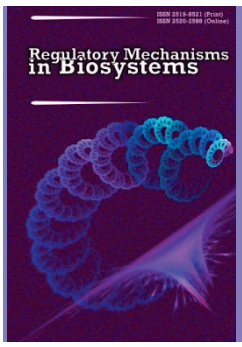

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Diagnostic traits of medicinal herbal raw material of species of *Thymus* genus

M. R. Hrytsyna*, M. I. Skybitska**, O. T. Novikevich*

**Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies, Lviv, Ukraine*
***Ivan Franko National University of Lviv, Lviv, Ukraine*

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Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies, Pekarska st., 50, Lviv, 79010, Ukraine. Tel.: +38-097-255-17-98. E-mail: hrytsynamr@lvet.edu.ua

Ivan Franko National University of Lviv, Cheremshyny st., 44, Lviv, 79000, Ukraine. Tel.: + 38-097-097-73-27. E-mail: mariaskyba@gmail.com

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The paper presents the peculiarities of morphological-anatomical structure of the organs, which are diagnostic traits of herbal raw materials (HRM) of the commonest species of *Thymus* L. genus in the moderate climatic zone. We determined that the studied species develop a subshrub life form, which grows monopodially in *Th. pulegioides* and *Th. marschallianus*, and sympodially with mono-, di- and polycyclic types of monocarpic shoots in *Th. serpyllum*. Inflorescence is of thyrsoid type with opposite partial inflorescences, which are constituents of simple dichasia with monochasia. Macroscopic and microscopic diagnostic features of the HRM are structure and pubescence on the stem, leaves and flowers, the anatomical structure of which was studied on 20 μm thick transversal sections using a light microscope. In *Th. pulegioides*, stems are tetrahedral, pubescent on ribs with unicellular and two-cellular elbow-shaped hairs. Leaves are ovoid, covered by multiangular epidermis that bears diacytic stromas, sharpened capitate unicellular and two-cellular hairs and 10–12 cellular essential oil glandules. In *Th. marschallianus*, the stems are poorly tetrahedral, rounded, pubescent on the entire surface. Leaves are elliptic, the cells of the epidermis are elongated, significantly tortuous, with 10–12 cellular glandules, diacytic stromas, capitate and unicellular hairs. In *Th. serpyllum*, the stems are cylindric, pubescent all round. The leaves are elongated-elliptic, epidermis cells are oval, tortuous, stromas are diacytic, essential oil glandules are 8–10 cellular, number of papilla-like hairs is low. The petioles and lamina base of the studied species are pubescent on the sides with short, coned and elbow-shaped, 2–3 cellular hairs, among which there are long, 4–8 cellular hairs; the hairs in *Th. serpyllum* are multicellular, interrupted. In mesophyll, there are schizogenous essential oil reservoirs. In *Th. marschallianus* and *Th. pulegioides*, the calyx is campanulate, bilabiate, and pubescent. The flower corolla is purple-violet, bilabiate, has tube and outward bend, pubescent inside with coned unicellular hairs, has essential oil glandules and glandular capitate hairs, and pubescence in the fauces comprises long unicellular hairs. In *Th. pulegioides*, cells of the corolla epidermis are multiangular-shaped with insignificantly wavy walls, in *Th. marschallianus* – rectangular with significantly wavy walls. Cells of the tube epidermis are rectangular, tortuous. Surface of the corolla of *Th. serpyllum* is pubescent on both sides with short, cone-like and three-cellular, interrupted hairs, and also capitate hairs, and has essential oil glandules. Epidermis cells are multi-angular, tubes – rectangular and narrow. The obtained results may be used for diagnostics of HRM of morphologically similar species during their preparation.

Keywords: *Thymus pulegioides*; *Th. marschallianus*; *Th. serpyllum*; epidermis; diacytic type of stroma apparatus; pubescence; essential oil glandules; schizogenous essential oil reservoirs.

Introduction

To identify herbal raw material (HRM), it is important to study morphological-anatomic features of the structures of the organs of plants, on which the systematics of flora is based. Official medicine allows the use of Breckland thyme (*Thymus serpyllum* L.) and Mediterranean species *Th. vulgaris* L. and *Th. zygis* L., introduced to Ukraine (European Pharmacopoeia, 2011; State Pharmacopoeia of Ukraine, 2014). However, for further processing, pharmaceutic industries receive raw materials of *Thymus* L. species other than *Th. serpyllum*, united under the general name *Herba serpylli*. This happens for several reasons. In particular, 37 species of *Thymus* genus grow in the territory of Ukraine, and *Th. serpyllum* is only distributed on sandy soils under coniferous forests in Polissia and Roztocze. The systematics of the genus is quite complex, the species are characterized by high polymorphism and hybridize with one another, and also there are many subspecies and synonyms of species.

For the flora of Ukraine, Mosyakin & Fedoronchuk (1999) distinguish the following synonyms, subspecies and hybrids in the examined species *Th. pullegioides*, *Th. marschallianus* and *Th. serpyllum*: *Th. marschallianus* Willd. (*Th. amictus* Klokov, *Th. latifolius* (Besser) Andrz.), *Th. marscliallianus* Willd. var. *latifolius* Besser (*Th. pannonicus*), *Th. pannonicus* All. subsp. *marschalliamis* (Willd.) Soo (*Th. marschallianus*); *Th. pulegioides* L. aggr. (*Th. pullegioides* L subsp. *ucrainicus* Klokov & Des.-Shost., *Th. ucrainicus*, *Th. clandestinus* Schur., *Th. ovatus* Mill.); *Th. serpyllum* L. (*Th. angustifolius* Pers.). In the western region of Ukraine, there sporadically occurs a hybrid of *Th. pulegioides* L. × *Th. serpyllum* L. emend. Mill., described as nothospecies *Th.* × *oblongifolius* Opiz. Another hybrid, parental forms of which belong to different sections, is *Th. pannonicus* × *Th. serpyllum*, their hybrids only occur on northern slopes of the Podillia Upland (Nachychko & Honcharenko, 2016).

The quality of herbal raw material depends on growth conditions, which affect the size of plants, sex (gynodioecy) and age structure of populations, and also the resource potential of species of *Thymus* genus (Stakelien & Ložien, 2014; Kryvtsova et al., 2019; Talebi et al., 2019; Penkovska, 2020). Furthermore, not only morphologically close species, but also various populations of one species have different quantitative and qualititative compositions of essential oils, which sometimes reduces the quality of HRM (Sárosi et al., 2012; Vaičiulytė et al., 2016; Wester et al., 2020; Boyko & Brygadyrenko, 2021).

During preparation of herbal raw material called *Herba serpilly*, there occur – other than official species *Th. serpyllum* – other closely related unofficial species – *Th. pulegioides* and *Th. marscliallianus*, which are commonest in natural populations in the moderate climatic zone in the

territory of Ukraine. The objective of our study was macro- and microscopic analyses of species *Th. serpyllum* L., *Th. pulegioides* L. and *Th. marscliallianus* Willd. in order to determine morphological-anatomical traits that would be diagnostic for HRM. Such studies would allow the possibility of identifying plants collected in the wild and selection of material to prepare medicinal drugs.

Materials and methods

The objects of the study were species of *Thymus* L. genus: Breckland thyme *Thymus serpyllum*, HRM of which is in the European Pharmacopoeia (2011) and State Pharmacopoeia of Ukraine (2014) and unofficial species – broad-leaved thyme *Th. pulegioides* L. and *Th. marschallianus* Willd. In Ukraine, *Th. pulegioides* occurs on forest edges and glades, on grassy slopes in the Prykarpattia. Plants of *Th. serpyllum* are confined to groups of sandy soils under coniferous forests, sometimes they are found on outcrops of crystalline rocks in the Polissia Lowland, Lesser Polissia and Roztocze. *Thymus marschallianus* grows on stepped slopes, outskirts and outcrops (Flora of Ukraine, 1960; Flora Europaea, 1976). Material for the study was collected in the territory of Lviv Oblast in populations of the Yavoriv National Nature Park (Roztocze), on the outskirts of pine forest of the geological nature relic the Outcrops of Tortonian Sandstones with Accumulation of Fossil Tortonian Fauna (Trostianets, Mykolaiv District), in the Znesinnia Regional Landscape Park (Lviv) and outskirts of the city Pustomyty.

Herbal raw material from species of *Thymus* genus is grass, i.e. above-ground herbaceous part of the plant during blossom. To conduct macro- and microscopic analyses, we took generative herbaceous shoots in the phenophase of complete blossoming (3–5 shoots of 10 individuals of each species). Using the macroscopic analysis, we identified the integral HRM according to the morphological features: type of life form and shoot, peculiarities of the external structure, pubescences, presence of essential oil-bearing structures of the stem, leaves, flowers and structure and type of inflorescence. For morphological studies, we used binocular microscope MBS 1. In the microscopic study, we analyzed the internal structure, epidermis structure, pubescences and essential oil-bearing structures of the stem, leaves and flowers (European Pharmacopoeia, 2011). On the trasversal section of the leaf, we examined mesophyll structure, mechanic, vascular and secretory tissues.

We also analyzed the structure of the upper and lower epidermis of leaf, in particular: shape, sizes of cells and structure of their membranes, types of stroma apparatuses; structure of cuticle, hairs and glandular structures. In the generative sphere, we studied: structure of calyx and corolla of flower and bracts, structure of epidermis, presence of hairs and essential oil-bearing glands. Macro- and microscopic studies of the raw material were performed according to the methods of State Pharmacopoeia of Ukraine 2.8.23 "Microscopic study of herbal raw material" and methods of Pausheva (1988) using a Leica DM 500 microscope. Freshly collected raw material was fixed in the mixture of 96% ethanol P – glycerol P – purified water P (1:1:1). To study peculiarities of anatomic structure of leaves and stems, we prepared their 10–20 μm thick transversal sections using microtome and blade. We used glycerol to illuminate the temporary preparations prepared of fresh plants, and stained them using iodine solution in iodine potassium and acid fuchsin. Microimages were taken using a digital camera and Bresser camera eyepiece.

Results

For medical purposes, according to the State Pharmacopoeia of Ukraine (2014), fresh or dried herbs of *Th. vulgaris* or *Th. zigis* (*Herba Thymi vulgaris*) are used, cultivated only. To prepare drugs, grass *Th. serpyllum* – *Herba Thymi serpilli* are also used. Herbal raw material of *Th. pulegioides* and *Th. marschallianus*, like all the official species, is "herb" (Herbae), i.e. blossoming shoots, collected during blossom without rough parts of stems. Raw material of *Th. serpyllum* has a strong aroma, which reminds one of thymol, increasing during fragmentation in mortar. Raw material of *Th. pulegioides* has a lemon aroma, which indicates high content of α-terpinelol, and raw material of *Th. marschallianus* has a spicy aroma (Sárosi et al., 2012; Vaičiulytė et al., 2016; Wester et al., 2020).

Diagnostic systematic traits within the *Thymys* genus are the following: type of life form, pubescences on stem and leaves, shape and way of veination of leaf lamina, degree of expression of petiole, type of inflorescence and form and pubescences on the calyx (Nachychko et al., 2015, 2017). The identification of HRM of species of *Thymus* genus was performed according to the external and internal structures of leaf and flower, their pubescence, presence of essential oil-bearing structures (European Pharmacopoeia, 2011; State Pharmacopoeia of Ukraine, 2014).

The life form of *Th. pulegioides* and *Th. marschallianus* is subshrub that grows sympodially, with main root (Berko, 2013). Skeletal plagiotropic axes of *Th. pulegioides* end with a generative shoot (Fig. 1a). The main structure-forming element is mono-cyclic, mono-carpic, often wintering, orthotrophic, herbaceous generative shoot, which develops from lateral buds of stiffened shoot. In *Th. marschallianus*, the form-developing shoot is di- and tricyclic (Fig. 1c). Plants of *Th. serpyllum* form small vegetatively semimobile shrubs of creeping type with stiffened multi-year shoots and system of the main root (Fig. 1b). Stiffened skeletal axes grow monopodially, i.e. are vegetative, polycyclic, and thrive plagiotrophically. Generative shoots, which develop from lateral buds of skeletal axes, are herbaceous, orthotrophic, monocarpic, often wintering.

Thymus pulegioides has 15–30 cm high flower-bearing shoots, green-purple stems, which are distinctly tetrahedral, pubescent on the ribs with two-cellular, bent hairs, among which there are also coned, unicellular hairs (Fig. 2a). Under the inflorescences, the pubescence is denser and noticeably longer (Fig. 2b). In *Th. marschallianus*, the stem is up to 15–25 cm high, green, inexpressively tetrathedral and pubescent with mostly long, 2–4 cellular elbow-shaped hairs, among which there are simple short hairs and glandular hairs with unicellular capitulum (Fig. 2c). Under the inflorescence, the stem is rounded, densely covered on the entire surface by long, distant hairs (Fig. 2d). In *Th. serpyllum*, the height of the stem is 5–10 cm. Generative stems are herbaceous, green, become reddish brown toward the top, cylindrical, uniformly pubescent on the entire surface with uni- and two-cellular retrorse hairs (Fig. 2e).

Important aspects during macroanalysis of HRM are structure and pubescence of stem, leaves and flowers. In the studied species, the leaves are opposite, differ by structure within one generative shoot, and their laminae are slightly curved downward. The lower leaves of *Th. pulegioides* are petioled, ovoid, with wedge-shaped base and rounded apex, with entire margins. Above the shoot, the petioles gradually become shorter, and the leaves become oval, almost sessile, ranging 7–10 to 15–20 mm in length and 2.5–4.0 to 10 in width, the petioles are 1–3 mm long. The leaves of *Th. marschallianus* change from being short-petioled (1.0–1.5 mm) to sessile, elongated-eliptic, with wedge-shaped base and insignificantly sharpened apex, with entire margins, thin, light-green, 15 to 24–30 mm long and 2.5 to 5.0–7.5 mm wide. In both species, poorly noticeable lateral branchlets begin to develop in the leaf angles (Fig. 3a). The leaves of *Th. serpyllum* are elongated-elliptical, up to 10 mm long, 1.5–3.0 mm wide, short-petioled, becoming sessile and elliptical up the shoot. Their laminae are dark-green have sharpened apices, wedge-shaped bases and entire margins (Fig. 3c). All the examined species have camptodromous veination. The central vein protrudes in keel-like manner above the lamina, resembling a wedge. The lateral veins are elongated arch-wise, gradually become thinner toward the margin of the leaf lamina and disappear (Fig. 3a, b, c).

Leaves of all species of genus *Thymus* are covered by numerous epidermal formations, in particular: non-glandular covering and glandular capitate hairs and essential oil glandules. The petioles and leaves of the studied species are – from the margins to the third part of the length of the laminae – covered by unicellular, coned, sharpened and two- and three-cellular, bent, elbow-shaped hairs (Fig. 3d, e, f, g). Among them, there are long, multi-cellular – 4–8-cellular hairs, one-rowed, slightly deflated in conjunctions, with moderately thickened, warty membranes and quite wide base. In *Th. serpyllum*, among short hairs, there are long, 7–8-cellular hairs with cell collapsed (Fig. 3h, i) (European Pharmacopoeia, 2011). The surface of the lamina is covered by unicellular, coned (papillary) hairs. In *Th. pulegioides* and *Th. marschallianus*, the number of essential oil glandules located on the adaxial (lower) side of the leaf lamina is about 12–15 per 25 mm^2, in leaves of *Th. serpyllum* – about 8–10 essential oil glands per 25 mm^2, on the abaxial (upper) side, their number is slightly

lower (Fig. 3d, e, f). Furthermore, on the surface of lamina of *Th. marschallianus*, there are small hairs, with unicellular leg and unicellular ball-like or ovid-shape capitulum, which are absent in *Th. pulegioides*, whereas *Th. serpyllum* has them in low number.

Fig. 1. Life forms of species of *Thymus* genus: *a* – *Th. pulegioides*; *b* – *Th. serpyllum*; *c* – *Th. marschallianus*: *1* – skeletal axis, *2* – vegetative shoots, *3* – generative shoots, *4* – inflorescence; bar: *a*, *b* – 2 cm; *c* – 1 cm

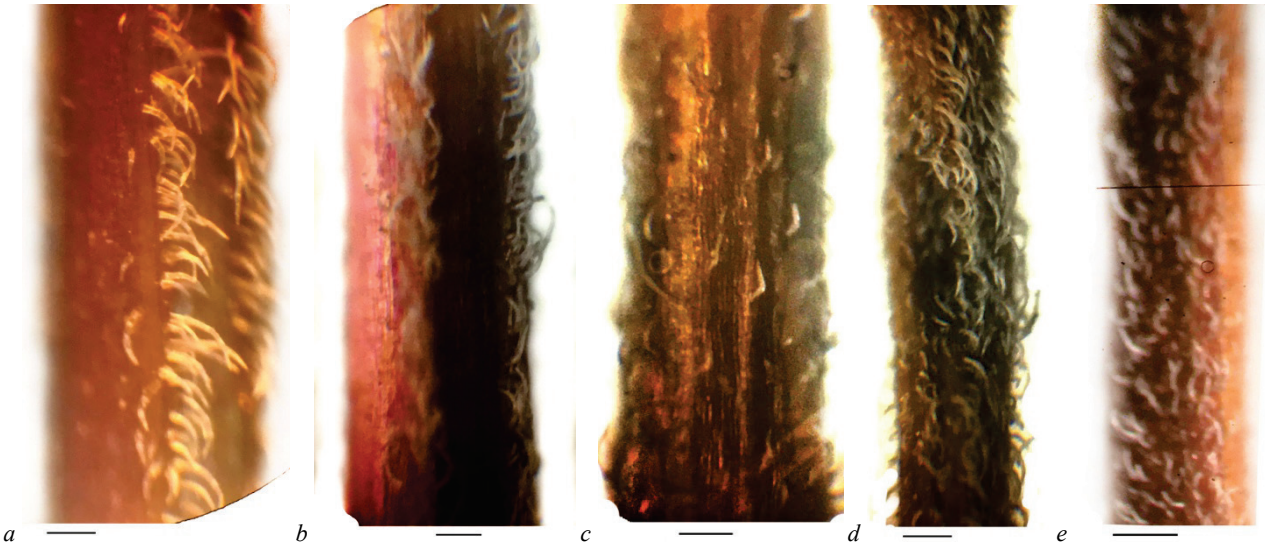


Fig. 2. Pubescence and form of the stem of species of *Thymus* genus: *a* – lower part of the stem of *Th. pulegioides*; *b* – upper part of the stem of *Th. pulegioides*; *c* – lower part of the stem of *Th. marschallianus*; *d* – upper part of the stem of *Th. marschallianus*; *e* – the stem of *Th. serpyllum*; bar – 1 mm

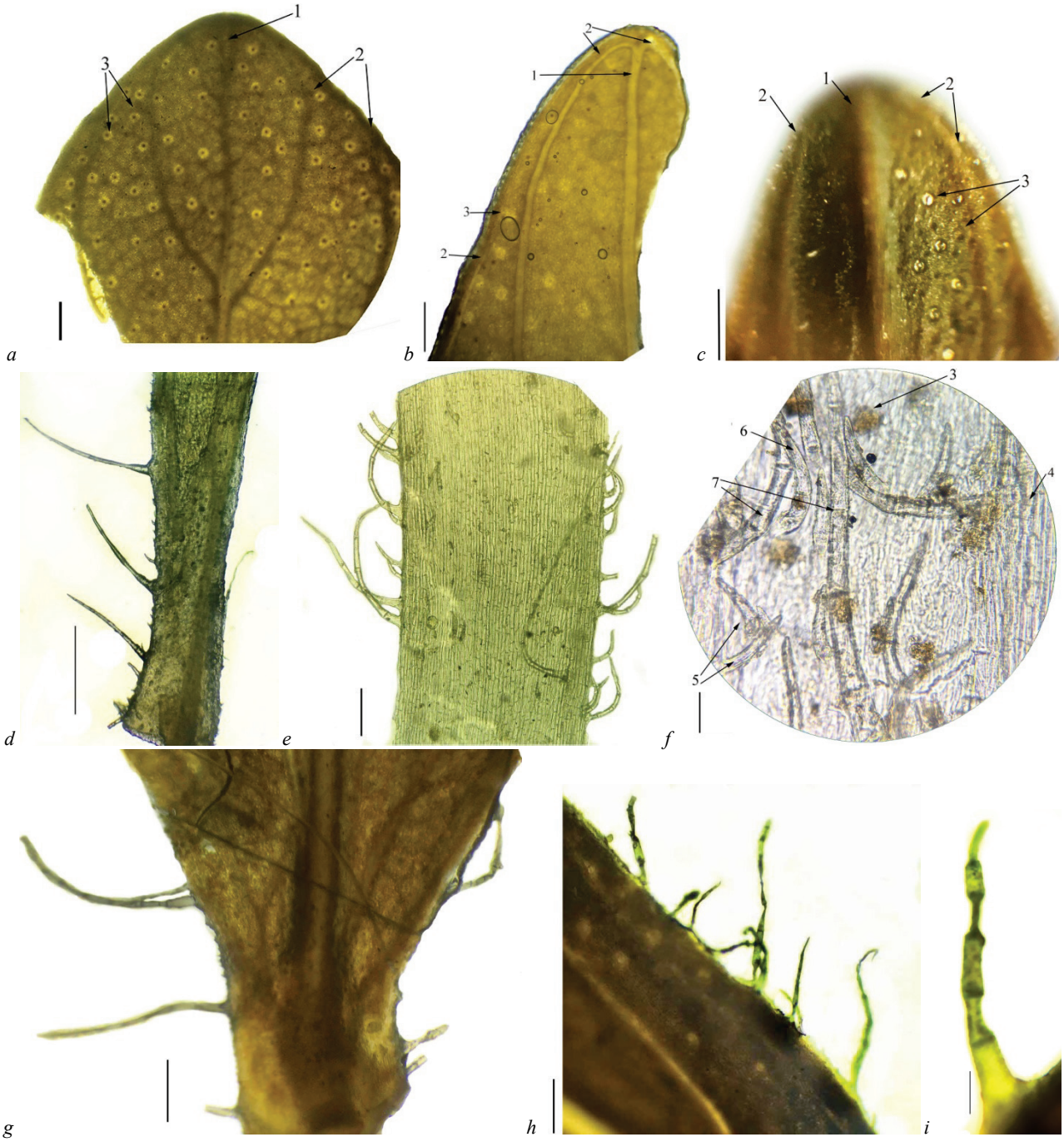


Fig. 3. Morphological structure of the leaf and petiole of species of *Thymus* genus: *a* – the upper part of the leaf lamina of *Th. pulegioides*, on the lower side; *b* – the upper part of the of the lower side of the leaf lamina of *Th. marschallianus*; *c* – the upper part of the of the lower side of the leaf lamina of *Th. serpyllum*: *1* – central vein, *2* – lateral veins, *3* – essential oil glands; *4* – elongated cells of epidermis; *5* – unicellular, papilla-type hairs; *6* – two-, three-cellular, sharpened, elbow-shaped hairs, *7* – multicellular hairs; *d* – leaf petiole of *Th. pulegioides*; *e* – epidermis and indumentum of the petiole of leaf in the middle region of *Th. pulegioides*; *f* – fragment of epidermis and pubescence of leaf petiole on the lateral side of *Th. pulegioides*; *g* – pubescences on the petiole of *Th. marschallianus*; *h* – margin of the lower part of the leaf lamina of *Th. serpyllum*; *i* – multicellular hair with cell collapsed of *Th. serpyllum*; bar: *a*, *b*, *c*, *d*, *g* – 1 mm; *e*, *h* – 400 μm; *f*, *i* – 250 μm

To systematize species of *Thymus* genus, an important feature is the structure of inflorescence. All the studied species form inflorescences of thyrsoid type, the apex of which grows monopodially, while lateral partial inflorescences grow sympodially. Partial inflorescences are located in the leaf angles of opposite bracts, forming false rings. The bracts are similar to the upper leaves, only smaller. By the end of the blossoming, the inflorescence of *Th. pulegioides* reaches more than 10 cm in length (Fig. 4a), is interrupted at the base, the first 6–9 false rings are spread. The inflorescence of *Th. marschallianus* is elongated, reaches up to 15 cm in length by the end of blossom, separated into false spread rings (7 and more). The inflorescences of *Th. serpyllum* have apical capitula, formed from dense lateral inflorescences (Fig. 4b). At the base of the partial inflorescence, there is growth axis of the first order, which has flower and single-node pedicel, with opposite arrangement of flower buds in the node (Fig. 4b, d). From those buds, there develop two opposite axes of ramification – 2 and 3, which blossom simultaneously, forming a dichasium.

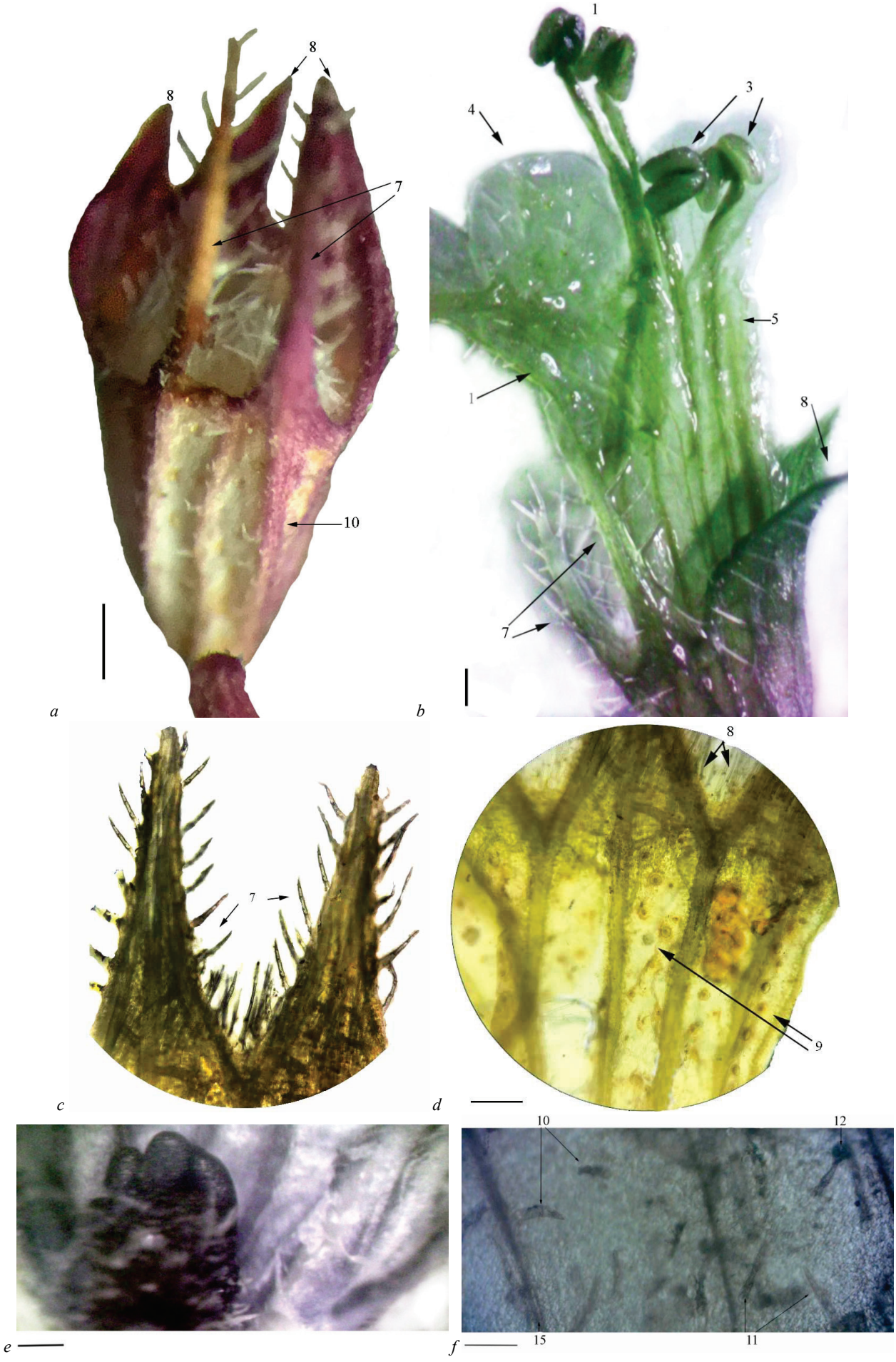
Each of the axes of the second order (2 and 3) has flower and single-node pedicel with one flower bud of the third order. In highly vital plants, those growth axes (4 and 5) blossom. The processes of growth and ramification result in partial inflorescences, with dichasium at the center, and its lateral growth axes – which form on ramification axes – are monochasia.

Therefore, in the angles of whorled pedicels of species of *Thymus* genus, there form partial (lateral) inflorescences, complex dichasium with monochasia. Such inflorescences form thyrsoid inflorescences. In *Th. pulegioides*, there forms a complex thyrsoid raceme, interrupted at the base, with opposite partial inflorescences, which are complex dichasia with monochasia, and in *Th. marschallianus*, there forms simple thyrsoid raceme with spread partial opposite inflorescences, which are also complex dichasia with monochasia. Up to the apex of the inflorescence, all flowers in partial inflorescences do not develop and are dichasia. In *Th. serpyllum*, compact, capitate raceme develops with dense partial inflorescences, which are mostly simple dichasia.

In diagnostics of herbs, which in case of species of *Thymus* genus are collected during blossom phenophase, important features are the morphological structure of the flower, as well as the structure of its epidermis, pubescence, and presence of essential oil structures. Therefore, in species of section *Th.* sect. *marginati*, to which *Th. pulegioídes* and *Th. marschallianus* belong, the calyx is campanulate, with 10 expressed veins, tube and two-lipped outward bend, which are equal by length (Fig. 5a). Along the veins, the tube is rarely covered by long sharp 1–2-cellular hairs, especially on the adaxial side, and has essential oil glandules. The upper lip of the calyx is to the middle divided into three lanceolate-triangular parts, pubescent with short sharpened unicellular hairs (Fig. 5b). The lower lip is divided almost to the base of the outward bend into two long sharpened parts, which along the margin of the serrations are covered by sharpened hairs, the number of which is no higher than 10–12 pairs (Fig. 5c). Inside, the calyx in both species has two-cellular hairs, which cover the pistil after blossom. The calyx of *Th. pulegioídes* is dark green-purple, up to 2.5 mm high, longer with fruits, the serrations of the upper lip are sharp, the calyx of female individuals is green (Fig. 5a, 8a).

Fig. 4. Morphology of inflorescences of species of *Thymus* genus: *a* – general view of the inflorescence of *Th. pulegioides*; *b* – inflorescence of *Th. serpyllum*; *c* – partial dichasium inflorescence with monochasia of *Th. pulegioides*: *1* – the axis bearing flowers of the first order in the central dichasium; *2* and *3* – opposite axes of ramification of the first-order growth; *4* and *5* – axis bearing flowers, located on axes 2 and 3; *6* – bract; *d* – partial simple dichasium inflorescence of *Th. serpyllum*; bar: *a*, *b* – 1 cm; *c*, *d* – 1 mm

The corolla of *Th. pulegioídes* is watering-can-like, 4–6 mm long, violet-purple, has a long tube and outward bend with sinuate upper and deeply three-lobular, downward bent lower lip (Fig. 5b). Inside, the corolla is sparsely pubescent with unicellular, coned, sharpened hairs (papillary), among which there are longer, 2–3 cellular bent hairs (Fig. 5f) and essential oil glandular structures of three types: glandules with 10-cellular capitulum, glandular wine-glass-like hairs and hairs with rounded unicellular capitulum and two-cellular leg (Fig. 5g, h, i). In the lip's fauces, there is dense pubescence, formed of sharpened two-cellular hairs (Fig. 5j). On the outer side, the corolla is very sparsely pubescent with coned unicellular hairs. Epidermis cells of the corrola's tube are rectangular with wavy walls (Fig. 5i), the corolla's lips are elongated, oval, with wavy walls (Fig. 5g). The androecium is formed by 4 erect stamena, spread from the base, is didymous – two stamena are shorter, two are longer and extend beyond the corolla, the anthers are dithecal, with quite wide connective tissue (Fig. 5b). Pollen grains are rounded with smooth exine (European Pharmacopoeia, 2011).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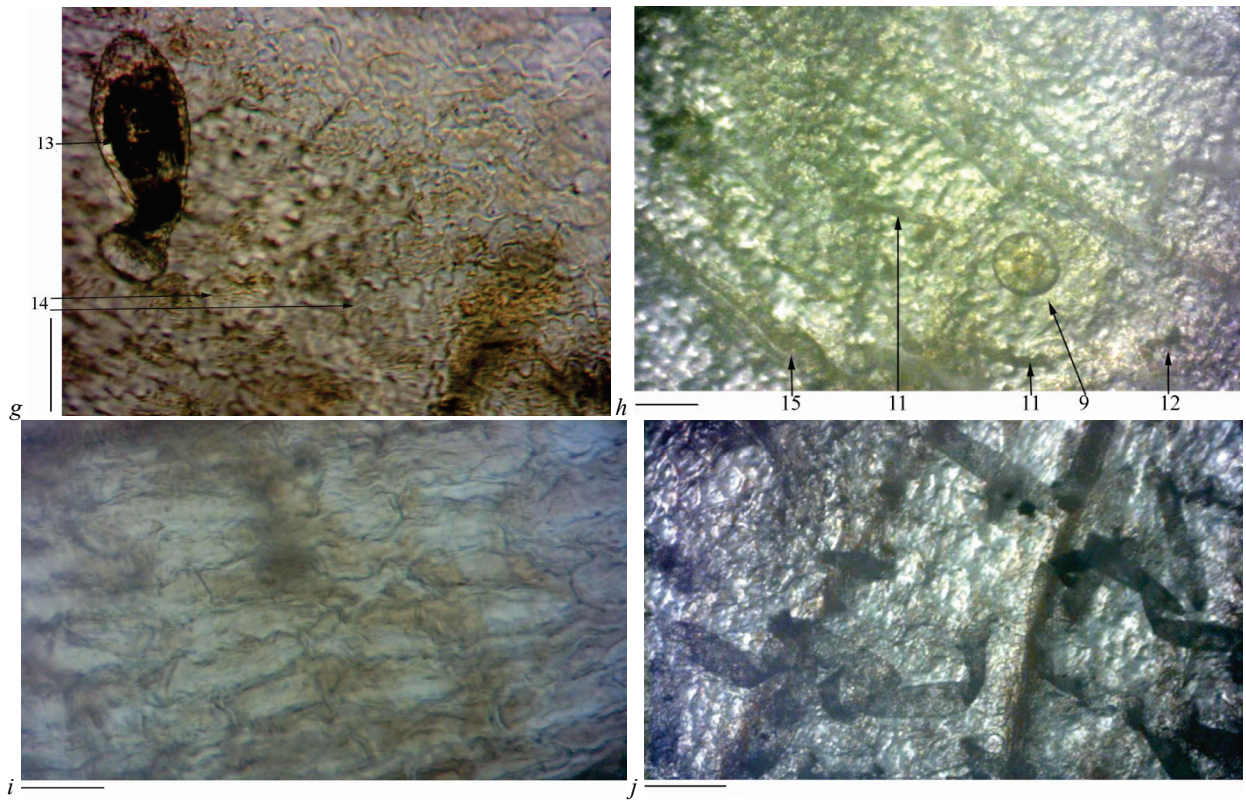


Fig. 5. Morphological-anatomic structure of the flower of *Th. pulegioides*: *a* – general view of the calyx; *b* – corolla and androecium: *1* – stigma of the pistil, *2* – longer stamens, *3* – shorter stamens, *4* – lower, three-lobed lip of the corolla, *5* – upper two-lobed lip of the corolla, *6* – tube of the corolla, *7* – lower, two-lobed lip of the calyx, *8* – upper, three-lobed lip of the calyx, *9* – essential oil-bearing gland; *c* – lower lip of the calyx; *d* – fragment of the tube and upper lip of the calyx; *e* – ovary of the pistil; *f* – fragment of the petal of the corolla's outward bend: *10* – short, papilla-like hairs, *11* – two-, three-cellular long hairs, *12* – essential oil capitate hairs, *13* – glandular wine-glass-like hairs, *14* – epidermis cells, *15* – corolla veins; *g* – structure of epidermis of the corolla lip with wine-glass-like gland; *h* – fragment of the petal with various hairs and essential oil glands; *i* – fragment of epidermis of the corolla tube; *j* – epidermis of the corolla fauces with multicellular hairs; *a*, *b*, *c*, *d* – 500 μm; *e*, *f*, *h*, *g*, *i*, *j* – 250 μm

The calyx of *Th. marschallianus* is campanulate, green, with denser pubescence than *Th. pulegioides*. The tube is shorter than the outer bend, which is pubescent on the entire surface with sharp hairs on the veins. The upper lip has sharp triangular-lancete serrations, pubescent with sharp short hairs. The lower lip is pubescent with sharp three-cellular hairs (Fig. 6a). Inside, the calyx has two-cellular hairs, which cover the pistil after blossom.

The corolla of *Th. marschallianus* is watering-can-like, lilac, up to 5 mm long, with poorly noticeable tube from the calyx. The upper lip is entire, plain on the top; the lower one is deeply three-lobular, retrorse. The androecium is the same as in *Th. pulegioides* (Fig. 6b, j). The corolla of *Th. marschallianus* has a white tube and an outward bend with rounded, plain lower lip. The tube is white, permeated by longitudinal veins, the epidermis is formed by rectangular cells. On the outside, it is pubescent with short coned hairs (Fig. 6b, c). The outward bend is lilac, rarely pubescent on both sides by unicellular, coned hairs, has essential oil glandules with 10-cellular capitulum and glandular hairs with unicellular capitulum and unicellular leg. In the fauces of the corolla, there are many long 2–3-cellular sharpened hairs (Fig. 6d). Epidermis cells are elongated, with wavy walls, filled by vacuoles with violet pigment anthocyan (Fig. 6d, e).

Thymus serpyllum, which belongs to section *Th.* sect. *serpyllum*, is characterized by tubular-campanulate calyx, 3.5–4.0 mm long, dark-green to slightly purple, two-lipped. On the adaxial side, the tube is more significantly pubescent than on the abaxial side, bearing 1–2-cellular hairs along the veins. The serrations of its upper lip are triangular, sharp, rarely hairy on the margins, serrations of the lower lip are pubescent with numerous two-three-cellular hairs, arranged in two rows (over 12 pairs). The inner surface is significantly pubescent with long hairs that cover the tube after blossom (Fig. 7a).

The corolla of *Th. serpyllum* has a long pale-pink thick tube and lilac outer bend with serrated lower lip (Fig. 7b). The surface of the corolla of *Th. serpyllum* is pubescent on both sides – more on the outer side – with short coned hairs (papillary), among which there are two-three-cellular, sharpened, multicellular hairs with one cell collapsed, capitate glandular hairs and rarely essential-oil glandules (Fig. 7c, e, f). Epidermis cells are rectangular on the corolla tube, and isodiametric on epidermis of the outward bend (Fig. 7d).

The studied species had one pistil with tetrathecal upper ovary, long style and two-lobular stigma (Fig. 5b, 6b, 7a, 8). The fruit is composed of four nutlets, placed in calyx, which remains with the fruits; the nutlets are about 0.6–0.8 mm long and 0.5 mm wide. The nutlets of *Th. pulegioides* are short-elliptical, with small hunch, in *Th. serpyllum* – dark-brown, rounded-eliptic, in *Th. marschallianus* – ball-like, almost black.

As a result of the studies conducted in two populations of *Th. pulegioides* and one of *Th. serpyllum*, we determined that hermaphrodite and female plants develop separately. The vegetative organs of hermaphrodite plants are more violet than in female plants. Unisexual flowers have reduced androecium, green colour and smaller sizes than hermaphrodite individuals, which have purple tone and greater sizes (Fig. 8). Therefore, length of bisexual flowers of *Th. serpyllum* equals 40–42 mm, whereas such of unisexual – 33–35 mm, of *Th. pulegioides* – 47–49 and 39–41 mm, respectively.

To identify herbal raw material in microanalysis, there are also used the internal structures of the stem (as additional feature) and leaf, structure of epidermis, pubescences, presence of essential oil-bearing structure.

In *Th. pulegioides*, the stem is distinctly tetrahedral. On the outer side, on the ribs, it is pubescent with short unicellular and long two- and three-cellular, bent hairs (Fig. 2a, b). On the transversal section, the bark is thick, formed by 3–4 rows of large cells with intercellular spaces. The stem ribs are filled with collenchyma. In the phloem, there are groups of sieve tubes. The central cylinder is of non-bundle type. The vessels are scattered, but more of them are located opposite the ribs, compared with the edges. A sclerification of parenchyma cells of the central cylinder is observed. The core has partly died (Fig. 9a, b, c). The stem of *Th. marschallianus*

has a structure similar to *Th. pulegioides*, poorly tetrahedral, with angles filled with collenchymas, becoming rounded in the upper part. On the outer side, the stem is significantly pubescent with two-, three-cellular hairs on the entire surface. The core dies, the central cylinder stiffens (Fig. 9d, e, f). In *Th. serpyllum*, the stem is rounded on the transversal section, pubescent with short unicellular and two-cellular bent hairs on the entire surface. The bark and the central cylinder are of almost the same thickness (Fig. 9g, h). There are no collenchyma-filled ribs on the bark, the parenchyma is formed by 4–5 rows of large cells with dense cytoplasmic content, with groups of sieve tubes underneath. The vessels are arranged in the circle, chaotically, and also there is seen sclerification of intercellular tissue. The core, formed by large parenchymal cells, begins to die inside.

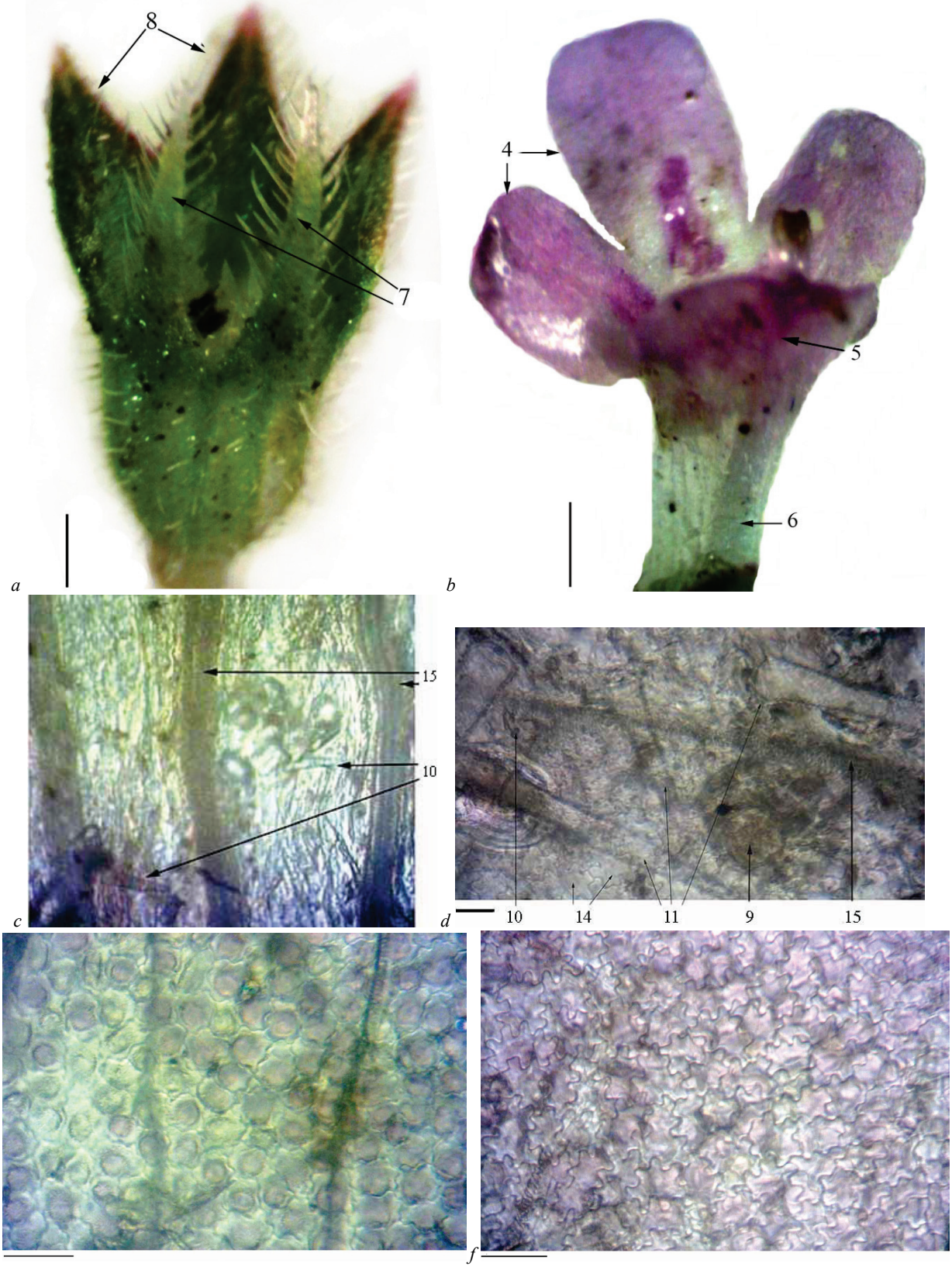


Fig. 6. Structure of flower of *Th. marschallianus*: *a* – flower calyx; *b* – flower corolla; *c* – fragment of the corolla tube; *d* – fragment of outer bend (lip) of corolla petal, closer to fauces; *e* – fragment of petal (lip) with vacuole, stained violet with anthocyanin; *f* – epidermis cells of the petals; other legends such as in Fig. 5; bar: *a*, *b* – 500 μm, *c*, *d*, *e*, *f* – 250 μm

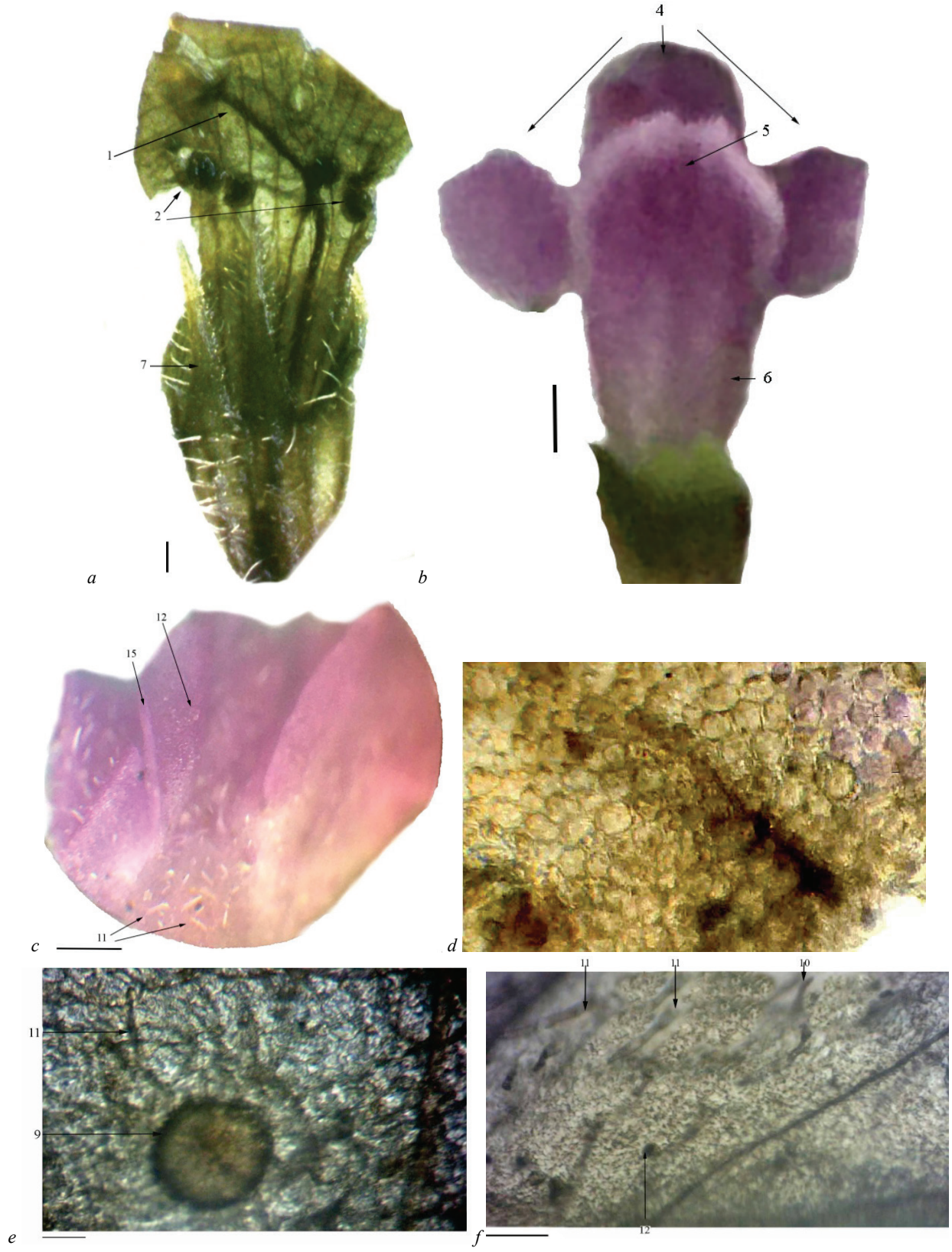
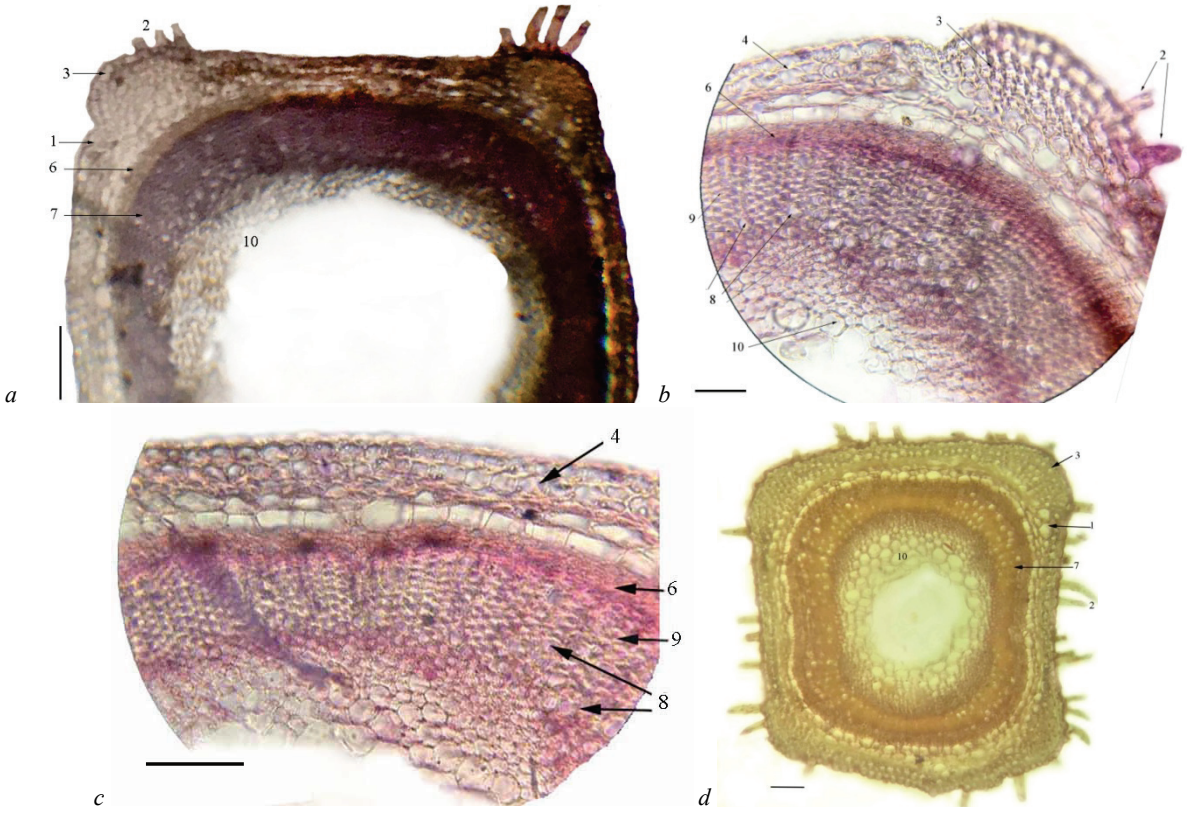


Fig. 7. Structure of corolla of the flower of *Th. serpyllum*: *a* – flower; *b* – flower corolla, *c* – fragment of corolla petal – lips of the outer bend, closer to the fauces, *d* – fragment of epidermis of the petal of the corolla outward bend; *e* – fragment of the petal (lip) with essential oil gland, *f* – epidermal cells of petals with hairs; other legends are the same as in Fig. 5; bar: *a*, *b*, *c* – 500 μm; *d*, *e*, *f* – 250 μm

During microscopic analysis of HRM, one of the main diagnostic features is the anatomic structure of the leaf. During identification of fragmented raw material – herbs, the structure of leaf epidermis is essential. The shape of epidermis cells of leaf lamina of *Th. pulegioides* varies. On the lower side of leaf lamina, between the leading bundles (large veins), epidermis cells are irregular-shaped, multi-angular, with poorly tortuous walls, between which there are stromas and multi-cellular essential oil glandules (Fig. 10a). Above the veins, epidermis cells become elongated-rectangular, the number of stromas is much lower (Fig. 11c). On the upper side of the lamina, epidermis cells are isodiametric, with slightly thickened walls (Fig. 10b). The stroma apparatus is of diacite type. It has two lateral, around-stromal cells, adjacent walls of which are per-

pendicular to the stromatal pore. On the upper side of leaf lamina, the stromas are singular, on the lower side they are located very densely, after each epidermis cell (Fig. 10a). The cuticle is radial-wavy. The diameter of essential oil glandules of *Th. pulegioides* is around 0.3 mm. They have a capitulum, formed of 10–12 rosette epidermis cells with essential oil under the cuticle (Fig. 10a, b) and located on both sides of the leaf lamina. On the surface of leaf lamina, there are unicellular, coned, sharpened hairs – papilla. The epidermis of the leaf calyx is formed of rectangular cells with diagonal walls, with no stromas, and with essential oil-bearing glandules on its surface (Fig. 10d). The pubescence is concentrated near the base and margins of the leaf lamina and on the sides of the calyx. It is formed by coned unicellular and two-cellular, sharpened, elbow-shaped hairs. Among them, there are long 3–7-cellular papilla-like hairs, with wide base, thickened on conjunctions, with sharpened apices (Fig. 10d).

Fig. 8. Structure of unisexual flower: *a* – *Th. pulegioides*; *b* – *Th. marschallianus*; bar: *a*, *b* – 1 mm



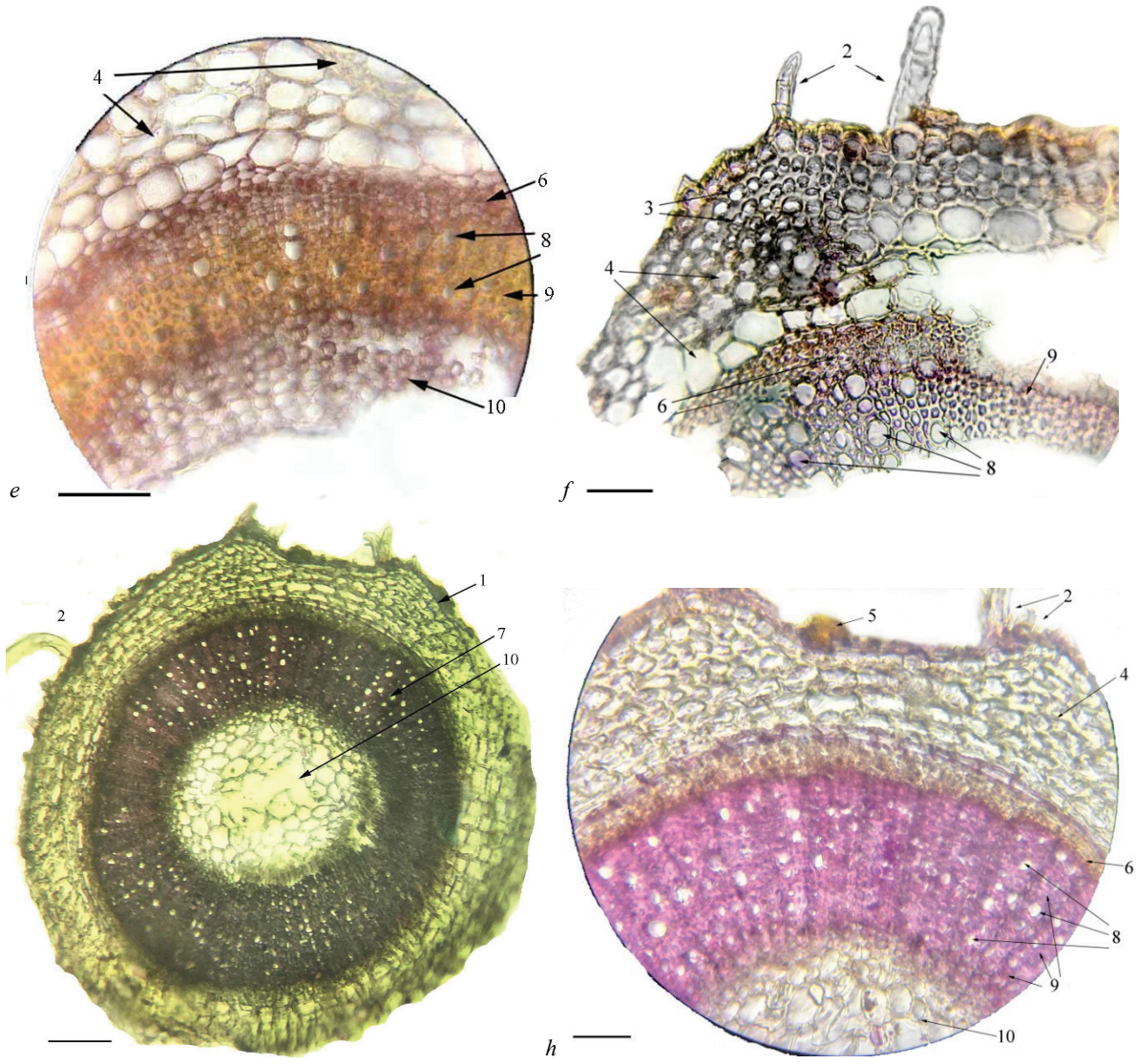


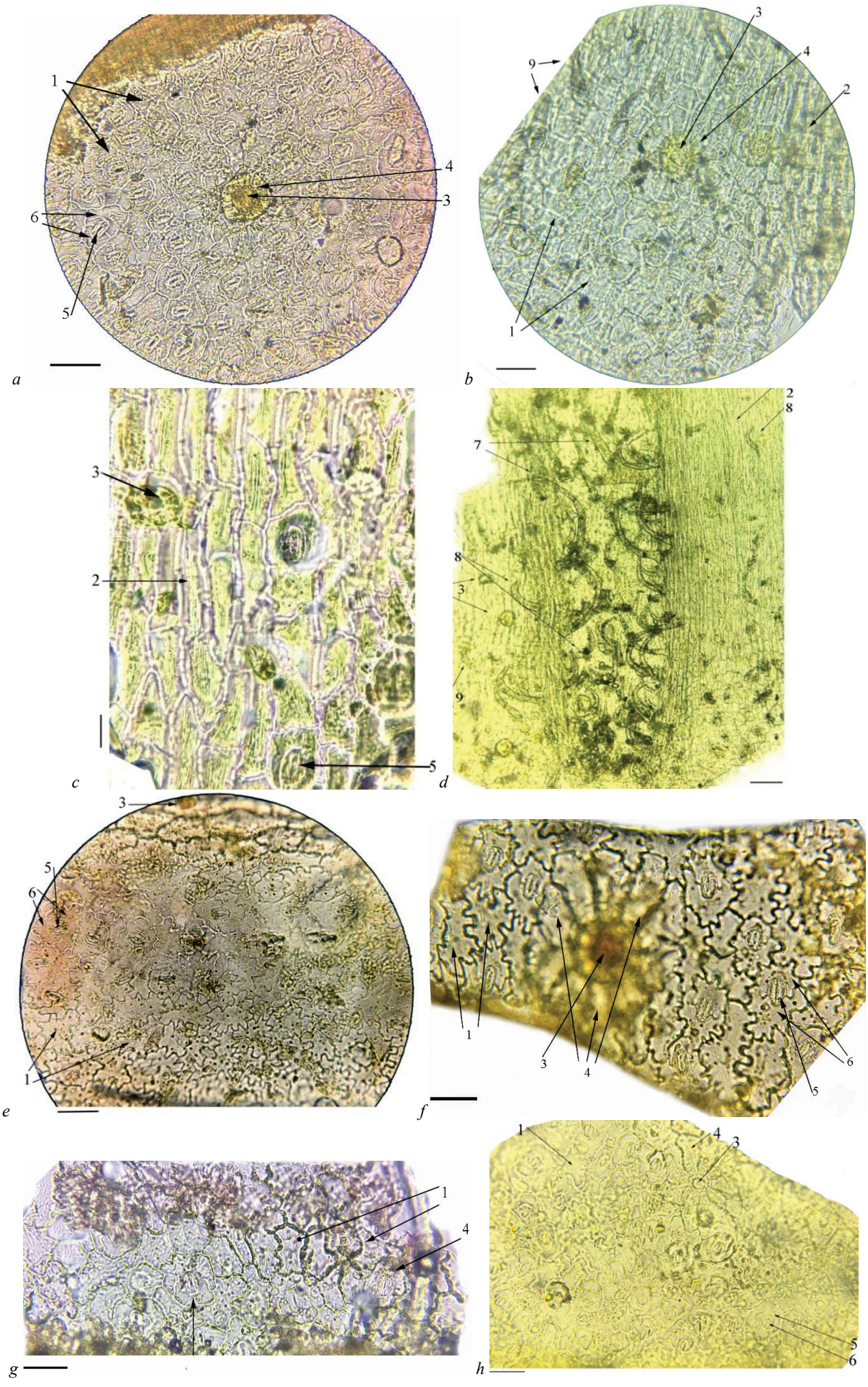
Fig. 9. Anatomical structure of the stems of species of *Thymus genus*: *a* – transversal section through the stem of *Th. pulegioides*; *b* – fragment of the stem in the region of the rib of *Th. pulegioides*; *c* – fragment of stem in the region of the edge of *Th. pulegioides*; *d* – transversal section through the stem of *Th. marschallianus*; *e* – fragment of the stem in the region of the rib of *Th. marschallianus*; *f* – fragment of the stem in the region of edge of *Th. marschallianus*; *g* – transversal section through the stem of *Th. serpyllum*; *h* – fragment of the stem of *Th. serpyllum*: *1* – bark, *2* – hairs, *3* – ribs of the stem, filled with colenchyma, *4* – bark parenchyma, *5* – glandular hairs, *6* – sieve tubes (phloem), *7* – central cylinder, *8* – vessels, *9* – parenchyma, *10* – core; bar: *a*, *d*, *g* – 700 μm; *b*, *c*, *e*, *h* – 250 μm; *f* – 100 μm

Epidermis of leaves of *Th. marschallianus* on the lower side of the lamina has oval cells with significantly tortuous walls, thickened on the sides, with stromas between them (Fig. 10e, f). On the upper side of the lamina, epidermis cells are smaller, and their walls are poorly tortuous, the stromas are single (Fig. 10g). Above the leaf veins, epidermis cells are rectangular, and walls are poorly tortuous. The arrangement of diacyte stromas is dense on the lower side of the leaf, and there is one epidermis cell between the stromas. The cuticle is radially-wavy. The peltate glands are up to 0.4 mm^2 in diameter, formed by 10–12 rosette epidermis cells, have capitulum with essential oil under the cuticle, and unicellular glandular hairs are located near (Fig. 10f). The petioles are short, pubescent on the margins with short hairs, with rare long multicellular hairs among them (Fig. 3j).

The cells of the leaf epidermis of *Th. serpyllum* are oval, with tortuous, often thickened lateral cellular walls. The stroma apparatus is of diacytic type, the stromas are much more densely located on the lower side of the leaf than on the upper one (Fig. 10h, i, j). On the surface of the leaf, there are essential oil glandules, capitate hairs with unicellular leg and ball-shaped capitulum and unicellular, coned, non-glandular hairs (papillae). The cuticle is radial-wavy. Essential oil glandules, with diameter of up to 0.4 mm, have sessile, oval, yellow capitulum, under the cuticle of which the essential oil accumulates, surrounded by 8–10 secretory cells (Fig. 10h).

The leaves of *Th. serpyllum* are hairy on the sides of the petiole and to the middle of the margin ofthe leaf lamina. The pubescence is formed by small, sharpened unicellular coned hairs. Between them, there are long 4–8-cellular hairs, constricted, with slightly deflated at the conjunction, averagely thickened, warty membranes and sharpened apices (Fig. 3k).

In the studied species of *Thymus* genus, essential oils accumulate not only in the epidermis glandules on leaf surface, but also in schizogenous essential oil reservoirs of mesophyll. On the transversal section through the leaf, we can see that mesophyll of *Th. pulegioides* is formed of columnar and spongy tissues. The columnar tissue is well developed, formed of two rows of rectangular cells with chloroplasts, and spongy tissue has large intercellular spaces (Fig. 11a). Under the lower epidermis, there are seen large intercellular spaces, which improve gas exchange. Cells of the upper epidermis have unicellular papillae-like hairs. Above the large veins in the mesophyll, there are schizogenous essential oil reservoirs (3–5). They have a large amount of cells with thickened walls, in which essential oils accumulate. Beneath them, there are vessels and sclerenchyma of vascular-fiberous leading bundle (Fig. 11b). *Thymus marschallianus* has similar anatomic structure of the leaf lamina, as described for other species of *Thymus* genus, though it has more expressed features of xerophytization – air chambers are present under the upper and lower epidermis, and pubescence with unicellular hairs is denser (Fig. 11c, d).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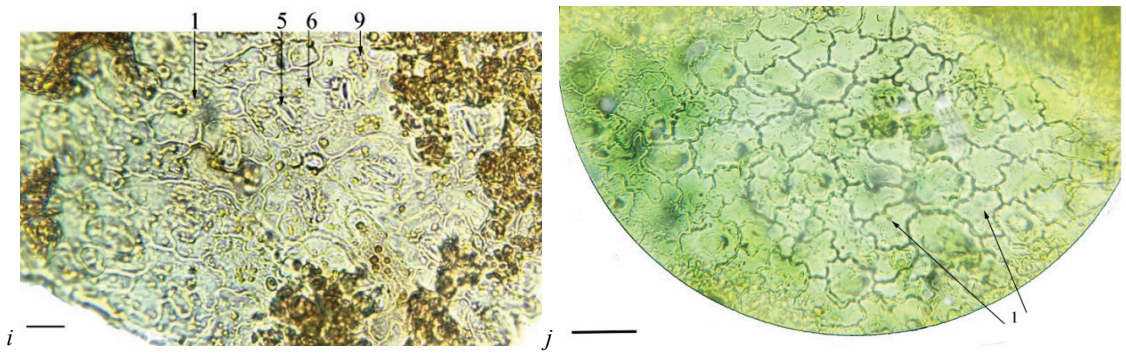

Fig. 10. Structure of epidermis and pubescences of leaves of species of *Thymus* genus: *a* – structure of epidermis on the lower side of the leaf lamina of *Th. pulegioides*; *b* – structure of epidermis on the upper side of the leaf lamina of *Th. pulegioides*: *1* – base cells of epidermis, *2* – rectangular cells of epidermis; *3* – capitulum of essential oil gland, *4* – rosette cells of essential oil glands, *5* – stromatal pore, *6* – around-stroma cells, *7* – multicellular hairs, *8* – two-cellular, sharpened, elbow-shaped hairs, *9* – unicellular, papilla-likei hairs, *10* – capitate glandular hairs; *c* – leaf lamina epidermis above the vein of *Th. pulegioides*; *d* – epidermis and pubescence of the petiole on lateral side of *Th. pulegioides*; *e* – lower epidermis of the leaf lamina of *Th. marschallianus*; *f* – upper epidermis of the lamina of *Th. marschallianus*; *g* – lower epidermis of leaf lamina with essential oil glandule of *Th. serpyllum*; *h* – lower epidermis of leaf lamina of *Th. serpyllum*; *i* – upper epidermis of leaf lamina of *Th. serpyllum*; bar: *a*, *b*, *c*, *d*, *e*, *f*, *g*, *h*, *i*, *j* – 250 μm

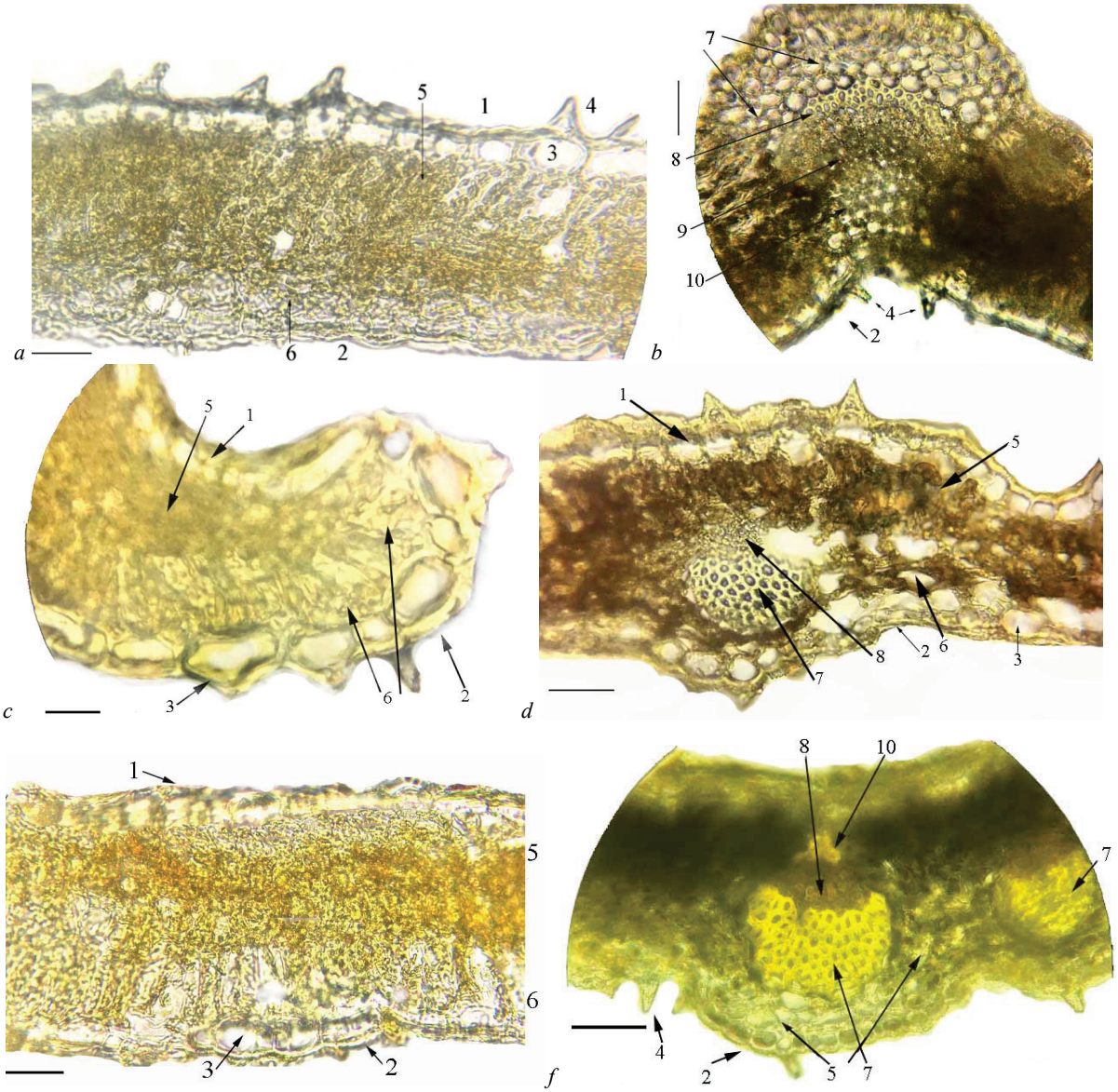

Fig. 11. Anatomical structure of leaf of species of *Thymus* genus: transversal section through the leaf lamina: *a* – *Th. pulegioides*; *c* – *Th. marschallianus*; *e* – *Th. serpyllum*; *b* – transversal section through the leaf in the region of the central vein and schizogenous reservoirs of *Th. pulegioides*; *d* – transversal section through leaf with schizogenous reservoir of *Th. marschallianus*; *f* – transversal section through the leaf in the region of the central vein and large schizogenous reservoir of *Th. serpyllum*: *1* – epidermis on the upper side of the leaf lamina, *2* – epidermis on the lower side of the leaf lamina, *3* – intercellular spaces, *4* – hairs, *5* – columnar mesophyll, *6* – spongy mesophyll, *7* – glandule, cells of which are filled with essential oils, *8* – sclerenchyma, *9* – sieve tubes, *10* – vessels; bar – 250 μm

On the transversal section through the leaf of *Th. serpyllum*, we can see that mesophyll is formed by columnar and spongy tissues. The columnar parenchyma is formed by two rows of rectangular cells with chloroplasts, whereas the spongy tissue has 2–3 rows of cells with large intercellular spaces (Fig. 11e). Under cells of the lower epidermis, there are air chambers and unicellular hairs. On the transversal section of the leaf, in the center, we can see one large schizogenous reservoir above the central leading bundle. Sideways from it, there are two smaller reservoirs, located above the smaller veins, two on each side. The schizogenous reservoir is formed of small cells, filled with dense cytoplasmic content (Fig. 11f). Above the epidermis, which surrounds the reservoir on the outside, there are unicellular hairs, and under the epidermis, there are located several rows of cells of spongy tissue with large intercellular spaces.

Discussion

According to the results of the conducted studies, there were determined diagnostic traits of the species of *Thymus* genus: *Th. pulegioides*, *Th. marschallianus*, *Th. serpyllum*, which are commonest in the Forest-Steppe zone of Ukraine and are a source of herbal raw material. In particular, we studied differences in the structure of life forms, types of shoots, forms, pubescence and structure of stem, leaves, presence of essential oil structures and peculiarities of the structure of inflorescence.

The National Pharmacopoeia of Ukraine (2014) and the European Pharmacopoeia (2011) provide descriptions and figures of diagnostic features of HRM of *Herba Thymi vulgaris* of Mediterranean thymes *Th. vulgaris* and *Th. zigis* and species of the moderate climatic zone *Th. serpyllum*. Since Mediterranean species in the conditions of Ukraine grow only in cultivated form, to prepare drugs, herbs of *Th. serpyllum* – *Herba Thymi serpilli* are used. The study indicated the diagnostic features of HRM of herbs (Herbae) *Th. pulegioides*, *Th. marschallianus* and *Th. serpyllum*, common in the wild and used as medical raw material. Unlike the Mediterranean species *Th. vulgaris* and *Th. zigis*, which have features of xerophytization, raw material of *Th. pulegioides*, *Th. marschallianus*, *Th. serpyllum* differs from them by colour, pubescences, amount and size of essential oil glandules. We should add that individuals of *Th. vulgaris* and *Th. zigis* cultivated in the Botanical Garden of the Ivan Franko National University of Lviv maintained features of xerophytization (Hrytsyna at al., 2019).

In macro- and microanalyses of HRM, important features were the structure and pubescences of stem, leaf, flowers. The examined species of the moderate climatic zone have saturated green colour with margins slightly rolled downward, which are much less hairy, mostly with coned unicellular and two-cellular, sharpened or elbow-shaped hairs on the petioles and margin of leaf lamina. Between them, there are long, 3–7-cellular, with broad base, thickened on the conjunction, papilla-like hairs with sharpened apex, which are interrupted in *Th. serpyllum*. On the surface of the laminae, there are rare singular papilla-like, unicellular sharpened hairs. Cells of the leaf epidermis of *Th. pulegioides* are multiangular, in *Th. marschallianus* – elongated, with the most tortuous walls. Epidermis cells of *Th. serpyllum* are ovally-tortuous, with cellular walls thickened on the sides. The upper epidermis of the leaf of *Th. marschallianus* and *Th. serpyllum* has slightly wavy walls. The stroma apparatus is the same in all the species – of diacytic type, located densely on the lower side of the lamina, and singularly on the upper side. The cuticle is radially-wavy. Essential oil glandules are 0.3–0.4 mm in diameter, with 8–10-cellular capitulum in *Th. serpyllum* and 10–12-cellular capitulum in the other species, located more densely on the lower side of leaf lamina than on the upper side. Other than them, there are glandular hairs with unicellular capitulum and two-cellular leg. Similar pubescence was earlier noted in *Th. vulgaris* L. (Boz et al., 2009). Glandular hairs are represented by basal cell, unicellular stem, and based on number of cells that form glandular capitulum, they are divided into secretory hairs with unicellular (capitate) and multicellular (peltate) capitula. We call peltate hairs essential oil glandules. Over time, the cuticle that covers the gland breaks off the cellular wall of multicellular capitulum and essential oil comes out to the surface. Secretory hairs with unicellular capitulum are located on all vegetative organs, with maximum density on leaves that are developing. Presence of glandular hairs with bicellular capitulum was only observed on lamina primordia. Glandular hairs with multicellular capitulum are on leaves and sepals. We also found the main hairs and essential oil glandules on flower petals. The calyx is two-lipped, serrations of its lower lip are more hairy in *Th. serpyllum*, compared with the other species. Epidermis structure of the petals repeats the patterns characteristic for the leaves. The petals have essential oil glandules, pubescent with unicellular sharpened hairs – papillae. Pubescence in the fauces of the corolla is dense, with two-cellular hairs, especially in *Th. pulegioides* and *Th. marschallianus*. In the studied species of *Thymus* genus, essential oils do not accumulate exclusively in the epidermal glands on the surface of the leaf, but also in schizogenous reservoirs of mesophyll (Hrytsyna et al., 2019).

In *Th. pulegioides*, by the end of blossoming, there form inflorescences, complex thyrsoid raceme, which reach more than 10 cm in length, interrupted at the base, the first 6–9 false rings are spread. By the end of blossoming, thyrsoid raceme of *Th. marschallianus* reaches up to 15 cm in length, fragmented into 7–10 and more spread false rings. In *Th. serpyllum*, there develops raceme (spike-like inflorescence) inflorescence, formed of densely located lateral inflorescences. In those species, complex thyrsoid inflorescence forms, the upper part of which grows monopodially, and partial (lateral) inflorescences are complex dichasium with monochasia. Such inflorescences are located in the angles of the pedicels, form whorls. We described similar type of inflorescence in species of *Verbascum* L. genus (Hrytsyna, 2013).

Environmental conditions influence size of plants, age structure of population (Penkovska, 2020), ratio between hermaphrodite and female species (gynodiasis) (Stakelien & Ložien, 2014), and qualitative composition of essential oils. All of this influences the quality of herbal material and resource potential of species (Tymchenko et al., 2007).

Morphometric parameters of *Th. serpyllum* and *Th. polessicus* Klok. vary and depend on growth conditions of species population (Penkovska, 2019). Formation of plants of *Th. serpyllum* under the canopy of pine forest is complicated; the population included only generative plants. The only ontogenetically complete population was of the meadow group *Elytrigioso (repenae)-thymosum (serpyllae)* (Penkovska, 2020). A negative effect on the number of the population of the genus *Th. marschallianus* and *Th. serpyllum* is caused by pascal pressure. Therefore, *Th. serpyllum* has resource value only at the beginning of inhabitation of disturbed habitats, and its population decreases as the level of sod increases. In populations of *Th. marschallianus* in formed growth locations, changes occur in the age structure of the population, negatively affecting the resource potential of species (Tymchenko et al., 2007). Plants of *Th. pulegioides* L. subsp. *effusus* Host, which grow on dolomite base on Manjača Mountain (Bosnia and Herzegovina), have short stems and shortened internodes, which resulted in shrub-like appearance, smaller leaves than plants that grow on limestone have (Janjić, 2008).

Generative reproduction of the species is also complex. In agricultural lands of Flanders (Belgium), populations of *Th. pulegioides* cannot exist for long without introduction of genes from the side (Vanden Broeck et al., 2015). One of the reasons for it is presence of gynodioecy. However, it provides xenogamy (cross-fertilization), which increases the vitality of the population. The literature contains data that gynodieoecy depends on growing conditions. Therefore, the higher the grass stand in a population of *Th. pulegioides* and the better the light in areas where heliophilous species *Th. serpyllum* grow, the higher the number of hermaphrodite and lower the number of female plants. Hermaphrodite plants of *Th. pulegioides* and *Th. serpyllum* and their hybrid *Th.* × *oblongifolius* Opiz have larger flowers and pistil compared with the female flowers due to specifics of fertilization (Stakelien & Ložien, 2014). In *Th. vulgaris*, high genetic variability (high number of female individuals) is characteristic for growing locations with disturbed plant cover, and by contrast, a stable environment is characteristic for large numbers of bisexual plants (Assouad et al., 1978).

The systematics of the *Thymus* genus is complex; the constituent species are characterized bya high level of polymorphism and hybridize with each other, and many subspecies and synonyms of species exist (Flora of Ukraine, 1960; Mosyakin & Fedoronchuk, 1999). In "Flora of Ukraine" (1960), *Th. pulegioides* L. belongs to the section *Goniothymus* Klok., *Th. marschallianus* Willd. – to the section *Verticillati* Klok., whereas *Th. serpyllum* L. – to the section *Euserpyllum* Klok. In "Flora of Europe"

(1972), the studied species belong to one section *Serpyllum* (Miller), subgenus *Thymus*. In the systematics of *Thymus* genus, Nachychko (2015) uses the Menitsky's classification of the genus, where it is divided into two sections: sect. *Serpyllum* (Mill.) Duby (*Th. serpyllum*) and *Th.* sect. *marginati* (A. Kern.) A. Kern. (*Th. pulegioides* and *Th. marschallianus*).

The phenomena of hybridization and polymorphism of species of *Thymus* genus is common because of the fact that genetic affinity, and therefore reproductive incompatibility, between species of section *Serpyllum* is very weak (Sostaric at al., 2012). Therefore, using markers of polymorphism of length of DNA fragments (AFLP), the author evaluated genetic relationships in 32 populations of seven species of *Thymus* genus, section *Serpyllum*, from Serbia. The collected species were identified to the three subsections: *Alternantes* Klokov (*Th. pulegioides* L.), *Isolepides* (Borbás) Halácsy (*Th. glabrescens* Willd., *Th. marschallianus* Willd., *Th. pannonicus* All.) and *Pseudomarginati* (Braun ex Borbás) Jalas (*Th. balcanus* Borbás, *Th. moesiacus* Velen., *Th. praecox* Opiz). Subsection *Alternantes* forms a well-supported clade, and genetic differentiation of *Th. pulegioides* is supported by morphological features, because it is the only taxon that has hairs only on the margins of the stem. Subsection *Isolepides* is polyphyletic. *Thymus glabrescens* is a species clearly distinguished from other two species of this subsection – *Th. marschallianus* and *Th. pannonicus*. Despite the fact that those species are morphologically different by leaf pubescences, studies have not confirmed the difference between them at molecular level. Species that belong to the third subsection *Pseudomarginati* formed a monophyletic clade with low affinity (Sostaric et al., 2012).

Other studies revealed morphological and genetic variability and presence of essential oils in seven species of *Thymus* genus. Morphometric studies indicated significant differences between species of *Thymus* according to all the parameters used in the experiment. The largest amounts of essential oil were found in *Th. vulgaris* (3.5%), *Th. serpyllum* (2.5%) and *Th.* × *citriodorus* (2%). Analysis of the dendrogram of phylogenetic affinity revealed that the highest level of affinity (67%) was between *Th. vulgaris* and *Th. serpyllum*; the level of affinity between *Th. serpyllum* and *Th. praecox*, *Th. vilosus*, *Th. serpyllum* 'Aureum', *Th.* × *citriodorus* і *Th.* × *citriodorus* 'Silver Queen' was 33%, and for the latter species and hybrids between each other – 100% (Smolik et al., 2009). Diploid set of chromosomes of *Th. pulegioides* equaled 28, 30; *Th. serpyllum* – 2n = 24, and in *Th. pannonicus*, which is considered synonym of *Th. marschallianus* (Mosyakin & Fedoronchuk, 1999), 2n = 28 (Mártonfi & Mártonfiová, 1996).

Scientists from different countries (Mockute et al., 2001; Svydenko et al., 2016; Vaičiulytė et al., 2016) determined that composition of essential oils of species of *Thymus* genus first of all depends on climatic conditions and geographic factor. Therefore, species *Th. vulgaris* and *Th. zygis*, which grow in countries with a warm climate, particularly in the Mediterranean regions, are characteristic for chemotype of thymol and carvacrol. Chemotype of monoterpene carbohydrates – p-cymene and γ-terpinene is characteristic for all species that grow in countries with a continental climate. The studies confirmed that the amount of essential oil from *Th. pulegioides* decreased during cold rainy weather and increased as the sunlight increased from 0.72% to 0.98%. The percentage of carvacrol ranged between 16.88% and 29.29%, p-cymene – 5.54–11.33%, γ-terpinene – 20.60–24.43% (Vaičiulytė et al., 2016). Species *Th. vulgaris* and *Th. pulegioides*, studied in Romania, had thymol chemotype, *Th. serpyllum* – with dominance of carvacrol, and *Th. glabrescens* – terpinyl-acetate chemotype (Varga at al., 2015). Concentrations of essential oil in two forms of *Th. serpyllum*, introduced to Southern Ukraine, in Kherson Oblast, equaled 1.10% and 0.67%, and the dominating components were thymol (40.70% and 40.29%) and γ-terpinene (12.88% and 23.31%). The main constituents of essential oil from *Th. pulegioides* form No. 2/6-07 are acyclic monoterpenes neral and geranial (50.25% in total) (Svydenko & Hlushchenko, 2016).

Therefore, the demarcation line between the morphologically relative species is quite unclear, as confirmed by genetic studies. The quality of their medicinal raw material is influenced by various surrounding factors. Presence of criteria accompanied by visual images, which allow morphologically relative species to be distinguished, would facilitate macro- and microscopic analyses of herbal raw material.

Conclusions

Diagnostic traits of species *Th. pulegioides*, *Th. marschallianus*, *Th. serpyllum* are differences in the structure of life form, type of shoots, structure of stem, leaves, flowers, and peculiarities of the structure of inflorescence. The studied species form a subshrub life form, growing monopodially in *Th. pulegioides* and *Th. marschallianus*, and sympodially in *Th. serpyllum* from mono-, di- and polycyclic types of monocarpic shoots. Thyrsoid-type inflorescences with opposite partial inflorescences make up simple dichasium with monochasia.

Macroscopic and microscopic diagnostic features of HRM are structure and pubescence of stem, leaves and flowers, presence of essential-oil structures. *Thymus pulegioides* has a tetrahedral stem, is pubescent with elbow-shaped hairs on the ribs, formed of bark colenchyma. Leaves are opposite, varying ovoid short-petioled to oval with wedge-shaped base and sharpened apex. On the lower side of the lamina, leaf epidermis has multiangular cells with poorly tortuous walls, between which there are diacyte stromas and essential oil glandules with 10–12-cellular capitulum. On the upper side of the lamina, epidermis cells are isodiametric, with no stromas, with glandules. The cuticle is radial-wavy. In *Th. marschallianus*, the stem is slightly tetrahedral, becomes rounded toward the top, significantly pubescent on the entire surface with two-cellular, bent hairs. Bark, ribs and stems are filled with colenchyma. Leaves of *Th. marschallianus* are opposite, sessile, elliptical with a wedge-shaped base and slightly sharpened apex, light-green. Leaf epidermis of *Th. marschallianus* on the upper and lower sides of the lamina has rectangular cells with significantly tortuous walls. In *Th. pulegioides* and *Th. marschallianus*, above veins and petioles, epidermis cells are of elongated-rectangular shape, even, with diagonal margins. Pubescence on the leaf concentrates near its base and along the petiole, comprising unicellular and curved two- and three-cellular hairs, among which there are long 7–8-cellular hairs. The surface of the lamina bears small papilla-like unicellular hairs.

The calyx is campanulate with three lancet-triangular parts of the upper lip and two long parts of the lower lip, pubescent with 10–12 pairs of two-, three-cellular, sharp hairs. In *Th. pulegioides*, the calyx is green-purple; in *Th. marschallianus*, it is green with denser pubescence on the lower lip. On the outside, the calyx is pubescent along the veins with short hairs and has essential oil glandules, inside – with longer, sharper, two-, three-cellular hairs. The corolla with the tube and two-lip outward bend, the upper lip of the outward bend is entire, the lower – deeply three-lobular, retrorse. The corolla in *Th. pulegioides* is purple-violet, has a long tube and outward bend with sinuate upper lip. On the outer surface, it is poorly pubescent with unicellular coned hairs, inside – denser, with unicellular coned and curved 2–3-cellular hairs, has glandules. Epidermis cells of the lip are oval, with poorly tortuous walls, tubes are rectangular, tortuous. The corolla of *Th. marschallianus* is watering-can-like, with poorly noticeable tube and straight upper lip, pale-lilac, pubescent with unicellular, two-cellular hairs, cells of epidermis are elongated, with significantly tortuous walls. The fauces of the corolla of *Th. pulegioides* and *Th. marschallianus* is pubescent with long sharpened hairs.

The stem of *Th. serpyllum* is cylindrical, pubescent on the entire surface with unicellular and two-cellular bent hairs. Leaves are opposite, with short petiole, elongated-elliptical, sessile along the shoot, elliptical, with sharpened apex, wedge-shaped base and entire margins. The pubescence on the leaves is similar to other species, bears multicellular interrupted hairs. Epidermis cells of the leaf of *Th. serpyllum* are oval, with tortuous membranes. The form of the flower calyx of *Th. serpyllum* is tubular-campanulate, two-lipped, the upper lip having triangular serrations, while the serrations of the lower lip are framed by numerous long hairs, the inner surface is significantly pubescent. The corolla of *Th. serpyllum* is pink-lilac, the surface on both sides is pubescent with short papilla-like and two-, three-cellular, sharpened, interrupted hairs. Nearby, there are capitate hairs and glandules. Epidermis cells of the outward bend are multiangular, the tubes are rectangular, straight.

On the transversal sections of the leaves of *Th. pulegioides*, *Th. marschallianus* and *Th. serpyllum*, in mesophyll, there are schizogenous essential oil reservoirs, their numerous cells contain dense cytoplasmatic content. The structure of mesophyll is seen to have features of xerophytization.

According to the results of macro- and microscopic analyses, we determined morphological-anatomic peculiarities of the structure of the organs of *Th. pulegioides*, *Th. marschallianus* and *Th. serpyllum*, which could be used for diagnostics of herbal raw material during its preparation.

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