

Pheasant's eye: a pharmacopoeial study of medicinal plant

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Abstract:

One of the fundamental steps in manufacturing of herbal drug products is the assessment of raw material authenticity. Usually, this is achieved by macroscopic and microscopic examination and allows to distinguish medicinal plant from related species and potential impurities/admixtures. Current edition of the State Pharmacopoeia of the Russian Federation includes "Microscopic characteristics" as a compulsory test for herbal raw material. It should be noted, that all parts of the plant must be described. In earlier editions of the State Pharmacopoeia only leaves of pheasant's eye (*Adonis vernalis*) were included in the corresponding monograph and flowers, fruit, and stem were omitted. The article describes anatomic and morphological characteristics of all parts of the plant, thus filling the knowledge gap. The results of the study can be used in routine quality control and for inclusion into an updated version of pharmacopoeial monograph on *A. vernalis*.

Keywords: *Adonis vernalis*, herb, microscopy, diagnostic features

INTRODUCTION

Macroscopic and microscopic analysis of herbal raw material is widely used to confirm its authenticity [1], which justifies the importance of morphological, anatomical, and diagnostic features study.

Authenticity assessment is one of the fundamental steps in herbal drug standardization. An optimal authenticity test should demonstrate the difference between the test object, related species and/or potential admixtures. The test should also be plant-specific and combine several methods depending on characteristics of the material under test.

In cases when drug products are based on medicinal plant material, especially pulverized one, the main method of authenticity assessment is a microscopic analysis. This method can reliably determine the authenticity of the herbal drug and the presence of impurities [2]. The Russian Federation State Pharmacopoeia and leading world pharmacopoeias use microscopy [1, 3, 4, 5, 6, 7, 8, 9, 10, 11] for herbal raw material authenticity assessment.

Current specifications for writing and revising of pharmacopoeial monographs on herbal drugs require "Microscopic characteristics" section, describing all relevant parts of the plant, to be included in the monograph, along with illustrations or photographs of characteristic morphological features. [1, 12, 13]. However, most world pharmacopoeias usually describe only powdered herbal raw material and lack photographs or, sometimes, even illustrations. The aim of the study was to assess anatomical and morphological characteristics of all parts of *A. vernalis* herb.

MATERIALS AND METHODS

Aerial parts (herb) of *A. vernalis* corresponding to the requirements of pharmacopoeial monograph was used as an object of the study [14].

Exact herbal raw material microslides preparation technique varies upon morphological group, and its fraction and the degree of fineness – uncut, milled, or powdered.

During microscopic analysis of the herb morphological group the following anatomical and diagnostic features were paid attention to [15]:

1. Anatomical and diagnostic features of leaves: character of the cuticle of the upper and lower epidermis, the form of cells of the upper and lower epidermis, sinuosity of the cells' walls of the upper and lower epidermis, sinuosity degree, thickening of the cells' walls of the upper and lower epidermis; presence of stomata, their shape (round, oval), size, frequency of occurrence

in the upper and lower epidermis, stomata type; presence and structure of the hairs on the upper and lower epidermis, their size, hair attachment (sockets), thickening of the walls, the nature of the cuticle; the presence of glands in the upper and lower epidermis, their structure, size; presence of secretory channels, laticifers, conceptacles (in parenchyma beneath the epidermis); presence and structure of crystalline inclusions and their location, size; presence of spare nutrients inclusions: mucus, inulin, etc., mesophyll structure, leaf structure; structure of leaf conductive system (form of the main vein, number, shape, and arrangement of vascular bundles in the vein, structure of vascular bundles - xylem and phloem location, presence of mechanical tissue); presence of mechanical tissue (collenchyma, sclerenchyma fibers, stone cells, bast fibers, etc.); structure of the stem: on a cross section of petiole its shape in the middle, basal, and apical parts (round, triangular, grooved, crescent-shaped, slightly webbed, wide-winged), number and location of the vascular bundles, presence of mechanical tissue (collenchyma, sclerenchyma) are indicated.

2. Anatomical and diagnostic features of flowers (petals, sepals, spatha leaves, epidermis of peduncles): nature of the cuticle of the upper and lower epidermis, shape of cells of the upper and lower epidermis, sinuosity of the cells of the upper and lower epidermis, thickening of the cells' walls of the upper and lower epidermis; presence of stomata, their shape, their size in the upper and lower epidermis, stomata type; number of peristome cells; presence and characteristics of hairs on the upper and lower epidermis, their size, hair attachment; presence and structure of the glands on the upper and lower epidermis, their sizes; presence of secretory channels, laticifers, conceptacles (in the parenchyma beneath the epidermis); presence and structure of crystalline and their size; presence of inclusions - mucilage, inulin, carotenoids, and others; pollen form, nature of its surface, size of pollen.

3. Anatomical and diagnostic features of fruits: characteristics of epidermis: nature of the cuticle (wax deposition), form of the epidermal cells (hypanthium, fruit, seed); sinuosity of the cells' walls of the epidermis; nature of the thickening of the walls of the epidermal cells; characteristics of the stomata: presence of stomata in the epidermis, their shape and size; type of stomatal apparatus, number of peristome cells; stomata absorption in the epidermis; presence of lenticels in the epidermis; presence and nature of trichomes (hairs), their size, hair attachment; secretory channels, laticifers, conceptacles; character of mesocarp parenchyma (cell shape and size, uniformity, density of location); aerenchyma presence; nature of

conduction system (location and structure of the vascular bundles); nutrients storages and their size; presence of mechanical tissue (stone cells, sclerenchyma fibers).

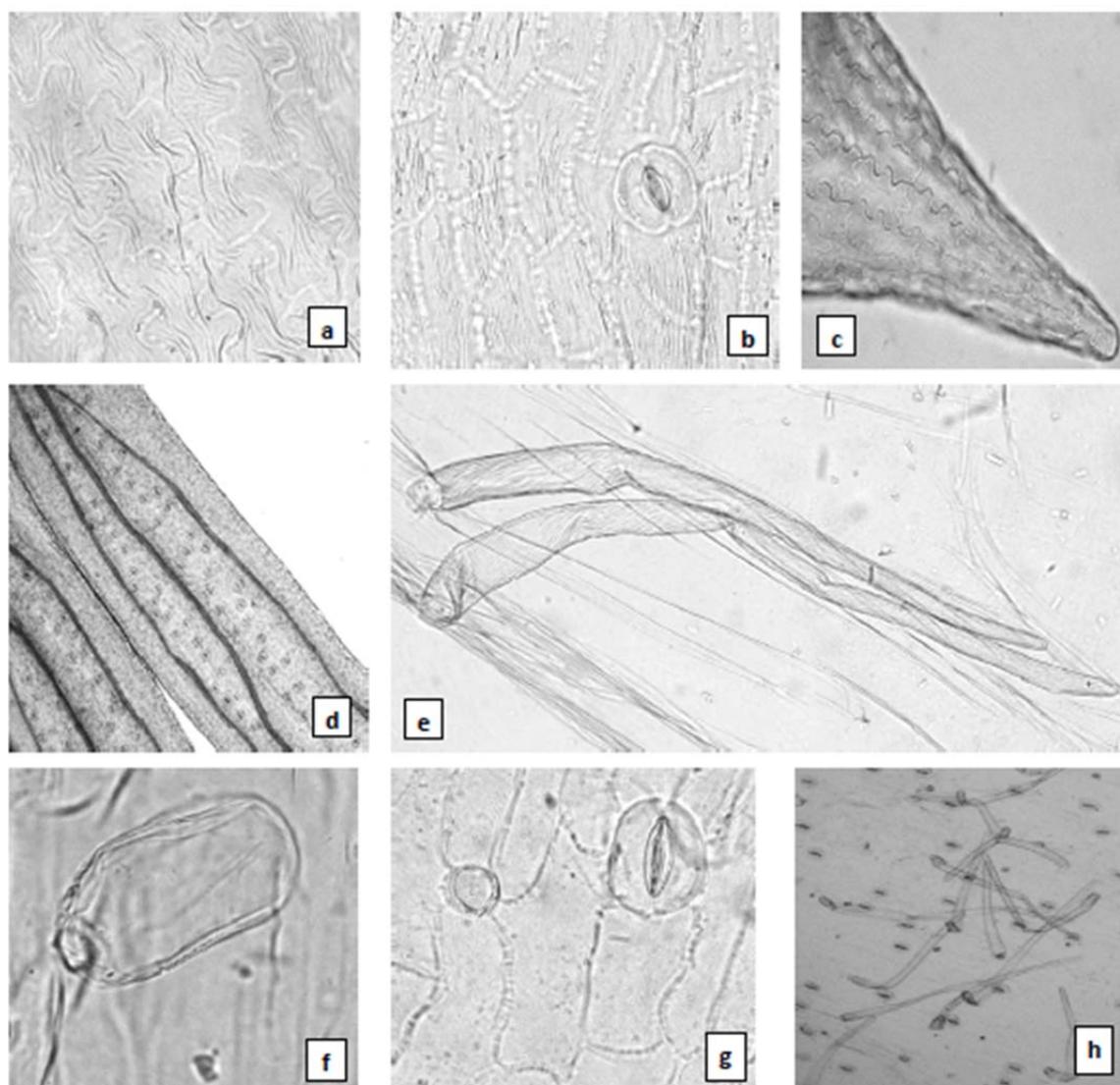
4. Anatomical and diagnostic features of stem: nature of the cuticle; form of the epidermal cells; sinuosity of the cells' walls of the epidermis and the degree of sinuosity; thickening of the cells' walls of the epidermis (presence of beaded thickening); presence of stomata and their shape, size; type of stomatal apparatus; presence, characteristics and size of the hairs, hair attachment (socket), walls' thickening (thick, thin walls), nature of the cuticle; presence and structure of glands, their sizes; presence of secretory channel, laticifers, conceptacles; presence of crystals, their structure and location, size; presence of inclusions: mucus, inulin, carotenoids and others, presence of the aerenchyma.

Method. Leaves, flowers, fruits, and stems of the *A. vernalis* were separately placed in a 50 ml beaker, then 5-10 ml of 5% sodium hydroxide solution were added, and the content of the beaker was boiled for 2-5 minutes using a hot plate. After that the solution was decanted, and the material was fractionally washed with purified water, waiting for the complete sedimentation of the particles before each next decantation. After clarification of the washings 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) with a 10× eyepiece and several lenses (4×, 20×, and 40×). The photographs were obtained using Canon PowerShot G1X digital camera (Canon, Japan) and the pictures were processed using Adobe Photoshop CS6 software (Adobe, USA).

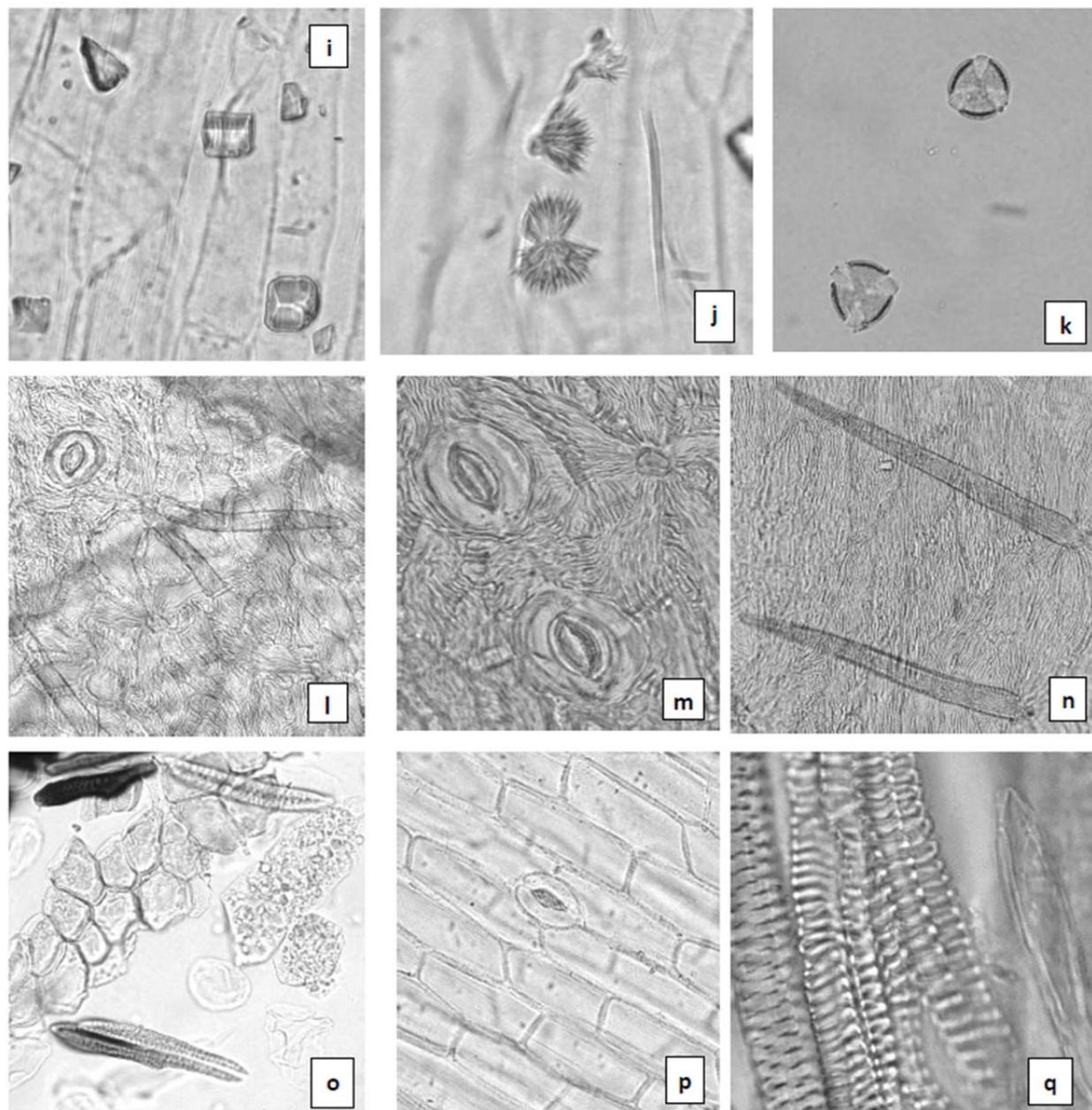
RESULTS AND DISCUSSION

The microscopic analysis of *A. vernalis* leaves have revealed large epidermal cells with very sinuous walls (Figure 1a, 1c), slightly stretched along segment. Some epidermal cells demonstrate beaded thickening (Figure 1b). The cuticle is strongly striated, wavy (Figure 1a, 1b). Large, oval anomocytic (surrounded by 4-5 epidermal cells) stomata are located only on lower surface of the leaf and are slightly risen above the surface (Figure 1b). The stomata are oriented along leaf blade (Figure 1d). Scarce unicellular hairs are located along leaf segments and at its base: long, ribbon hairs with rounded tip, narrow at the base (Figure 1e), and short clavate hairs, narrow near attachment place (Figure 1f). All hairs have spirally-striated cuticle (Figure 1e) and are fixed atop very small round epidermal cell (Figure 1f).



a – leaf epidermis fragment with very sinuous walls and striated cuticle (200×); b – leaf epidermis fragment: anomocytic stomatum and beaded thickening of the cell walls (200×); c – top of the leaf lobe (200×); d – stomata oriented along leaf blade (40×); e – simple unicellular hairs (200×); f – clavate hair (400×); g – sepal epidermis fragment with beaded thickening, anomocytic stomatum and hair attachment site (400×); h – epidermis fragment with simple unicellular hairs and stomata aligned lengthwise (squash preparation) (200×).

Figure - 1. leaves (a-f), sepal (g-h)



i – prismatic calcium oxalate crystals (400×); j – calcium oxalate spherocrystals (400×); k – pollen (200×); l – fruit epidermis fragment: thick-walled cells with beaded thickening, striated cuticle, hair (200×); m – fruit epidermis fragment: anomocytic stomatum and hair attachment site (400×); n – fruit epidermis fragment: single simple hairs (200×); o – fruit epidermis fragment (squash preparation): endocarp, stone cells and endosperm cells with starch and fatty oil drops (200×); p – stem epidermis fragment with rectangular elongated cells and anomocytic stomatum (200×); q – reticular (a) and helical (b) vessels of the stem (200×).

Figure - 2. Crystalline inclusions (i-j), pollen (k), fruit (l-o), stem (p-q)

Examination of sepals reveals rectangular elongated epidermal cells with straight or slightly sinuate cell walls and pronounced striated cuticle (Figure 1g), sometimes with beaded thickening. Anomocytic stomata are located on the outer surface (Figure 1g) and are aligned lengthwise. Characteristic hairs can be observed all over the sepal. Some long, ribbon hairs are fasciculate (2-3 hairs per bundle) (Figure 1h). Single prismatic calcium oxalate crystals (Figure 2i) and spherocrystals (Figure 2j) are found in mesophyll. Petals' epidermis consists of rectangular elongated cells with sinuate cell walls. Round pollen grains are triporate (Figure 2k).

An exocarp cells with beaded thickening of its thick walls can be seen in fruit squash preparation. The cuticle is pronouncedly striated (Figure 2l). Hairs are simple, unicellular, with spiral

striated cuticle. Epidermal cells at the base of the hairs have wrinkled cuticle (Figure 2m). Hairs are attached to the epidermis either alone or in pairs/triplets (Figure 2l, 2n). Separate sclereids and their layers are observed. Endocarp cells are polygonal. Endosperm consists of polygonal cells filled with aleurone grains and drops of fatty oil (Figure 2o).

A rectangular elongated epidermal cells with straight cell walls, some with beaded thickening (Figure 2p) can be observed in stem squash preparation. Anomocytic stomata are oriented along the stem (Figure 2p). Epidermis has pronounced lateral striated cuticle around the stomata. Pith parenchyma consists of oblong cells with porous thickened envelope. The vascular system is represented by helical, scalariform, and reticular vessels (Figure 2q).

CONCLUSION

The results of the study can be used in routine quality control and for inclusion into an updated version of pharmacopoeial monograph on *A. vernalis*.

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