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RESEARCH ARTICLE

Morphological, anatomical and phytochemical characterizations of *Lavandula stoechas* L. subsp. *stoechas* Growing in Turkey

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Abstract

In this study, *Lavandula stoechas* L. subsp. *stoechas* which is plant with economic and medicinal importance has comparatively been examined morphologically, anatomically and chemically. A detailed description of the species has been prepared and compared with those published in the Flora of Turkey of Davis in tabular form. The taxonomical, morphological and anatomical characteristics of the plant material have been described and illustrated with drawings. General appearance of leaves, branches, flowers, calyx, corolla and fruit shapes are described. Herbaceous and woody stem and leaves of the *L. stoechas* subsp. *stoechas* samples collected from Muğla, Ula, Portakallık were examined. Analyses of the hydrodistilled oil were performed on GC and GC/MS systems. Camphor (45.8%), α-fenchone (31.8%) and bornyl acetate (4.2%) were found as main constituents in the essential oil of *L. stoechas* subsp. *stoechas.*

Keywords: Lamiaceae, *Lavandula stoechas* subsp. *stoechas,* morphology, anatomy, essential oil, GC, GC-MS

Introduction

The genus *Lavandula* L. (Lamiaceae) is represented in Turkey by 3 taxa; *Lavandula stoechas* L. subsp. *stoechas, Lavandula pedunculata* subsp. *cariensis* (Boiss.) Upson & S.Andrews and *L. angustifolia* Mill. subsp. *angustifolia* in the Flora of Turkey (Mill, 1982; Dirmenci, 2012). *L. angustifolia* is locally known as "lavanta", L. *pedunculata* subsp. *cariensis* is known as "karan", L. *stoechas* ''karabaşotu, karan, gargan, keşişotu''(Baytop, 1984) and *L. stoechas* subsp. *stoechas* is locally known as "karabaş" (Mill, 1982; Dirmenci, 2012)). *L. stoechas* has been used as analgesic, antiseptic, sedative, expectorant, cardiotonic and to heal eczema (Baytop, 1984). *L. angustifolia* (lavender) is a cultivated plant and its flowers have diuretic and rheumatic pain-relieving properties. The flowers are fragrant and are used in the perfumery industry and in certain pharmaceutical preparations. Pain relief, antiseptic, wound healing, sedative (epilepsy and asthma), expectorant, urinary tract inflammation relieving, eczema remedy, nerve and heart strengthening effects have been registered (Baytop, 1984).

L. angustifolia known as medicinal lavender, is a well-studied and widely used species. Lavender grows easily in arid fields. This commercial plant is also known as English lavender or *L. officinalis*. Lavender is used in aromatherapy as sedative. Experimental studies in humans and animals have shown that its sedative effect comes from linalool and linalyl acetate. Tannins contained in flowers of lavender have antidiarrheal effect. Essential oil of lavender is used in various skin diseases and wounds. Essential oil of prolongs the sleeping period. In addition, it also shows antimicrobial, antiinflammatory, fungicidal, insecticidal and acaricidal effects (Zeybek et al., 2010).

In this study, the morphological, anatomical and chemical properties of *L. stoechas* subsp. *stoechas* were investigated.

Materials and Methods

Plant material

Plant material was collected from Turkey, Muğla, Ula, Portakallık in 25.3.2001. The material was identified by SK and voucher specimen is kept at the Herbarium of the Faculty of Pharmacy of Anadolu University, Eskişehir (ESSE 14113).

Morphological studies

A Wild M5 A stereo microscope with drawing tube was utilized for morphological drawings.

Anatomical studies

Permanent microscobic preparations were made of plant material fixed in the field in 70% alcohol. Cross and surface sections of the plant leaf and stem were made by hand and stained with Sartur reagent. Anatomical studies were made using a Leitz SM-LUX binocular light microscope with a drawing tube. Their photographs were taken with Olympus BX 51 trinocular microscope.

Hydrodistillation

The aerial parts of plants were water distilled for 3 h using a Clevenger-type apparatus to obtain the essential oil.

Gas chromatography mass spectrometry (GC/MS) analysis

The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60m x 0.25mm, 0.25μm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was at 250°C. MS were taken at 70 eV. Mass range was from m/z 35 to 450.

GC analysis

The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300°C. In order to obtain same elution order with GC/MS, simultaneous injection was done by using same column and appropriate operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms. The results of analysis are shown in Table 2.

Identification of the essential oil components

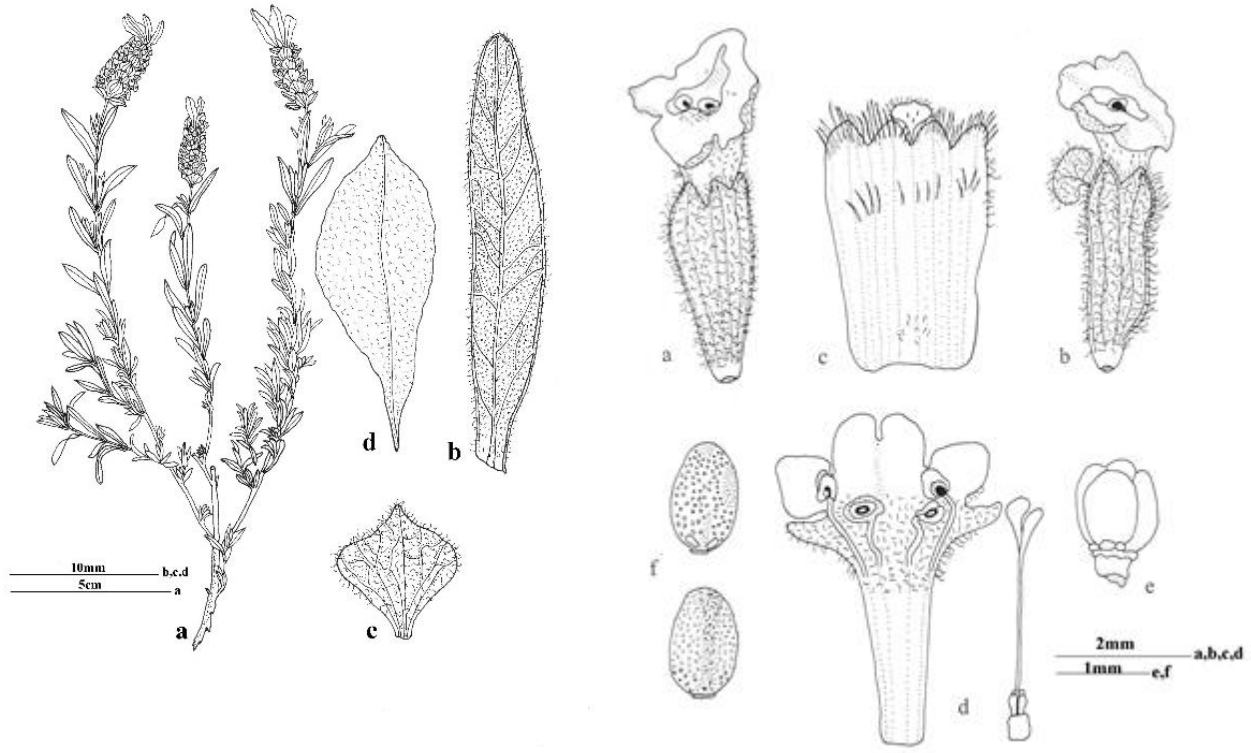
Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, Adams Library, MassFinder 2.1Library) (McLafferty et al., 1989; Joulain et al., 2001) and in-house “Baser Library of Essential Oil Constituents” built up by genuine compounds and components of known oils, as well as MS literature data (Jennings et al., 1980; Joulain et al., 1998; ESO 2000, 1999) was also used for the identification.

Results and Discussion

Morphological results

Morphological and morphometric findings related to *L. stoechas* subsp. *cariensis* are given in Table 2 and compared with descriptions given in the Flora of Turkey (Mill, 1982). In addition, in Table 2, new characters (indumentum of calyx and corolla, pistile and stamens, ovary and nectary, nux), important for the systematics of the species and genus have been determined (Figures 1A, 1B).

Figure 1. A: *L. stoechas* subsp. *stoechas* (ESSE 14113).



A **B**

a: Habit, b: Leaves, c: Fertile bracts, d: Upper (sterile) bracts; B: a: Flower (front view), b: Flower (side view), c: Inside of calyx, d: Inside of corolla, pistile and stamens, e: Ovary and nectary, f: Nux

Lavandula stoechas L. subsp. *stoechas*

Aromatic shrubs, with stellate indumentum, glandular and eglandular hairs. Stem erect or ascendens, branched, 10-39 cm, tomentose. Leaves simple, upper leaves lanceolate, elliptic to narrowly elliptic, margin entire, revolute, 10.5-25x(1-)2-6(-7) mm, obtuse at apex, both side tomentose hairy, veins prominent, sessile, with numerous smaller fastigiate strongly revolute leaves in their axils and at base of stem. Peduncle 0.3-3.5 cm, shorter or equalling than spike. Inflorescence, terminal dense spike in form. Spike pedunculate, 1-3(-)3.5 cm, oblong- ovoid, dense, not interrupted. Fertile bracts 4-8x5-10 mm, purple, rhombic-cordate, mucronate at apex. Upper (sterile) bracts (5-)8-18x(-2)4-8 mm, obovate, spathulate, purple, obtus at apex, outside tomentose, innerside glabrose. Bracteoles absent Verticillasters usually 5 sometimes 7 flowered. Calyx 3,5-9 mm, tubulate, 13-veined, veins prominent, with 5 teeth, upper tooth with spade-shaped to transversely oblong, entire or slightly lobed, upper¬most usually appendiculate. Appendage 0.5-1.5x1-2.5 mm, undulate,

reniform, margin entire, tomentose outside, throat straight hairy. Corolla blackish-purple, 5-9 mm, tubulate, bilabiate, upper lip 2 lobed, rotundate, throat straight hairy. Stamens 4, didynamous, inside corolla tube. Ovary 4 lobed, stylus bifid, inside corolla tube. Nutlets 1-1.5 mm, oblong-ovate, red-brown, trigonal. Fl. 3-6. Open *Pinus brutia* forest, macchie, phrygana, rocky limestone and granitic slopes, sandy places, roadsides, s.1.-700 m (Figures 1A, 1B) (Mill, 1982).

Table 1. The comparison of morphometric characteristic between Flora of Turkey (A) and the present study (B) with respect to *L. stoechas* subsp. *stoechas*.

	L. stoechas* subsp. *stoechas	
	A	**B**
Flowering stems	45 cm or more	10-39 cm
Leaves	14-40 x1-5 mm	10.5-25x(1-)2-6(-7) mm
Spike	1.5-4.5 cm	1-3(-)3.5 cm
Peduncles	0.5-2.5(-2.7) cm, shorter than spike	0.3-3.5 cm, shorter than or equalling spike
Fertile bracts	4-7x3-8 mm	4-8x5-10 mm
Upper (sterile)bracts	7-17x3-8 mm, obovate	(5-)8-18x(-2)4-8 mm, obovate
Calyx	4-6 mm	3.5-9 mm
Appendage	0.5-1x1-2 mm, entire	0.5-1.5x1-2.5 mm, entire
Corolla	(4.5-)5-8.5 mm	5-9 mm
Numbers of flowers	6-10	5-7
Nux	-	1-1.5 mm

Anatomical results

The sections taken from stem and leaves of *L stoechas* subsp. *stoechas* were examined and the anatomic features were determined, compared as shown in Figures 2-8.

Herbaceus stem (Figs 2,3)

Lavandula stoechas subsp. *stoechas*

In cross sections, the stem is rectangular (Figs. 2, 3A, 3B). Epidermis: one layered and with a thick cuticle, composed of roundish-oval. The outer and inner tangential walls are thicker than the anticlinal walls. There are covering and glandular hairs on the epidermis. Covering hairs: usually stellate, branched and multicellular, 2-7 celled with smooth cuticle. or seldomly uniseriat, straight or recurved. Glandular hairs are five types; head 1 stalk 1 celled, head 2 stalk 1 celled; head 1 stalk 2 celled, head 3 stalk 1 celled and head 8 celled (Labiatae type) (Figs. 2, 3A,3B, 8). 3-4 rows collenchyma tissue was found in the four angles. Cortex is parenchymatous to endodermis. Endodermis is consisting of flattened cells can hardly be distinguished from the cortex parenchyma. Pericycle composed of cylindric multi-layered sclerenchyma. Vascular system: consisting of a complete cylinder of xylem surrounded by phloem. Cambium: indistinct, Medullary rays: 1-2 cells wide. Pith: Parenchymatous and covers a large area. Starch grain was observed in some cells (Figs. 2, 3A, 3B).

Figure 2. *L. stoechas* subsp. *stoechas* (ESSE14113). Cross-section of herbaceous stem (Photographs Olympus BX 51 trinocular microscope).

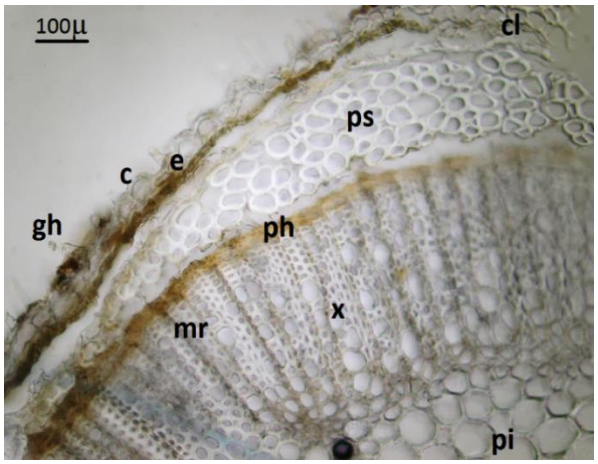
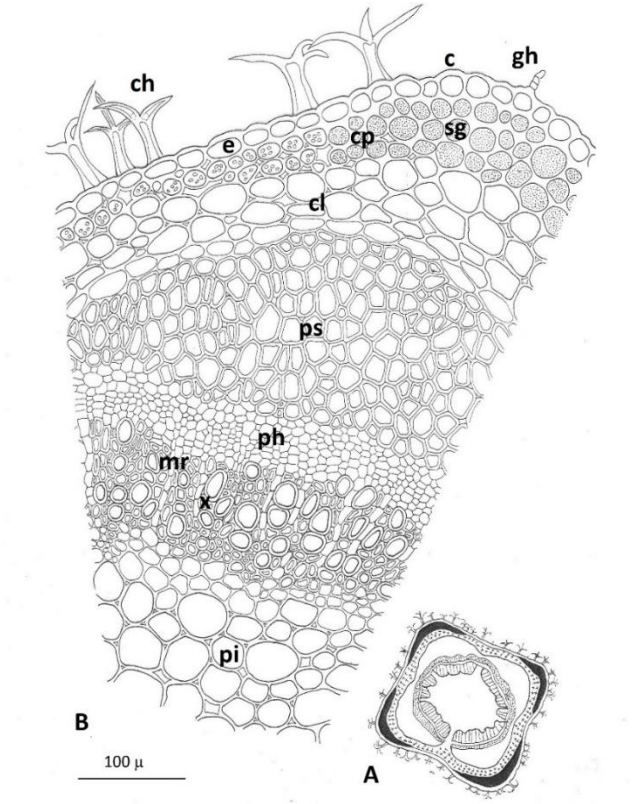


Figure 3. *L. stoechas* subsp. *stoechas* (ESSE14113). Cross-section of herbaceous stem.



A diagrammatic, B detail from (hand-drawn), c: cuticle, cl: collenchyma, ch: covering hair, cp: cortex parenchyma, e: epidermis, gh:glandular hair, mr: medullary ray, ph: phloem, pi: pith, ps: pericyclic sclerenchyma, sg: starch grain, x: xylem.

Woody stem (Figs. 4, 5A, 5B)

The root in both species is composed of periderm on the outside and phelloderm where 3-5 radial rows are broken down and phelloderm with 2-3 rows of tissue. Outer phelloderm cells are tissue debris of the primary cortex that has been shattered or crushed in place. Secondary phloem formed of elliptical-shapeless, round-shaped, irregular-arranged and 4-6 row cells under the periderm is taken part. Cambium is uncertain. The secondary xylem part covers a large area and consists of tracheal elements with large and small sizes in a

sclerenchymatic tissue. Medullary rays are 2-3 rows of cells. The pith region, covering a narrow area, is parenchymatic. Starch grain was observed in some cells (Figs. 4, 5A, 5B).

Figure 4. *L. stoechas* subsp. *stoechas* (ESSE14113). Cross-section of woody stem, (Photographs Olympus BX 51 trinocular microscope).

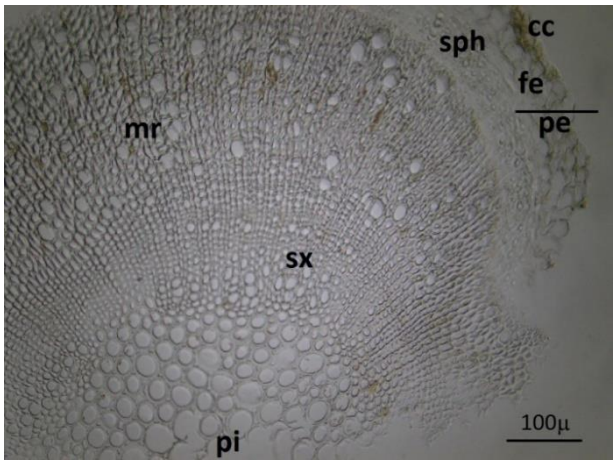
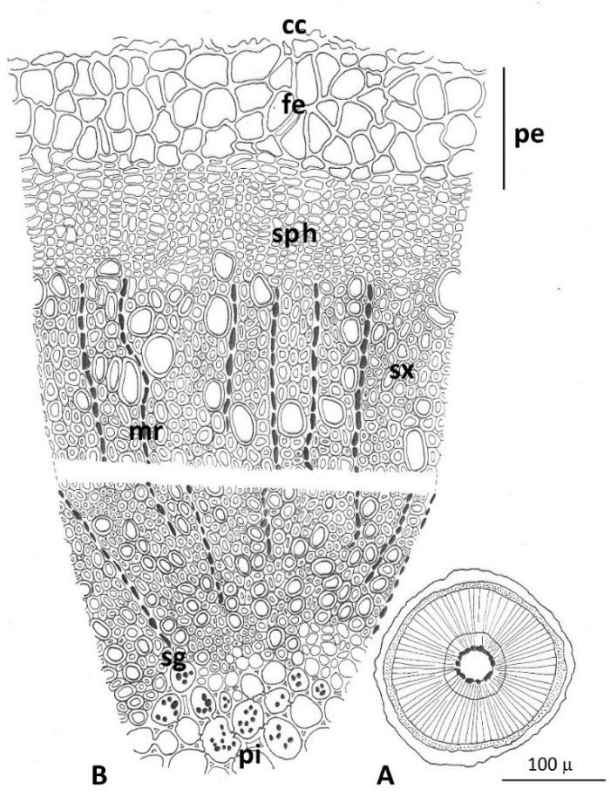


Figure 5. *L. stoechas* subsp. *stoechas* (ESSE 14113). Cross-section of woody stem.



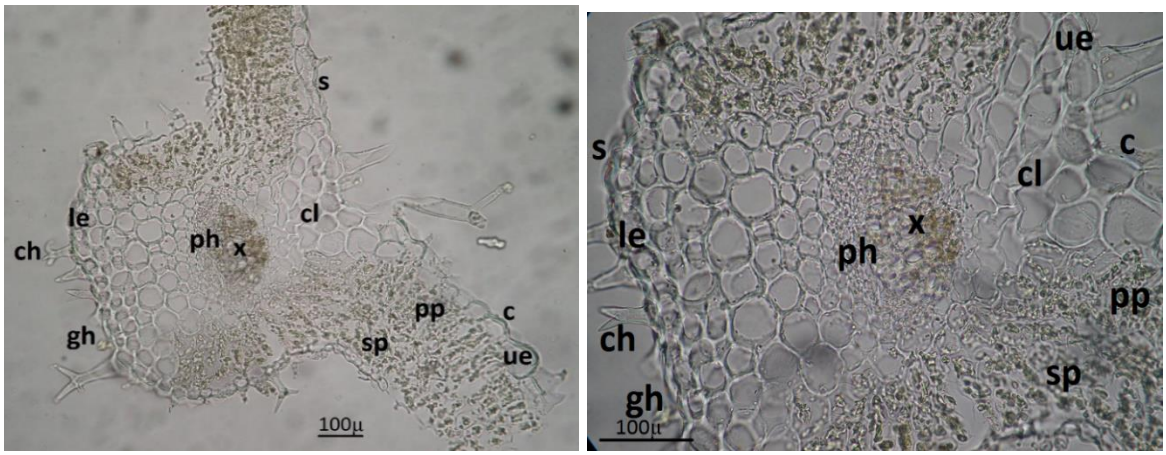
A diagrammatic, B detail from (hand-drawn), cc: crushed cells, fe: phelloderma, mr: medullary ray, pe: periderma, pi: pith, sg: starch grain, sph: secondary phloem, sx:secondary xylem

Leaf (Figs. 6, 7A, B, C, D, 8)

In cross sections, epidermis is one layered and has roundish or rectangular cells with a thin cuticle; the cells of upper side are larger than lower side and the outer tangential walls are thickened (Figs. 6, 7A, B, C, D), the anticlinal cell walls are sinuous in the lower side and are almost straight in the upper side. Covering and glandular hairs were observed on both upper and lower epidermis. Covering hairs: usually stellate, branched

and multicellular, 2-7 celled with smooth cuticle. or seldomly uniseriate, straight or recurved. Glandular hairs are five types; head 1 stalk 1 celled, head 2 stalk 1 celled; head 1 stalk 2 celled, head 3 stalk 1 celled and head 8 celled (Labiatae type). Stomata: the leaves amphistomatic, with diacytic stomata (Figs 4B-6B) superfacial or slightly raised above the epidermal level (Figs. 6, 7A,B,C,D, 8) and more numerous on the lower side of the leaf. Mesophyll: bifacial, palisade tissue 1-2 layered, spongy parenchyma 3-4 layered (bifasiyal) (Figs. 6, 7A,B,C,D). Midrib: prominent arced in lower side. Below the upper and lower epidermis, there is a collenchyma tissue a few layered. Vascular bundles are collateral and phloem in the abaxial, xylem in the adaxial direction. Collenchymatic bundle caps present in the xylem and phloem poles.

Figure 6. *L. stoechas* subsp. *stoechas* (ESSE14113).



Cross section of the leaf: detail from intervessel area; surface views of epidermis, in LM, respectively; ue: upper epidermis, m: mesophyll, le: lower epidermis, c:cuticle, cl: collenchyma, gh: glandular hair, ch: covering hair, p: parenchyma, pc: phloem collenchyma, ph: phloem, x: xylem, xc: xylem collenchyma, c: cuticle, pp: palisade parenchyma, s: stoma, sp: spongy parenchyma, vb: vascular bundle.

Figure 7. *L. stoechas* subsp. *stoechas* (ESSE 14113). Cross section of the leaf: A diagrammatic, B detail from (hand-drawn), C surface view of upper epidermis D surface view of lower epidermis in LM, respectively.

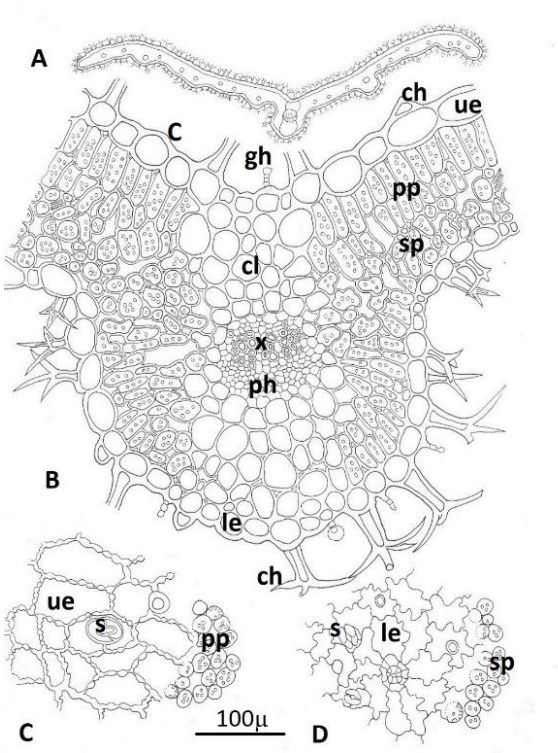
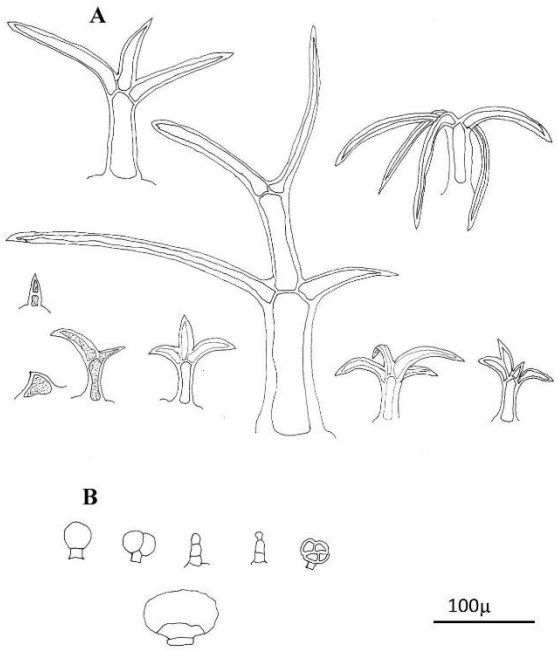


Figure 8. Stem and leaf hairs. *L. stoechas* subsp. *stoechas* (ESSE14113).



A: covering hairs, B: glandular hairs.

Chemical results

The yield of the hydrodistilled oil was 1.08%. Twenty nine compounds were identified by GC/MS, representing 87.1% of the total oil (Table 2). Camphor (45.8%), α-fenchone (31.8%) and bornyl acetate (4.2%) were found to be the main constituents in the essential oil.

Table 2. The Composition of the Essential Oil of *L. stoechas* ssp. *stoechas*

RRI[a]	Compound	%
1032	α–Pinene	0.30±0
1072	α–Fenchene	0.10±0
1076	Camphene	2.50±0
1135	Thuja-2,4(10)-diene	0.10±0
1203	Limonene	0.10±0
1213	1,8-Cineole	0.90±0
1255	γTerpinene	*tr*
1280	p-Cymene	0.30±0
1406	α–Fenchone	31.77±0.05
1450	*trans*-Linalool oxide (Furanoid)	*tr*
1452	α,p-Dimethylstyrene	0.10±0
1474	Camphenilone	0.10±0
1482	Fenchyl acetate	0.70±0
1532	Camphor	45.80±0.09
1553	Linalool	0.10±0
1586	Pinocarvone	0.10±0
1591	Bornyl acetate	4.20±0
1591	Fenchyl alcohol	1.23±0.05
1598	Camphene hydrate	0.10±0

1648	Myrtenal	0.57±0.05
1661	*trans*-Pinocarvyl acetate	1.03±0.05
1683	*trans*-Verbenol	0.50±0
1706	α–Terpineol	0.10±0
1719	Borneol	1.70±0
1725	Verbenone	0.40±0
1751	Carvone	0.20±0
1773	δ-Cadinene	0.10±0
1797	*p*-Methyl acetophenone	*tr*
1804	Myrtenol	0.60±0
1838	2-Phenylethyl acetate	0.10±0
1845	trans-Carveol	0.10±0
1849	Calamenene	0.17±0.05
1864	*p*-Cymen-8-ol	0.50±0
1900	epi-Cubebol	*tr*
1941	α–Calacorene	0.10±0
1953	Palustrol	0.10±0
1957	Cubebol	*tr*
2008	Caryophyllene oxide	0.10±0
2057	Ledol	0.27±0.05
2080	Cubenol	0.10±0
2088	1-epi-Cubenol	*tr*
2098	Globulol	0.10±0
2104	Viridiflorol	1.60±0
2209	T-Muurolol	0.10±0
2289	Oxo-α–Ylangene	0.20±0
	Total	**97.13±0.29**

[a] RRI Relative retention indices calculated against *n*-alkanes on the HP Innowax column; [b] mean % calculated from Flame Ionization Detector (FID) data ± SD (n=3); [c] tr Trace (< 0.1 %)

Discussion

This study was aimed at comparing morphological, anatomical and chemical features of *L. stoechas* subsp. *stoechas* with a view to establish further proof to its taxonomical identity.

In this study, descriptions of the *L. stoechas* subsp. *stoechas* have been defined more detailed than those in Flora of Turkey and also lacking properties like habit, leaves, fertile bracts, upper (sterile) bracts; flower, calyx, corolla, pistil, stamen, ovary and nectary, nux have been completed. On the other hand, all detailed figures which help to define the taxa have been given first time in this study. Our findings about *L. stoechas* subsp. *stoechas* contradict with some findings of Miller as stated above (Table 1).

According to our morphological findings, height of upper (sterile) bracts, calyx, appendage and corolla were different. We have observed that the upper (sterile) bracts were spathulate.

Anatomic structure of the stem and leaves of *L. stoechas* subsp. *stoechas* samples has been given in this study for the first time. Most anatomical properties generally match with the findings of Metcalfe and Chalk for the genus (Metcalfe et al., 1950).

Analysis of the hydrodistilled oil was performed on GC and GC/MS systems. The composition of the essential oil is given in Table 2. with relative retention indices (RRI) and relative percentages (%). Camphor (45.8%), α-fenchone (31.8%) and bornyl acetate (4.2%) were found as main constituents in the essential oil of *L. stoechas* subsp. *stoechas*.

The quality of *Lavandula* essential oils is regulated by ISO standards (The International Organization for Standardization). Also various international standarts such as European Pharmacopoeia (PhEur) contain monographs on *Lavandula angustifolia* and *L. latifolia* securing pharmaceutical grade quality. The monograph on *Lavandula aetheroleum* ex *L. angustifolia* in PhEur indicates the percentage limits of main components as linalool 20.0-45.0% and linalyl acetate 25.0-47.0%, limonene: max. % 1.0, 1,8-cineole: max. % 2.5, 3-octanone: % 0.1- 5.0, camphor: max % 1.2, terpinen-4-ol: % 0.1- 8.0, lavandulyl acetate: min % 0.2, lavandulol: min % 0.1, α-terpineol: max. % 2.0. (Council of Europe European Pharmacopoeia, 2014; Kırımer et al., 2016). There is no monograph in the European Pharmacopoeia on the taxon subject to this study.

In previous studies, Karaca et al. [Karaca et al., 2018) identified the main components of *L. stoechas* leaf and flower essential oil as camphor (46.7 %) and α-fenchone (28.9 %); Kirmizibekmez et al. (2009) reported α-fenchone (42 %) ,1,8-cineole (16 %), camphor (12 %) and Sertkaya et al. (2010) α-thujone (66 %), camphor (18 %), 1,8-cineole (8 %) as main constituents of *Lavandula stoechas* L. ssp. *stoechas*. Our findings complement the previous reports.

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