**Acmella oleracea**: A Comprehensive Study of Anatomical and Diagnostic Characteristics

Alexey Arkad’evich Matyushin, Olga Vladimirovna Evdokimova

Sechenov First Moscow State Medical University, 119991, Moscow, Russian Federation, Trubetskaya Street, 8-2

**Abstract**

The article describes anatomical and diagnostic characteristics of a promising source of biologically active substances – *Acmella oleracea*. *A. oleracea* belongs to Asteraceae family and is native to Brazil and Peru, grows in India; it also can be introduced in humid continental climate of Moscow region, but only as an annual plant due to its sensitivity to frost. It is a well-recognized traditional remedy for toothache and gum diseases. Previous studies have demonstrated its antioxidant, antiinflammatory, antibacterial, hepatoprotective, and analgesic activity. The plant is very rich in various chemical substances, namely N-alkamides, flavonoids, coumarins, polysaccharides, and terpenoids. Recent publications have introduced several methods of quantification of these components. However, no studies were specifically devoted to comprehensive description of anatomic and diagnostic features of all aerial parts of *A. oleracea*, which can be important for authentication of herbal drug products containing this herb. Therefore, the aim of the study was to describe anatomical and diagnostic characteristics of *A. oleracea* that can be used to distinguish the plant from contaminating species and prevent adulteration and falsification of herbal drugs. The study was conducted using dried aerial parts of the herb which were separated into flowers, leaves, and stems. The slides for microscopic examination were prepared and examined according to the Russian State Pharmacopoeia requirements. The authors conclude that the results of the study can be used for identification purposes and in regulatory documents development.

**Keywords**: acmella olerace, spilanthes, diagnostic features, microscopy

**INTRODUCTION**

The need for new sources of biologically active substances is driven by the growth of herbal drug products consumption and increasing demand for novel drug products. One of the possible ways to find novel promising drug products is the thorough study of herbs and herbal remedies, that are used in traditional medicine of different countries.

One of such herbs is *Acmella oleracea* (L.) R.K. Jansen, which is native to Brazil and Peru, but also grows in India, China, Taiwan, and can be introduced in humid continental climate, such as Moscow region as an annual plant [1, 2]. *A. oleracea* is a perennial (annual in some regions due to frost intolerance) herbaceous plant which is also known throughout the world as para cress, toothache plant, Brazilian cress, sechuan button, and eyeball plant. A large number of botanical synonyms, numerous reclassifications, and an ongoing discussion among botanists add to the general confusion about the proper naming of the plant. For example, The Plant List and several other sources list various synonyms for this plant, namely *Anacyclus pyrethra*ria* (L.) Spreng., Bidens fusca Lam., Spilanthes oleracea L., Spilanthes acmella Jacq., and Spilanthes acmella auct. non (L.) Murr.; however, they all seem to be of the same plant, erroneously attributed to different genera [3, 4, 5, 6].

The plant is about 15-30 cm in height, stems decumbent to usually erect, glabrous to sparse pilose near the top, green to red in color. Petiole is glabrous, narrowly winged. Leaves are opposite, simple, green to reddish; leaf blade is broadly ovate to deltate, glabrous, with dentate margins, truncate bases, and acuminate to acute apexes. Inflorescences are discoid, globe to short conical, yellow with central disk flowers emerging maroon to red, turning yellow-golden as they mature. Water extracts of the plant have specific, pungent taste [2]. *A. oleracea* shows a wide range of pharmacological properties. For example, its extracts are used in odontalgia and parodontitis [7, 8]; have analgesic [9, 10] and antiinflammatory properties [11]. A DPPH assay confirmed strong antioxidant activity of *A. oleracea* [12], along with sufficient hepatoprotective properties, both in paracetamol-induced hepatic damage [13], and in aflatoxin B1-induced damage [14].

Several studies investigated chemical composition of *A. oleracea*, and it was found, that its main chemical constituents are flavonoids, coumarins, polysaccharides, and terpenoids [15, 16]. Spilanthal, an N-alkamide which is also found in several other species of the Asteraceae family, is recognized as the constituent responsible for the local anesthesia induced by different parts of the plant [17].

One of the main steps in herbal drug standardization is the proper identification of herbal raw materials. The previous studies have investigated the anatomical and diagnostic characteristics of *A. oleracea* to some extent [18, 19]; however, current pharmacopoeial quality control criteria needed for ensuring the identity of herbal raw materials require description of macro- and microscopic characteristics of all aerial parts, i.e. leaves, flowers, and stems of medicinal herbs to distinguish them from related species and possible impurities [20, 21]. Therefore, the aim
of the study was to comprehensively describe anatomic and
diagnostic characteristics of *A. oleracea* herb – a
promising source of biologically active substances.

**MATERIALS AND METHODS**

Herbal raw material (*Acmella oleracea* herb) was cultivated
and harvested in the Moscow region in the summer 2016,
then aerial parts of the plant were shade dried for 10-12
days on drying frames with frequent stirring in order to
provide adequate air circulation.

After the end of the drying period the material was
separated into flowers, stems, and leaves, then the slides for
microscopic examination were prepared using the
following method: about 0.1 g of the material were 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 10-20 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 Petri dish where it was separated into
different parts according to the General Pharmacopoeial
Monograph “A technique of microscopic and
microchemical study of herbal medicinal raw materials and
herbal medicinal products” [21].

Separated parts of the material were 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 description of slides was performed according to the
requirement of the general pharmacopoeial monographs on
the corresponding morphological groups. All shots are done
in surface view.

The microscopic analysis of *A. oleracea* leaves have
revealed cells of the lower epidermis with thin sinuous walls
(Figure 1a), cells with evenly thickened cell walls (Figure
1b), and anomocytic stomata of various sizes, surrounded
by 5-7 cells. Upper epidermis is represented by cells with
thin, slightly sinuous walls.

A large simple multicellular thick-walled scarce hairs
(Figure 1c), sometimes with brown content (Figure 1d), can
be observed at the edge of the leaf blade. The hair is
surrounded by subsidiary cells (Figure 1e).

The microscopic analysis of *A. oleracea* flower petals have
revealed upper epidermal cells with papillae and drops of
volatile oil (Figure 1j), and lower epidermal cells with
sinuous walls (Figure 1k).

The mesophyll of petals also contains drops of volatile oil.
The vascular system is represented by helical vessels
marginated by slit-like pores (Figure 1l); vesicles with
brown content can be seen along the vessels (Figure 1m).
Ground tissue of unripe achenes is composed of thick-
walled cells (Figure 2n).

The microscopic analysis of *A. oleracea* flower sepals
have revealed polygonal rectangular thickened cells of
upper epidermis located over vein with striated cuticle
(Figure 2o) and polygonal cells (Figure 2p). Scarce
anomocytic stomata can be seen in the upper epidermis,
and cells with very sinuous walls and multiple anomocytic
stomata – in the lower epidermis (Figure 2q).

The following types of hairs can be observed on the surface
of the sepal:

- simple multicellular thick-walled (sometimes with
  brown content) hairs located along the edges of sepal
  and bent to the surface of the sepal (Figure 2r);
- simple multicellular thin-walled hairs (subsidiary cells
can be seen) (Figure 2s);
- bifarious hairs forming bundles (Figure 2t).

Lower epidermal hairs are simple, without content.
The parenchyma of sepals is represented by rounded thin-
walled cells. Vesicles with uniform and non-uniform
content (Figure 2u) are located along the veins (after
histochemical reaction with Sudan III the content was
partially colored orange), drops of volatile oil are also
present. The pollen (Figure 2v) is tricolpate, with spiny
surface and smooth intina; lypophilic content is found
inside the pollen.

The microscopic analysis of *A. oleracea* stem have
revealed polygonal rectangular epidermal cells with straight
thin walls (Figure 2w) and oblong cells with sinuous walls
and anomocytic stomata (Figure 2x). Scarce simple thin-
walled unicellular hairs with brown content can be
observed (Figure 2y).

Vesicles with non-uniform content and drops of volatile oil
(Figure 2z) can be seen in in the mesophill, along with
separate drops of volatile oil. The vascular system is
represented by helical and annular vessels (Figure 2aa).
Figure 1. Leaves (a – i):

a – lower epidermis cells, anomocytic stomata of various sizes (200×);
b – cells with thickened walls (200×); c – simple hair (200×); d – fragment of a simple thick-walled hair with brown content (200×); e – fragment of an epidermis with subsidiary cells (200×); f – parenchymal cells (200×); g – secretory vesicles along the vessels (200×); h – annular and scalariform vessels (400×); i - helical and scalariform vessels (400×). Petals (j – m): j – upper epidermal cells with papillae (200×); k – lower epidermis cells (200×); l – helical vessels (200×); m – vesicles along the vessels (200×).
Figure 2. Petals (cont., n): n – achene ground tissue (200×).

Sepals (o – v): o – polygonal rectangular cells of upper epidermis over vein with striated cuticle (200×); p – upper epidermis polygonal cells (200×); q – lower epidermis cells with very sinous walls and anomocytic stomata (200×); r – simple multicellular hair with brown content (200×); s - simple multicellular thin-walled hairs (200×); t – bifarious hairs (200×); u – vesicles along vessels (200×); v – tricolpate pollen (400×). Stem (w – aa): w – polygonal rectangular epidermal cells (200×); x – oblong epidermal cells (200×); y – thin-walled hairs (200×); z – vesicles with non-uniform content (200×); aa – vascular system (200×).
CONCLUSION
Anatomical and diagnostic characteristics of *A. oleracea* leaves, flowers, and stems which allow proper identification of this herbal raw material are described. The results of the study can be used for standardization and quality control of *A. oleracea* herbal raw material and herbal drug products containing this plant.

REFERENCES