



Macroscopic and Microscopic Diagnostic Features of the potential Herbal Drug *Crataegus almaatensis* Pojark Endemic in Kazakhstan

Elmira N. Bekbolatova^{*a}, Natalya V. Kurbatova^b, Zuriyadda B. Sakipova^a, Liliya N. Ibragimova^a, Saltanat I. Alpysbayeva^c, Akmaral T. Kabdenova^c, Wirginia Kukula-Koch^d, Fabio Boylan^e

^aSchool of Pharmacy, Asfendiyarov Kazakh National medical University, Almaty, Kazakhstan, ^bBiodiversity and Bioresources Department, Al-Farabi Kazakh National University, Almaty, Kazakhstan, ^cDepartment of Specialized Expertise of Medicines, National Center of Expertise of Medicines and Medical Devices, Almaty, Kazakhstan, ^dDepartment of Pharmacognosy with Medicinal Plants Unit, Medical University of Lublin, Lublin, Poland, ^eSchool of Pharmacy and Pharmaceutical Sciences, Panoz Institute, Trinity College, Dublin 2, Ireland

Abstract

This study presents the results of anatomic-morphological examination of vegetative (leaves) and generative (flowers, fruits) organs of the endemic plant *Crataegus almaatensis* Pojark – family Rosaceae, growing in the foothills of Ile Alatau mountains, Almaty region of the Republic of Kazakhstan. The plant *Crataegus almaatensis* Pojark was examined during the phenological phase of flowering and fruit ripening periods. The macroscopic and microscopic diagnostic features were established, which will allow for the standardization of this herbal plant in order to ensure its quality, safety, and efficacy and to avoid adulteration, misuse in the process of medicines' preparation on its basis. For the first time significant diagnostic macroscopic and microscopic features of the dried vegetative and generative organs of *Crataegus almaatensis* Pojark were described in detail. Currently not much is known about this species and this manuscript brings the information which are studied for the first time, according to the authors' knowledge.

Key words: anatomy, morphology, *Crataegus almaatensis*, hawthorn, macroscopy, microscopy.

Corresponding Author: Elmira N. Bekbolatova, School of Pharmacy, Asfendiyarov Kazakh National medical University, Almaty, Kazakhstan

Tel: (+7) 707 1177706

E-Mail: elmirajumagulova@gmail.com

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1. Introduction

Nowadays the interest in the drugs of natural origin is increasing significantly and it is estimated to cover 25% of all the prescribed medicines in the world market [1, 2]. According to the World Health Organization (WHO), more than 80% of the world's population prefer traditional medicine as their

first choice of medication treatment [3]. Phytomedicines are easily assimilated in the human body and sometimes can possess lower toxic effect in comparison to synthetic analogs. Moreover, medicines from plants are easily available, economically affordable, however they are prone to being adulterated [4,5,6]. Adulteration usually may take place by substitution with another plant material or by the addition of low quality substances. The therapeutic effect of medicinal plants is only exhibited due to the presence of a certain quantity of the biologically active compounds [7]. Usually the misuse of the herbal drugs plant can be as a result of their wrong botanical identification. All these problems can be solved out by setting up right pharmacognostic specifications for a certain herbal drug. Pharmacognostic studies will help in authentication of a herbal drug and will ensure the reproducible quality, safety and effectiveness of the medicines on this basis [4]. Macroscopic, microscopic examinations are one of the pharmacognostic indices in ensuring the quality of herbal material [8].

The herbal drug which requires full attention in this study is the endemic *Crataegus almaatensis* Pojark (*C. almatensis*) from Kazakhstan. *Crataegus L.* is native to Northern temperate zones with approximately 280 species. Each *Crataegus L.* species are native to certain region, for example *Crataegus pinnatifida* (Chinese hawthorn), *C. pubescens* (Mexican hawthorn), *C. cuneata* (Japanese hawthorn), *C. laevigata* and *C. monogyna* (Europe), *C. oxyacantha* and *C. aronica* (Middle East), *C. phaenopyrum*

(American hawthorn) and *C. ambigua* (Russian hawthorn). Hawthorn is usually grown as large shrubs or small trees, and in most cases is covered by thorns. Mostly they produce dense white coloured flowers, bright to dark green leaves with nearly entire to deeply lobbed leaves and berries with colour ranging from yellow to dark red [9-12]. *Crataegus L.* species are rich in different biologically active compounds to which their pharmacological activity could be attributed. Numerous laboratory tests and clinical trials have demonstrated Hawthorn's efficacy in treating various cardiovascular diseases and its positive effects on chronic congestive heart failure [13,14]. Chinese hawthorn is more famous for its ability to reduce total blood cholesterol [15], while in Mexico along with heart problems, it is also used for the treatment of cough [16]. There are numerous studies proving positive pharmacological activities of *Crataegus L.* such as antihypertensive, antiarrhythmic, antioxidant, anticataract, anti-inflammatory, and antimicrobial activities [17-20]. The main active compounds of *Crataegus L.* are proanthocyanidins and glycosylated flavonoids [21, 22]. Additionally, Hawthorn flowers, leaves and fruits contain sugars, phenolic acids, terpenes, essential oils, phenylpropanoids, fatty acids among other less important compounds [9, 23-25].

Several species of hawthorn are officially registered in the pharmacopoeias of several countries, such as China, USA, Germany, France, England, the European Union [26], and in the Republic of Kazakhstan [27]. There are seven species of hawthorn growing in

Kazakhstan, from which *C. almaatensis* is an endemic one (28). Up to date *C. almaatensis* is not an official herbal drug and there are no full scale pharmacognostic studies with it, except for the few attempts on biochemical evaluation of cultivated samples [29, 30]. The aim of this work is to present the results of macro and microscopic evaluations on *C. almaatensis* leaves, flowers, and fruits as a part of full scale pharmacognostic studies. The purpose of diagnostic morpho-anatomical studies is to set up standardization criteria to ensure the quality, safety, and efficacy of the raw herbal drug and therefore the medicines developed using *C. almaatensis*.

2. Materials and Methods

2.1. Plant Material

The leaves, flowers, and fruits of *C. almaatensis* were collected at the foothills of Ile Alatau Mountains, in Medeo valley, Almaty region, Kazakhstan in September 2015 (fruits, leaves) and May 2016 (flowers) and authenticated in the Institute of Botany and Phytointroduction, Almaty, Kazakhstan, by the head of the High Plant Flora Laboratory, Dr. G. Kudabayeva and confirmed by the general director Dr. G. Sitpayeva (reference letter 01-04/456 from 10.11.2015). The harvested plants were dried in a predetermined condition and stored in a bag made of kraft paper (31-32).

2.2. Macroscopic Studies

The macroscopic study is the morphological description of the plant parts which was carried out by a naked eye placing the plant material on a white paper surface.

Organoleptic features such as shape, size, colour, odour, taste of leaves, flowers, and fruits were evaluated.

2.3. Microscopic Study

The microscopic study is the anatomical study which is done by taking appropriate sections of the plant parts to be further studied. Transverse sections of plant parts (leaves and flowers) were treated with a solution of chloral hydrate for 1hr, then were placed on to the microscopic slide, divided into two pieces by the dissecting needle and stained with phloroglucinol followed by addition of 1-2 drops of conc. HCl. Microscopic sections of fruits were prepared by means of deep freezing microtome. The thickness of the materials was 10-15 μm .

The slides were covered by cover slip, and were observed from both sides under the microscope first through small (x100), then on high (x400) magnification with means of Olympus BX41 microscope at the School of pharmacy and pharmaceutical sciences, Trinity College, Dublin, Ireland. The photos were taken simultaneously with DP 25 digital camera, and processed by cellⁿB –Analysis Image processing system. Microscopic studies of stamen and pistil were carried out on Leica DM 6000 M microscope. The well established methods in the anatomical evaluation of plants were applied during preparing and describing the preparations [33-35].

3. Results and Discussion

3.1. Morphological Features

C. almaatensis is a small tree up to 3-4 m high, with a wide crown. Young sprouts are cherry-red coloured, glabrous, shiny, with very rare 1-1.5 (3) cm long thorns. Also, the old branches are variegated, brownish-grey coloured (Figure 1,2).

When studying the external features of *C. almaatensis* leaves following specific characteristics, it was possible to observe: leaves are dark-green coloured on the adaxial surface, fainter green colored on the abaxial surface, 4 to 6 cm long, 3 to 5 cm wide, ovoid shaped, incised into 5(7) lobes, on the adaxial surface up to $\frac{1}{3}$ depth, on the abaxial surface up to $\frac{1}{2}$ depth of leaf blade. The lobes are ovoid shaped, in rare cases tapered into short curly sharp apexes, roughly sharp-toothed along the edges, with cuneated basis. The single trichomes were observed on the adaxial surface along the veins of the leaf blade, the

lower blade is covered in a dense number of trichomes. Reticulated venation, red-brown coloured vein can be observed on the abaxial blade (Figure 3).

The flowers are in crumbly, compound, multiflorous, bare corymb, shorter than leaves. Triangular sepals are sharp, reaching the middle of ovary, reflected in the fruits. Corollas are up to 18 mm in diameter, with 3 to 5 columns. Choripetalous corolla yellowish-white coloured. Twenty stamens with purple anthers, with 2 to 5, more often 3 to 4 columns. Pistil consists of 3 to 5 carpel, inosculated with concave receptacle (Figure 2, 4).

Fruits (berries) are wide-ovoid, black-purple, 11 to 14 mm long, 5 to 8 mm wide, shiny, with reticulated surface. There are 3 to 5 elongated, smooth, shiny, triangular seeds in a reddish fleshy pulp. The seeds are triquetrous with smooth sides, on a spiral side with 2 to 3 superficial grooves and sharp-



Figure 1. *C. almaatensis* fruits.



Figure 2. *C. almaatensis* flowers and leaves.

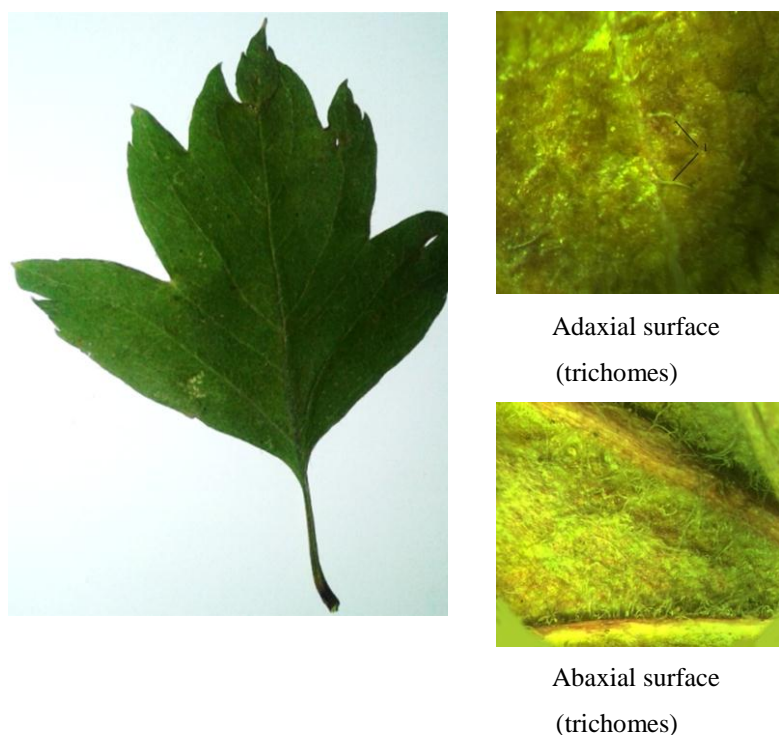


Figure 3. Morphological features of *C. almaatensis* leaves.



Figure 4. Structure of *C. almaatensis* stamen and pistil.

triangular hypostyle, which is $\frac{1}{3}$ shorter than seeds. The Odourless one was slightly sweet in taste (Figure 1).

3.2. Microscopic Features

C. almaatensis leaves possess deeply located central vein and less deepened numerous lateral veins. The epidermal cells have undulated anticlinal walls with numerous stomata, surrounded by 3 to 5 subsidiary cells.

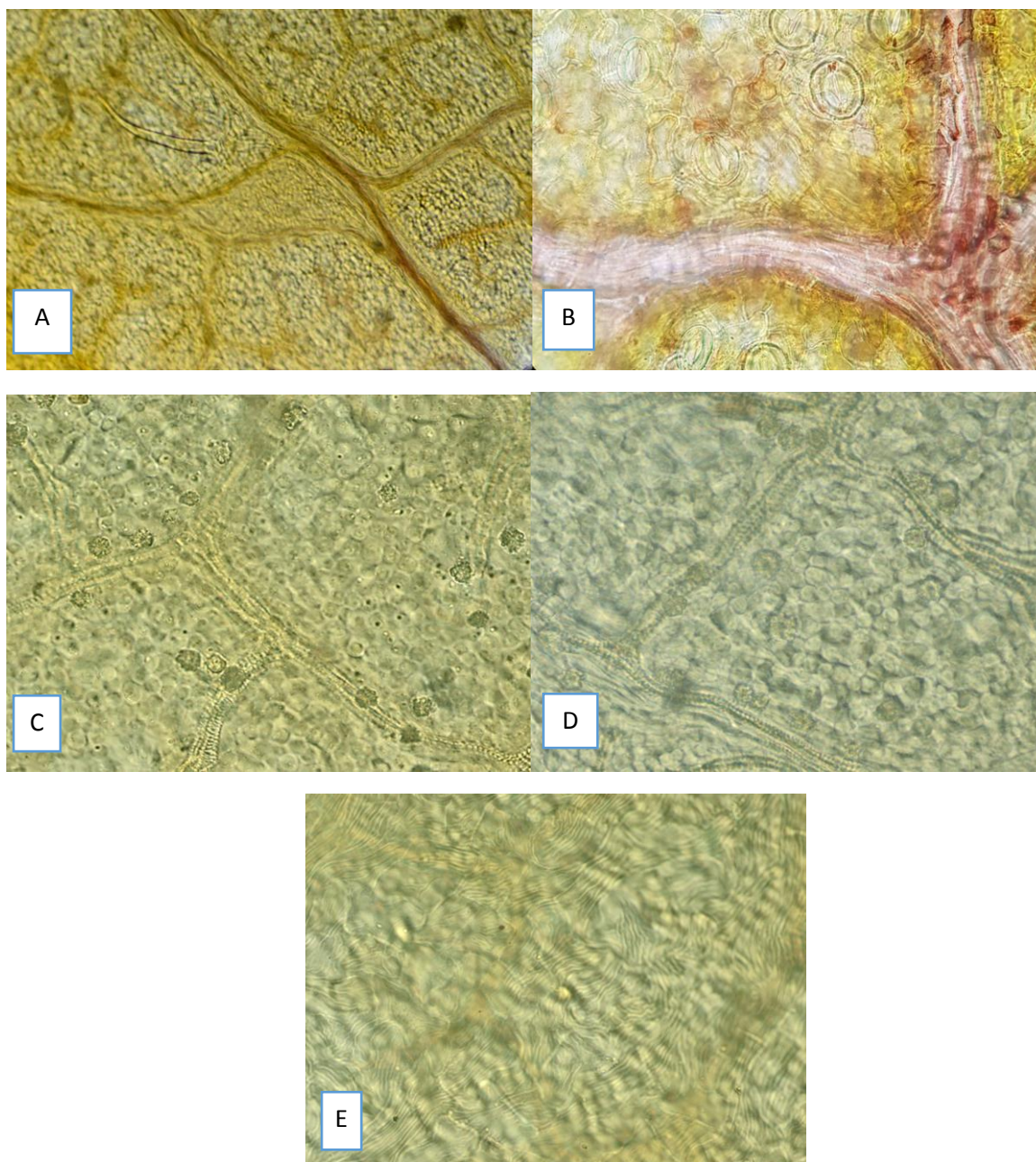


Figure 5. Anatomic features for the authentication of *C. almaatensis* leaves (a) trichomes, (b) stomata, (c) parenchymatous cells, (d) calcium oxalate clusters, (e) cuticular striations.

Upon carrying microscopic studies the following anatomical features were observed: fragments of lamina have simple single cell trichomes, widening towards the base (Figure 5.1), epidermal cells with undulate walls, veins surrounded with a sheath of calcium oxalate prism crystals, numerous anomocytic stomata and numerous globular type glands (Figure

5.2). Stomata guard cells are surrounded by cells, which are not different from other epidermal cells. The parenchymatous cells of the mesophyll contain calcium oxalate clusters in general sized from 5 to 15 micrometres, associated with veins (Figures 5.3, 5.4). Fragments of weakly sinuous polygonal epidermal cells, of which individual cells

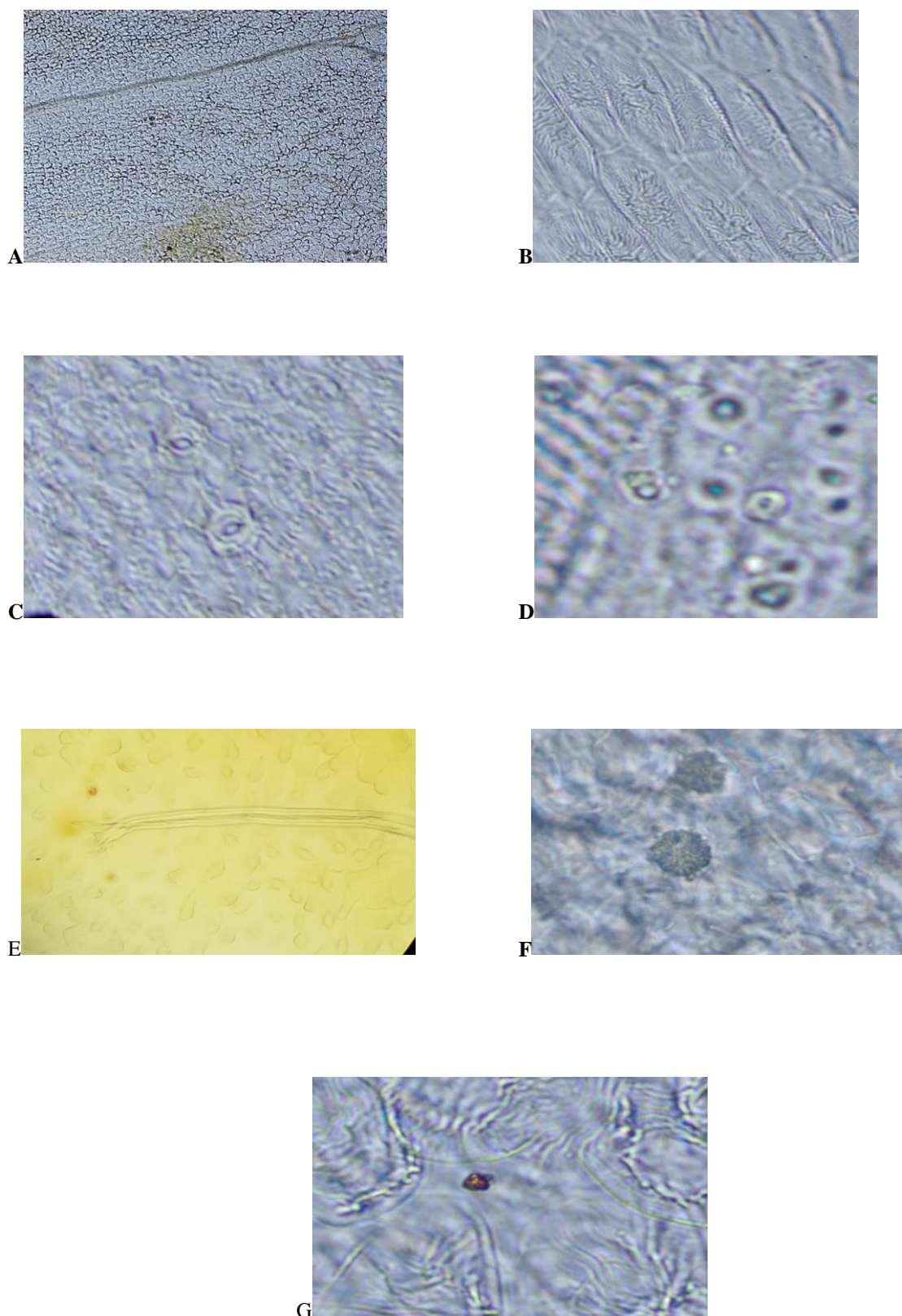


Figure 6. Anatomic features for the authentication *C.almaatensis* flowers (a) petal cells, (b) epidermal cells, (c) stomata, (d) globular glands, (e) trichomes, (f) calcium oxalate clusters, (g) pollen grains

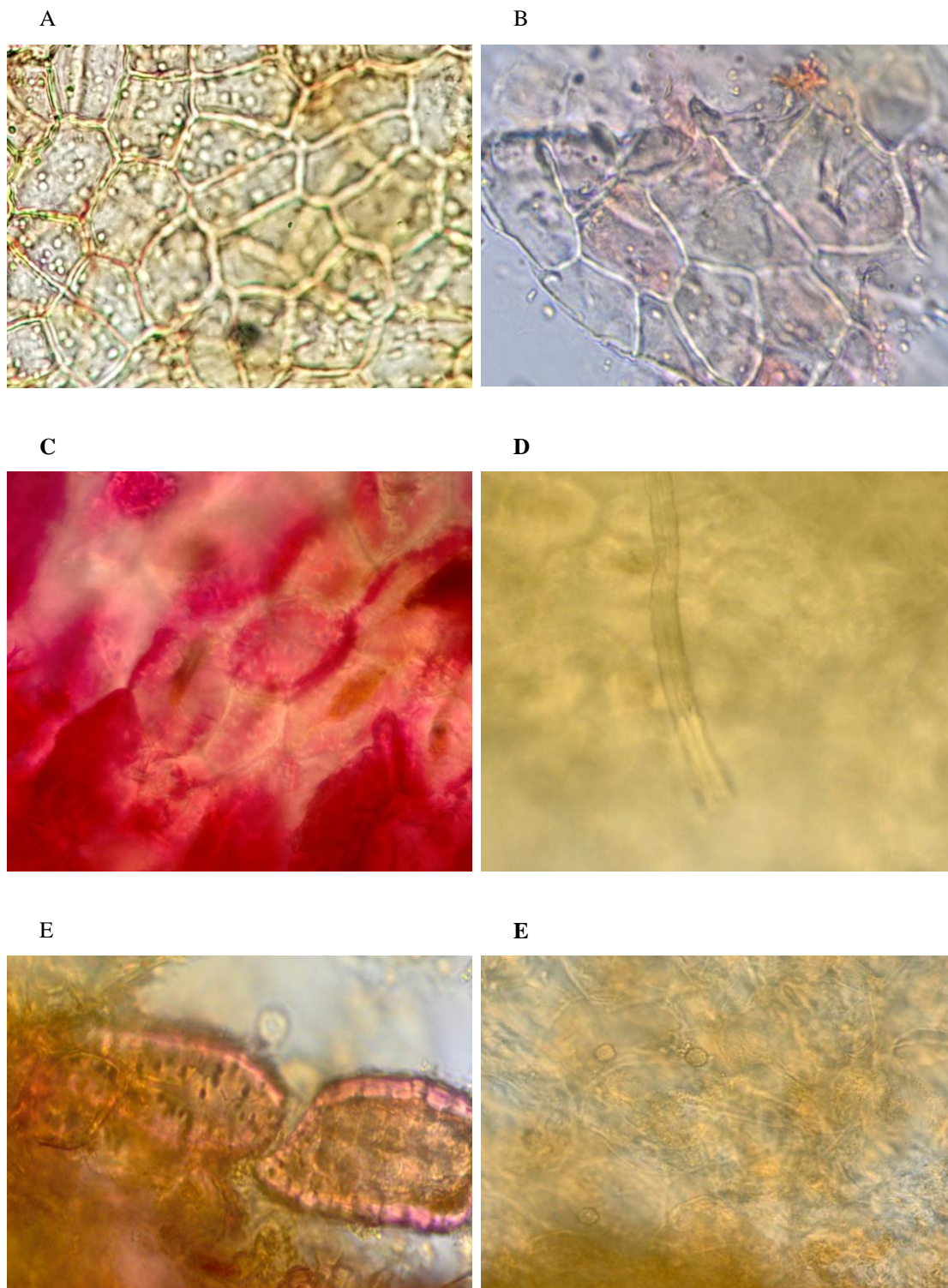


Figure 7. Anatomic features for the authentication *C.almaatensis* fruits (a) parenchyma cells, (b) aleurone grains, (c) sclereids, (d) trichomes, (e) parenchyma cells with reddish-brown content, (f) calcium oxalate prisms.

contain drops of oil and are covered by wavy cuticular striations (Figure 5.5).

Flowers. Anatomic fragments of petals show the presence of the fine elements of

vascular tissues, which form single and branched veins through the whole surface of the petal. The petal cells contain rounded polygonal epidermal cells, with thin walls and with cuticle which has weakly striated appearance (Figure 6.1).

When examining *C. almaatensis* flowers, the following anatomic features were observed: the sepals and petals from surface view have the epidermal cells with slightly thickened walls on its outer side and striated cuticles (Figure 6.2), rare presence of large anomocytic stomata located on the outer surface of sepals (Figure 6.3). The whole surface of sepals contain multicellular globular glands (assidenous and on multicellular “stipes”) with yellowish-brown content (Figure 6.4), on the surface – there are rare, simple, unicellular trichomes with thick walls, smooth, sharpening towards the apex, straight or weakly curved, and widened at the base (Figure 6.5), in the sepal mesophyll there are calcium oxalate clusters (Figure 6.6), few pollen grains of various shapes with 3 germinal pores (Figure 6.7).

Fruits (berries). Microscopic examination of fruits revealed the following anatomic features: the fragments of fruits reflect the parenchyma cells of the fruits pulp, with red coloured outer surface, 4 to 6 angular with uniformly thickened walls and with yellow-brownish content in some individual cells (Figure 7.1), parenchyma cells containing aleurone grains (Figure 7.2), fragments including thick walled sclereids with attached cells (Figure 7.3), long, unicellular, tapering to a point, warty trichomes (Figure 7.4),

fragments of parenchyma cells with reddish-brown content on an outer surface (Figure 7.5), come cells of inner layer, containing large calcium oxalate prisms (Figure 7.6). In rare case the fragments of seed cover can be observed, under which there is a yellow layer, containing the thick walled parenchyma endosperm cells with aleurone grains.

4. Conclusion

Authenticity of any raw herbal material needs to be standardized using approved techniques in order to avoid possible adulterations and the misuse of wrong drugs. Macroscopic and microscopic studies are one of the important tools composing the botanical identification of a herbal drug. *C. almaatensis* is an endemic plant from Kazakhstan and as no detailed standardized work has been reported in the literature for this plant so far this work provides unique authenticity features, which will be useful for the drafting of a monograph enabling the inclusion of *C. almaatensis* into the State Pharmacopoeia of the Republic of Kazakhstan.

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