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Comparative morphological and anatomical analysis of *Thymus serpyllum* L., gathered in different parts of Karaganda region

At the article the results of comparative morphological and anatomical analysis of herb *Thymus serpyllum* L. are given in article for identification of the main distinctive signs of a species. The raw materials of thyme are gathered in Karkaraly Mountains (Karkaraly region) and at the territory of Korneev forest (Bukhar-Zhirau region). Results of researches have allowed defining that the *Thymus serpyllum*, collected in different areas of the Karaganda region, is presented by two chemo-types. Chemo-types of Thymus serpyllum have external similarity, however under the influence of various factors of the environment essential differences are determined by the external characteristic of stalks, leaves, inflorescences, a structure and omission of a cup and a nimbus that allows using them for identification of this species. In the course of conducting researches micro diagnostic signs for a leaf *Thymus serpyllum* are specified and also new signs for a stalk, a scape, a nimbus and a cup of whole raw materials are revealed. Diagnostic signs of morphological structure are form and size of stalk, leaf, degree of omission of cup. Diagnostic sings of anatomical structure for leaf, stalk, cup and nimbus are degree of omission, structures of trichomes and their composition, also presence of crystals of oxalate of calcium in tube of nimbus, essential oil glandular, nipples of outgrowths of cells of epidermis for a leaf of a cup, a nimbus.

Keywords: *Thymus serpyllum* L., morphological analysis, anatomical analysis, Karkaraly Mountains, Korneevka forest, chemo-type, comparative investigation, identification, micro-signs.

Improvement of techniques of determination of authenticity of raw materials on morphological and anatomical signs, both for whole raw materials, and for the crushed raw materials is provided by modern requirements to quality of medicinal vegetable raw materials. Now as official medicinal species are used *Thymus vulgaris* and *Thymus serpyllum*.

Thymus serpyllum L. of genus thyme (*Thymus* L.) treats valuable herbs, thanks to the pharmacological properties: expectorant, antioxidant, antimicrobial and anti-neoplastic activity [1].

Thymus serpyllum meets in a temperate climate of Eurasia, from Scandinavia to the Mediterranean and from the British Isles to Eastern Siberia, in the Karaganda region meets in Ku Mountains, Korneev woods, mountains Kent, Karkaraly Mountains. It grows in a steppe zone, on dry and fresh sandy soils, in the coniferous and deciduous forests, on forest edges and glades, cuttings, meadows, in young landings of the wood, on the southern slopes, rocks, in the pine woods, on slopes of hills in shrubby thickets [2].

The analysis of literary data shows that *Thymus serpyllum* in the nature, depending on the place of growth, is presented by several chemo types that was the cause for detailed studying of features of a structure of *Thymus serpyllum*, collected in different areas of the Karaganda region.

The purpose of our work was studying of morphological and anatomic features of *Thymus serpyllum* collected from two points, for identification of the main distinctive signs.

Material and methodology

Object of a research were elevated vegetative (leaves and year escapes) also generative (flowers) bodies of *Thymus serpyllum* L., collected in the territory of the Karaganda region: Korneev woods (Bukhar-Zhyrausky district) and Mountains Karkaraly (Karkaraly district).

Sampling was made during the vegetative period of 2016 year in a phase of budding-blossoming. Studying of morphological features of a grass of *Thymus serpyllum* collected from two points, was carried out on herbarium samples, fresh-gathered plants and the dried-up raw materials, in comparison with the description stated in the article «Thymus serpyllum» of the State Pharmacopeia of the Republic of Kazakhstan [3].

Studying of micro-diagnostic signs of raw materials was carried out on the temporary micro medicines prepared by the technique included in SP of RK with the subsequent micro photographing. Fresh-gathered bodies fixed in mix alcohol 70 %: glycerin: the water distilled in the ratio 1:1:1 (Straus-Fleming solution).

During determining anatomic features of a sheet plate of the studied speices selected the intact most developed leaves in a middle part of stalks; analyzed fragments (surface preparations and cross-cuts) in a middle part — between the main vein and edge. For flowers studied surface medicine of a nimbus, cross medicine of a pedicel. Cross cuts of year stalks did on all its length, through each 2–3 cm.

The anatomic research of plants is conducted according to methodical instructions of M.N. Prozina [4], A.A. Dolgova and E.Ya. Ladygina [5], V.N. Vekhov and L.I. Lotova [6]. Production of temporary preparations (surface and crushed preparations, cross cuts) was made by the standard techniques [7–9] with use of the freezing microtome «Tekhnon MZP-01». Clarification of micro preparations was carried out by means of glycerin. For receiving surface medicines leaves boiled in 10 % potassium hydroxide solution.

The received medicines studied by means of the scanning microscope of «MT 4310 L» of Melji-Techno, the Bision Cam V 500 B camera. Digital photos are received at increase in an eyepiece and lens 10×4, 10×10, 10×40 when using the Bisual Bio program. At the description of an anatomic structure the terminology offered K. Esau [10, 11], N.A. Aneli [12], L.I. Lotova [13] was used.

Results and discussion

Morphologically *Thymus serpyllum* is presented by herbs or semi-low shrubs with the lying or ascending lignified stalks and the upright or rising grassy floriferous branches.

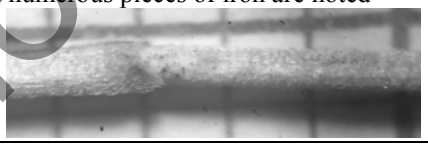
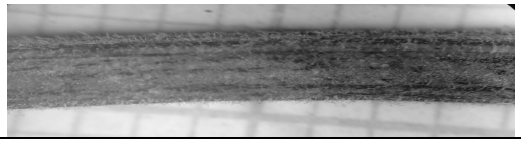
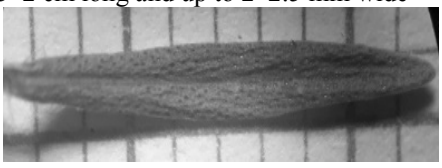
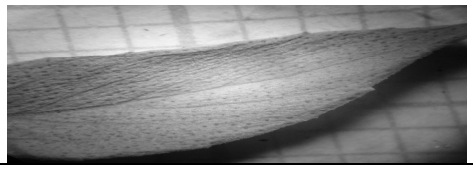
It is an integral or partially crushed thin stalks, leaves and flowers. Stalks are tetrahedral, thin (up to 0.5 cm), greenish or yellowy-brown color, sometimes with a violet shade. Leaves are the short scape, lancet, elliptic or longitude — elliptic, reaching length about 15 mm, low omission or naked with sharply being veins on the lower side of a leaf. On all surface of a leaf numerous yellowish-brown points (essential oil glandular) are visible; at the basis of leaves long bristle hairs are often visible.

Flowers are small, single or collected on some pieces in semi-verticils. Each flower consists of a two-lip cup and a two-lip nimbus. A cup about 4 mm long, a nimbus 5–8 mm long, stamens — 4, a pestle with a four-separate top ovary.

Color of leaves is green or gray-green; cups are hemna-red; a nimbus are bluish-violet. The smell is fragrant, taste of water extraction bitter and spicy. Data on the comparative analysis of morphological features of *Thymus serpyllum* are presented in Table.

Table

Comparative characteristics of morphological signs of raw materials of *Thymus serpyllum* from two points of gathering

Diagnostic signs	Korbeev woods	Karkaraly Mountains
1	2	3
Cross cut of stalk	Cylindrical or not clearly tetrahedral	Round-tetrahedral
Character of omission of stalk	The stalk is trimmed small trichomes, located perpendicular to a stalk from all directions, not numerous pieces of iron are noted 	The stalk is trimmed the small bulged trichomes, are noted by not numerous essential oil glandular 
Form of leaves	Linear or narrowly-elliptic, a smooth edge, 1.5–2 cm long and up to 2–2.5 mm wide 	Narrow-elliptic, smooth edge, 1.8–2.2 cm long and 2–2.5 mm wide 
Presence of scape	Leaves are sedentary or there is very short scapes	Leaves are sedentary or there is very short scapes
Omission of leaves	To the middle or in the lower third ciliate on edge, a leaf surface almost naked, clear and ferruterous. The pieces of iron shipped in a leaf epiderma	Leaves are naked, with insignificant omission on edge and the main vein, on both sides clear and glandular. Glandular are shipped in a leaf epiderma

Continuation of Table

1	2	3
Inflorescence	Heady	Heady
The direction of growth of hairs on a pedicel	Trichomes are well expressed, axes of a pedicel or slightly down directed are perpendicular	Trichomes are white, well expressed, perpendicularly bulged, more rare slightly rising over a surface
Form of cup	Narrow-jingled	Jingled or narrow-jingled
Color of cup	Lilac	Lilac, lilac-violet
Omission of cup	It is noted dense omission by long whitish trichomes; an arrangement the trichomes are mainly on cup ribs 	It is noted dense omission with long whitish trichomes; an arrangement trichomes are mainly on cup ribs 
Characteristic of teeth of an upper lip of a cup	Cup is 3-teeth, teeth are small (medium tooth is more large), sharply triangular, unbent	The cup is 3-teeth, teeth of almost identical size, small, triangular, unbent
Omission of upper teeth of cup	At the edges it is ciliate	On edge it is dense — ciliate
Color of nimbus	Pink-violet, bright pink, seldom white	Pink-violet

The analysis of descriptions of the raw materials collected in the Korneev woods and in Karkaraly Mountains shows similarity in a structure of vegetative and generative bodies though some differences which are connected with ecological conditions of growth are noted.

So, for raw materials of *Thymus serpyllum*, collected in the Korneev woods, the cross section of a stalk almost cylindrical or not clearly tetrahedral; for the raw materials collected in Karkaraly Mountains, clear rounded tetrahedral. For both points of collecting it is noted that the stalk is trimmed small trichomes which are located perpendicular to a stalk axis.

Leaves are usually narrow and elliptic, is more rare linear with a smooth edge, sedentary or with very short scapes. The size of leaves at raw materials from the first point of collecting — 1.5–2 cm long and 2–2.5 mm wide; for raw materials from the second point of collecting — 1.8–2.2 cm long and 2–2.5 mm wide. A surface of leaves almost naked with well expressed numerous essential oil glandular; for raw materials from the first point of collecting — on edge ciliate, for raw materials from the second point of collecting — with insignificant omission on the main vein.

Flowers are small; collected in semi-verticils on several pieces forming a heady inflorescence in the lower part of an inflorescence verticils settle down, there can be single flowers less often. On a pedicel the white painted trichomes forming not dense omission are noted. For raw materials from the first point of collecting a cup is narrow-jingle, lilac, densely trimmed, 4–4.5 mm long. Three teeth of an upper lip of a cup are small (average larger), acute-angled, unbent. For raw materials from the second point the cup is jingle, rarer narrow jingle, lilac or lilac-violet; 3-gear with teeth of almost identical size, triangular shape and slightly unbent teeth. In both places of collecting the cup is densely trimmed trichomes on ribs and edges of teeth. Color of a nimbus pink-violet, bright pink, is more rare white — for raw materials from Korneev woods, pink-violet — for raw materials from Karkaraly Mountains.

Raw materials smell fragrant, taste of water extraction bitter and spicy.

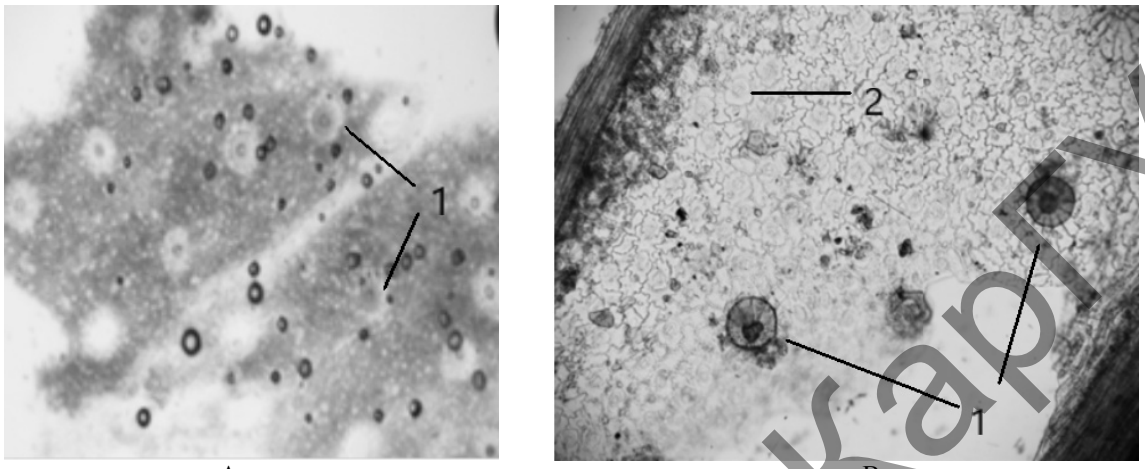
Thus, the obtained data demonstrate that in the territory of the Karaganda region two chemo types of *Thymus serpyllum* are revealed.

Diagnostic signs of raw materials of *Thymus serpyllum* it is possible to consider the following: — for a stalk — a form of a cross cut of stalks, type of omission; — for a leaf — the general form of a sheet plate,

ciliate edge, existence of a large number, slightly shipped in an epidermis of a leaf, essential oil glandular; — for a cup — a form and color of a cup, a structure of teeth, extent of omission, placement by trichomes.

Similar comparative researches are conducted at the microscopic level.

On the top epidermis and the folds of a cuticle and a necklace-shaped thickening of cellular walls is sometimes noticeable by the sheet edge; cells of the top and lower epidermis with twisting sidewalls (Fig. 1).



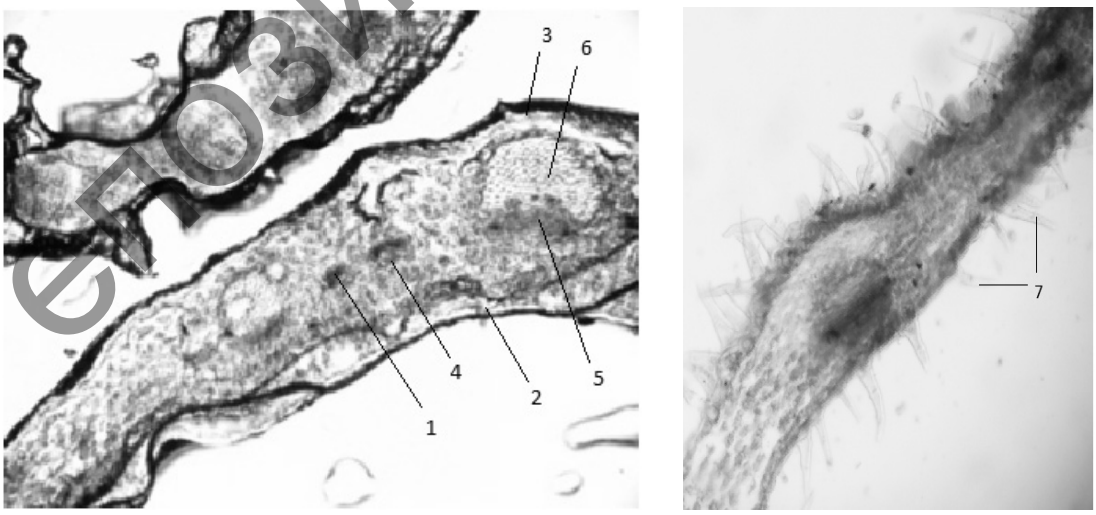
A B

A — upper epidermis; *B* — lower epidermis with rise glandular;
1 — essential oil glandular; *2* — main cells of epidermis

Figure 1. Fragment of structure of upper and lower epidermis of leaf of *Thymus serpyllum*. Zoom 10×16

Stomata are available on both parties of a leaf, on their lower party much more; stomata are followed by two near stomata cells which adjacent cells are located perpendicular to stomata crack (diacytes type). Essential oil glandular are large, consist of 8 secret cells located radially; cells of epidermis around the place of attachment of essential oil glandular often form the socket.

Hairs are several types: very large, multi cellular, warty, located at the leaf basis («bristle hairs»); above on the sheet edge meet smaller simple two — three-cellular hairs a warty surface; heady hairs very small with a mono-celled oval head on a mono-celled short leg, meet on all surface of a leaf; nipple outgrowths of epidermis, smooth or slightly warty, meet on the top party and on the sheet edge (Fig. 2) more often.

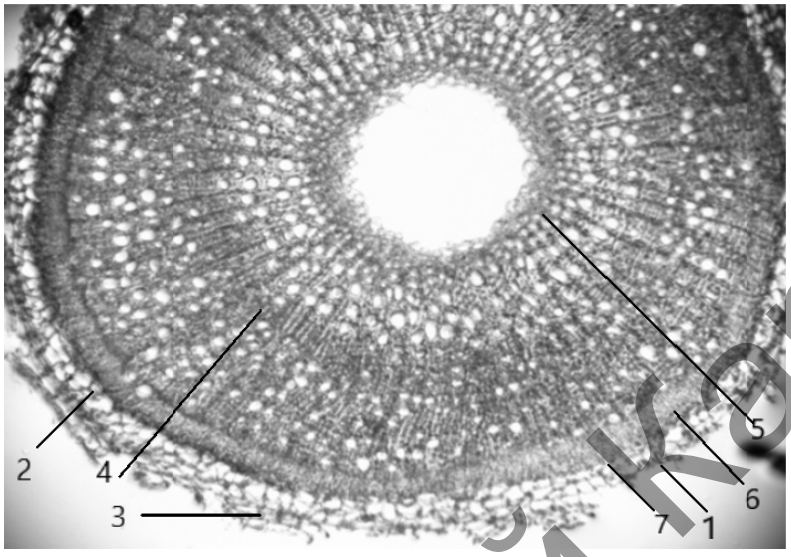


A B

A — Karkaraly Mountains; *B* — Karneev wood; *1* — lower epidermis; *2* — upper epidermis; *3* — spongy mesophyll; *4* — phloem; *5* — palisade mesophyll; *6* — xylem; *7* — trichomes

Figure 2. Cross-cut of leaf of *Thymus serpyllum*. Zoom 10×16

Cells of epidermis of a stalk are rectangular, extended, with straight lines or with the slanted ends, with a necklace-shaped thickening of cellular walls; stomata are diacytes type, are located generally on edges. Epidermis is trimmed simple, curved-knee two-, five-cellular hairs with a warty cuticle, heady hairs are on a mono-celled short leg with a mono-celled oval head, nipple outgrowths of epidermis meet a smooth cuticle or slightly warty less often; the essential oil glandular includes 8 secret cells located radially (Fig. 3).

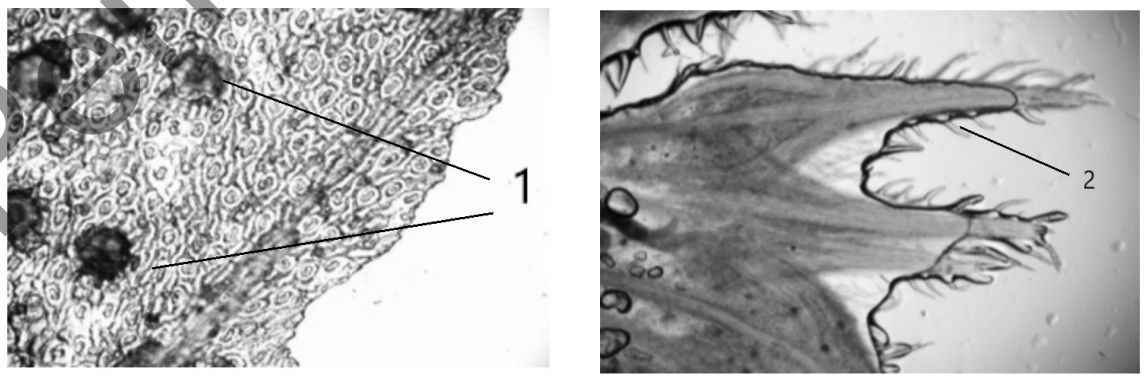


1 — epidermis; *2* — cork parenchyma; *3* — collenchymas; *4* — secondary xylem; *5* — primary xylem; *6* — phloem; *7* — endoderm

Figure 3. Cross-cut of stalk of *Thymus serpyllum*. Fragment. Zoom 10×16

Epidermis of nimbus tube is direct wall, with longitudinal folds of a cuticle with straight lines or with the slanted ends (Fig. 4). In a pharynx and bending of a nimbus epidermis cells are sinuous walls, with nipples outgrowths, is trimmed various hairs: simple than two — four — is more rare five-cellular with a warty cuticle and head hairs of two types: on a mono celled short leg with a mono celled oval head, on a two-cellular leg with a mono celled oval head. On epidermis of leaflets of a cup and a nimbus the large essential oil glandular, sometimes with yellow-brown contents consisting of radial located 8 secretor cells meet; in places of attachment the essential oil glandular of a cell of epidermis form the socket.

On epidermis of stalk are nipple outgrowths with smooth or slightly warty cuticle and multi-cellular hairs with a warty cuticle settle down. Cells of external and internal epidermis of leaflets of a cup strongly sinuous; there are hairs of three types: simple, two — five-cellular with a warty cuticle, nipple outgrowths of epidermis with smooth or slightly warty cuticle, head on a mono-celled short leg with a mono-celled oval head (Fig. 4).



A B

A — nimbus of flower; *B* — cup; *1* — essential oil glandilar; *2* — trichomes

Figure 4. Epidermis of nimbus of flower and cup of *Thymus serpyllum*. Preparation from surface. Zoom 10×16

The conducted morphological and micro diagnostic analysis of *Thymus serpyllum* has revealed the general and distinctive diagnostic signs. The morphological features important for diagnostics of raw materials of thymes are:

1. For a stalk — the cross section of a stalk: tetrahedral, rounded tetrahedral, cylindrical; the nature of omission of a stalk — is trimmed the short, pressed to a stalk hairs from all directions; it is trimmed the short hairs perpendicularly located to a stalk from all directions; it is trimmed from all directions down the located hairs; it is trimmed on edges the pressed long hairs down; it is trimmed long perpendicular the standing hairs.

2. For leaves — leaves linear or linearly-shovel, flat; lancet or oblong and elliptic; linear or it is narrow — elliptic; ovoid or it is wide — elliptic; existence of stalk: sedentary leaves; short scape leaves; petiole leaves; leaves are almost sedentary; leaves almost sedentary or there is a short scape.

3. For generative bodies. An inflorescence form is an inflorescence head, compact; head or extended with 2–7 moved apart verticils or interrupted; faltering extended with 3–7 placed verticils: it is extended-head or faltering, at *Thymus serpyllum* the inflorescence also is short — hairy. The direction of hairs on a pedicel hairs are directed down; perpendicular to a pedicel or slightly up; perpendicular to a pedicel; perpendicular to a pedicel or slightly down. Omission of a cup — the cup is trimmed; it is densely trimmed; naked. Characteristic of teeth of an upper lip of a cup: sharply triangular; triangular, rather stupid, three teeth, small (average larger); triangular, delayed; lancet, sharp; triangular-lancet, sharp.

Diagnostic signs of anatomical structure for a leaf, a stalk, a cup and a nimbus are degree of omission, character of trichomes and their combination and also existence of crystals of oxalate of calcium in a nimbus tube, essential oil glandular, nipples of outgrowths of cells of epidermis for a leaf of a cup, a nimbus.

Conclusion

Our investigations have shown that the studied chemo types of *Thymus serpyllum*, collected in different areas of the Karaganda region, are characterized by xero-mesophytes and xerophytes type of a structure that is expressed in a small cellular structure of epidermis of a leaf, existence of numerous essential oil glandular and cuticles. The received results of a morphological and microscopic structure allow including these signs for identification of specific accessory of *Thymus serpyllum*.

In spite of the fact that there are distinctions on morphological features of *Thymus serpyllum*, comparison of anatomic drawings doesn't show a difference on a structure of *Thymus serpyllum*, collected in two different places growth.

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Қарағанды облысының әртүрлі аудандарында жиналған *Thymus serpyllum* L. салыстырмалы морфологиялық және анатомиялық талдау

Мақалада түрлердің негізгі ерекшеліктерін анықтау мақсатымен тасшөптің (*Thymus serpyllum* L.) салыстырмалы түрде морфологиялық және анатомиялық көрсеткіштерін талдау нәтижелері келтірілген. Тасшөп шикізаты Корнеевка (Бұқаржырау ауданы) және Қарқаралы таулы аймақтардан (Қарқаралы) ауданы жиналды. Зерттеу нәтижелері Қарағанды облысының әртүрлі өңірлерінде жиналған шелектердің екі түрдегі хемотүрлері бар екенін анықтауға мүмкіндік берді. Зерттеу барысында тимьянның жапырақтары үшін микродиагностикалық белгілер тазартылды, бағаналар, петиол, королла және тұтастыққа арналған жаңа белгілер анықталды. Пішіні мен мөлшерін түр түнып, жұмыс парағын, табақша құрылымның өсу белгілерін анықтау морфологиялық көрінісі болып табылады. Анатомиялық құрылымына, сипаты мен дәрежесі және оларды үйлестіру, сондай-ақ өсу трихомдар гүл тәжінің табақша парағы үшін диагностикалық түр болып есептеліп, белгілері, қабықты ағаш бездері темірлерлерді эфир-майлану гүлдің тәжінде кальций оксалаты болуы, анатомиялық құрылым белгілері болып табылады.

Кілт сөздер: *Thymus serpyllum* L., морфологиялық талдау, анатомиялық талдау, Қаркаралы таулары, Корнеевка ормандары, хемотүр, салыстырмалы талдау, идентификациясы, микробелгілер.

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Сравнительный морфологический и анатомический анализ тимьяна ползучего *(Thymus serpyllum* L.), собранного в разных районах Карагандинской области

В статье приведены результаты сравнительного морфологического и анатомического анализа лекарственного растения тимьяна ползучего *(Thymus serpyllum* L.) для выявления основных отличительных признаков вида. Сырье тимьяна было собрано в горах Каркаралы (Каркаралинский район) и на территории Корнеевских лесов (Бухаржырауский район). Результаты исследований позволили определить, что тимьян ползучий, собранный в разных районах Карагандинской области, представлен двумя хемотипами. Хемотипы тимьяна ползучего имеют внешнее сходство, однако под влиянием различных факторов окружающей среды определены отличия по внешней характеристике стеблей, листьев, соцветий, строению и опушению чашечки и венчика, что позволяет их использовать для идентификации вида. В процессе проведения исследований уточнены микродиагностические признаки для листа тимьяна ползучего, а также выявлены новые признаки для стебля, черешка, венчика и чашечки цельного сырья. Диагностическими признаками морфологической структуры являются форма и размеры стебля, листа, опушенность чашечки. Диагностическими признаками анатомической структуры для листа, стебля, чашечки и венчика являются степень опушенности, характер трихом и их сочетание, а также наличие кристаллов оксалата кальция в трубке венчика, эфирно-масличных железок, сосочковидных выростов клеток эпидермиса для листа чашечки, венчика.

Ключевые слова: *Thymus serpyllum* L., морфологический анализ, анатомический анализ, горы Каркаралы, Корнеевские леса, хемотип, сравнительное исследование, идентификация, микропризнаки.

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