DIAGNOSTIC SIGNS OF MORPHOLOGICAL AND ANATOMICAL STRUCTURE OF *Medicago falcata* L. RAW MATERIALS

**Aim.** To study the diagnostic signs of morphological and anatomical structure of *Medicago falcata* L. raw materials from the flora of Azerbaijan.

**Materials and methods.** Plant samples were fixed in a solution made in 0.1 M phosphate buffer (pH = 7.4), containing 2.5 % glutar-aldehyde, 2 % paraformaldehyde, 4 % sucrose, and 0.1 % picric acid. The fixed materials have been submitted to the Electron Microscopy Laboratory of the Azerbaijan Medical University for study by electron microscopy. Samples have been postfixed in 1 %-osmium tetraoxide solution prepared in phosphate buffer (pH 7.4) within two hours after being left in the same fixer for one day. Araldite-Epon blocks made from materials using general methods adopted in electron microscopy. The semi-thin (1-2 μm) sections from the blocks taken on an EM UC7 (Leica, Germany) ultramicrotome, stained with methylene blue, azure II, and basic fuchsin were observed under microscope Primo Star (Zeiss, Germany) and images of required parts were shot with EOS D650 (Canon, Chine) digital camera.

**Results and discussion.** Multicellular trichome covered on both leaf sides. Adaxial epidermis a sheet plate have sinuous cells, and abaxial epidermis have differed slightly sinuous cells with clear-shaped walls. In leaflet anatomy of plant vascular bundles are of the collateral type. Stomata are located from both sides of a leaf. Stomata belong to anisocytic structure. The epidermis of the stem consists of elongated thick-walled cells with anisocytic stomata. The calyx is densely covered by trichomes. The outer epidermis of cross-section of the keel petals cells is the wing horns cells form. And inner epidermis consists of oval cells. Mesophyll cells inside the leaf consist of loose spongy cells on the underside with several conducting bundles (dorsoventral).

**Conclusions.** Morphological and anatomical studying of raw material *Medicago falcata* L. has shown, that there are prominent features of a structure: four large conductive bundles are located on the cross section of the stem at the corners of the ribs on two sides. Between angular conductive bundles, there are three conductive bundles, and the other two sides are located along one conductive bundle. Consequently, *M. falcata* differs from *M. Sativa* L. The epidermis of the stem consists of elongated thickened-walls cells with anisocytic stomata. The indicated diagnostic morphological and anatomical characters could be used in the compilation of a monograph and in identifying plant materials on the raw material of *Medicago falcata* L.

**Key words:** anatomy; morphology; raw material; *Medicago falcata* L.; diagnostic signs
Результаты и их обсуждение. Верхний и нижний бок листа вкритий багатоклітинними волосками. Верхній епідерміс складається зі шлільно прилеглих один до одного сильнозівистих клітин і продихів. Нижній епідєрміс містить слабко зівисті клітини з чітко розрізненими стінками і продихами. В анатомії листка судинні пучки мають колатеральний тип будови. Продихи розташовані на обох боках листка і відносяться до анизоцитного типу. Епідерміс стебла складається з великих витягнутих товстостінних клітин з продихами анизоцитного типу. Чашечка квіткі густо покрита волосками. На епідермісі зовнішнього боку вічок є клітини трикутної форми, що нагадують пікі, з внутрішнього боку великі округлі клітини чергуються з дрібними клітинами. Мезофіл листка складається з пухків губчастих клітин на нижньому боці з декількома провідними пучками (доровинними).

Висновки. Морфологічна і анатомічна вивчення лікарської рослинної сировини трави M. Falcata L. показало, що є такі характеристики освітленості будови, на поверхневому зрізі стебла по кутах рібер знаходяться чотири великих провідних пучків, на двох боках між кутовими провідними пучками присутні по три провідних пучки, а на двох інших боках розташовано по одному провідному пучку. Саме цим M. Falcata L. відрізняється від M. Sativa L. Епідерміс стебла складається з витягнутих товстостінних клітин з анизоцитними продихами. Зазначені морфологічні і анатомічні діагностичні ознаки можуть бути використані при складанні монографії на сировину і при ідентифікації рослинної сировини трави Medicago falcata.

Ключеві слова: анатомія; морфологія; рослинна сировина; Medicago falcata L.; діагностичні ознаки

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Діагностичні признаки морфологічного і анатомічного строєння сиров'я Medicago falcata L.

Актуальність. Флора Азербайджану розподіляється разнообразным растительным покровом и отличается богатством выбора. Однако эти источники сырья на сегодняшний день недостаточно полно используются для удовлетворения потребностей медицины и народного хозяйства. Нами проведено ресурсное рекогносцировочное обследование видов рода Medicago на территории Азербайджана и выявлены регионы их массового произрастания. Новые ареалы расположены в различных экологических условиях, поэтому возможности изменения диагностических признаков морфологического и анатомического строения сырья, а также его химического состава. Совокупность дополнительных морфологических и анатомических признаков позволяет более точное определение подлинности сырья.

Цель работы. Целью работы явилось определение диагностических признаков морфологического и анатомического строения сырья Medicago falcata L. на территории Азербайджана.

Материалы и методы. Материал для исследований фиксировался смесью 2,5 % раствора глютаралdehyde гида, 2 % раствора параформальдегида и 0,1 % раствора пикриновой кислоты на фосфатном буфере (рН = 7,4). Последующая постфиксация проводилась в 1 % растворе четырехокиси осмия и в 1,5 % растворе феррицианида калия на 0,1 М фосфатном буфере (рН = 7,4). Дальнейшая обработка материала – обезвоживание и заливка в Аралдит и Эпон-812 проводилась по общепринятой методике. В последующем срезы (1-2 μm) окрашивались метиленовым синим, азур II, фуксином) и были просмотрены под микроскопом Primo Star (Zeiss, Germany). Изображения были сфотографированы цифровой фотокамерой EOS D650 (Canon, Japan).

Результаты и их обсуждение. Верхний и нижний бок листа покрыты многолетними волосками. Верхний эпидермис состоит из плотно прилегающих друг к другу сильнозиговистых клеток и продыхов. Нижний эпидермис имеет слабозиговистые клетки с четко различными стенками и продыхами. В анатомии листа существуют пучки, имеющие колатеральный тип. Устьицы расположены на обеих сторонах листа и относятся к анизоцитному строению. Эпидермис стебля состоит из вытянутых толстостенных клеток с продыхами анисоцитного типа. Чашечка ветвей густо покрыта волосками. На эпидермисе наружной стороны венчика имеют ся клетки треугольной формы, напоминающие шпильки, с внутренней стороны крупные округлые клетки чередуются с дрбнми клетками. Мезофил листа состоит из пухків губчатых клеток на нижней стороне с несколькими проводящими пучками (доровинными).

Выводы. Морфологическое и анатомическое изучение лекарственного растительного сырья травы M. Falcata L. показало, что имеют следующие характерные особенности строения: на поперечном срезе стебля по углам рёбер находятся четыре крупных проводящих пучка, на двух сторонах между угловыми проводящими пучками, присутствуют по три проводящих пучка, а на двух других сторонах расположено по одному проводящему пучку. Этим M. Falcata L. отличается от M. Sativa L. Эпидермис стебля состоит из вытянутых толстостенных клеток с анисоцитными устьицами. Указанные морфологические и анатомические диагностические признаки могут быть использованы при составлении монографии на сырье и при идентификации лекарственного сырья травы Medicago falcata.

Ключевые слова: анатомия; морфология; растительное сырье; Medicago falcata L.; диагностические признаки

INTRODUCTION
Flora of Azerbaijan has a diverse vegetation cover and is rich in choice. However, these sources of raw materials today are not fully used to meet the needs of medicine and national economy. The main reason for this is the insufficient study of the chemical composition and biological activity of plants and the lack of information about the raw material base [1].

The genus Medicago a perennial herbaceous plant from the legume family (Leguminosae) consists of more than 100 species common in Europe, Africa, Asia. In Azerbaijan, the genus Medicago L. is represented by 21 species [2].
Species of the genus *Medicago*, growing in various regions of the world, have been studied in chemical composition and biological activity [3, 4, 5].

For the first time, flavonoids larcitrin, genistein, and biochanin A were isolated from the aerial part of *M. littoralis* [6]. The quantitative content of isoflavonoids for monarchin, genistein and irilone in the plant species *M. orbicularis*, *M. doliata* and *M. arabica* was significantly higher than in raw material soybean and red clover [7]. Triterpene saponins were isolated from the roots of *M. sativa* and *M. truncatula* and identified as medicagoic acid, hedagenin, soyasapogenol B and E [8]. Extract of the species *M. minima*, *M. tornata*, *M. truncatula*, *M. scutelata*, *M. segalis* and *M. sativa* were screened for antioxidant, cytotoxic and antimicrobial activity. *M. segalis* showed the highest antioxidant activity. *M. rigidula* showed the least antioxidant activity [9].

*M. sativa* extract has hepatoprotective and antioxidant properties under stress [10]. Microscopic features and physicochemical parameters for standardizing plant materials of *M. sativa* were established [11].

We conducted a resource reconnaissance survey of species of the genus *Medicago* in Azerbaijan and identified regions of mass growth. We conducted a preliminary phytochemical analysis of the aerial mass of *M. falcata* harvested during the flowering phase and established the presence of flavonoids, coumarins, triterpene saponins and other, less important substances in it.

New natural habitats are located in different environmental conditions and it is possible to change the diagnostic features of the morphological and anatomical structure of plant materials, as well as the chemical composition.

In some monographs on raw materials, a limited number of characters are given, and sometimes features characteristic of a family or genus are indicated. The plant material “herb” is harvested in the flowering phase and it is desirable to give signs regarding pollen, seeds, compounds, the base, the upper and the lower surface is hairy. The length of leaflet is 8-20 mm. The apex of the leaflet is acute. Stipules entire or toothed at their base. The stem, leaf rachis and peduncle are covered with hairs also (Fig. 1). Petioles are 0.3-1.5 mm. Stipules are 0.4-0.9 mm, lanceolate. Inflorescence is 1.1-2.5 cm, dense raceme. It has raceme of small yellow flowers followed by fruits containing 10-18 seeds. Seeds are flat, yellow brown, 10-12 mm long, semi-spiral, glabrous or slightly hairy.

Anatomical characteristics
Leaflet anatomy
The transverse sections of leaflets show that the upper and lower epidermis consist of uniseriate, oval or isodiametric cells with adaxial and abaxial cuticles. The lower epidermal cells are similar to those on the adaxial surface there are some slightly sinusous cells with clear-shaped walls. Both leaf surfaces have stomata (Fig. 2). Epidermal cells are tightly linked to each other and stoma belongs to the anisocytic structure. Mesophylic - chlorenchyma tissue consists of 1-2 layered the palisade parenchyma cells and 4-5 layered spongy parenchyma cells (Fig. 3). The mesophyll tissue separated into a distinct palisade zone in the adaxial portion of the leaf, and spongy mesophyll in the abaxial portion. The spongy parenchyma cells are present isodiametric spongy parenchyma cells with large intercellular spaces. There are more trichomes present both surfaces, especially along the veins.

Steam anatomy
The stem is about square in cross section. The stem epidermis is composed of large elongated thick-walled cells with stomata of the anisocytic type. Vascular bundles are located in the angles of the axis. Vascular bundles in *M. Falcata* L. are collateral. The epidermis is underlain by chlorenchyma and collenchyma and the innermost layer of the cortex is the starch sheath. Collenchyma tissue is composed of cells with thick cell walls. Pith is composed of large, compactly arranged parenchyma cells. Phloem and xylem are well developed. Phloem parenchyma and companion cells are present in the phloem. The center of the is occupied by the pith. The pith region of the stem consists of parenchymatic cells and some of them include dark secretions (Fig. 4).

**RESULTS AND DISCUSSION**

**Morphological characteristics**

*M. falcata* L. is perennial plant belonging to the genus *Medicago* of the family Leguminoase. The plant is erect or stems prostrate to upright, arising from the crown, up to 40-60 (100) cm, roots well-branched, rarely taproot or rhizomatous. Leaves and stems covered sparsely to densely with hairs. Leaves are oblong or obovate with serrations, compound, the base is narrowed, the upper and the lower surface is hairy. The length of leaflet is 8-20 mm. The apex of the leaflet is acute. Stipules entire or toothed at their base. The stem, leaf rachis and peduncle are covered with hairs also (Fig. 1). Petioles are 0.3-1.5 mm. Stipules are 0.4-0.9 mm, lanceolate. Inflorescence is 1.1-2.5 cm, dense raceme. It has raceme of small yellow flowers followed by fruits containing 10-18 seeds. Seeds are flat, yellow brown, 10-12 mm long, semi-spiral, glabrous or slightly hairy.

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**Fig. 1.** Morphological characteristic of *M. falcata* L. a) hairs of lower side of leaves; b) stem with hairs; d); f) the upper and lower side of leaves; c); e) inner side of calyx with hairs

**Fig. 2.** Leaf and stem epidermises: a); d) upper epidermis of leaf with stomata; c); f) lower epidermis of leaf with stomata, c); e) stem epidermis with stomata
Petal anatomy

The papilionaceous corolla is consists of five petals: a large standard or banner, two lateral wing petals and two fused petals that form the keel. The keel petals is composed of outer epidermis and inner epidermis. Outer epidermis changes their shape to wing horns cells. The transverse section of the keel petals consists of scattered vascular bundles (Fig. 5).

Morphological and anatomic studying of raw material Medicago falcata L. has shown, that there are prominent features of a structure. Some of the microscopic features for standardizing plant materials of M. Sativa L. were established [11]. This is the first report on the examined characteristics of the M. Falcata L. in flora Azerbaijan. Transverse sections of stems of the plant were studied for microscopic characters. Multicellular trichome covers on both leaf sides. Adaxial epidermis a sheet plate have sinuous cells, and abaxial epidermis have differed slightly sinuous cells with clear-shaped walls. The transverse sections of stem of M. Falcata L. are made up three main tissues from outside to inside and their names are epidermis, vascular bundles and pith region. Transverse section in the main stem some Medicago sativa L. cultivars were carried out; stained and seventy anatomic characters were examined by light microscope [14]. In leaflet anatomy of plant vascular bundles are of the collateral type. Stomatas are located from both sides of a leaf and are surrounded by three or four unequal cells. Stomata belong to anisocytic structure. The epidermis of the stem consists of elongated thick-walled cells with anisocytic stomata. The calyx is densely covered by trichomes. The outer epidermis of cross-section of the keel petals cells is the wing horns cells form. And inner epidermis consists of oval cells.

Morphological and anatomical characteristics might be useful in the definition of the species investigated. These characteristics will be more valuable if other species of Medicago are also investigated.

CONCLUSIONS

Morphological and anatomic studying of raw material Medicago falcata L. has shown, that there are prominent features of a structure: four large conductive bundles are located on the cross section of the stem at the corners of the ribs on two sides. Between angular conductive bundles, there are three conductive bundles, and the other two sides are located along one conductive bundle. Therefore, M. Falcata L. differs from M. Sativa L. The epidermis of the stem consists of elongated thick-walled cells with anisocytic stomata.

The indicated diagnostic morphological and anatomic characters could be used in the compilation of a monograph and in identifying plant materials on the raw material of Medicago falcata L.

Conflicts of interest: authors have no conflict of interest to declare.
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