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Promising *ex situ* essential oil from *Thymus camphoratus* (Lamiaceae)

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The search for new medical fines herbs among species of foreign flora is a relevant issue of today. The article describes the morphological-anatomical, phytochemical peculiarities and antimicrobial action of an endemic species of the Iberian Peninsula *Thymus camphoratus* Hoffmanns. & Link (Lamiaceae Martinov) introduced to the Botanical Garden of Ivan Franko National University of Lviv. It was determined that the diagnostic peculiarities of medical herbal raw material (herbs) are the structure and woolliness of the stem, leaves and generative organs. *Th. camphoratus* is a small subshrub with the stele of the stem of bundle type. Leaf lamina is oval, with slightly bent downward, large-crenate margins. Their lower part is woolly with simple crook-like hairs, the upper part is almost bare. The lamina from both sides is densely covered by peltate essential oil-bearing trichomes, between which there are lots of capitate hairs. Peltate trichomes have 4–6 cellular head and are surrounded by 12–16 cells of the epidermis. The epidermis is formed of elongated cells, the stomatal apparatus is of diacytic type, the stromas are densely located on the abaxial side of the leaf. The mesophyll of the leaf is formed of palisade and spongy tissues with no essential oil containing reservoir. The calyx is tubular-campanulate with almost similar short teeth of the upper and lower lip, the corolla has two lips, light-violet, or pink. The perianth is uniformly, woolly externally with single-cellular simple and capitate essential oil-bearing hairs. The inflorescence is compound, raceme-like thyrsoid type. Its opposite, angle partial inflorescences are complex dichasia formed of 7 flower-bearing axes at different stages of development. Determination of qualitative composition of essential oils in the raw material of *Th. camphoratus* was performed using the method of gas chromatography with gas chromatograph Carlo Erba Vega on capillary columns DB-WAX, 30 m and GC-SCION 456, column SOLGEL-WAX, 60 m with mass spectrometric detector (GCMS) in the Calendula laboratory of the University of Prešov. The standards for the comparison of essential oils were provided by Extrasynthese Ltd. In the raw material, the content of essential oil equaled $0.40 \pm 0.05\%$ of air-dried mass, in which 48% was borneol and fenchol, and 20% – linalool and α -terpineol. Essential oil composition of the studied plants is different from the populations in Portugal, where the dominating constituents, except borneol and fenchol, were 1,8-cineol, α -pinene, camphene. The obtained essential oil exerts bactericidal properties against *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, *Enterococcus faecalis* and has slightly lower antimicrobial activity towards clinical isolates of these microorganisms. Against single-celled fungi *Candida albicans*, essential oil from the plants exhibited no inhibition of growth of their colony. According to the results of the conducted studies, it was determined that in the Botanical Garden, *Th. camphoratus* subsp. *congestus* was introduced, which due to the content of borneol and fenchol, that have bactericidal effect, can be used as an anti-inflammatory medical preparation.

Keywords: camphor thyme; morphological-anatomical structure of stems; simple and crooked non-glandular hairs; peltate and capitate glandular trichomes; essential oil; antimicrobial effect.

Introduction

Introduction and study of the phytochemical composition and pharmacological properties of species of essential oil bearing plants of the global flora is important for the enlargement of the assortment of the medical preparations of natural origin. A promising species for the introduction to Ukraine is the Mediterranean plant *Thymus* L., the composition of essential oil of which is distinct from others growing in the continental climate. Also, the content of essential oils in plants differs in various ecological conditions of one territory.

An interesting species for use is essential oil-bearing species camphor thyme (*Thymus camphoratus* Hoffmanns. & Link) of the sage family Lamiaceae Martinov or Labiatae Juss. with strong aroma of camphor, introduced to the collection Medical Plants of the Botanical Garden of Ivan Franko National University of Lviv. In the natural conditions, *Th. camphoratus* is endemic to the South and South-West Coast

of Portugal, where it is common in shrubs on dry sandy and calcium soils. The species is included in the Red Book of Europe (The IUCN Red List of Threatened Species, 2010).

In Portugal, *Th. camphoratus* is broadly used along with the official species *Th. vulgaris* and *Th. zygis* included in the European Pharmacopoeia (2011). It contains essential oils and exerts bactericidal, nematocidal, antioxidant and antiseptic properties, making it an important preparation for treating diseases of the respiratory system (Boyko & Brygadyrenko, 2016; Zuzarte et al., 2018), and also aromatherapy and cuisine. In the above-ground part of the plants of *Th. camphoratus* growing in Portugal, the main constituents of essential oils are: 1,8-cineol, linalool, borneol, α -pinene, camphene, trans-sabinene hydrate, terpinen-4-ol and two new oxygenated sesquiterpenes for *Thymus* genus: intermedol and homalomenol-D (Salgueiro et al., 1997).

The medicinal herbal raw material of *Th. camphoratus*, as in other species of *Thymus* genus, is the above-ground part in the blossoming

phase. Its diagnostic peculiarities include the structure of the stem, leaves and generative organs and their woolliness (Tykhonenko & Kotov, 2010). Research has revealed that the leaves, stem and the calyx of *Th. vulgaris*, *Th. pulegioides* (Blažeković et al., 2006; Boz et al., 2009) bear numerous non-ramified covering and glandular trichomes. The latter are divided into peltate (shield-shaped) and capitate (headlike).

Due to the distinctiveness of *Th. camphoratus* from the species of *Thymus* genus traditionally used in medicine: *Th. vulgaris*, *Th. serpyllum*, *Th. pulegioides*, there is a need for its detailed study in Ukraine, where this species grows in cultivated as well as experimental conditions. The objective of our study was determining the morphological-anatomical peculiarities which will become diagnostic for the medicinal raw material of the Iberian species *Th. camphoratus* during cultivation in the conditions of the forest-steppe zone of Ukraine, and also determining the quantitative content and qualitative composition of its essential oils and other bactericidal properties.

Materials and methods

The surveys on the morphological-anatomical, phytochemical and bactericidal properties of the plants of the Iberian species *Th. camphoratus* were performed in the vegetative period of 2018–2019 in the Botanical Garden of Ivan Franko National University of Lviv. In the phenological stage of full blossoming, we collected immature non-lignified stems, leaves of the middle formation which were located on them, inflorescences and flowers with bracts. Anatomical studies were conducted according to the method of Pausheva (1988) using Leica DM500 microscope. For the survey of the peculiarities of the anatomical structure of the leaves and stems, on the microtome their cross-sections of 10–20 µm thickness were prepared. The structure of the epidermis of the upper and lower side of the lamina was studied on native preparations. For the morphological studies, we used MBS 1 binocular microscope. Microphotography was performed using a digital camera. In the study, we used terminology from the European Pharmacopoeia (2011).

Essential oils were extracted from the freshly collected raw material of *Th. camphoratus* during the period of mass blossoming of plants using hydrodistillation (2 h) in the Clevenger-type apparatus according to the European Pharmacopoeia (2011). A mixture of hexane and diethyl ether (1:1) was used for the extraction of essential oil. Prior to determination, essential oil was maintained in a dark place under N₂ at the temperature of +4 °C.

Essential oils were analysed using gas chromatograph GC-SCION 456. The following operating conditions were used: column SOLGEL-WAX, 60 m × 0.25 mm i.d., thickness of the film: 0.25 µm, carrier gas: nitrogen regulated to the current of 1 mL/min, the temperature of injection to the detector FID: 220 °C, respectively, 250 °C.

Constituents of essential oil were determined by the time of their release (in minutes), and the obtained values were compared to the literature. The standards of the constituents of essential oils were provided by Extrasynthese Ltd. (France). The results of the studies are demonstrated as percentages. The percentage of single chromatographic peaks was measured by the ratio of the area of single peaks to the total area of the peaks.

Analysis of GC/MS was conducted using gas chromatograph Varian 450-GC connected with Varian 220-MS. The division was made using FactorFourTM: capillary column DB-WAX, 30 m × 0.25 mm i.d., the thickness of film was 0.25 µm. The injector of 1177 type was heated in the temperature of 220 °C. The injection equaled 1 µL of the solution of 1:1,000 n-Hexane/diethyl ether. Helium was used as carrier gas during constant velocity of the current in the column of 1.2 mL/min. Temperature of the column was set as follows: the initial temperature during 10 min was 50 °C, then to 100 °C at 3 °C/min; isothermic temperature – during 5 min, and then continued to 150 °C at 10 °C/min. Total time for the analysis of one sample was 54.97 min. The constituents were identified by comparing their mass-spectrum with the spectra kept in NIST 02 (library of software) or mass-spectra from the literature (Adams, 2007), and also the comparison of their indices obtained with the standards was made. Statistical analysis was conducted using reli-

able intervals at the level of $P < 0.05$ with our calculation through mean statistical deviation and standard error.

Antimicrobial activity of essential oils was studied at the Microbiological Laboratory of the Department of Genetics, Physiology of Plants and Microbiology of the Biological Faculty of Uzhhorod National University. The susceptibility of the microorganisms to essential oils were determined using the method of diffusion in agar with reference strains *Candida albicans*, *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Streptococcus pyogenes* and clinical isolates (Balouiri et al., 2019). As positive control, we used gentamicin (10 mg/disk) – for Gram-negative bacteria, ampicillin (10 mg/disk) – for Gram-positive bacteria, nystatin (100 U) – for *Candida*. As negative control, we used DMSO. In the experiment, 100 µL of bacteria in normal saline was adjusted to the equivalent of 0.5 McFarland standard and uniformly distributed on the surface Mueller-Hinton agar (incubated in 37 ± 2 °C during 24 h). Microscopic fungi of *Candida* genus were distributed on the surface of Sabouraud agar (incubated in 35 ± 2 °C during 48 h). Essential oils in 20 µL amount were added into the wells of 6 mm diameter. The diameters of the inhibition zones were measured in millimeters, including the diameter of the well. Antimicrobial effect was assessed by the presence of growth inhibition zone. Each antimicrobial analysis was made in at least three replications. The obtained data were expressed as mean ± standard deviation of three measurements. To calculate mean values, errors and mean deviation of the results of the experiment, statistical software was used.

Results

In the conditions of the Botanical Garden, *Th. camphoratus* is a ramified subshrub of up to 30–60 cm height. The stems are upright, lignified at the base, their young parts are quadrangular; woolly around with short, simple one-, two-cellular straight or crook-like hairs, between which capitate glandular hairs with single-celled leg are located (Fig. 1a).

The stem is quadrangular, has bark on the cross-section, central cylinder and the pith. The bark is thin, covered on the outer side with the epidermis with hairs formed by large parenchyma cells, a thickening is seen on the ribs of the stem, formed by collenchyma (Fig. 1c, e, f). Vascular bundles are gathered in four cords opposite the ribs of the stem bark. Cambium ring is complete, with bundle and intrafascicular cambia distinguished in it. The vascular bundles have phloem, cambium and vessels are located in rows. The spring vessels are of large diameter, thin-walled, the summer ones are small, thick-walled, and sclerification of their inter-vascular parenchyma is seen (Fig. 1b, g, i). Between the bundles, large pith parenchyma rays are located, which also partly lignify (Fig. 1b, h). In the center of the stem the large-cellular pith is located.

In *Th. camphoratus*, the arrangement of leaves is opposite decussate, in the leaf angle, lateral shoots develop. In the lower part of the shoot, the leaves have short petioles, in the upper part, they are sessile. With the similar pattern, the form of the lamina changes from oval with dull apex to rhombus-like with sharpened apex, the margins are large-crenate, slightly bent downward (Fig. 2a, c). The venation of the leaves is noteworthy. The species under survey has camptodrome venation with clearly manifested central and lateral veins which disappear to the margin. The veins protrude only on the abaxial surface of the lamina (Fig. 2c, d).

Woolliness of *Th. camphoratus* is formed by non-glandular single- and two-cellular straight and bent (crook-like) and glandular hairs. On the veins, the leaves are covered by simple, straight and crook-like hairs, mostly on the lower side of the lamina, their apical surface is insignificantly covered by simple hairs. Glandular hairs are divided into capitate (head-like) and peltate (shield-shaped, Fig. 2h, g). The capitate hairs have single-cellular head, two-cellular leg and are sessile. In the peltate hairs of 0.2 mm in diameter, the head is sessile, formed of 4–6 cells and surrounded by 12–16 rosette cells of the epidermis (Fig. 2h). They are located more densely on the lower (abaxial) side of the lamina than the adaxial (Fig. 2b, d).

Cells of the epidermis on both sides of the lamina in *Th. camphoratus* are of irregular oval shape with tortuous walls (Fig. 2e, f). The stomatal apparatus is of diacytic type, the stomas are oval, located more densely on the abaxial side of the leaf than on the adaxial. On cells of the epidermis, drops of wax are observed.

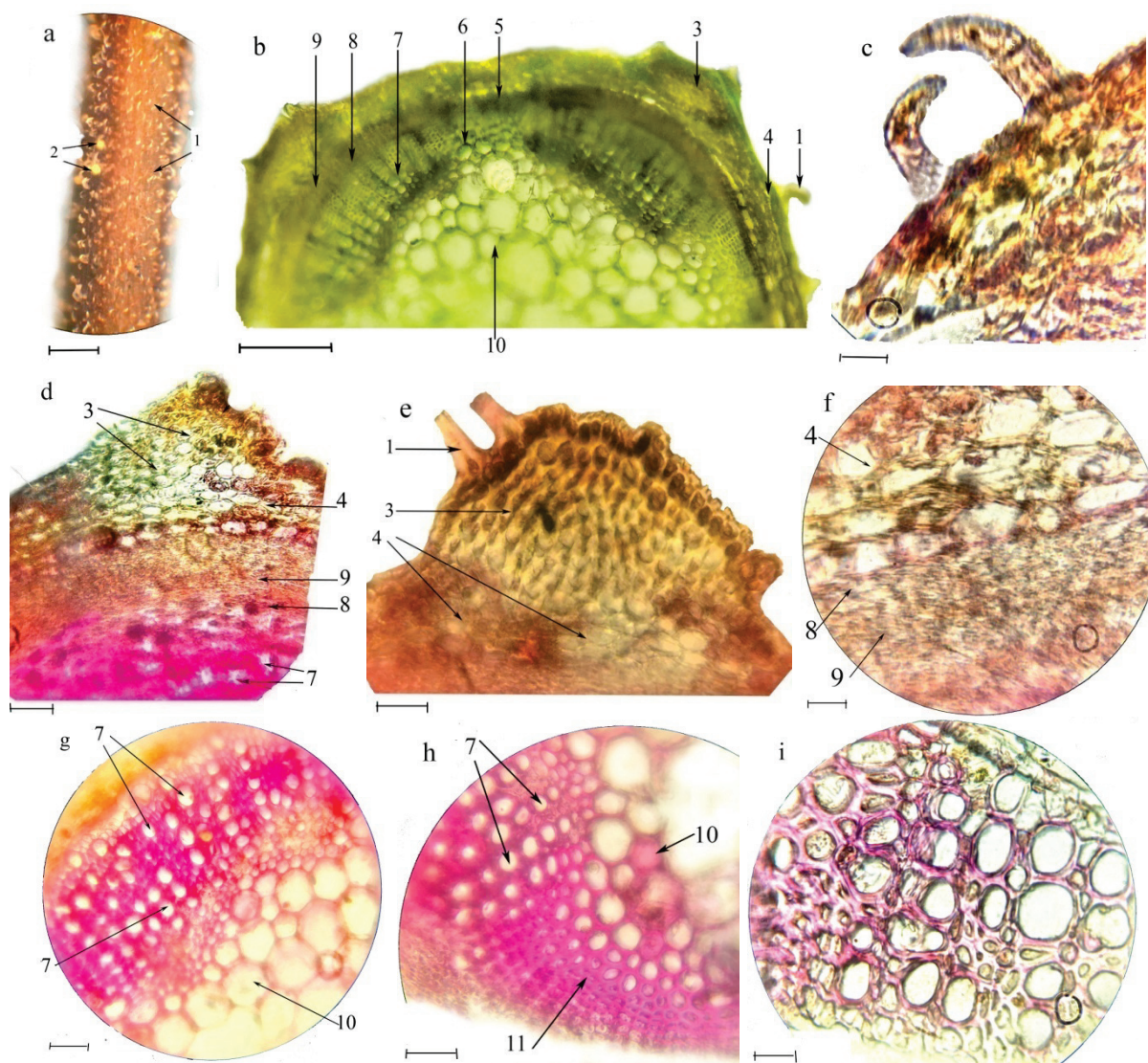


Fig. 1. Cross-section of the stem of *Th. camphoratus*: *a* – external view of the stem; *b* – cross-section of the stem: 1 – simple hairs, 2 – capitate hairs, 3 – ribs of the stem with collenchyma, 4 – parenchyma of the bark, 5 – intrafascicular cambium, 6 – pith ray, 7 – xylem (vessels), 8 – bundle cambium, 9 – phloem, 10 – pith, 11 – sclerenchyma; *c* – crook-like hairs on the stem; *d* – cross-section of the rib of the stem; *e* – fragment of the cross-section of the stem in the area of the edge of the stem; *f* – fragment of cross-section of the stem through the bark; *g* – cross-section through the vascular bundle; *h* – cross-section of the stem through the pith ray; *i* – fragment of the xylem (vessel); bar: *a* – 620 μm ; *b*, *c*, *d*, *g*, *h* – 250 μm ; *f*, *e*, *i* – 100 μm

On the cross-section of the leaf, mesophyll is divided into palisade and spongy parenchymas (Fig. 3). On the margin, the leaf is bent downwards and under the epidermis, capitates, single-cellular, sessile essential oil-bearing trichomes are seen (Fig. 3c, d). Above the epidermis, on the external side, capitate trichomes with single-cellular leg are located. Near, straight and bent simple hairs are located (Fig. 3a, b). *Th. camphoratus* has no essential oil reservoirs. The vascular bundle has easily distinguished small vessels positioned in rows. On the external side, the bundle is surrounded by large sclerenchyma cells with dense cytoplasmic content (Fig. 3a, b).

In *Th. camphoratus*, in the conditions of cultivation, a complex raceme-like inflorescence of thyrsoid type forms, the apex grows monopodially, and angle partial inflorescences – sympodially. Partial inflorescences are compound dichasia arranged in opposite decussate way in the angles of the bracts (Fig. 4). The module of such inflorescence is the flower-bearing axis formed of pedicel and flower. The pedicel has one node and two internodes. In this node, two flowers in the angles of the bracts are located in opposite decussate way. In one partial inflorescence, there form up to 7 flower-bearing axes at different stages of the development to the youngest, embryonic ones. The bracts of the partial inflorescences are similar to the leaves, being sessile, mostly ovoid, with margins slightly bent downward and sequencing secondary veins ramifying from the midrib, densely covered by glandular hairs. Another important diagnostic

feature of the medicinal herbal raw material of species of *Thymus* genus is the structure of the generative organs (Fig. 5). The calyx of *Th. camphoratus* is tubular-campanulate, grown together in the bases of the sepals, quinque-dentate, two-lipped, with almost identical short teeth of the upper and the lower lips, uniformly, densely covered by single-cellular simple and capitate, essential oil-bearing hairs. The corolla is light-violet or pink, two-lipped, 5 petals grow together at the bases into a tube, the upper lip is complete, slightly concave, the lower is trilobite, deflexed. The corollas are covered on the outer surface by short single-cellular and capitate essential oil-bearing hairs, while being almost bare on the internal surface of the lateral lobes.

In natural populations (Flora Europaea, 1972), as in the cultivated ones, hermaphrodite flowers form in the inflorescence, which differ from one another. Some flowers have long (5–8 mm) corollas, other have approximately corollas half as short. There are four upright stamens located in the tube of the corolla; anthers are dithecal, violet with quite large connective. In long flowers, two stamens are longer, with stamen filaments of up to 8 mm long, coming beyond the upper lip of the corolla, and two filaments twice as short (Fig. 5a, b). In short flowers, not all stamens protrude out of the lower lip of the corolla (Fig. 4e, f). There is one pistil with four pollen sacs, the stigma is long (6.0–7.5 mm), comes out of the corolla. The fruits are nuts of ellipsoid or almost rounded shape, more rarely ovoid.

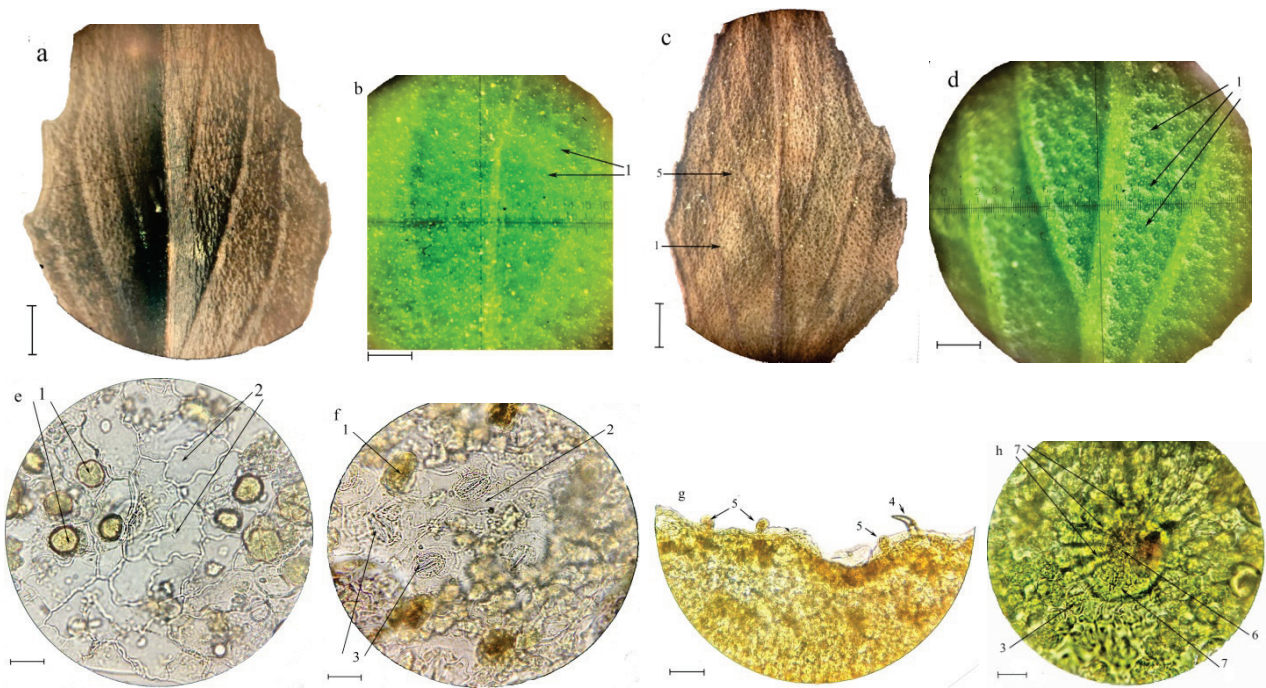


Fig. 2. External structure of the leaf of *Th. camphoratus*: *a* – external view of the leaf (adaxial); *b* – its enlarged fragment; *c* – external view of the leaf (abaxial side); *d* – its enlarged fragment; *e* – structure of the epidermis of the upper side; *f* – structure of the epidermis of the lower side of the lamina; *g* – hair coverage of the lamina; *h* – structure of peltate hair: 1 – peltate hairs, 2 – cells of the epidermis, 3 – stomas, 4 – bent hairs, 5 – capitate hairs, 6 – head, 7 – cells (rosette) surrounding the capitate hairs of the epidermis; bar: *a*, *c* – 620 μm ; *b*, *d* – 310 μm ; *e*, *f*, *g* – 250 μm ; *h* – 100 μm

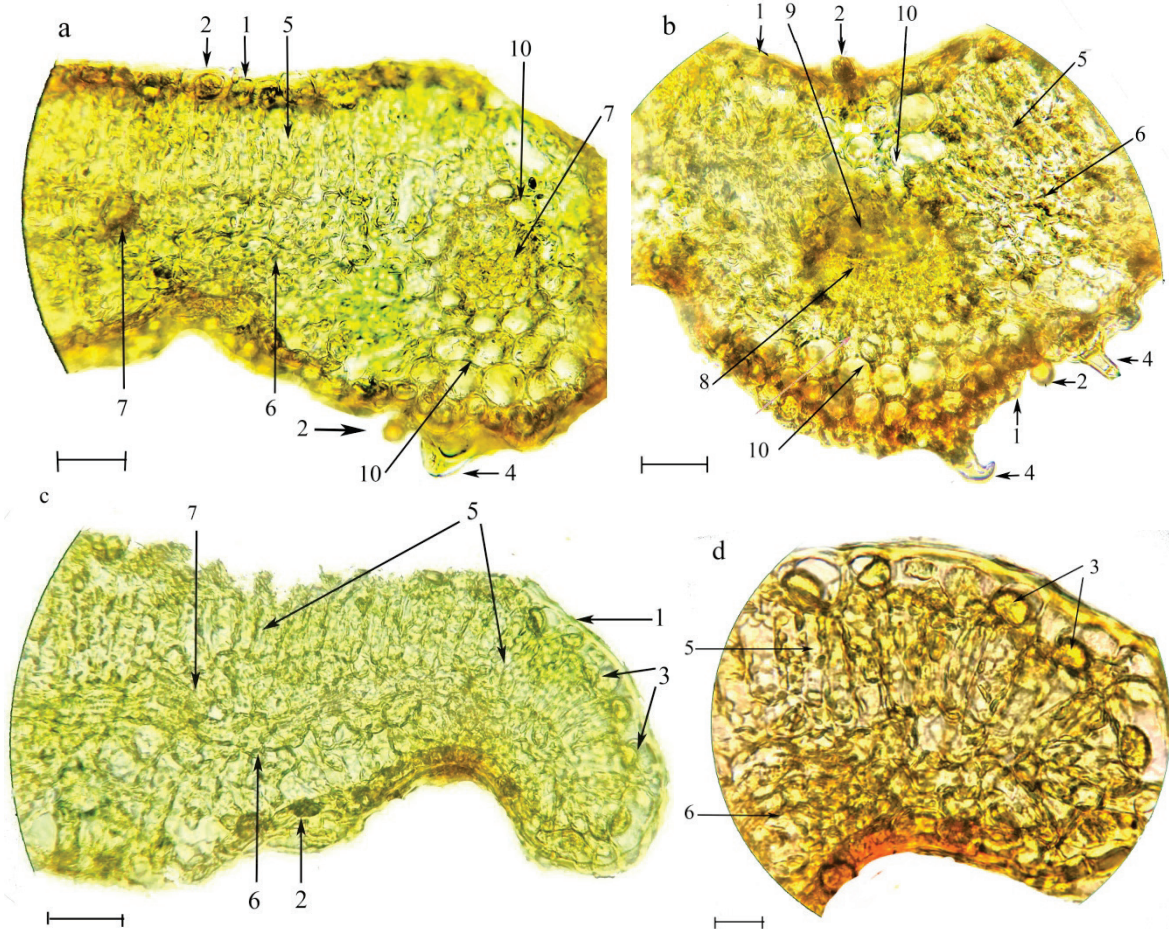


Fig. 3. Cross-section of the leaf of *Th. camphoratus*: *a* – of the lamina; *b* – areas of the midrib of the leaf; *c*, *d* – through the margin of the lamina: 1 – epidermis, 2 – headlike (capitate) hairs, 3 – subepidermal capitate hairs, 4 – simple hair, 5 – palisade mesophyll, 6 – spongy mesophyll of the leaf, 7 – vascular-fibrous bundle, 8 – xylem, 9 – phloem, 10 – sclerenchyma cells of the bundle; bar: *a*, *b*, *c* – 250 μm ; *d* – 100 μm

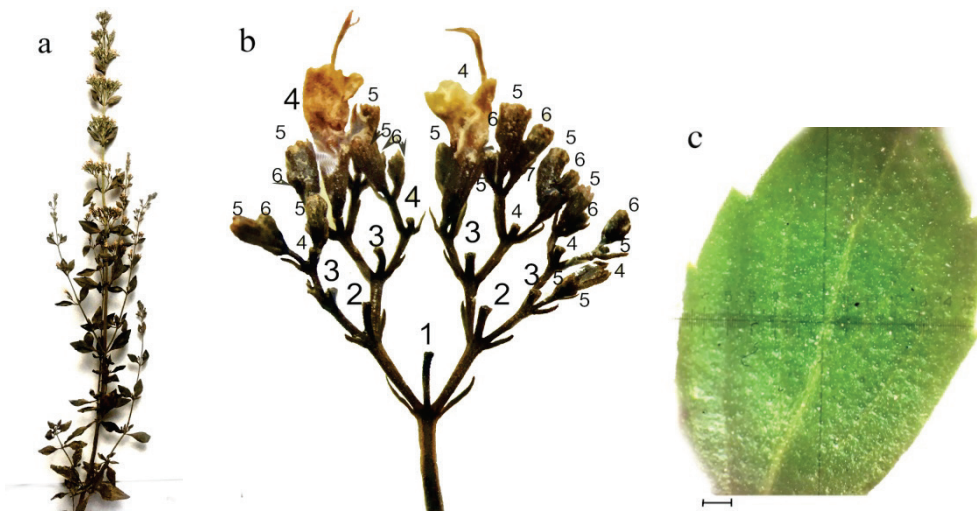


Fig. 4. Structure of the inflorescence of *Th. camphoratus*: a – view of synflorescence; b – structure of partial inflorescence; c – bract; bar: c – 620 μ m

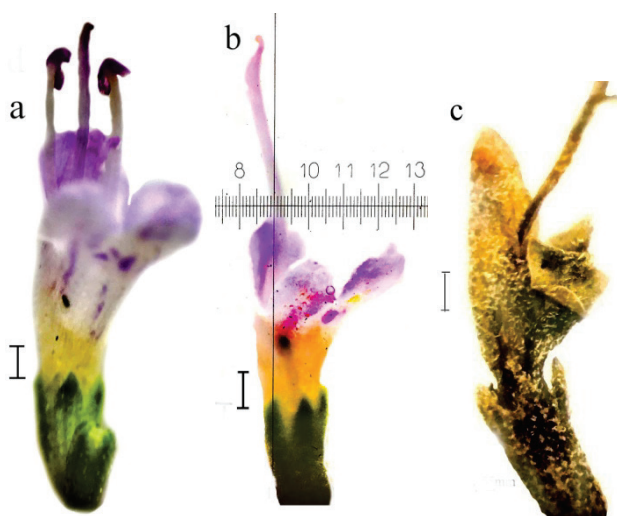


Fig. 5. Structure of the flower of *Th. camphoratus*: a – long flower; b – short flower; c – flower of the herbarium sample; bar: a, b – 620 μ m; c – 310 μ m

Figure 6 demonstrates a chromatogram composed in relation to the time of bearing a certain essential oil in *Th. camphoratus* made using GC-SCION 456 gas chromatograph, SOLGEL-WAX column, 60 m, connected with mass spectrometric detector. On the abscissa axis, the time of release of certain essential oil is shown, while on the ordinate axis – quantitative content of the oils. Each oil had certain time of peak occurrence, and the height indicates its quantitative content. The standards of essential oils are given by Extrasynthese Ltd. (France) Company.

The percentage content of each essential oil (A_n , %) was measured by the ratio of the size of one peak (A_n) to the sum of all the sizes of all peaks ($A_1 + A_2 + \dots + A_{n+1}$). Therefore, for example, borneol appeared at the minute 28.73. Its amount as a percentage equals 50309/180086, which accounts for 28% (Table 1. II variant).

In *Th. camphoratus* introduced to the Botanical Garden, the content of essential oil accounted for $0.40 \pm 0.05\%$ (4 g/kg) of air dried mass. In qualitative composition of essential oils, half consisted of bicyclic monoterpene: borneol and fenchol comprised around a quarter. Acyclic linalool and monocyclic monoterpene α -terpineol comprised around 10% of each oil, eucalyptol accounted for around 2% (Table 1). The rest of the constituent composition of essential oil from *Th. camphoratus* comprised: p-myrcene, limonene, α -terpinene, p-cymene, terpinolene, thujone, bornyl acetate, β -caryophyllene, geraniol, thymol, carvacrol.

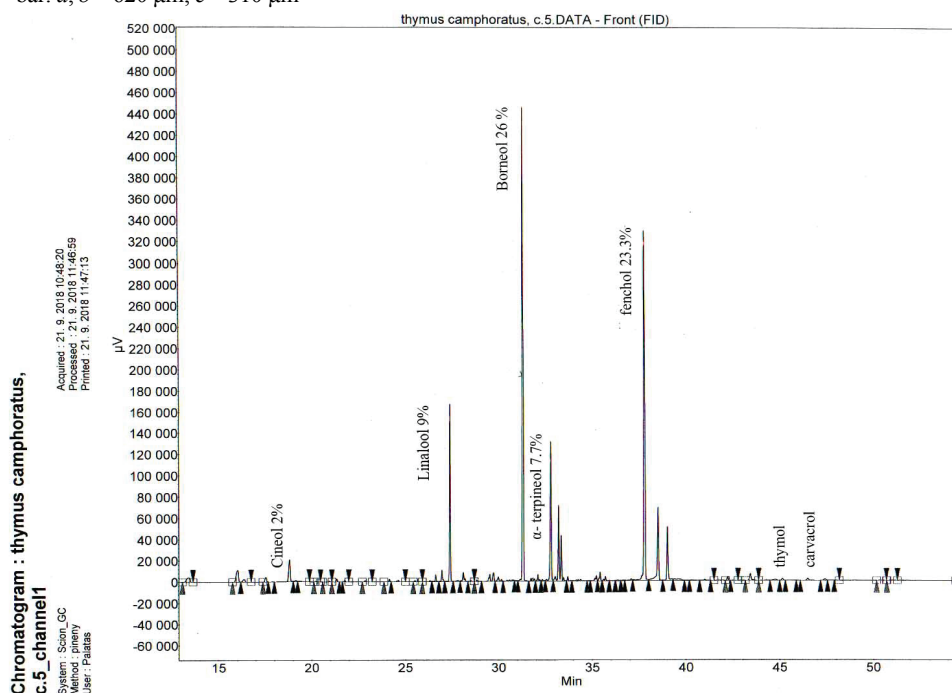


Fig. 6. Chromatogram of the constituent composition of essential oils of *Th. camphoratus*

Table 1Composition of the essential oil from medicinal plant raw material of *Th. camphoratus* ($x \pm SD$)

Constituents of the essential oil	I variant*		II variant**		area, BC
	time of release, min	quantitative content, %	time of release, min	quantitative content, %	
β -myrcene	16.0	traces	10.99	traces	2512.01
cineol	19.0	2.0 \pm 0.5	14.36	1.5 \pm 0.2	2899.01
limonene	20.0	traces	20.97	traces	134.01
α -terpinene	21.0	traces	22.66	traces	184.01
p-cymene	23.0	traces	23.39	traces	587.01
terpinolene	25.0	traces	23.89	traces	586.01
thujone	26.0	traces	24.07	traces	594.01
linalool	27.5	9 \pm 1.0	24.29	9 \pm 1.0	16476.01
bomyl acetate	29.0	traces	25.42	traces	291.01
β -caryophyllene	30.0	traces	26.65	traces	797.02
borneol	31.5	26 \pm 2.0	28.73	28 \pm 2.0	50309.03
α -terpineol	33.5	7.5 \pm 0.5	30.08	11 \pm 1.0	19619.02
fenchol	38.0	23 \pm 2.0	35.88	25 \pm 2.0	45154.02
geraniol	39.0	traces	37.76	traces	773.01
thymol	45.0	traces	41.25	traces	1480.01
carvacrol	48.0	traces	44.28	traces	327.01
Unidentified peaks	–	32.5	–	25.5	37364
Total amount	–	100	–	100	180086

Note: * – gas chromatograph GC-SCION 456, capillary column SOLGEL-WAX, 60 m; ** – gas chromatograph Carlo Erba Vega, capillary column DB-WAX, 30 m.

Essential oil obtained from *Th. camphoratus* contained 48% borneol and fenchol and exerted bactericidal properties against *Staphylococcus aureus* ATCC 25923 – growth inhibition zone measured 11.33 \pm 0.58 mm, *Escherichia coli* ATCC 25922 – 11.83 \pm 0.7 mm, *Enterococcus faecalis* ATCC 29212 – 11.50 \pm 0.25 mm and *Streptococcus pyogenes* ATCC 19615 – 16.50 \pm 0.5 mm (Table 2). Also, antimicrobial activity of the essential oil was determined against clinical isolates of these microorganisms which was insignificantly lower compared with reference strains (on average 0.48–1.58%) and only for *S. pyogenes* was the difference the highest – 5.20 mm compared with corresponding reference strain. Against microscopic fungus *Candida albicans*, essential oil from *Th. camphoratus* displayed no antimicrobial activity.

Table 2Antimicrobial activity of essential oil from *Th. camphoratus* against type and clinical conditionally pathogenic bacteria ($x \pm SD$, $n = 3$)

Test cultures	Inhibition zone, mm
<i>Staphylococcus aureus</i> ATCC 25923	11.33 \pm 0.58
<i>S. aureus</i> (clinical isolates)	10.85 \pm 0.55
<i>Escherichia coli</i> ATCC 25922	11.83 \pm 0.70
<i>E. coli</i> (clinical isolates)	10.25 \pm 0.25
<i>Enterococcus faecalis</i> ATCC 29212	11.50 \pm 0.25
<i>E. faecalis</i> (clinical isolates)	10.50 \pm 0.33
<i>Streptococcus pyogenes</i> ATCC 19615	16.50 \pm 0.50
<i>S. pyogenes</i> (clinical isolates)	11.30 \pm 0.33
<i>Candida albicans</i> ATCC 885-653	0

Discussion

For diagnosing medicinal plant material of species of the *Thymus* genus, the following parameters are used: structure of the stem, the flowers and their woolliness, and also presence of essential oil structures. Anatomical structure of the stem of *Th. camphoratus* is distinct from other species of *Thymus* genus (Hrytsyna et al., 2019). In the studied species, the vascular bundles are gathered in four cords opposite the ribs, between which large pith rays are located, whereas in other species (*Th. vulgaris*, *Th. zygis*, *Th. callieri*) the central cylinder of the stem is of non-bundle type.

Woolliness of the leaves of *Th. camphoratus* is distinct from the earlier described species *Th. vulgaris*, *Th. zygis*, *Th. callieri* (Hrytsyna et al., 2019) by much lower intensity and the number and arrangement of glandular trichomes. The woolliness is formed by non-glandular single- and two-cellular simple and crook-like hairs, between which pel-

tate and capitate trichomes are located. The latter have a single-cellular head, single-cellular leg or are sessile. Peltate trichomes in the literature are called “essential oil glands” or spheroid glands (Flora Europaea, 1972) and are identified to excretory tissues of external selection. They are sessile and have multicellular head surrounded by rosette cells of the epidermis. When essential oil accumulates under the head-covering cuticle, it separates from the cellular wall and the secretion releases to the outer surface (Blažeković et al., 2006). The density of the glands is a species-specific feature. Essential oil glands of *Th. camphoratus* are 0.2 mm in diameter, located denser on the lower side of the lamina (on average 5–6 glands per 1 mm²), than on the upper (3 glands per 1 mm²). In other species of the genus, the glands are located on the leaf in lower number on both sides of the lamina. Therefore, in *Th. vulgaris* they are larger in size (0.3–0.4 mm), located in the quantity of 2–4 per 1 mm², in *Th. serpyllum* – smaller in size, located more rarely in the quantity of 1 per 1 mm², and in *Th. pullegioides* – only 0.3 per 1 mm² on the surface of the leaf (Hrytsyna et al., 2018). Unlike other species of the genus (*Th. vulgaris*, *Th. zygis* and *Th. callieri*), in *Th. camphoratus* under the epidermis of the leaf, capitate single-cellular essential oil-bearing sessile trichomes are located, and in the mesophyll of the leaf, no internal essential oil reservoir is present (Hrytsyna et al., 2019).

In *Th. camphoratus*, in the conditions of cultivation, elongated ringed inflorescence forms. Its synflorescence (united inflorescence) formed of the main inflorescence and annual lateral vegetative-generative non-dormant ramifying shoots (Fig. 4a, b). Detailed analysis revealed that in all species, raceme-like thyrsoid type inflorescence forms, the apex of which grows monopodially, and the partial opposite angle inflorescences are sympodially formed of the collection of simple dichasia. Such type of partial inflorescence could be named “compound multiple dichasia”, similarly to the species of *Verbascum* L. genus (Hrytsyna, 2013).

In natural conditions, *Th. camphoratus* is a polymorphic species. The studies by Carlos Pinto Gomes confirmed presence of its subspecies: *Th. camphoratus* subsp. *congestus* distributed in sandy soils and *Th. camphoratus* subsp. *camphoratus*, which tends to live in limestone marine coasts (Gomes, 2006). The species living in the collection of the Botanical Garden has tubular calyx of the flower, with almost flat short teeth of the upper and lower lips, light-violet or pale-pink corolla, two-lipped, 2.5–5.0 mm long. The calyx and the corolla are woolly only on the external side, having short single-cellular simple and capitate sessile hairs (Fig. 5a, b, c). According to all the above indicated morphological features of the structure of the flower, we can state that the species introduced to the Botanical Garden is *Th. camphoratus* subsp. *congestus*.

For *Th. camphoratus*, as well as for other species of *Thymus* genus, different chemotypes of the populations are characteristic. In the above-ground part of *Th. camphoratus*, growing in different places in Portugal according to the main constituents of essential oil, 4 chemotypes were distinguished (Salgueiro et al., 1997): linalool (I chemotype), borneol (II), 1,8-cineol (III) and 1,8-cineol / borneol (IV). In another study conducted in Portugal, in one population of *Th. camphoratus*, the dominating components were trans-sabinene hydrate (20%), in another – borneol (13%) and linalool (11%) and in third – 1,8-cineol (33%) (Miguel et al., 2007). In the conditions of the Southern Coast of Crimea, the study (Korsakova, 1998) revealed that mass share of essential oil from *Th. camphoratus* varied within large boundaries of 2.1–10.1%. In its composition, 1,8-cineol (80.0%), α -terpineol (6.4%) and linalool (3.6%) dominated.

As for our studies conducted in the conditions of the forest-steppe zone of Ukraine, the content of essential oil in the above-ground part of *Th. camphoratus* was 4.0 g/kg of air dried mass. Half of its composition comprised alcohols borneol and fenchol which are bicyclic, oxygen-bearing monoterpenes, the derivatives of camphane and fenchane. Essential oils, acyclic monoterpenes – linalool and α -terpineol accounted for around 20% of the composition, whereas oxygen-bearing cineol, derivative of monocyclic menthane which dominated in the populations from Crimea and Portugal, in the conditions of Lviv Oblast accounted for about 2%.

On the example of other species of the *Thymus* genus, the composition and quantitative content of essential oil were determined to be de-

pendent to a large extent on the ecological conditions. Therefore, in the studied samples of medicinal herbal raw material of *Th. vulgaris* grown on farms of Poltava Oblast and Crimea, the content of essential oil ranged 0.60–1.25%. According to the requirements of the European Pharmacopeia (European Pharmacopoeia, 2011), the total content of thymol and carvacrol in essential oil should be no less than 40%. Such value was found only in plants from Crimea (Tykhonenko & Kotov, 2010). In *Th. serpyllum* introduced to Kherson Oblast (Steppe zone of Ukraine), the dominating constituents of essential oil are thymol and γ -terpinene, and the main constituents of essential oil from *Th. pulegioides* are neral and geranial (50.3% in total) (Svydenko et al., 2016).

The reasons for different composition of essential oil from species of *Thymus* genus are due to meteorological and soil conditions, and also the stage of the development of plants. Therefore, in *Th. vulgaris*, a quite close correlation between the type of soil and structural chemotype of populations exists (Gouyon et al., 1986). It was also proved that during rainy weather with insignificant solar radiation, in *Th. pulegioides*, the precursors of carvacrol synthesize - p-cymene and γ -terpinene, and during sunny weather – mostly carvacrol (Vaičiulytė et al., 2016). In Campania (South Italy), the essential oil from *Th. pulegioides* was found to comprise 63 compounds, among which γ -terpinene, p-cymene, thymol and carvacrol varied 57.3–62.5% of the total content, depending on the phenophase of the plant. The best time for the preparation of the raw material of *Th. vulgaris* is the phenophase of full blossoming (Senatore, 1996; Hudaib et al., 2002).

The use of radioactive isotopes, on the example of *Th. vulgaris*, for the first time gave convincing proofs of the fact that thymol biosynthesizes by aromatization of γ -terpinene to p-cymene with following hydroxylation of hydroxylation of p-cymene to thymol (Poulose & Creteau, 1978).

The French scientists (Gouyon et al., 1986) also indicate that the high level of diversity of chemotypes is due to the conditions of the environment. In a stable environment, low generative diversity was observed, whereas in disturbed environments the genetic diversity was high, allowing, through individual selection, a clearly determined specialization of each population in the environment.

It is known from the literature that *Th. camphoratus*, depending on the composition of essential oil, exerts anti-inflammatory, antifungal and antioxidant properties. Therefore, essential oil from *Th. camphoratus* rich in 1,8-cineol and borneol (Zuzarte et al., 2018) in the experiment exhibited anti-inflammatory properties, inhibiting the expression of two determinant anti-inflammatory proteins iNOS and COX-2. Bioactive concentrations of essential oils were not toxic to macrophages and hepatocytes, opening new ways of development of effective anti-inflammatory medical plant medications.

Among the studies on antioxidant action of eight essential oil-bearing Iberian species of plants, essential oil from *Th. camphoratus* containing 1,8-cineol (21%) exerted the best anti-acetylcholinesterase activity (Albano et al., 2012). *Th. camphoratus* with slightly different composition of essential oil: linalool (17%), linalyl acetate (15%) and 1,8-cineol (11%) also displayed antioxidant activity (Miguel et al., 2004, 2007).

The oil from *Th. camphoratus*, rich in 1,8-cineol and α -pinene exhibited fungicidal properties against *Cryptococcus neoformans* and *Candida albicans* to a larger extent than fluconazole are broadly used in clinical practice as an antifungal preparation. It is worth noting that the effect was greater when the essential oil was used as a whole than its separate constituents (Alves et al., 2019). In our study, the essential oil from *Th. camphoratus*, half of the composition of which was borneol and fenchol, had no antimycotic activity towards microscopic fungi *Candida albicans*.

Essential oil from *Th. camphoratus* introduced in the conditions of Lviv Oblast exhibited bactericidal impact on *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus pyogenes*, *Escherichia coli* and had somewhat lower antimicrobial activity against clinical isolates of these microorganisms. In the previous studies (Kryvtsova et al., 2019), antimicrobial activity of essential oil from *Th. vulgaris* was determined against type and clinical isolates of these microorganisms, which is explained by the high content of thymol and carvacrol in it.

Obviously, the results we obtained which are somewhat different from the literature data could be explained by the fact that borneol took up a large share of essential oil (26% and 28%, respectively, in each experiment) from which dehydration camphora is obtained, which has not only antiseptic properties, but also irritating, analgesic, cardiotoxic and sedative effects. In the future, it is intended to study in detail the effect on the nervous and cardio-vascular system of the plants of *Th. camphoratus* growing in the Botanical Garden of Ivan Franko National University of Lviv.

Conclusions

According to the results of the studies we performed, it was determined that the diagnostic features of medical herbal raw material *Th. camphoratus* are the structure of the stem, leaf and generative organs and their woolliness. The shape of the lamina changes from oval with dull apex in the lower part of the stem to rhombus-like with sharpened apex in the upper part. The lamina has slightly downward bent large-crenate margins, its lower part is covered by simple, straight and crook-like hairs, mostly along the veins. The epidermis is formed of elongated cells, the stomatal apparatus is of diacytic type, on the abaxial the stomas are located densely. The surface of the leaf is covered by peltate trichomes with many glandular hairs between them. Peltate hairs have 5–6 cellular head and are surrounded by 12–16 rosette cells of the epidermis. The mesophyll is formed of palisade and spongy tissues and has no essential oil reservoirs.

The calyx of *Th. camphoratus* is tubular-campanulate with almost similar short teeth of the upper and lower lip, entirely covered by uniformly wooly with single-cell simple and capitate essential oil bearing hairs. The corolla has two lips, light-violet, or pink. The inflorescence of *Th. camphoratus* is compound, raceme-like thyrsoid type. Its opposite, angle partial inflorescences are compound dichasia formed of 7 flower-bearing axes at different stages of development.

On the basis of the morphological-anatomical analysis, we determined that the species introduced to the Botanical Garden of the Ivan Franko National University of Lviv is *Th. camphoratus* subsp. *congestus*.

In the herbal raw material of *Th. camphoratus*, the content of essential oil accounts for $0.40 \pm 0.05\%$ of air dried mass, in which 48% of the composition is borneol and fenchol. The obtained essential oil exerts bactericidal properties against *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, *Enterococcus faecalis* and their clinical isolates. Towards single-cell fungi of *Candida albicans*, it displayed no growth inhibition of their colony.

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