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Comparative Morphological and Anatomical Studies of *Artemisia absinthium* and *Artemisia austriaca*

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ABSTRACT

A comparative analysis of the morphological, anatomical and histological features of *Artemisia absinthium* L. (Family: Asteraceae) and *Artemisia austriaca* Jacq. (Family: Asteraceae) were carried out. The basis of the study was a microscopic analysis of raw materials of *A. absinthium* L. (Family: Asteraceae) and *A. austriaca* Jacq. (Family: Asteraceae). The study was conducted to establish the diagnostic signs of raw materials of *A. absinthium* L. (Family: Asteraceae) and to determine the anatomical and histological features of raw materials of *A. austriaca* Jacq. (Family: Asteraceae). As a result of the morphological, anatomical and histological studies additional diagnostic features of the herb of *A. absinthium* L. (Family: Asteraceae) and the herb of *A. austriaca* Jacq. (Family: Asteraceae) were identified. Based on the results, the criteria of differential diagnosis, allowing to reliably identifying the medicinal raw materials of these plants were developed. These criteria can be used in scientific practice in the further development of the methods for identification of these raw materials.

Keywords: *Artemisia absinthium* L., *Artemisia austriaca* Jacq., Asteraceae, Herb, Leaves, Microscopic characteristics.

INTRODUCTION

Artemisia L. (Family: Asteraceae) has long been used as an anti-inflammatory, antihelminthic, antifungal, bactericidal, choleric, appetite-stimulating agent. Preparations of *Artemisia absinthium* L. (Family: Asteraceae) and preparations of *A. absinthium* L. (Family: Asteraceae) (infusion, tincture, herbal infusion for appetite, gastric herbal infusion, thick extract, complex bitter tincture) are used as appetite-stimulating classic bitter-spicy agents.

There are many species of *Artemisia* L. (Family: Asteraceae), among them *A. absinthium* L. (Family: Asteraceae), *A. austriaca* Jacq. (Family: Asteraceae) and other species, that are growing on the territory of the Orenburg region. In fact *A. absinthium* L. (Family: Asteraceae) can be found in the European part of Russia, in the territory of the CIS countries, in the Caucasus, in South-Western Siberia, in Eastern Siberia, in Kazakhstan. This plant is naturalized in North America. *A. absinthium* L. (Family: Asteraceae) is a weed plant which may grow mainly of steppes and forest-steppes. *A. austriaca* Jacq. (Family: Asteraceae) grows in the territory from South-Western Europe to North-West China. This plant prefers dry meadows, steppes, forest edges, saline areas. These plants are closely related species, but they have different morphological and anatomical features [1-7]. Due to the fact that currently the official is raw material from *A. absinthium* L. (Family: Asteraceae) relevant is the question of diagnosis of this type of medicinal plant. Now the State Pharmacopoeia of Russia, XIV edition contains the Pharmacopoeia article on herb of *A. absinthium* L. (Family: Asteraceae). This article contains anatomical and histological features of raw materials of *A. absinthium* L. (Family: Asteraceae). However this article has no information about impurities of closely related plants [8].

Purpose of the study is comparative anatomical and histological research for herb of *A. absinthium* L. (Family: *Asteraceae*) and herb of *A. austriaca* Jacq. (Family: *Asteraceae*).

MATERIALS AND METHODS

An object of the study was raw materials which had been prepared on June 2018 in Orenburg, Orenburg region. We used a light microscope (digital microscope «Motic DM111» with the possibility of increasing 4×10; 10×10; 40×10; 100×10) for anatomical and histological research. We used this technique to prepare micropreparations of plant material samples. Herb was placed in a flask or in a test-tube, then a solution of Natrii hydroxydum 5% diluted with water (1: 1) was added, then this mixture was boiled for 2 min. Contents of the glass were transferred with the small amount of water to a Petri dish. The pieces were removed from the water with a scalpel or spatula and placed on a glass slide in a drop of the solution of chloral hydrate or the solution of glycerin 33% [8].

RESULTS AND DISCUSSION

Morphological features of the herb of *A. absinthium* L. (Family: *Asteraceae*) are the following: whole or partially crushed leafy tops of flowering stems not more than 25 cm long. The flowering stems are slightly ribbed, ending with a leafy sprawling complex panicle, the branches of which carry small spherical baskets 2.5-4 mm in diameter. Baskets are drooping and come out in one or two of the sinuses of lanceolate covering leaves. From the outside baskets are covered with a wrapper of tiled-shaped linear, outside woolly leaflets, the inner leaves are elliptical, dull, and filmy. The receptacle is convex and covered with white ribbon-like scaly films. The flowers are small, the outer tubular flowers are pistillate and inner funnel flowers are bisexual. Upper bracts are sessile, oblong, entire, lower on peduncle are trifoliate, less often twice or tripartitely divided. There are non-flowering leafy shoots. The color of the stems is greenish gray, the leaves are grayish-green from above, silver-gray from below, and flowers are yellow. The smell of raw materials is fragrant, peculiar, strong, the taste is bitter [1].

Morphological features of the herb of *A. austriaca* Jacq. (Family: *Asteraceae*) are the following: whole leafy stems bearing flowers or parts of them which are no longer than 25 cm long, devoid of coarse vegetative organs. Stems are erect and covered with pubescence and grayish color with a reddish tinge. The lower leaves are double-divided, the upper leaves are three-fold, the lower leaves are petiolate, the middle and upper leaves are sessile. Flower-bearing stems end in the panicle bearing an egg-shaped basket. From the outside the baskets are covered with the double-row wrapper: the outer leaves are pubescent and linear, the inner ones are membranous and elliptical. The flowers are all tubular, median flowers are bisexual, marginal flowers are pistillate. The flowers are yellow or red. The smell of raw materials is fragrant [1-3,6-12] (Figure 1).

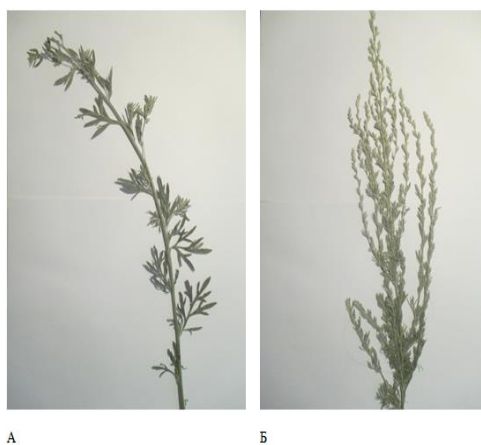
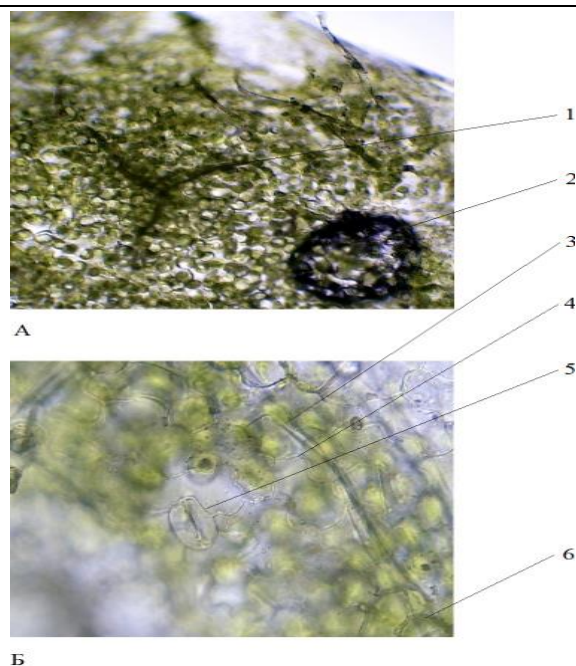


Figure 1: A–herb of *Artemisia absinthium* L. (Family: *Asteraceae*); B–herb of *Artemisia austriaca* Jacq. (Family: *Asteraceae*)

In the study of the anatomical features of the upper epidermis of the leaves of *A. absinthium* L. (Family: *Asteraceae*) we defined that the cells of the epidermis have tortuous shape. Stomata on the upper epidermis of the leaves of *A. absinthium* L. (Family: *Asteraceae*) are presented in a small amount, they are oval. Stomata of *A. absinthium* L. (Family: *Asteraceae*) have anomocytic type (they are surrounded by 3-5 cells of the epidermis). The upper epidermis of the leaves of *A. absinthium* L. (Family: *Asteraceae*) is characterized by the presence of simple and T-shaped filaments. Places of attachment of filaments have the form of rounded rollers. The upper epidermis of the leaf of *A. absinthium* L. (Family: *Asteraceae*) contains large oval essential oil glands with a transverse septum (Figure 2).



A–longitudinal section of the upper epidermis of the leaf of *Artemisia absinthium* L. (Family: Asteraceae), fragment with gland and T-shaped filaments (x 100);
 B–longitudinal section of the upper epidermis of the leaf of *Artemisia absinthium* L. (Family: Asteraceae), fragment (x 400).
 Designations: 1–T-shaped filament; 2–gland; 3–simple filament; 4–cuticle tortuosity; 5–stoma; 6–place of attachment of filament

Figure 2: Longitudinal section of the upper epidermis of the leaf of *Artemisia absinthium* L. (Family: Asteraceae)

In the study of the anatomical features of the lower epidermis of the leaves of *A. absinthium* L. (Family: Asteraceae) we define that the cells of the epidermis have the more tortuous shape (Figure 3). Stomata are represented in great number, but they are similar to the anatomical features of the upper leaf epidermis of this type of wormwood. Simple filaments on the lower epidermis of the leaves of *A. absinthium* L. (Family: Asteraceae) are represented in larger quantities.



Designations: 1–simple filament; 2–cuticle tortuosity; 3–stoma; 4–essential oil inclusions

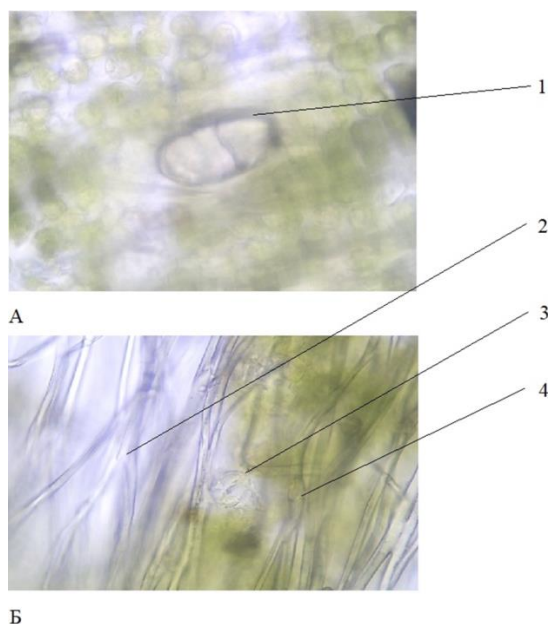
Figure 3: Longitudinal section of the lower epidermis of the leaf of *Artemisia absinthium* L. (Family: Asteraceae) (x 400)

Features of the anatomical structure of *A. austriaca* Jacq. (Family: Asteraceae) do not suggest the possibility of differentiation of the upper and lower epidermis due to the strong pubescence of vegetative organs of the plant. The anatomical features of the upper and lower epidermis are characterized by significant similarity. In the study of anatomical features of the longitudinal sections of the upper and lower epidermis of leaves of *A. austriaca* Jacq. (Family: Asteraceae) the presence of T-shaped and simple filaments was determined. Simple filaments are represented in smaller quantities. The place of attachment of filaments has a characteristic shape in the form of the rounded rollers. The stomatal apparatus has anomocytic type, the stomata are represented in small quantities, they are oval-shaped and characterized by location on both sides of the leaf. We noted the content of essential oil inclusion with the oblong shape. There are a few essential oil glands. The glands have an oval shape, they are large. Cuticle tortuosity can't determine (Figures 4 and 5).



Designations: 1–stoma; 2–the place of attachment of filament; 3–simple filament; 4–T-shaped filament; 5–essential oil inclusions

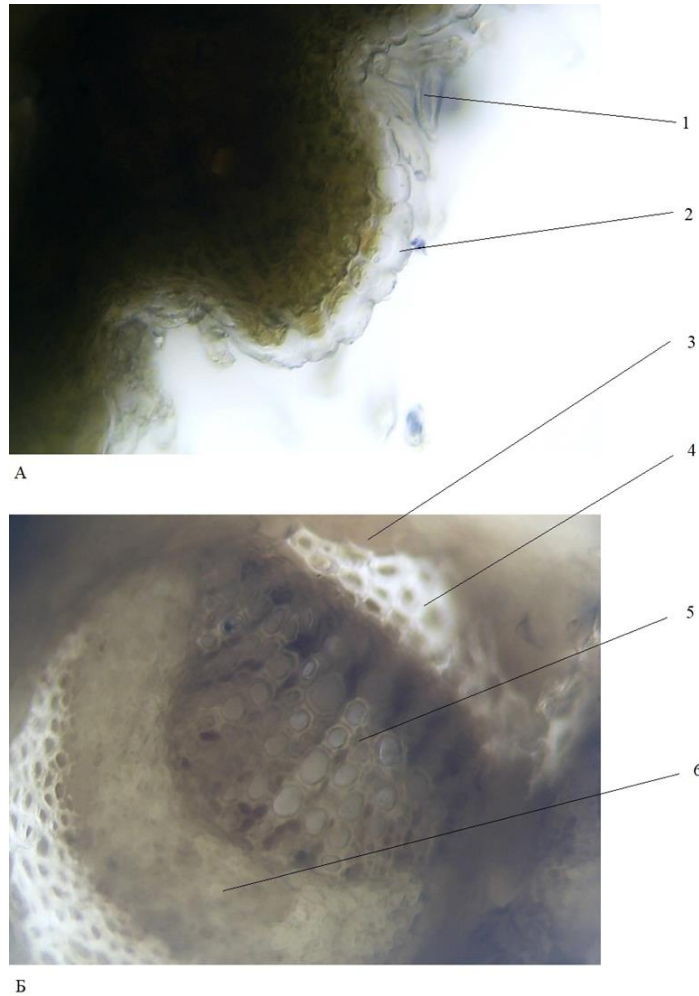
Figure 4: Longitudinal section of the upper epidermis of the leaf of *Artemisia austriaca* Jacq. (Family: Asteraceae) (400x)



A–longitudinal section of the lower epidermis of the leaf of *Artemisia austriaca* Jacq. (Family: Asteraceae), fragment with gland (x 400); B–longitudinal section of the lower epidermis of the leaf of *Artemisia austriaca* Jacq. (Family: Asteraceae), fragment (400x); Designations: 1–essential oil gland; 2–T-shaped filament; 3–stoma; 4–the place of attachment of filament

Figure 5: Longitudinal section of the lower epidermis of the leaf of *Artemisia austriaca* Jacq. (Family: Asteraceae)

The cross section of the leaf of *A. austriaca* Jacq. (Family: Asteraceae) (Figure 6) shows the oblong shape of the leaf of the isolateral type, since the presence of the palisade layer defining a similar type of leaf is found on both sides of the leaf. In the center of the leaf, there is a vascular bundle characterized by a collateral type of structure (represented by the xylem and phloem in such way that the xylem lies higher than the phloem), this vascular bundle is open. The cells of the palisade and spongy mesophyll are clearly visible; these cells are large, oblong in shape, with the cells of the palisade mesophyll bordering on both the upper and lower epidermis of the leaf. On the cross section of the studied leaf, the presence of T-shaped filaments and oil-bearing glands protruding above the surface of the leaf cell is confirmed.



A—cross section of the leaf of *Artemisia austriaca* Jacq. (Family: *Asteraceae*), fragment with T-shaped filaments (100x); B—cross section of the leaf of *Artemisia austriaca* Jacq. (Family: *Asteraceae*), fragment (x 400); Designations: 1—simple filament; 2—upper epidermis; 3—palisade layer; 4—spongy layer; 5—xylem; 6—phloem

Figure 6: Cross section of the leaf of *Artemisia austriaca* Jacq. (Family: *Asteraceae*)

CONCLUSIONS

1. Morphological, anatomical and histological features of the herb of *A. absinthium* L. (Family: *Asteraceae*), the herb of *A. austriaca* Jacq. (Family: *Asteraceae*) were studied.
2. As a result of morphological and anatomical and histological studies of the leaves of *A. absinthium* L. (Family: *Asteraceae*) the classical structure of the leaf was confirmed and the structural features of the leaves of this species were determined
3. *A. austriaca* Jacq. (Family: *Asteraceae*) is characterized by the following distinguishing features: an abundance of T-shaped filaments of characteristic shape, structural features of the essential oil glands and inclusions.
4. *A. absinthium* L. (Family: *Asteraceae*) is characterized by the following distinguishing features: the cuticle tortuosity, the characteristic structure of the essential oil glands, the abundance of not only T-shaped but also simple filaments.
5. On the basis of the results of morphological and anatomical and histological studies, developed the criteria of differential diagnosis of the herb of *A. absinthium* L. (Family: *Asteraceae*) and the herb of *A. austriaca* Jacq. (Family: *Asteraceae*) which allow to identify the corresponding medicinal raw materials reliably.

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