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PHYTOCHEMICAL AND MORPHO-ANATOMICAL PROPERTIES OF *ALCHEMILLA MOLLIS* (BUSER) ROTHM. GROWING IN TURKEY

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Abstract

Stem, leaf and petiol morphoanatomy were studied in an important medicinal plant *Alchemilla mollis* (Buser) Rothm. for the pharmacognostic and taxonomic species identification. There are eglandular and glandular hairs on the stem, petiole and leaf. Leaf is bifacial. Cluster and rosette crytals of calcium oxalate are seen in the leaf and stem. Stomata type is anomocytic. Tanninsare found in both anatomical and prechemical studies. In addition, phytochemical evaluation revealed the presence of carbohydrate, flavonoids and coumarins.

Introduction

Alchemilla mollis (Buser) Rothm. (Rosaceae) is a perennial plant and growsin the mountains of Europe especially in Bulgaria where the species is critically endangered (Vitkova *et al*. 2011). However, the species has a further wide distribution particularly in theNorth Anatolia regions of Turkey (Davis 1974). A few studies have been found in the literature on the anatomy and morphology of *Alchemilla* species (Violetta 2011a,b,c). However,morphological and anatomical properties are important for the taxonomy of related species. The species belonging to the genus *Alchemilla* has variable forms which presents interesting taxonomical properties. Most of them are apomictic, so descriptions of some species based only on morphological characters is difficult (Violeta 2011a). The aim of the present research was to study the morpho-anatomical properties of *A. mollis* because prechemical and physicochemical studies are required for the identification of the plant medicinally.

Material and Methods

Samples of *A. mollis* were collected during the flowering period (June, 2012) in the Kayseri province of Turkey (38.64544 N - 35.5371 E). Voucher specimens (AEF 26256) are deposited in the Herbarium of the Faculty of Pharmacy, Ankara University, Ankara, Turkey and Faculty of Pharmacy, Erciyes University, Kayseri, Turkey (Fig. 1).The collected fresh herba were divided into two parts. One part was preserved in the 70% ethanol for anatomical studies and other part was dried in shade for pharmacognostic studies.

For the morpho-anatomical studies, transverse sections of mid-parts and surface (dorsal and ventral) of leaves and stems and petioles were made by freehand sectioning from the materials preserved in chloral hydrate solution R and Sartur reagent (Celebioglu and Baytop 1949). The characteristic elements were determined and photomicrographs were taken (ZEISS Primostar

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415500 with AxioCam ICc3 camera, Germany). In addition, camera lucida drawings were made with a drawing tube attachment (Zeiss Stereo Microscope Discovery V8).Flower and leaf specimens were mounted onto Scanning electron microscope (SEM) stubs using double-sided adhesive tape and coated with gold. Photmicrographs were taken with a scanning electron microscope (SEM LEO 440 Polaran SC 7620 Sputter Coater, Germany).

The preliminary phytochemical identification was carried out on *A. mollis* leaf powder by using phytochemical tests to detect the presence of different phytochemical constituents (Tanker and Sakar 1991). The total and insoluble hydrochloric acid ash values and water amount of plant were determined by the standard methods described in the pharmacopoeia (EP, 2008; TP, 1974).

All the chemicalswere of used analytical grade and were obtained from Merck (Darmstadt, Germany).

Results and Discussion

Morphological properties of different parts of *A. mollis* shown in Figs 1-3 have been summarized in Table 1. Similarly, Table 2 contains the anatomical description of different plant parts which are shown in Figs 4-6.

Table 1. Morphological details of the stem, leaf and inflorescence of *A. mollis*.

Plant parts	Morphological details
Stem	35 - 40 cm, densely patent-hairy throughout
Leaf	Densely hairy on both surfaces, orbicular or orbicular reniform (4-)-5× 10(-13) cm, lobed to 1/2- 8/11 and 9-11 lobes arcuate or semi orbicular rounded but not truncate, (4-)5-7 teeth unequal, petiole densely patent-hairy.
Inflorescence	Large, 34 - 87 flowered, 3.5 - 5.0 mm wide, hypanthia mostly patent–hairy, epicalyx lobes ovate or ovate lanceolate often one denticle usually equal with ovate sepals and sometimes longer than sepals,pistil glabrous 1.4- 2.0 mm, pedicel glabrous (1-)2-3 mm.

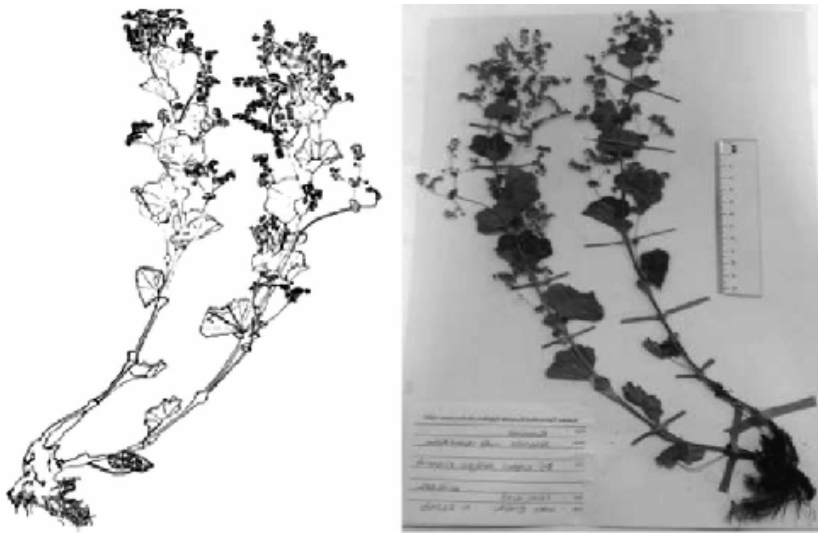

Fig. 1. Herbarium sample and drawing of *A. mollis*.

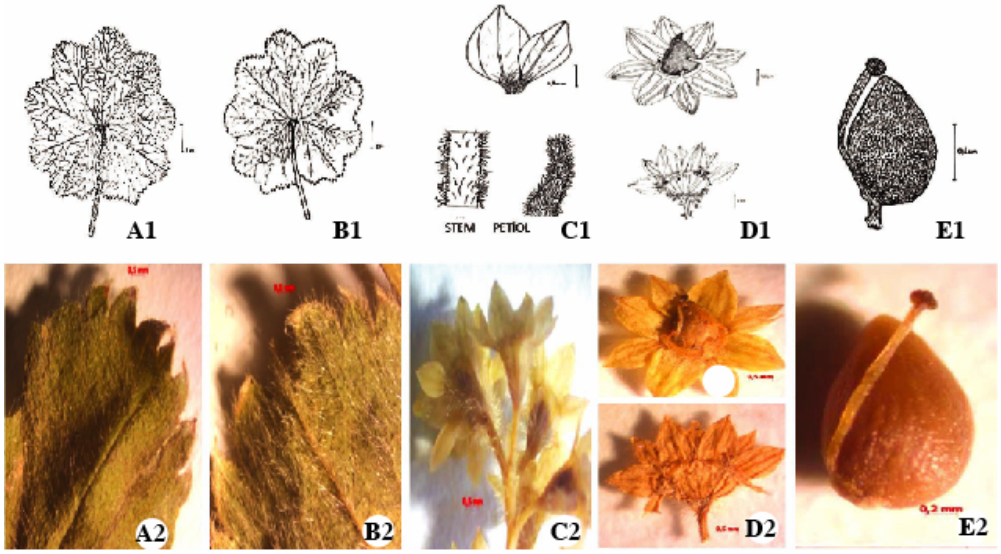

Fig. 2. Morphological drawings and microphotographs of *A. mollis*. (A1-A2: Adaxial surface of leaf, B1-B2:Abaxial surface of leaf, C1: Calyx and epicalyx, C2:Inflorescence, D1-D2: General views of flowers, E1-E2:Capitate stigma)

Leaf of *A. mollis* is bifacial and upper epidermis is covered with thin cuticle. Palisade parenchyma are composed two layers.Parenchyma cells long and cylindirical or partly short and irregular. Spongy parenchyma cells are loose and have got a thin wall and irregular shape.Upper and lower epidermis covered with glandular and aglandular hairs. Aglandular hairs which are unicellular narrow and blunty at the apex, densely in lower epidermis. Glandular hair is stalked capitate. Stomata is anomocytic type and more intense in lower epidermis. There are 4-10 palisade cells under the each cells of upper epidermis. While upper epidermis cells are moderately wavy, lower epidermis cells are zigzag. Vascular bundle are scattered throughout the mesophyll. Vascular bundle of midrib are enclosed with 1-2 layers endodermis cells and consisting of a ring of xylem and phloem. Solitary or rosette cyristals are presents in mesophyll. (Figs. 3-4).

Petiole has got a triangular visible shape, adaxial face almost flat and abaxial one is strongly convex. The epidermis is single layered. There are many aglandular hairs on epidermis. Collenchymatic cells are 2-3 layered. There are three vascular bundle in the median region of the petiole. Each vascular bundle surrounded with endodermis. There are big cellulosic parenchymatic cells in the central area of petiole. Strach grain is observed in parenchymatic cell especially on upper surface of endodermis. There are a few rosette and prismatic cluster crystalsin parenchyma (Fig. 5).

The epidermis is composed of small izodiametric cells and surrounded thicker striated cuticle.The glandular and aglandular hairs are present. There are 3-4 layered collenchymatic cell under the epidermis. Under the collenchyma 4-5 layered cellulosic parenchyma which compose polygonal-rounded cells. Endodermis is unilayered and well-defined.Under the epidermis sometimes3-4 layered pericyclic scleranchyma continues. Phloem is outside and consists mainly of vessels. Xylem is inside and surrounded by the multi-tiered schleranchyma. Vascular bundles seperate with radial pith beam. Big cellulosic parenchymatic cells in the pith. Tannin cells and strach grain are very common in parenchymatous tissues (Fig. 6).

Table 2. Anatomical structures of leaf, petiole and stem of *Alchemilla mollis*.

Leaf	Petiole	Stem
Bifacial [Figure 4B] Mesophyll [Figure 4B] Palisade parenchyma two layered (first layer is long and cylindirical the second layer is short and irregular) Spongy parenchyma is loose with thin wall and irregular shape Midrib [Figure 4A] Upper epidermis is one layered; under the epidermis is 1-2 layers of collenchymatic cells Several centric bundles, each surrounded by an endodermis (1-2 layers) and consisting of a ring of xylem and phloem enclosing pith-like tissue. Adaxial epidermis Cells with irregular shape, and side walls moderately wavy [Figure 4F] Stomata of anomocytic type [Figure 4G]. Under each cell of the upper epidermis there are 4-10 palisade cells[Figure 4F]. Abaxial epidermis Arrangement of cells is zigzag[Figure 4C]. Stomata are of anomocytic type, with density much higher than on the adaxial surface and hairs too. Trichome and Glandular hair Unicellular, narrow trichome [Figure 4D] and galandular hair [Figure 4E]	Triangular visible shape, adaxial face almost flat and abaxial one is strongly convex [Figure 5A]. The epidermis consists of small cells, under the epidermis is 2-3 layer collenchymatic cells. Three stele, (the two steles which are placed opposite the abaxial epidermis are smaller than the other stele which is placed opposite the adaxial epidermis) [Figure 5A]. Each stele is surrounded by the endodermis, followed by sclrenchymatic ring but sometimes sclerenchyma does not consisting like a ring. Big cellulosic parenchymatic cells are presentin the central area of the steles[Figure 5B]. Starch grain is present in parenchyma especially in the upper endodermis [Figure 5A]. Prismatic cluster crystal are present [Figure 5C-5D] Aglandular trichomes among the epidermal cells which are narrow and blunty at the apex [Figure 5B].	Epidermis consists of small izodiametric cells and is surrounded by thicker with striated cuticle Collenchymatic cells are under the epidermis and cellulosic parenchyma are composed of polygonal-rounded cells [Figure 6A-6B]. Endodermis is unilayered and well defined [Figure 6B]. Under the endodermis sometimes pericyclic sclerenchyma continues (3-4 layer) but sometimes ring is absent. Phloem is outside and xylem is inside. Phloem consists mainly of vessels. Xylem is surrounded by the multi-tiered sclerenchyma. . Vascular bundles are separeted with radial pith beam. Big cellulosic parenchymatic cells are present in the pith[Figure 6A].. Unicellular, narrow trichcomes which are blunty at the apex and glandular trichomes is stalked capitate [Figure 6E-6C]. Rosette crystals are foung in parenchyma cell [Figure 6F]. Tannins are very common especially in parenchymatous tissues[Figure 6D]. Starch grains are very common in the tissues[Figure 6A].

In this study, the morphological and the anatomical features of the used parts of *A. mollis*were examined.Theresults agree, in general, with the description given in the Flora of Turkey and the other literatures (Davis1966, EP 2008, Metcalfe1965).

Regarding the data obtained, the flowers of *A. mollis*wereabout 3-4 mm in diameters; in the Flora of Turkey,dimension of the flowers was given as 3-6 mm in diameter.The pedicels were glabrous according to the Flora but we observed sparce hairs on the pedicels(Davis, 1974).Petals are absent. The epicalyx is present in all species. The calyx is double with 4 small segments of epicalyx alternating with 4 larger sepals. Four short stamens are present and a single carpel with a capitate stigma (Table 1 and Fig. 2).These features are similar with *A. xanthochlora*in the pharmacopoeia (EP 2008). In addition, the hypanthium is quite hairy and this feature is similar to the *A.pseudeomollis* species (AyazandInceer 2009).

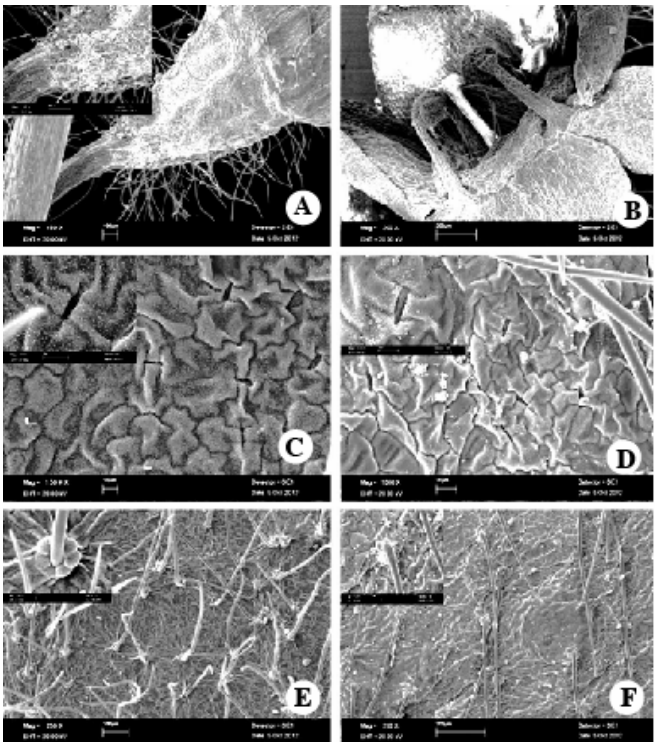

Fig. 3. Anatomic and morphologic photographs of *A. mollis* taken with SEM (A: Hypanthium, B: Stamens, C: Undersurface (epidermis) of leaf and stomata, D: Upper surface (epidermis) of leaf and stomata, E: Trichomes on underepidermis,F: Trichomes on upperepidermis).

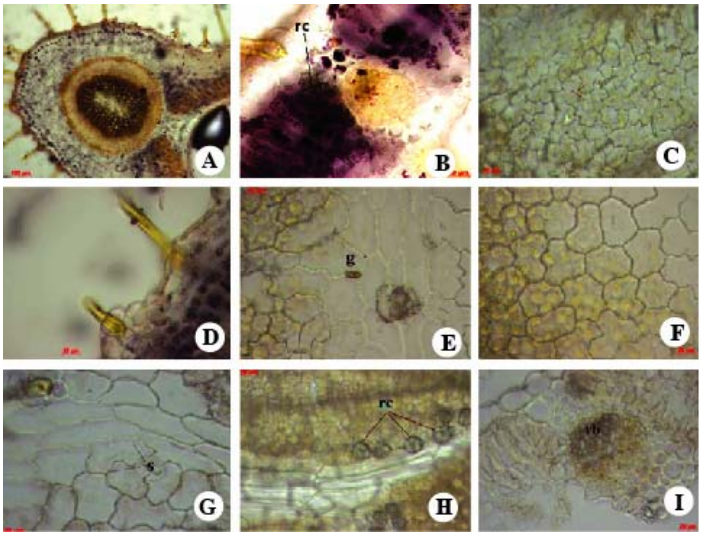

Fig. 4. Microphotograps of *A. mollis* leaf (A: Midrib, B: Mesophyll (rc: rosette crystal), C: Lower epidermis, D: Eglandular hair, E: Glandular hair(g), F: Upper epidermis, G: Anomocytic type stomata (s) H: Rosette crystals (rc), I: Vascular bundles (vb).

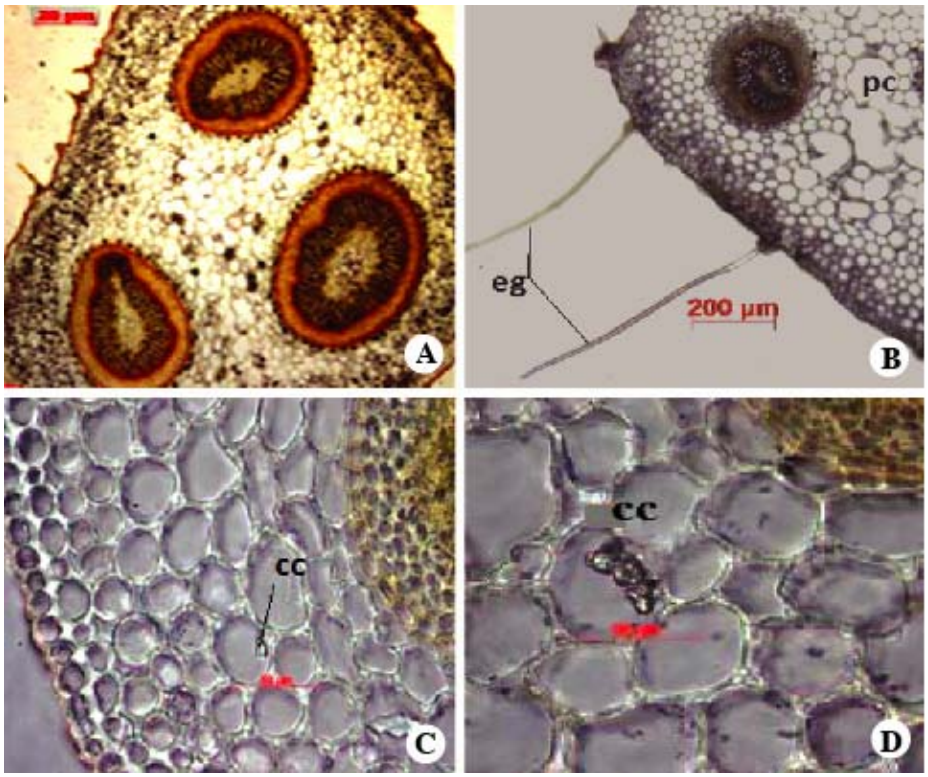

Fig. 5. Microphotographs of *A. mollis* petiole (A: Transverse section of petiole, B: Eglandular hair of petiole (pc: Big cellulosic parenchymatic cells), C, D: solitary and clustered crystals).

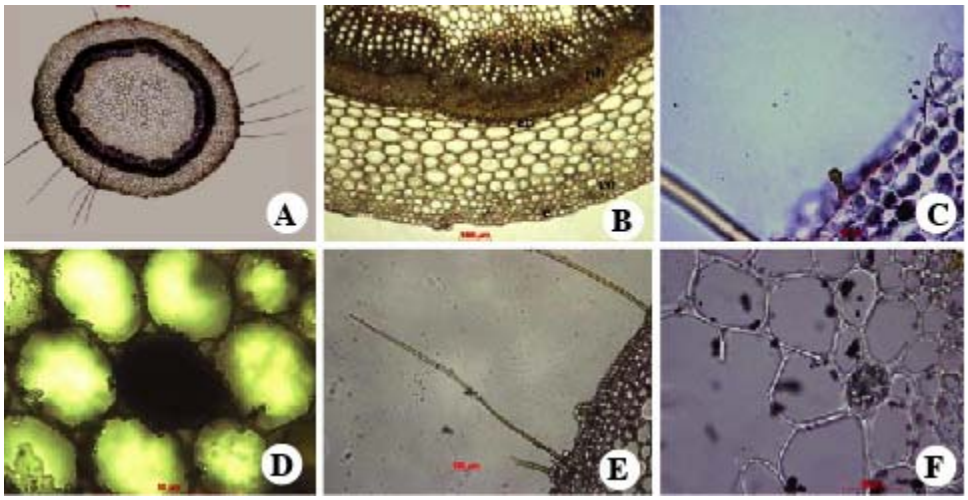

Fig. 6. Microphotographs of stem (A: General view of stem, B: Anatomical viewof a portion of stem e:epidermis, co: collenchyma, en: endodermis, ph: phloem, xyl: xylem, C: Glandular hair, D: Tannin cells, E: Eglandular hairs F: Rosette crystals).

A. mollis was qualitatively analyzed for the major chemical groups (alkoloid, flavanoid, saponin, tannin, coumarins, carbonhydrate) and the results are shown in Table 3.

Table 3. Phytochemical analysis of *A. mollis*.

Tests	*Alchemilla mollis*
Alkaloids	-
Saponins	-
Flavonoids	+
Cyanidin	+
Anthocyanins	-
Tannins	+
Anthraquinones	-
Cardiacglycosides	-
Coumarins	+
(+): positive, (-): negative	

The results obtained from various determinations of physico-chemical analysis are shown in Table 4.

Table 4. Physico-chemical determination of *A.mollis.*

Parameters	Values
Total ash	11.8 % (w/w)
Acid insoluble ash	4.45 % (w/w)
Loss on drying	4 % (w/w)

The leaves are bifacial and incisions from the midrib of leaf showed that the upper surface is channeled and the lower surface is convex. Anomocytic stomata are seen on both surfacesbut are more intense on the lower surface. Lower and upperepidermiscellsare differentin shape(Fig. 4 and Table 2).

In the present study, calcium oxalate is identified in the form of solitary and clustered crystals (Fig. 4H, Fig. 5C-D, Fig. 6F).These data arecomplied with the European Pharmacopoeia and Anatomy of the Dicotyledons (EP 2008,Metcalfe 1965).On the other hand, in some previous studies on *A. mollis* no information is available oncrystals in the stem, petiole or leaf (Violeta 2011a, c).

The stems are densely pubescent, hairs are generally unicellular, narrow and blunty pointed at the apex. Stalked capitate glands are observed rarely (Fig. 6).

The endodermis is well defined instem of *A.mollis.* The pericycle continues as sclerenchymatous ring (Fig. 6B).Tannin cells are very common in parenchymatous tissue and are very important for this species (Fig. 6D).That's why*Alchemilla* has got therapeutic effects generally due to tannins. Rosette crystals have been observed in the parenchymatic cell of the stems (Fig. 6F). Occurrence of rosette crystals in dicotyledonous stem has not been reported before.In the present investigation large medulla was found (Fig. 6A), but Violeta (2011b) reported the occurrence of narrow medulla in the stem of *A. flabellata*.

Total ash (11.8%)and acid insoluble ash (4.45%) are considered to beimportant and useful parameters for detecting the presence of inorganic substance. Ash values of drug also give an idea of earhty matter and other impurities present along with drug. Values presented in Table 4, are comparable with those mentioned in Pharmacopoeia (EP 2008).In the present investigation, *A. mollis*was found to contain flavonoids, glycosides, tannins and coumarins (Table 3).The morphoanatomical and phytochemical data presented in this paper on *A. mollis* is the first report and thus can serve as a basis of knowledge suitable for identification of this plant material for future investigations and application.

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