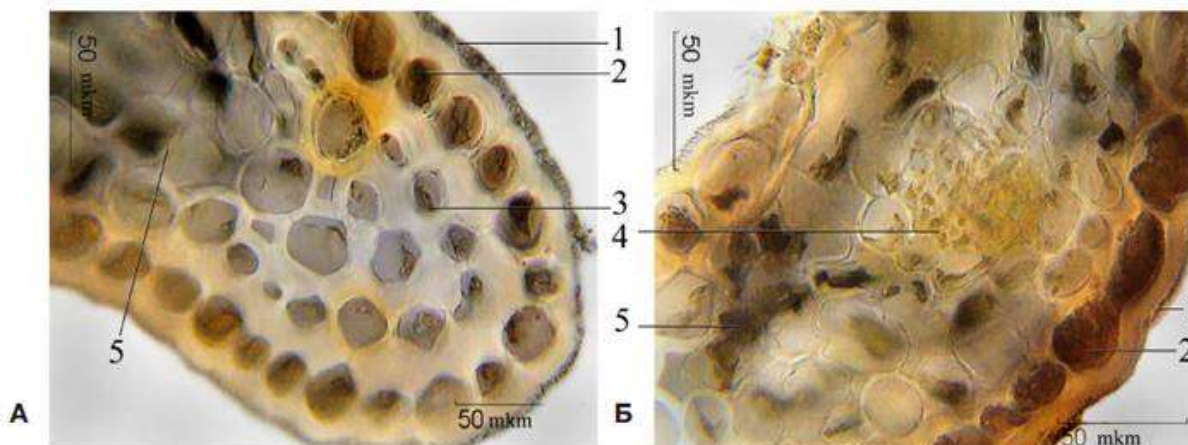


Rice. 4. Trichomes of the leaf petiole (x400): A - the end of the hair; B - hair base.
 Legend: 1 - terminal broken off trichome cell, 2 - epidermal cell, 3 - cell trichome bases, 4 - protoplast.

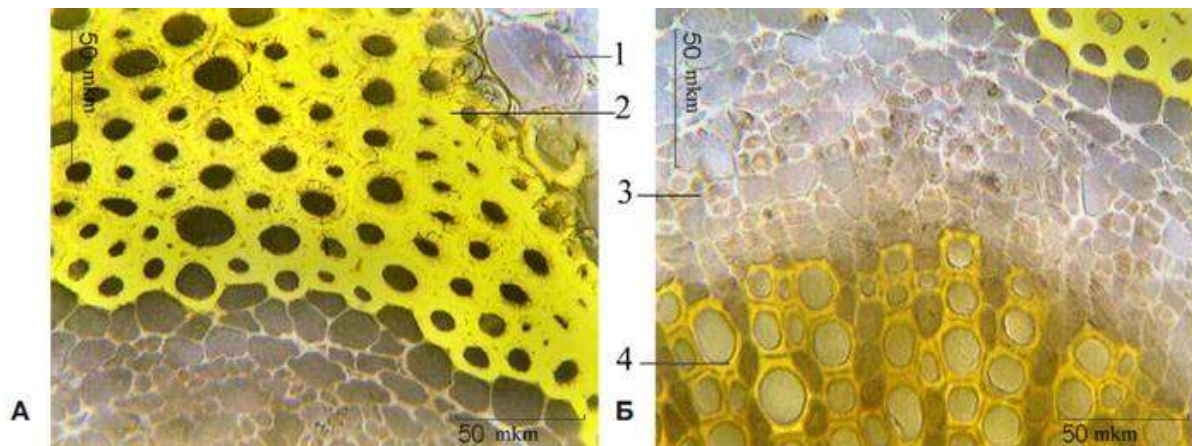
The remnants of the lamina described above have a similar epidermis. The mesophyll of the remnants of the leaf plate is represented by cells of a rounded shape with a green protoplast. In this case, mesophyll cells are located chaotically with a large number of intercellular spaces (loose chlorenchyme). In the mesophyll, closed collateral vascular bundles can occasionally occur (Fig. 5B). At the edges of the leaf plates, an area of angular collenchyma is observed. Collenchyma cells are alive, which is confirmed by the presence of a protoplast in them (Fig. 5A).

In the parenchyma of the petiole, closed collateral vascular bundles are localized. The number and size of the beams varies depending on the location of the cross-section. On average, the petiole contains about seven large bundles reaching 0.3 mm in diameter (Fig. 1). In this case, the largest bundle is the central vein. It is localized in the corner of the "diamond" from the abaxial side (Fig. 1). The structure of the bundles has no peculiarities, however, it should be noted the presence of powerful sclerenchyma with lignified cell membranes (Fig. 6).

In the future, in a comparative aspect, it is planned to study the diagnostic features of the structure of the leaf petiole of other types of echinacea. The data obtained are of great practical interest, since they allow one to judge the authenticity of the raw materials, as well as the presence of closely related plant species in the crushed raw *Echinacea purpurea*.



Rice. 5. Cross section of the leaf petiole. The edge of the leaf blade (x400): A - a fragment of the edge of a sheet plate; B - a fragment of a sheet plate with a conducting beam. Designations: 1 - cuticle; 2 - epidermis cell; 3 - collenchyma cell; 4 - elements of the conducting beam; 5 - leaf mesophyll cell.



Rice. 6. Conductive bundle of leaf petiole (x400): A - fragment of the bundle with sclerenchyma, B - fragment of the bundle with phloem. Designations: 1 - parenchyma cell; 2 - sclerenchyma cell; 3 - cage phloem; 4 - xylem cell.

conclusions

Thus, as a result of our studies, we identified the main diagnostic features of the leaf petiole of *Echinacea purpurea*:

1. The shape of the cross-section of the petiole is a rhombus with a V-shaped cutout from the adaxial side.

2. The leaf petiole is made by the main parenchyma, the cells of which in the cross section have an isodiametric rounded shape. Cell walls have pores; in the main parenchyma, cells with lignified membranes are found.

3. Epidermal cells of the leaf petiole from the surface are elongated, polygonal, different sizes. The stomatal apparatus are rare and are surrounded by four to five peristomatal cells, similar to the cells of the main epidermis (anomocytic type).

4. The epidermis of the petiole has trichomes, represented by simple multicellular hairs. The shape of the cells at the base is rounded, flattened, the terminal cell is longer than all the others and strongly narrowed.

5. Mesophyll of the remnants of the descending leaf plate is represented by cells of a rounded forms with a green protoplast. Mesophyll cells are located chaotically with a large number of intercellular spaces.

6. In the parenchyma of the petiole, an average of 6–7 closed collateral conductive beam. The largest bundle, the central vein, is localized in the corner of the rhombus from the abaxial side. The presence of a powerful sclerenchyma with lignified cell membranes is characteristic.

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