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Morphological and anatomical diagnostic features of *Aristolochia clematitis* L. herb

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ABSTRACT

Diagnostic macro- and microscopic features of *Aristolochia clematitis* L. herb were established as an obligatory element for the standardization of medicinal plant raw materials. They are as follows: Stems are cylindrical, unbranched, leaves are simple, petiolar, leaf blade is entire, and broadly ovate to rounded-triangular. Leaf base is deeply reniform, tip pointy concave. Leaf epidermis cells tortuous, stomata of anomocytic type, and glands with essential oil are present. The stem and petiole have fascicles of open collateral type.

Keywords: *Aristolochia clematitis* L., diagnostic features, epidermis, identification, morphological and anatomical research

INTRODUCTION

Medicinal plants and herbal remedies play a significant role in the world pharmaceutical practice, as they are often considered as drugs of choice in preventive medicine, pediatrics, and gerontology.[1,2] However, an obligatory requirement for the introduction of medicinal plants in official medical practice (including the production of herbal remedies) is its standardization, i.e., ensuring compliance with the requirements of regulatory documents. One of the mandatory elements of medicinal plant raw material standardization is establishment of macro- and microscopic diagnostic features, which can serve for confirmation of authenticity (identification) of raw material. An indicator of authenticity is particularly important in the identification of medicinal plants containing toxic substances. One of such plants is *Aristolochia clematitis* L. from the family *Aristolochiaceae* Juss., which applies in folk medicine for diseases of gastrointestinal tract, genitourinary system, fungal lesions, and as an antibacterial agent.[3-5] At the same time, there is experimental data on neuropathic and carcinogenic properties of *Aristolochia*.[6-11] Drugs with *A. clematitis* L. are prohibited on the territory of the Russian Federation due to their high toxicity. Nevertheless, an aerial part of *Aristolochia* is a prospective medicinal plant raw material.

A. clematitis is the only species of the family *Aristolochiaceae* growing in the territory of the Russian Federation as a wild plant. In the Russian Federation, the area of growing of *A. clematitis* L. is limited by the territories of Belgorod, Voronezh, Kursk, Lipetsk, and Tambov regions, but the plant forms large vines and therefore the raw material base is sufficient. The studying of diagnostic characteristics of this type of raw material is necessary for its implementation in medical practice. Therefore, the purpose of the study was to identify macroscopic and anatomical characteristics of raw materials of the herb *A. clematitis* L., growing on the territory of Russia, for the characterization of identity and development of the section "identity" for the project of pharmacopeial monograph.

MATERIALS AND METHODS

Leafy aerial parts of *A. clematitis* L. up to 30 cm long were used for the study. They were harvested during the flowering period at the end of June 2017 in Novy Oskol (Belgorod region, 50.778519° N, 37.847453° E). The identification of the raw materials was carried out using the description given in literature.[12] The raw material was dried by natural drying in a shadow. The voucher specimen of *A. clematitis* L. (LE 01051846) is deposited in the Herbarium of the Komarov Botanical Institute of the Russian Academy of Sciences, St. Petersburg, Russia.

Macro- and microscopic analysis was carried out in accordance with requirements of the Monograph 1.5.1.0002.15 "herbae," OFS.1.5.3.0003.15 "Technique of microscopic and microchemical studies of herbal drugs and herbal medicinal products" State Pharmacopoeia of the Russian Federation XIII edition (GF RF XIII).[13] For the studies, three fragments of the middle third of the leaf from different three samples of *A. clematitis* were taken for averaging and unification of obtained results. Micropreparations were treated with alkali and examined from the surface. Identification and localization of essential oil were carried out by histochemical reaction with Sudan III.

Anatomy of the leaf, petiole, and stem was studied on cross-sections. For this purpose, the samples were fixed in 3% glutaraldehyde solution within 2 days. Postfixation was performed with 2% osmium solution during the night. The material was dehydrated in a series of acetone solutions of increasing concentrations (from 30% to 100%). Samples were embedded in Epon-Araldite mixture.[14-16] Semi-thin sections (2 and 4 μm) were obtained using ultramicrotome Ultracut E (Reichert-Jung). Cutting was performed with a diamond knife. The preparations were stained with toluidine solution. To determine lignified areas transverse sections of stem and petiole were treated with an 0.5% alcoholic solution of phloroglucinol.

Preparations were viewed and photographed using the Axio scope A1 (Zeiss) light microscope with the AxioCam MRc5 digital camera and Zen 2011 software. To improve image quality, we used the program Helicon Focus with a light microscope by Carl Zeiss Axio Lab.A1.

RESULTS AND DISCUSSION

Morphology of *A. clematitis* L. herb

Raw material represented by dried leafy tops of shoots, individual leaves and their parts, pieces of stems. Stems are cylindrical, slender, unbranched (rarely subramose), slightly angled, and glabrous. Color of the stems is light green. Leaf position on the stem is alternate [Figure 1]. Leaves are simple, thin, entire, petiolar, and without stipules. Leaf blade is broadly ovate and/or rounded-triangular, up to 10 cm long, up to 8 cm wide. The base of the leaf is deeply reniform, tip pointy concave, the leaf margin is entire or slightly rough. Veins are better visible on the lower side. Dried leaves become brittle. The color of the leaves is matte green; on the upper side the color is brighter. The flowers are zygomorphous, gathered for several flowers at the axils of the leaves. The perianth is a simple corollaceous in the form of a long tube with a slanted bending, swollen at the base. The flowers have a light-yellow color. The smell of raw materials is specific, unpleasant. Taste is not determined due to information about toxicity of the plant.

As can be seen from the quoted results, morphological characteristics of *A. clematitis* L. harvested in the territory of the Russian Federation are not species-specific and have the same main characteristics as other members of the family have. The only difference is the size and shape of the leaf blade and the characteristic of the flowers.

Anatomical Research

Leaf

Leaf epidermis cells are tortuous, both on the upper and lower sides [Figure 2a and c]. Stomata occur on the lower side of the leaf blade (hypostomatous type) and are accompanied by 5–6 cells near the stomata (anomocytic type). On the surface of an epidermis, there are glands with essential oil, consisting of 4–6 excretory cells. The contents of glands when treated with Sudan III solution is colored orange [Figure 2b]. This reaction confirms the presence of terpene substances. An epidermis is represented by a single layer of cells. The outer cell wall of the upper epidermis is thicker than the inner one. Cells of the lower epidermis have papillae [Figure 3a]. On the transverse section of the leaf blade, there are many small conductive fascicles. A leaf has a dorsoventral type [Figure 3b].

Petiole

From the surface of petiole epidermal cell is short polygonal, over the veins cells are elongated rectangular. Stomata of

Figure 1: Raw material sample of *Aristolochia clematitis* L.

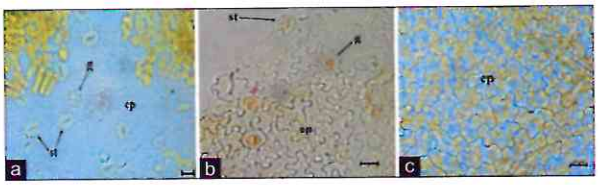

Figure 2: Leaf surface of *Aristolochia clematitis* L.: (a) Lower epidermis.; (b) lower epidermis after treatment with Sudan III solution; (c) upper epidermis Scale bar 20 μm. ep – epidermis, g – essential-oil gland, st – stomata

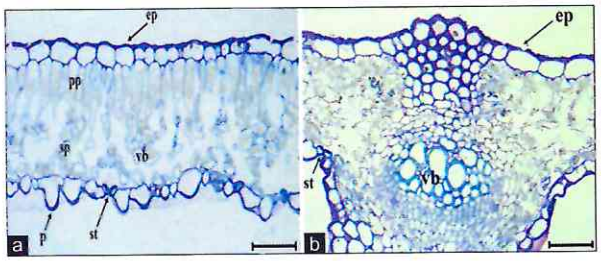

Figure 3: Cross-sections of the leaf *of Aristolochia clematitis* L.: (a) leaf blade; (b) leaf vein; scale bar 50 μm. ep – epidermis, p – papilla, pp – palisade parenchyma, sp – spongy parenchyma, st – stomata, vb – vascular bundle

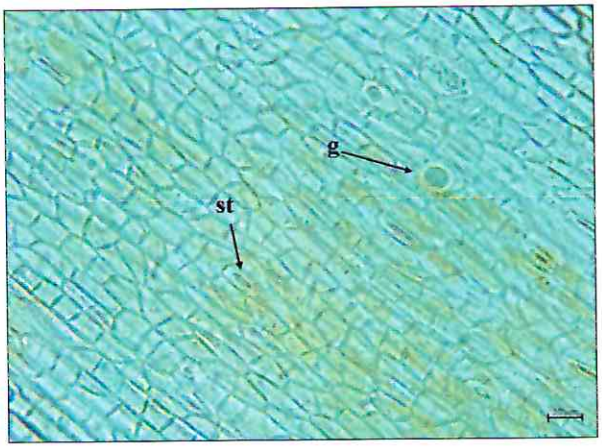

Figure 4: The epidermis of the petiole of *Aristolochia clematitis* L. Scale bar 20 μm. g – essential-oil gland, st – stomata

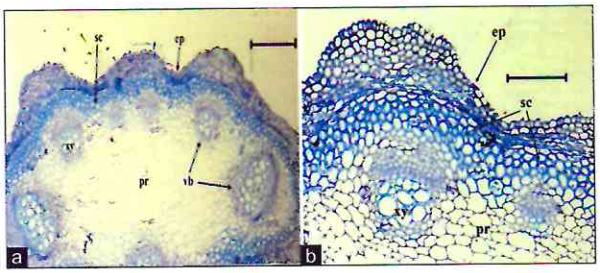

Figure 5: Cross-section of petiole *Aristolochia clematitis* L.: (a) General view of the cross-section; (b) vascular bundle of the open collateral type; scale bar a – 200 μm, b – 100 μm. ep – epidermis, pr – parenchyma, sc – sclerenchyma, vb – vascular bundle, xy – xylem

anomocytic type with 4–6 cells near stomata. Glands with essential oil are rare [Figure 4]. A petiole is cylindrical in a cross-section. An epidermis is represented by cells of almost rectangular form. Parenchyma cells (endoderm) are located under the epidermis: In 1–2 rows in interfascicular space and in 4–6 rows over fascicles. A conductive cylinder starts with a ring of sclerenchyma elements of pericyclic origin [Figure 5]. An upper border of sclerenchyma is flat, and the lower border is wavy, slightly sinking between the fascicles. Most of sclerenchyma cells are evenly thickened. A thin-walled parenchyma is located under the ring of

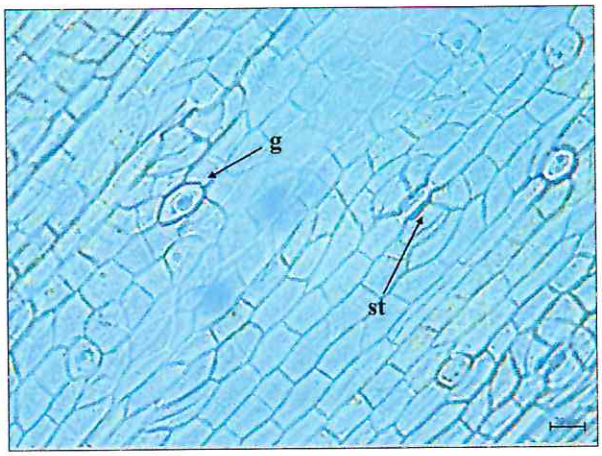

Figure 6: The epidermis of the stem of *Aristolochia clematitis* L. Scale bar 20 μm. g – essential-oil gland, st – stomata

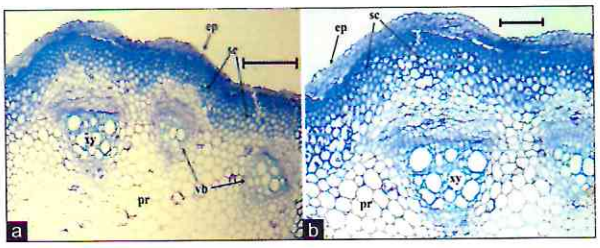

Figure 7: Cross-section of the stem of *Aristolochia clematitis* L.: (a) General view of the cross-section; (b) vascular bundle of the open collateral type; scale bar a – 200 μm, b – 100 μm. ep – epidermis, pr – parenchyma, sc – sclerenchyma, vb – vascular bundle, xy – xylem

sclerenchyma. The core consists of large parenchymal cells. Vascular fascicles are arranged inward from the pericycle in a circular row. A shape of fascicles is round or oval. The petiole has fascicles of open collateral type. Xylem consists of large vessels and a small number of parenchymal cells surrounding the xylem.

Stem

From the surface of stem, epidermal cells are rectangular in shape, larger, and more elongated compared to cells of an epidermis of petiole. Stomata of anomocytic type with 4–6 cells near stomata. Glands with essential oil are more common than on a petiole [Figure 6]. In a cross-section, the stem is grooved [Figure 7a]. The epidermis is represented by almost rectangular cells. A conductive cylinder starts with a ring of sclerenchyma elements of pericyclic origin [Figure 7b]. The cells of sclerenchyma are different in size, with progressive cavities in the direction of the core. Vessels and cell walls of the sclerenchyma are stained with an alcoholic solution of phloroglucinol into a crimson-red color, and the ring of sclerenchyma from above is colored more brightly than from below, this indicates their greater lignification. A thin-walled parenchyma is located under the ring of sclerenchyma. The core consists of large parenchymal cells. In the same way as on a transverse slice of the petiole, the vascular fascicles are located inside from a pericycle in one row. The xylem consists of large vessels and a small number of parenchymal cells

surrounding the xylem. Since the young part of the stem was taken for the sample, the phloem is poorly differentiated.

CONCLUSIONS

Morphological diagnostic features of *A. clematitis* L. herb were established: stems are winding, cylindrical, up to 30 cm long. Leaf blade is broadly ovate and/or rounded-triangular, up from 7 cm to 10 cm long, up from 5 cm to 8 cm wide, the leaf margin is entire or slightly rough, matt green color. The smell of the raw materials is unpleasant, specific. It is shown that these characteristics are not species-specific and define the belonging of a plant to the family *Aristolochiaceae*.

Anatomic diagnostic features of the raw materials are defined as anomocytic stomata can be found on the underside of the leaf blade (hypostomatic type), essential-oil-bearing glands, cells of the lower epidermis with papillae can be found on the surface of the lower epidermis. The cells of the epidermis of the petiole and stem have a prosenchymatous shape, while the cells of the epidermis of the stem are more elongated. Conducting bundles of the petiole and stem have open collateral type, located inward from the pericycle in a row in a circle. Mechanical cells have various sizes and slowly growing cavities toward the pith.

The obtained data will be included in the section "identification" in the development of the draft regulatory documentation on the *A. clematitis* L. herb.

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