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Comparative morphological, anatomical, ecological and chemical studies on endemic *Satureja parnassica* subsp. *sipylea* from Turkey

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Satureja. parnassica Heldr.et Sart. subsp. *sipylea* P.H. Davis is a subspecies endemic to Turkey. The anatomical, morphological, ecological and chemical features of *S. parnassica* subsp. *sipylea* have been investigated. Plant samples were collected from different regions in Turkey. The morphological features of various organs of the plant such as leaf and flower are described in detail. In anatomical studies, transverse sections of the plant stem and leaf have been examined and supported by illustration and photographs. Ecological studies provide information about the physical and chemical structure of soil types in Spil Mountain and Marmara Island. The chemical composition of the essential oil of aerial parts of the plant was examined.

Key words: Anatomy, morphology, ecology, essential oil, Lamiaceae, Satureja parnassica

Introduction

The genus *Satureja* (*Lamiaceae*) is distributed in an area stretching from the Mediterranean region to Europe, West Asia, North Africa, the Canary Islands and South America (HEGI 1964, BAYTOP 1991, STRID and TAN 1991).

Systematic studies on the genus *Satureja* are presented in various flora and publications (TUTIN et al. 1972, FEINBRUN-DOTHAN 1978, DAVIS et al.1988, STRID and TAN 1991). The first detailed information on the species of *Satureja* growing in Turkey was given in Flora Orientalis by BOISSIER (1879). The author described 15 species, 8 of which are distributed in Turkey. Later, HAYEK (1931) provided some further information on *Satureja* species collected in Turkey.

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The genus *Satureja* consists of perennial herbs except for one species and is represented by 14 species in the flora of Turkey (DAVIS et al. 1988). The number of *Satureja* species in Turkey rose to 15 after a recent investigation in the Aegean and Marmara regions (TÜMEN et al. 2000).

There have been very few studies on the morphology and anatomy of *Satureja* species in Turkey (KAYA et al. 1994) but the morphological and chemical features of *Satureja* species collected from Thrace and West Anatolia were investigated by BAŞER et al. 2000.

This study was carried out in order to provide insight into the distribution, morphology, anatomy, ecology and chemical components of essential oils of *Satureja parnassica* subsp. *sipylea* from Marmara Island and Spil Mountain.

Material and Methods

S. parnassica subsp. *sipylea* was collected from Marmara Island in Balikesir and Spil Mountain in the Manisa province of Turkey in August 1996. Voucher specimens are deposited in the Herbarium of the Faculty of Pharmacy, Anadolu University in Eskişehir Turkey (Acronym ESSE). Morphological features were determined on living and herbarium materials. Approximately thirty specimens were used to determine the morphological characteristics. Transverse sections and surface preparations of stem and leaves were made manually for anatomical studies. A Wild M5 stereomicroscope with drawing tube and a Nikon Eclipse E 600 research microscope were used in morphological and anatomical studies.

The soil samples were collected from the localities between July and August at a depth of between 0–20 cm. The texture, total salt, $CaCO_3$, pH, P_2O_5 Kg/dk, K_2O Kg/dk and organic matter were determined.

Essential Oil Distillation

The aerial parts of the air-dried plants including the inflorescence were subjected to water distillation for 3 h using a Clevenger apparatus to yield essential oils.

Gas Chromatography / Mass Spectrometric (GC/MS) Analysis

The oils were analyzed by GC/MS using a Hewlett Packard GCD system. An Innowax FSC column (60 m × 0.25 mm ϕ, with 0.25 μm film thickness) was used with helium as carrier gas (1 ml min^{-1}). The GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C at a rate of 4 °C min^{-1}, then kept constant at 220 °C for 10 min and then programmed to 240 °C at a rate of 1 °C min^{-1}. Alkanes were used as reference points in the calculation of relative retention indices (RRI). Split ratio was adjusted at 50:1. The injector temperature was at 250 °C. MS were taken at 70 eV. Mass range was from 35 to 425 *m/z*. Library search was carried out using the Wiley GC/MS Library and TBAM Library of Essential Oil Constituents. Relative percentage amounts were calculated from TIC by the computer.

Results

Morphological Studies

Satureja parnassica Heldr.et Sart. subsp**. *sipylea*** P.H.Davis

The species was first described by Boissier in Fl. Or. 4:563 (1879) and the subspecies by Davis in Notes R.B.G. Edinb. 38:52 (1980). Small shrub, 6–16 cm long. Root with stout woody stock. **Stem** 1–4 cm diameter, woody at base, much branched from basal part to upwards, terete, bark dark brown, falling off in small pieces (Fig. 1). Upper stem densely patent hirsute all round, hair 0.2–0.6(–4) mm, especially longest near nodes (4–6 mm). Leafless below, densely leafy above. **Cauline leaves** greyish-green, cuneate-oblanceolate, outer leaves big in size, 7.5–14 × 2.5–4.5 mm, inner leaves small, 2.5–8 × 1–3 mm, sessile, entire, strongly mucronate, cuneate-oblong at base, without visible lateral veins beneath, densely hispidulous-pubescent on both surfaces, especially dense, hirsute long hairs at base, oil dots numerous, embedded in both surfaces, usually red. **Floral leaves** spathulate-oblanceolat, 4.5–7.5 × 0.5–1.5 mm, sessile, strongly mucronate, as long as 1–1.5 × the length of calyx, entire, cuneate at base, scabrid-pubescent, long hirsute hairs at base, sparsely glandular hairs with stalk on both surfaces (*S. parnassica* which is the only species having glandular hairs with stalk in floral leaves). **Bracteoles** (1.5)2–3(–3.2) mm, not longer than ½ calyx, sometimes as long as the calyx. **Inflorescence** not long, 1.5–3.5 cm, verticillasters gradually becoming dense towards apex 3–9, 2-flowered, petiole to 8 mm. **Calyx** greyish-green, (2.5)3–3.5(–4) mm, subactinomorphic, gamocephalous, slightly bilabiate, 10 veins, five teeth, teeth 1–1.5 mm, lanceolate. 1/3–1/2 calyx, outer scabrid-hirsute, glandular, inner dense hirsute in teeth. **Corolla** white, exserted calyx, 4–4.5 mm,

Fig. 1. *Satureja parnassica* subsp. s*ipylea* (ESSE 12147): Habit

tubulate, gamopetalous, bilabiate, upper lip erect and large, apex obtuse, sometimes bifid, lower lip 3-lobed, margin slightly undulate, dense pubescent, sparsely glandular outside, short pubescent inside. **Stamens** 4, didinamous and exserted from upper lip, anthers purple, 0.4–0.8 mm, dorsifix, filaments white (1.5–2)–6.5 mm, style white, 4.5–8 mm, gynobasic, subulate, exserted from corolla. **Nutlets** light brown, 0.8–1.2 × 0.6–0.9 mm, obovate-oblong, apex obtuse, pubescent-glandular (Figs. 2, 3).

Distribution

Type: Turkey, B1 Manisa: Spil Mountain, 16.07.1854, Balansa 309 (holotype Kew Herbarium, isotype British Museum, Geneva Herbarium – *S. spinosa*)(Fig. 4).
Turkey, A1 Balıkesir: Marmara Island, above Çınarlıköy, 600 m, E. Tuzlacı (ISTE 38783)!

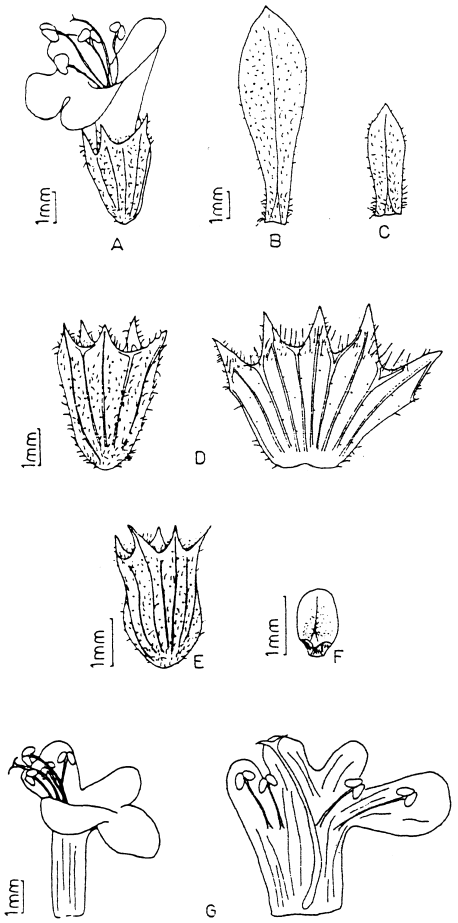

Fig. 2. *Satureja parnassica* subsp. s*ipylea* (Marmara Island): A-Flower, B-Leaf, C-Bracte, D-Calyx, E-Fruiting calyx, F-Nutlet, G-Corolla

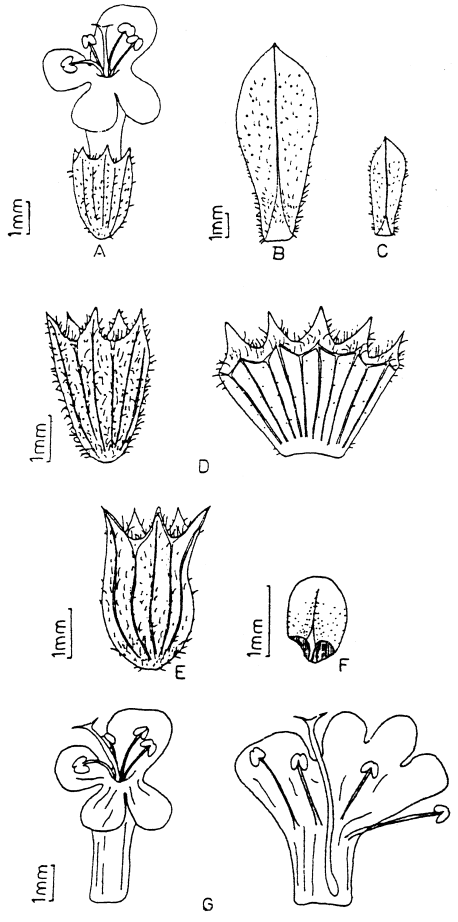

Fig. 3. *Satureja parnassica* subsp. s*ipylea* (Spil Mountain): A-Flower, B-Leaf, C-Bracte, D-Calyx, E-Fruiting calyx, F-Nutlet, G-Corolla

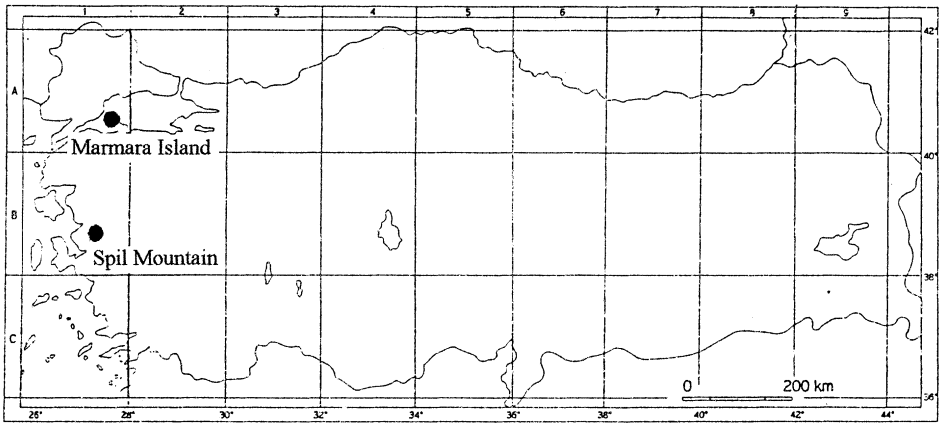

Fig. 4. Map showing the distribution of *Satureja parnassica* subsp. s*ipylea* (•)

Turkey, A1 Balıkesir: Marmara Island, Çınarlıköy 150 m, 30.08.1996, T. Dirmenci, F. Satıl 1039.

Turkey, B1 Manisa: Spil Mountain, fire tower, 1500 m, 31.08.1996, F. Satıl, G. Tümen 1040 (Fig. 4).

Anatomical Studies

Stem: Transverse sections taken from the middle part of the stem (annual) were observed as follows (Figs. 5–9): The epidermis is composed of a single layer of cells, and covered with a thick and undulated cuticle containing glandular and eglandular hairs. Eglandular are more common than glandular hairs. Covering trichomes are usually multicellular (1–5) and covered with a thin cuticle. Unicellular hairs are rare. Two types of glandular hairs are encountered; head and stalk unicellular, and *Labiatae* type (Fig. 7A). The collenchyma tissue, located immediately under the epidermis, is 4–6-layered on the corners and 1–3-layered in between the corners. Parenchyma tissue is 2–5-layered and usually squashed. Single layered endodermis consists of rectangular cells and is absent place to place. There is a 4–5-layered cork tissue above the endodermis. The pericycle is in-

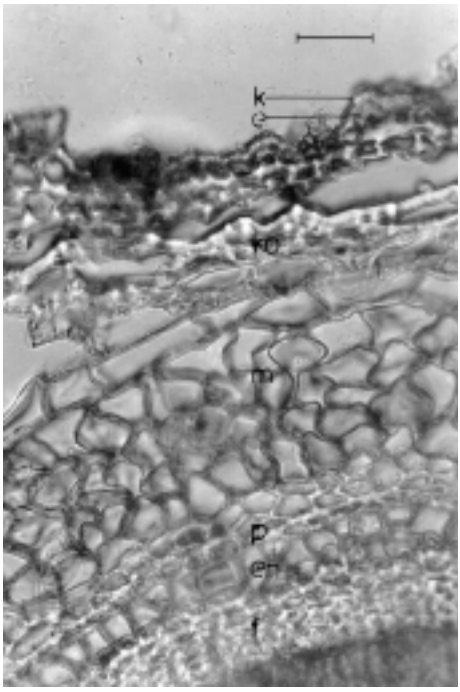

Fig. 5. *Satureja parnassica* subsp. s*ipylea* (Marmara Island): Cross-section of stem. k-cuticula, e-epidermis, ko-kollenchyma, m-cork, p-parenchyma, en-endodermis, f-phloem. Bar = 20 μm

distinguishable. Phloem is 6–8-layered, cells pressed and squared. The cambium is also not distinguishable. The xylem comprises of tracheas and tracheids. The tracheas are orbicular or ovoid while tracheids are polyhedral. The xylem forms a large ring divided with uniseriate rays. The pith consists of large orbicular or polyhedral parenchymatous cells and the centre of pith often breaks into pieces (Figs. 8, 9).

Leaves: Transverse sections of the leaf lamina (Figs. 10–13) and the midrib and surface preparations of both the epidermis layers (Fig. 7B) revealed the following elements.

In transverse section upper and lower epidermis comprise uniseriate square and rectangular cells. Outside walls are thicker than internal and lateral walls. Both epidermis are covered with a thick undulate cuticle. There are dense glandular and eglandular hairs on the surfaces of both epidermis. The covering eglandular trichomes are 1–5-cellular. Unicellular hairs are usually on the lateral side of leaf. There are two types of glandular hairs; *Labiatae* type and

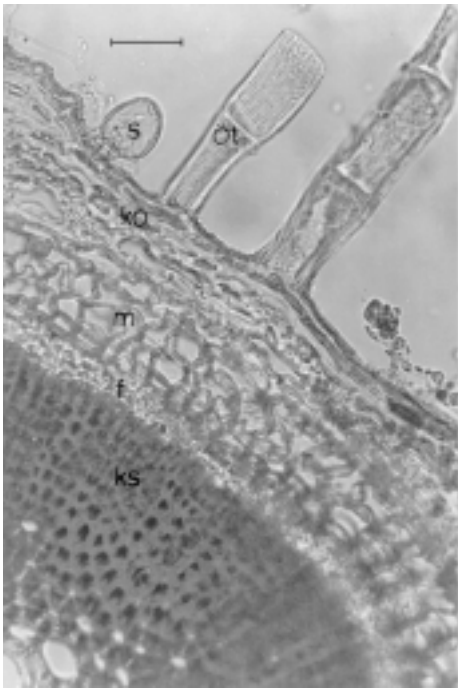

Fig. 6. *Satureja parnassica* subsp. s*ipylea* (Spil Mountain): Cross-section of stem. s-glandular hair, öt-eglandular hair, ko-collenchyma, m-cork, f-phloem, ks-xylem. Bar = 20 μm

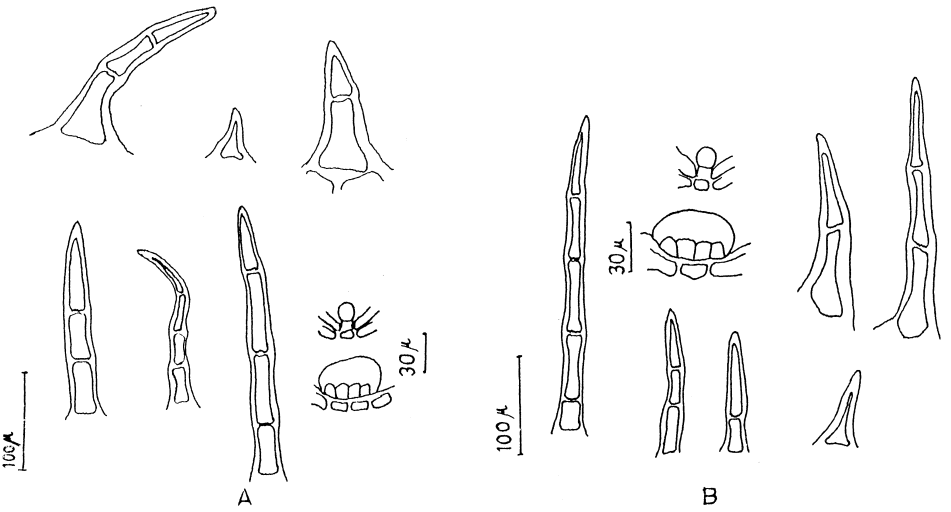

Fig. 7. *Satureja parnassica* subsp. s*ipylea*: Hair types A) in stem B) in leaf

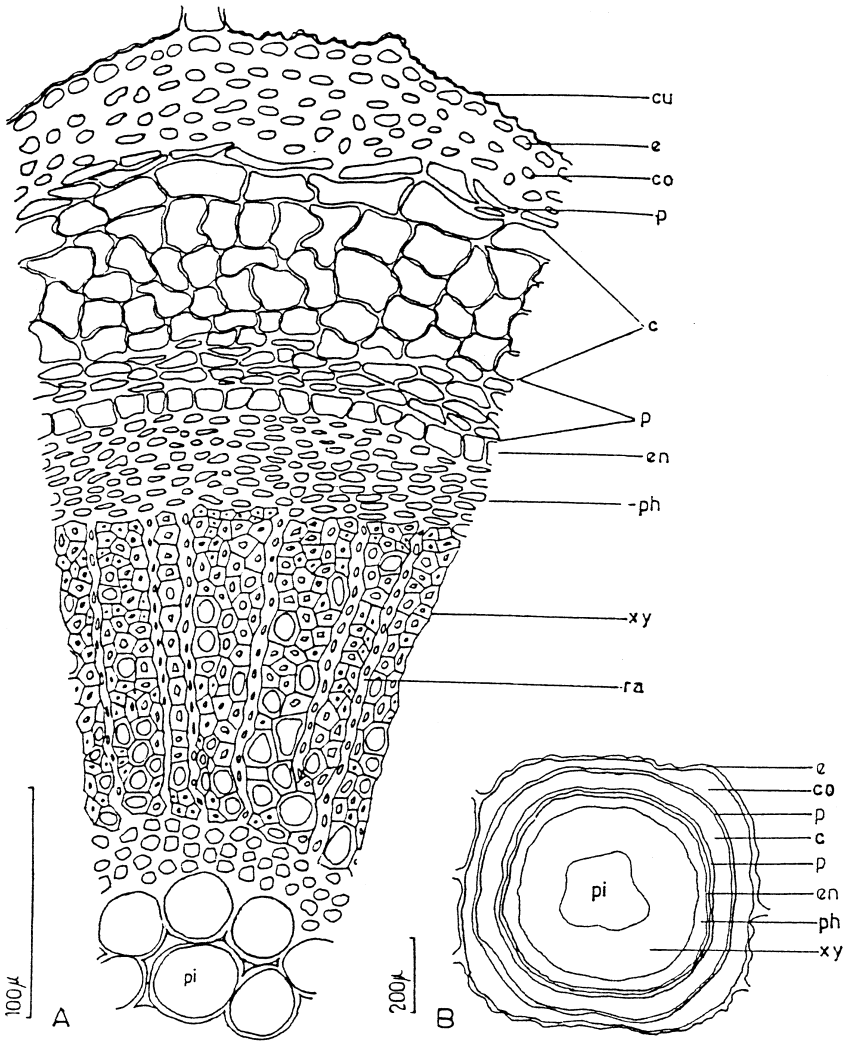

Fig. 8. *Satureja parnassica* subsp. *sipylea* (Marmara Island) A–B) Cross-section of stem, cu--cuticula, e-epidermis, co-collenchyma, p-parenchyma, c-cork, en-endodermis, ph-phloem, xy-xylem, ra-rays, pi-pith.

head and stalk unicellular type (Fig. 7B). They are embedded in the surfaces of both epidermal layers. Stomata type is diacytic (METCALFE and CHALK 1979) and occurs on the surfaces of both epidermal layers, being more abundant on the lower surface. Stomata are located almost on the same level as teh epidermal cells. The leaf is isolateral. Mesophyll occurs as 1-seriate palisade tissue under both epidermises and 2–4-seriate spongy parenchyma in between. Both parenchyma tissues contain starch and ergastic substance. The central vessel is better developed than the lateral vessel. As expected, the xylem faces the upper surface while the phloem faces the lower epidermis. Vascular bundles are surrounded by parenchymatous bundle sheathings (Figs. 10, 12, 13).

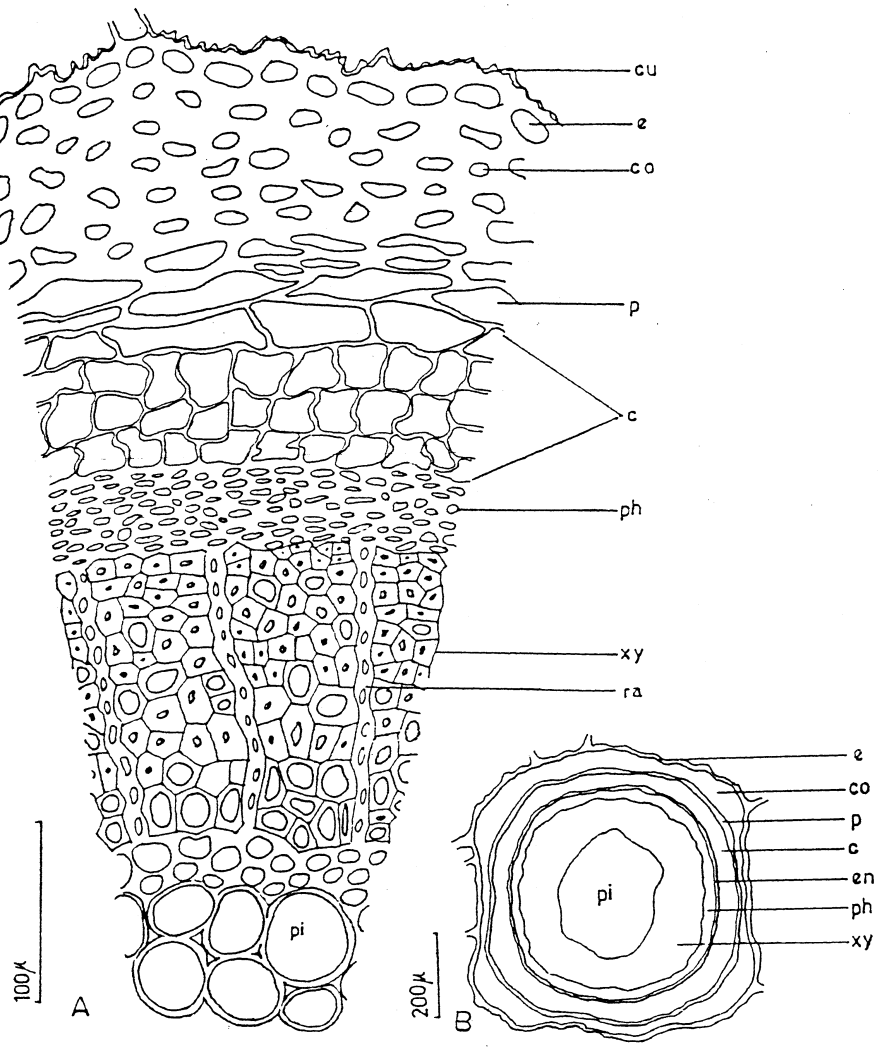

Fig. 9. *Satureja parnassica* subsp. s*ipylea* (Spil Mountain) A–B) Cross-section of stem, cu-cuticula, e-epidermis, co-collenchyma, p-parenchyma, c-cork, en-endodermis, ph-phloem, xy-xylem, ra-rays, pi-pith.

Ecological Characteristics:

S. parnassica subsp. s*ipylea* grows on exposed limestone rocks and stony slopes. It shares a habitat with *Quercus* sp., *Sideritis* sp., *Salvia* sp., *Thymus* sp. and *Gramineae* sp.

Flowering period: August-October.

The results of the soil analysis collected from suitable habitats for *S. parnassica* subsp. s*ipylea* are given in Table 1.

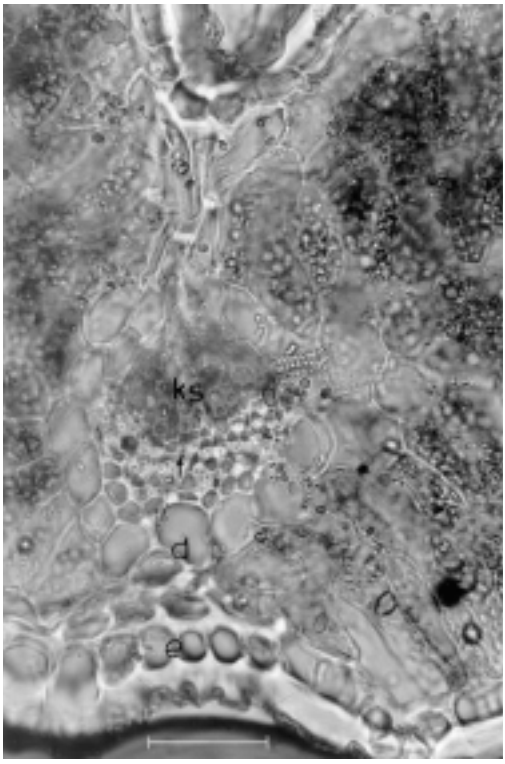

Fig. 10. *Satureja parnassica* subsp. s*ipylea* (Marmara Island): Cross-section of leaf ks-xylem, f-phloem, d-bundle sheath, e-epidermis. Bar = 50 µm

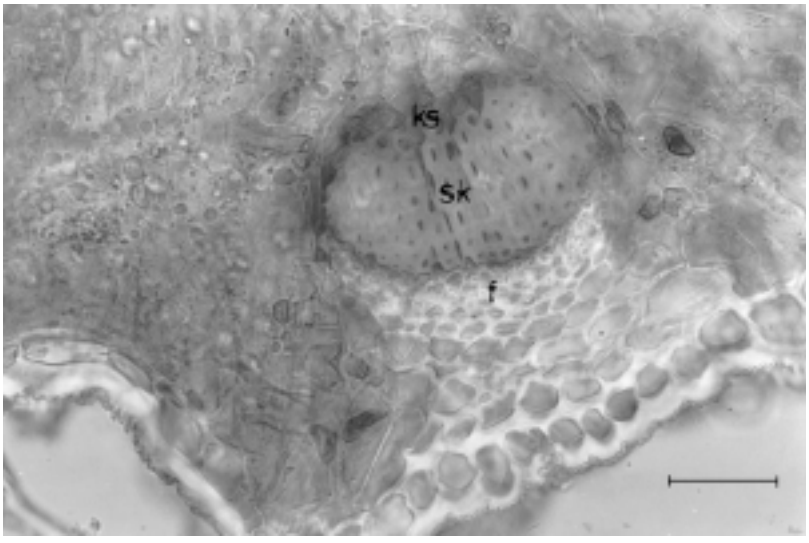

Fig. 11. *Satureja parnassica* subsp. s*ipylea* (Spil Mountain): Cross-section of leaf ks-xylem, sk-sclerenchyma, f-phloem. Bar = 50 µm

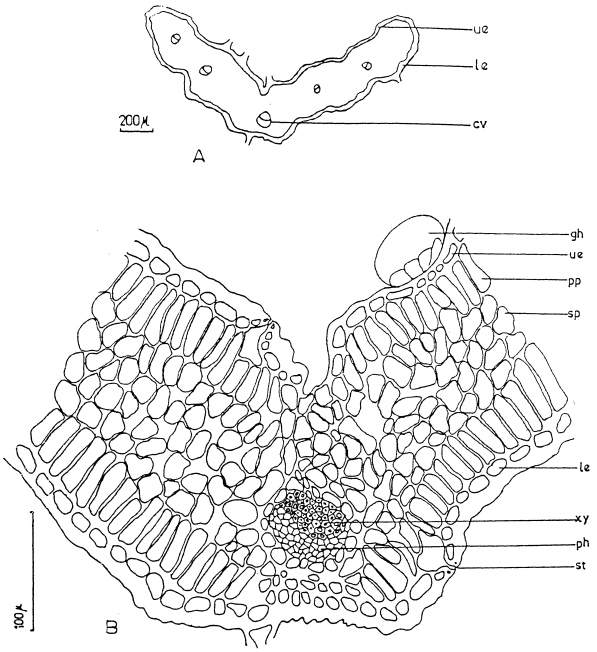

Fig. 12. *Satureja parnassica* subsp. s*ipylea* (Marmara Island): A–B) Cross-section of leaf, ue-upper epidermis, le-lower epidermis, cv-central vessel, gh-glandular hair, pp-palisade parenchyma, sp-spongy parenchyma, xy-xylem, ph-phloem, st-stomata

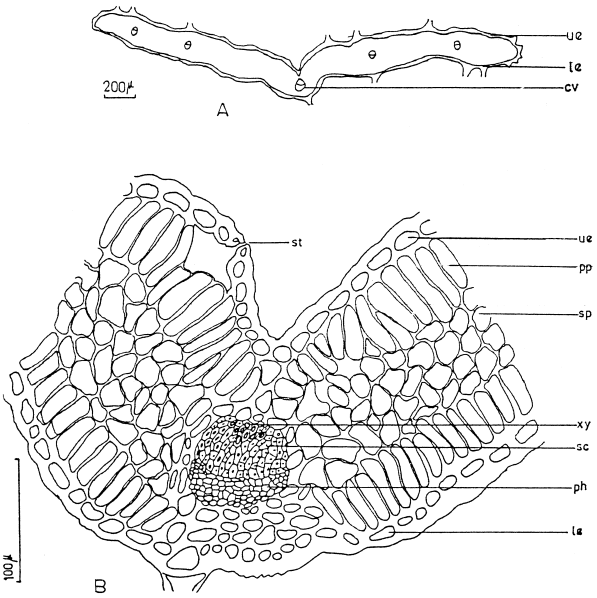

Fig. 13. *Satureja parnassica* subsp. s*ipylea* (Spil Mountain): A–B) Cross-section of leaf. ue-upper epidermis, le-lower epidermis, cv-central vessel, st-stomata, pp-palisade parenchyma, sp-spongy parenchyma, xy-xylem, sc-sclerenchyma, ph-phloem

Tab. 1. Soil characteristics of *Satureja parnassica* subsp. s*ipylea* growing regions

	Spil Mountain	Marmara Island
Texture	Clayish	Clayish
Salinity (%)	0.11	0.08
PH	7.0	7.0
$CaCO_3$ (%)	33.6	15.2
P_2O_5 Kg/dk	25.2	9.6
K_2O Kg/dk	116	104
Organic matter	Abundant	Abundant

Chemical Characteristics:

The oil yields, localities and collection data of the samples are given in Tables 2 and 3

Tab. 2. The oil yields of *Satureja parnassica* subsp. *sipylea*

Material	ESSE No	Locality	Collection dates	Oil yield (%)
A	9222	Turkey: Balikesir-Marmara Island	30.08.1996	0.11
B	12147	Turkey: Manisa-Spil Mountain	24.08.1996	1.19

Tab. 3. Main components of the essential oil of *Satureja parnassica* subsp. *sipylea*

Main Components	A%	B%
p-cymene	6.6	4.25
Terpinen-4-ol	7.42	12.12
Borneol	5.90	5.23
trans-sabinenehydrate	0.72	18.49
Linalool	**23.17**	**20.59**

Discussion

In this study, *Satureja parnassica* Heldr. et Sart. subsp. *sipylea* P.H.Davis samples were collected from 2 different localities, on Marmara Island and Spil Mountain, and the morphological, anatomical and chemical characteristics of the species were investigated. *S. pilosa* Velen. is similar to *S.parnassica* Heldr. et Sart., as described in Flora Europaea (BALL and GETLIFFE 1976) but it differs from *S. parnassica* Heldr.et Sart in having flowering stems up to 25 cm; cauline leaves 10–20 × 3–8 mm, usually sparsely hispid; calyx 4–5.5 mm, teeth slightly shorter than the tube, corolla 6–8 mm. Furthermore, the anatomical features of stem and leaves of *S. parnassica* were found to be similar to those of *S. pilosa.*

There is no significant difference in the morphological features of the samples grown in the two regions. Yet the leaf lengths (6–15 mm) of samples collected from Marmara Island are longer than those of (5–12 mm) in Spil Mountain (Tab. 4).

Tab. 4. The comparison of *Satureja parnassica* subsp. *sipylea* features in different regions

		Spil Mountain	Marmara Island
Morphological Difference	Leaf	5–12 mm	6–15 mm
Anatomical differences	Stem		
	Collenchyma:	Lyzigenous is present	Lyzigenous isn't present
	Cortex :	The parenchyma isn't present	The parenchyma is present
	Leaf		
	Space of stoma below:	Larger	Large
	Glandular hair:	Densely unicellulars	Densely *Labiatae* type
	Unicellular hair:	Dense	Rare
	Bundle sheath:	Inconspicuous	Conspicuous
	Central vessel:	Sclerenchyma is present	Sclerenchyma isn't present
Ecological differences	$P_2 O_5$ Kg/dk:	25.2	9.6
	Lime (%):	33.6	15.2

Unicellular gland hairs and dentate trichomes of species in Spil Mountain are denser, but *Labiatae* type glands are denser in Marmara Island.

The morphological characteristics might be dependent on the type of habitat. This species was collected from Spil Mountain at 1500 m among the rocks and from Marmara Island at 100 m on rocks exposed to northern winds.

There is no difference in stem anatomy of the samples from the two regions, but the trachea and tracheids of the species in Spil Mountain are larger than those of in Marmara Island.

The differences in leaf anatomy are as follows: The space of the stoma in specimens collected from Spil Mountain is larger. The bundle sheath is inconspicuous, but they are conspicuous in the Marmara Island specimens. Sclerenchymatic tissue is not present in the vascular bundle of samples from Marmara Island, while it is found in that from Spil Mountain (Tab. 4).

S. parnassica grows on rocky slopes and limed soil. According to soil analysis results, the soil features of the samples grown in the two regions are similar to each other, although lime and phosphorus is higher on Spil Mountain.

We have been extensively studying the essential oils of the plants belonging to the *Lamiaceae* family in Turkey. Some of the most studied genera include *Satureja, Thymus, Origanum, Thymbra* and *Corydothymus.* A common feature of all these genera is that the main components in the oils are either carvacrol or thymol or both (TUMEN et al. 1992). At the same time, the essential oils of these species are devoid of phenol.

Numerous studies on the essential oil of the *Satureja* species growing in Turkey exist in the literature (TÜMEN 1991, TÜMEN et al. 1993, 1996, 1997, 1998a, b, c, BAŞER et al. 1995, 2001, KÜRKÇÜOĞLU et al. 2001). These studies have shown that the genus *Satureja* may or may not contain phenols. Phenol-containing species are divided into »carvacrol type«and »thymol type« species, while those not containing phenols are divided into: The »Monoterpenic-keton«, »Monoterpenic-hydrocarbone« and also »Sesquiterpenic hydrocarbone« types.

In the studies considered, of about 15 species of *Satureja,* only *S. parnassica* subsp. *sipylea* and *S. spinosa* L. are included in the »Monoterpenic alcohol type« group.

Linalool was found to be the main component in the essential oil of *S. parnassica* subsp. s*ipylea* (23.17%, 20.59%) and *S. spinosa* L. (62%). Although *S. parnassica* subsp. *sipylea* includes less linalool than *S. spinosa*, the amount of essential oil in it is considerably higher (0.11%, 1.19%) than that of *S. spinosa* (TÜMEN et al. 1997).

A difference was found in the second main component of subspecies in the two regions, such that the second main component in the samples from Marmara Island was Terpinen-4-ol whereas it was *trans*-sabinenehydrate in those from Spil Mountain.

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