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Essential oil composition and anatomical characteristics of *Stachys megalodonta* Hausskn. & Bornm. ex P. H. Davis subsp. *mardinensis* R. Bhattacharjee endemic in Turkey

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Abstract

Stachys L. (Deliçay) (Lamiaceae) is presented in Turkey by 89 species. 46 species are endemic in Turkey. *Stachys megalodonta* Hausskn. & Bornm. ex P. H. Davis subsp. mardinensis R. Bhattacharjee is locally known as "gevrek deliçay". Stachys spp. are aromatic perennial subshrubs used as spices or herbal teas in the Turkish folk medicine.

The aerial parts of *S. megalodonta* subsp. *mardinensis* (Lamiaceae) were water distilled for 3 h using a Clevenger-type apparatus and the oil was analyzed. The essential oil was analyzed by GC-FID and GC/MS, simultaneously. The main constituents were identified as a-pinene (17.7%), germacrene D (7.2%), 1,8-cineole (6.1%), β-caryophyllene (4.8%), limonene (3.1%), camphene (3.1%) and camphor (2.9%). It's morphological, morphometric and anatomical properties are described in the present study. Data obtained after investigations were compared with those in the Davis's "Flora of Turkey". Morphological and anatomical results are supported by photographs and illustrations.

1. Introduction

Stachys L. is among the largest genera of the Lamiaceae and it contains about 350 species (435 taxa) worldwide. Species of the genus are concentrated in the warm temperate regions of the Mediterranean region and southwestern Asia, with secondary centres in North America, South America, and southern Africa. Native species are absent from Australia and New Zealand. The Asiatic centre contains 2 phytogeographical regions (Mediterranean and Irano-Turanian). The European centre is Mediterranean and Euro-Siberian. The first is confined to southern and eastern Anatolia, Caucasia, north-western Iran, and northern Iraq, and the other to the Balkan Peninsula (Bhattacharjee, 1974). *Stachys* was revised by Bhattacharjee for the Flora of Turkey[2]. Since then, 18 new species have been described from Turkey. *Stachys* (Deliçay) has 93 species (118 taxa) belonging to 15 sections and 2 subgenera in Turkey. Of the 118 taxa, 57 are endemic in Turkey. *Stachys megalodonta* has two subspecies: subsp. *megalodontha* is distributed in Northern Iraq and subsp. *mardinensis* R. Bhattacharjee. subsp. *mardinensis* is considered as a taxon in between *S. megalodonta* Hausskn. et Bornm & ex P.H. Davis and *S. mardinensis* (Post) R.R. Mill. (Baser and Kirimer, 2018; Bhattacharjee, 1974; 1980; 1982; Celep and Dirmenci, 2017; Davis *et al.*, 1988; Duman, 2000; Dinc and Dogu, 2015; Akcicek *et al.*, 2012; Renda *et al.*, 2017; Salmaki *et al.*, 2012). *S. megalodonta* Hausskn. and Bornm. ex P.H. Davis subsp. *mardinensis* R. Bhattacharjee is locally known as "gevrek deliçay" (Akcicek, 2012).

Whole plant or leaves are used in phytotherapy due to sedative, antispasmodic, stomachic and diuretic properties in tea preparations (Baytop, 1999; Goger *et al.*, 2016).

S. balansae subsp. *balansae*, *S. mardinensis* and *S. megalodonta* subsp. *mardinensis* were evaluated. The antimicrobial activity of methanol extracts were determined by minimum inhibitory concentrations (MIC) method, the best activity was observed with *S. megalodonta* subsp. *mardinensis* against *E. coli* and *S. aureus*. In addition, flavonoids content was analyzed by HPLC. The highest phenolic content was found in *S. mardinensis* and *S. megalodonta* subsp. *mardinensis* (79.09 mg QE/g). According to results, *S. mardinensis* had higher quercetin (26.5 mg/g) and catechine (11.00 mg/g) contents than the other *Stachys* species (Karaboduk *et al.*, 2014).

To the best of our knowledge, this is the first report on the GC and GC/MS determination of the essential oil composition of *Stachys megalodonta* subsp. *mardinensis*.

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2. Materials and Methods

2.1 Plant material

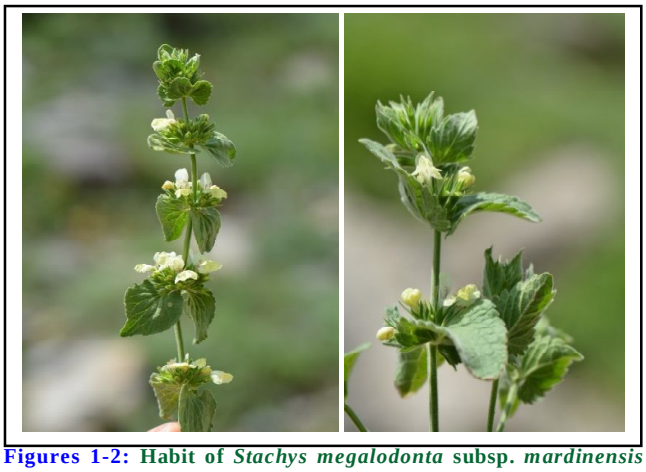
The flowering aerial parts of *Stachys megalodonta* subsp. *mardinensis* was collected from B8: Bitlis: Kambos Mountain on June 26, 2016 (Figures 1-2). Voucher specimens of *Stachys megalodonta* subsp. *mardinensis* are deposited in the Herbarium of the Bitlis Eren University (Voucher Specimen code: M.K. 6082) and Herbarium of the Faculty of Pharmacy, Anadolu University in Eskisehir (ESSE: 15295), Turkey. Of the *S. megalodonta* subsp. *mardinensis*, botanical identification was carried out by one of us (M. Kursat) (Bhattacharjee, 1980).

2.2 The isolation of essential oil and GC-GC/MS analysis

The essential oil obtained from aerial parts of the *Stachys megalodonta* subsp. *mardinensis* by analyzed simultaneously using Gas Chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) systems. Volatiles were identified according to their retention indices and mass spectral properties (Kucuk *et al.*, 2019). The results of analysis are shown in Table 1.

2.3 Anatomical studies

For anatomical studies, samples have been collected from the irnatural habitats and kept in 70% alcohol. Root, stem and leaves of mature and flowered plants have been utilized in the study. Investigations were performed on the cross-sections of the root, the flowering stem and the leaf. All sections were embedded in glycerin jelatine and stained by Sartur reactive and photographs were taken through a light microscope (Olympus BX51T).



Figures 1-2: Habit of *Stachys megalodonta* subsp. *mardinensis* (Photo: M. Kursat).

3. Results and Discussion

The aerial parts of *Stachys megalodonta* subsp. *mardinensis* (Lamiaceae) was water distilled for 3 h using a Clevenger-type apparatus. The essential oil was analyzed by GC and GC-MS, simultaneously. Eighty-three compounds representing 91.8 % of the essential oil were characterized from *S. megalodonta* subsp. *mardinensis*. The main constituents were identified as α-pinene (17.7%), germacrene D (7.2%), 1,8-cineole (6.1 %), β-caryophyllene (4.8%), limonene (3.1%), camphene (3.1%) and camphor (2.9%).

According to previous research report by Kaya and steel, the water distilled essential oil from dried aerial parts of *Stachys iberica* subsp. *stenostachya* (Lamiaceae) was analyzed by GC/MS. Seventy-one compounds were characterized representing 96% of the oil. The main constituents were found as linalyl acetate (42.2%), linalool (18.9%), geranyl acetate (8.2%), and α-terpineol (5.3 %) (Kaya *et al.*, 2001).

The main constituents for *S. laetivirens* Kotschy & Boiss. were found to be nonacosane (23.1%) and phytol (17.9%) (Duman *et al.*, 2005).

S. cretica ssp. *lesbiaca* Rech. f. (germacrene D 20.3, β-caryophyllene 9.5, α-pinene 8.6, β-pinene 6.2) and *S. cretica* ssp. *trapezuntica* Rech. f. (germacrene D 12.9, octacosane 7.2, linalyl acetate 5.2) were determined by GC and GC-MS (Serbetci *et al.*, 2010).

Chemical compositions of the essential oils of twenty-two *Stachys* species were evaluated (*S.balansae* Boiss. and Kotschy subsp. *balansae*, *S. balansae* Boiss. & Kotschy subsp. c*arduchorum* R. Bhattacharjee, *S. bayburtensis*, R. Bhattacharjee, *S. cretica*, subsp. *anatolica* Rech. f., *S. cretica* subsp. *bulgarica* Rech. f., *S. cretica* subsp. *cassia* (Boiss.) & Rech.f., *S. cretica* subsp. *garana* (Boiss.) Rech. f., *S. cretica* subsp. *kutahyensis* Akcicek, *S. cretica* subsp. *lesbiaca* (Boiss.) Rech.f., *S. cretica* subsp. *symrnaea* (Boiss.) Rech.f., *S. germanica* subsp. *bithynica* (Boiss.) R. Bhattacharjee, *S. germanica* subsp. *heldreichii*(Boiss.) Hayek, *S. huber-morathii* R., Bhattacharjee, *S. huetii* Boiss, *S. longispicata* Boiss. & Kotschy, *S. obliqua* Waldst. & Kit, *S. pinetorum* Boiss. & Bal., *S. sericantha* P.H. Davis, S. *spectabilis*, Choisy ex DC, *S. thirkei* C. Koch, *S. tmolea* Boiss, *S. viticina* (Boiss.). Thirty-nine compounds, which accounted for 70.5-97.8% of the total composition of oils, were reported. Germacrene-D (2.9-45.3%), β-caryophyllene (2.3-62.3%), caryophyllene oxide (trace to 12.8%), spathulenol (trace to 7.8%) and α-cadinene (1.4-8.5%) were identified as the major components of the essential oil of species. Additionally, α-cadinol, α-bisabolol, α-copaene and bicyclogermacrene were determined (Goren *et al.*, 2011).

The *S. lavandulifolia* Vahl. subsp. *lavandulifolia* essential oil obtained by hydrodistillation were analyzed both by GC-FID and GC/MS, simultaneously. Thirty seven compounds representing 98.3% of the oil were characterized. β-phellandrene (27%), α-pinene (18.5%) and germacrene-D (13%) were found as major components of the oil (Iscan *et al.*, 2012).

S. macrantha (C. Koch) Stearn, *S. sylvatica* and *S. annua* ssp. *annua var. annua* were analyzed by a solid phase microextraction (SPME) method coupled with gas chromatography-flameionization detector (GC-FID) and gas chromatography/masss pectrometry (GC-MS). The major volatile constituents of the investigated three *Stachys* species were; ± - pinene (11.2%), *p*-cymene (18.2%) and carvacrol (28.8%) in *S. macrantha*, *S.*muurolene (10.2%), ± -cedrene (11.2%) and limonene (37.0%) in *S. sylvatica* and ± -pinene (11,4%), 2-pinene (23.1%) and (Z)-2-ocimene(24.8%) in *S. annua* ssp. *annua* var. *annua* (Renda *et al.*, 2017).

Water distilled essential oil of three species of *Stachys* was analysed by Kaya and steel. The major components found were hexadecanoic acid (23.7%), dodecanoic acid (11.3%), and caryophyllene oxide (10.7%) for *S. sericantha* P.H. Davis (endemic); α-pinene (53%), β-pinene (8.2%) for *S. gaziantepensis* M. Dinc and for *S. mardinensis* (Post) R. Mill. menthyl acetate (15.3%), isomenthone (15.0%), pulegone (10%), spathulenol (7.0%), and caryophyllene oxide (6.7%) (Kaya *et al.*, 2017). Karaboduk and steel studied antimicrobial and antioxidant activities of *S. balansae* subsp. *balansae*, *S. mardinensis* and *S. megalodonta* subsp. *mardinensis* were evaluated (Karaboduk *et al.*, 2014).

Table 1: The Composition of the essential oil of *Stachys megalodonta* subsp. *mardinensis*

RRI	Compounds	%	IM
1014	Tricyclene	0.8	MS
1032	**α-Pinene**	**17.7**	t_R, MS
1076	Camphene	3.1	t_R, MS
1118	*β*-Pinene	1.7	t_R, MS
1132	Sabinene	0.8	t_R, MS
1174	Myrcene	0.5	t_R, MS
1188	α-Terpinene	0.1	t_R, MS
1203	Limonene	3.1	t_R, MS
1213	**1,8-Cineole**	**6.1**	t_R, MS
1225	(*Z*)-3-Hexenal	0.3	t_R, MS
1255	γ-Terpinene	0.3	t_R, MS
1280	*p*-Cymene	0.2	t_R, MS
1290	Terpinolene	0.1	t_R, MS
1450	*trans*-Linalool oxide (*Furanoid*)	t	MS
1452	1-Octen-3-ol	1.0	t_R, MS
1474	*trans*-Sabinene hydrate	0.1	t_R, MS
1479	*cis*-Linalool oxide (*Furanoid*)	0.1	MS
1484	Bicycloelemene	0.6	MS
1485	α-amorphene	0.2	MS
1497	α-Copaene	2.9	MS
1529	α-Bourbonene	0.1	MS
1532	Camphor	2.9	t_R, MS
1535	β-Bourbonene	1.5	t_R, MS
1544	α-Gurjunene	0.1	MS
1550	β-Cubebene	0.2	MS
1553	Linalool	1.6	t_R, MS
1577	β-Ylangene	0.7	MS
1594	*trans*-β-Bergamotene	0.2	MS
1600	β-Elemene	0.9	MS
1602	β-Copaene	0.8	MS
1611	Terpinen-4-ol	1.2	t_R, MS
1612	β-Caryophyllene	4.8	t_R, MS
1641	iso-isopulegyl acetate	0.3	MS
1661	Alloaromadendrene	t	MS
1668	(*Z*)-β-Farnesene	1.1	MS
1684	γ-Gurjunene	0.2	MS
1704	γ-Muurolene	0.2	MS
1706	α-Terpineol	0.5	t_R, MS
1719	Borneol	0.2	t_R, MS
1726	**Germacrene D**	**7.2**	MS
1740	α-Muurolene	2.1	MS
1751	Bicyclogermacrene	1.1	MS
1772	δ-Cadinene	1.5	t_R, MS
1776	γ-Cadinene	0.1	MS
1784	(*E*)-α-Bisabolene	0.1	MS
1786	*ar*-Curcumene	0.1	MS
1787	Aromadendra-1(10), 4(15)-diene	0.2	MS
1849	Cuparene	0.3	MS
1882	1-Isobutyl 4-isopropyl-2,2-dimethyl succinate	0.3	MS
1900	Epicubebol	0.2	MS
1941	α-Calacorene I	0.2	MS
1957	Cubebol	0.6	MS
1958	(E)-β-Ionone	0.1	MS
1984	α-Calacorene II	0.1	MS
2008	Caryophyllene oxide	1.5	t_R, MS
2037	Salvial-4(14)-en-1-one	0.2	MS
2041	(*E*)-Nerolidol	0.7	t_R, MS
2057	Ledol	0.2	MS
2069	Germacrene D-4-ol	0.3	MS
2096	Elemol	0.2	MS
2104	Viridiflorol	0.4	MS
2123	Salviadienol	0.2	MS
2130	1-α-Himachal-4-en-1-β-ol	0.2	MS
2131	Hexahydrofarnesyl acetone	0.2	t_R, MS
2144	Spathulenol	2.1	t_R, MS
2162	Muurola-4,10(14) dien-1-ol	0.4	MS
2192	Copaborneol	0.7	MS
2193	Eugenol	t	t_R, MS
2209	T-Muurolol	0.3	MS
2232	α-Bisabolol	1.4	MS
2243	Torilenol	0.3	MS
2255	α-Cadinol	0.1	t_R, MS
2287	8,13-Epoxy-15,16-dino r-labd-12-ene	0.5	MS
2312	9-Geranyl-p-cymene	1.3	MS
2316	Caryophylladienol I	0.1	MS
2333	Isobicyclogermacrenal	0.7	MS
2353	Caryophyllenol I	0.1	MS
2369	Eudesma-4(15), 7-dien-1β-ol	t	MS
2376	Manoyl oxide	1.3	MS
2396	*epi*-Manoyl oxide	5.9	MS
2622	Phytol	0.1	MS
2676	Manool	0.4	MS
2736	Labda-7,14-dien-13-ol	0.6	MS

RRI: Relative retention indices calculated against n-alkanes. %: calculated from the FID chromatograms. t: Trace (<0.1 %). Identification method (IM): t_R, identification based on the retention times (t_R) of genuine compounds on the HP innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the Wiley and Mass Finder libraries and comparison with literature data.

3.1 Anatomical results

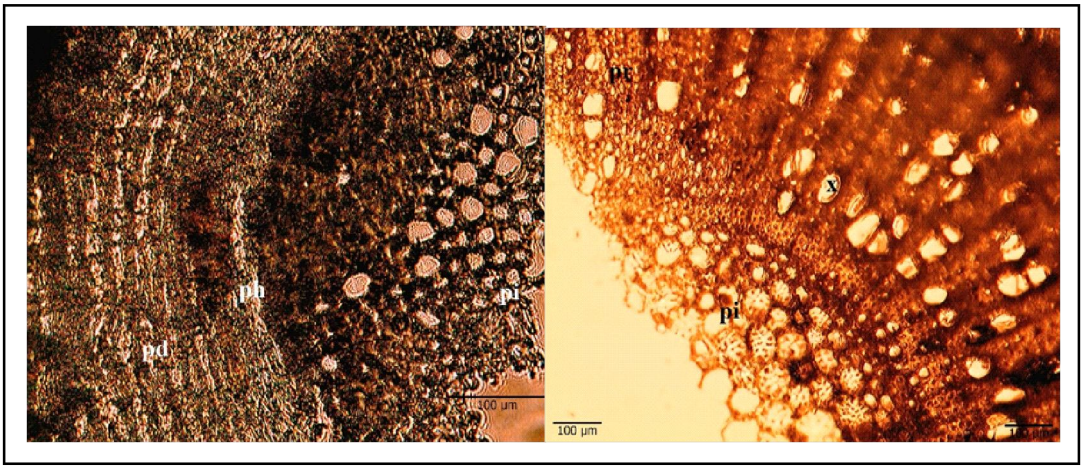
The sections taken from root, stem and leaves of *Stachys megalodonta* subsp. *mardinensis* were examined and the anatomic features belonging to plants were determined, compared and showed in Figures 3-13.

3.2 Root

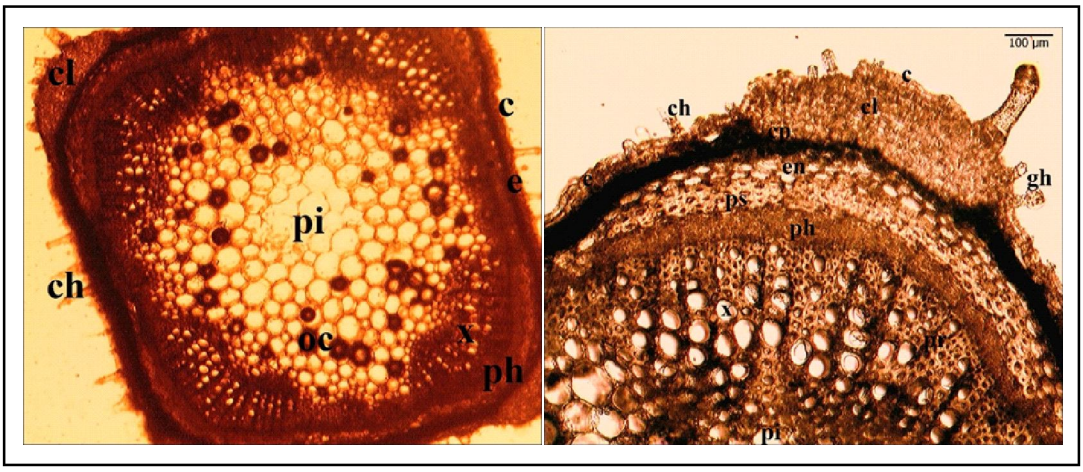
Root is formed by periderm on the outside and phelloderm where 4-5 radial rows are broken down and phelloderm with 2-3 row tissues. Outer phelloderm cells are broken up or tissue residues belonging to primer cortex is, time to time, crushed are found. Secondary phloem formed of elliptical-shapeless, round shaped, irregular arranged and 4-6 row cells under the periderm is taken part. Cambium is uncertain. Seconder xylem part cover a large area and consist of tracheal elements with big and small sizes in a sclerenchymatic tissue. Pith branches are 2-3 row cells (Figures 3, 4).

3.1.1 Herbaceous stem

In cross sections, the stem is rectangular. Covering hairs: multicellular, glandular hairs are four types; head 1 stalk 2 celled, head 1 stalk 1 celled, head 2 stalk 1 celled and head 8 celled (Labiatae type 3-4 rows collenchyma tissue was found in the four angles. Cortex is parenchymatous to endodermis. Endodermis consists of flattened cells can hardly be distinguished from the cortex parenchyma. Pericycle is composed of cylindiric scleranchyma with a lot of layer medullary rays: 1-2 cells wide. Pith branches are in the form of large polygon or round shaped and parenchymatic cells where its walls are lignified. Druse crystals are clearly found in these cells. Phloem forms a narrow ring. Cambium is indistinct, Branches in both types of seconder xylem are especially getting narrow towards the primery xylem. Medullary rays: 1-2 cells wide. Sclerenchymatic cells in this part are formed from trache and tracheids in both types have been created regular rows in radial direction. Druse crystals are clearly found in these (Figures 5, 6).



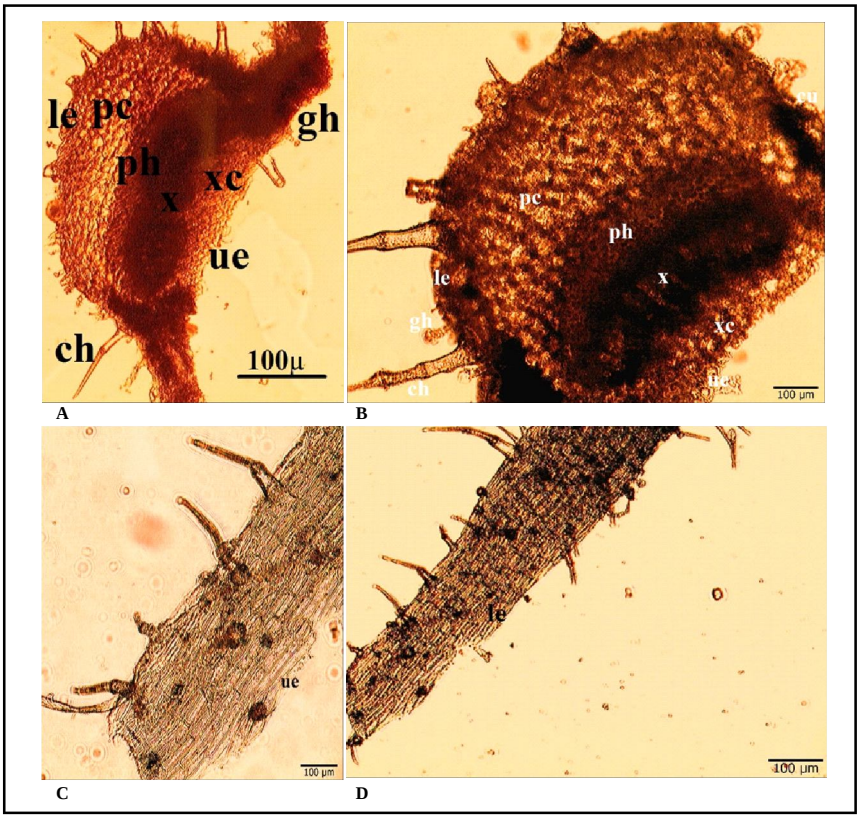
Figures 3-4: ***Stachys megalodonta*** **subsp.** ***mardinensis*** **(ESSE 15295). Cross-section of root. pd-peridermis; ph-phloem; pi-pith; pr-pith ray; x-xylem.**



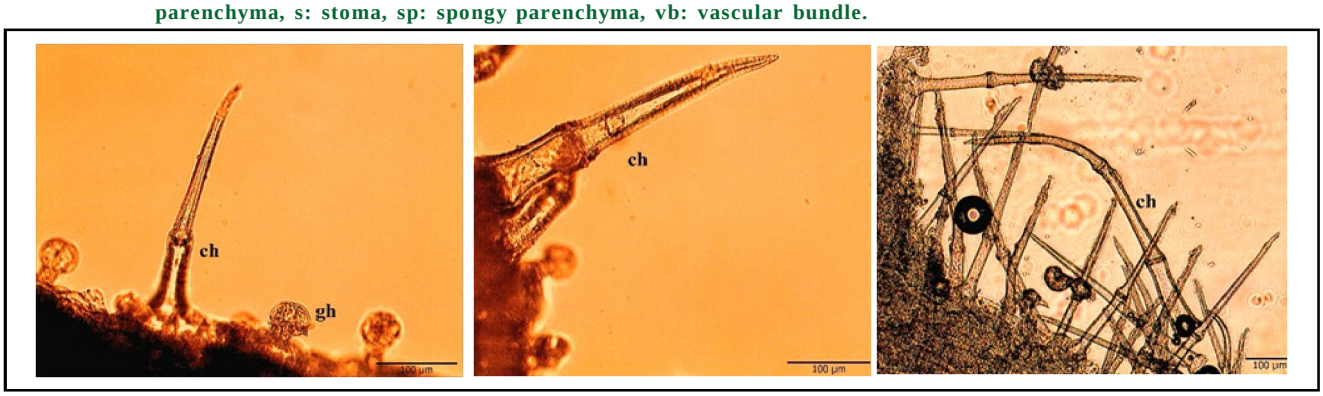
Figures 5-6: Stachys megalodonta subsp. mardinensis (ESSE 15295). Cross-section of herbaceous stem. A: diagrammatic, B: detail from, c: cuticle, ch: covering hair, e: epidermis, en:endodermis; cl: collenchyma, cp: cortex parenchyma, gh: glandular hair, oc: oil cells, x: xylem, ps: pericyclic scleranchyma, ph: phloem, pr: pith ray, pi: pith.

3.1.2 Leaf

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, 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: uniseriate, straight or recurved, 1-4 celled with smooth cuticle. Glandular hairs: head 1 stalk 1-2 celled and head 8 celled (Labiatae type); head 1 stalk 1-3 celled, head 2 stalk 1 celled. Stomata: the leaves amphistomatic, with diacytic superfacial or slightly raised above the epidermal level and more numerous on the lower side of the leaf. Mesophyll: bifacial, palisade tissue 1-2 layered, spongy parenchyma 3-4 layered (bifasiyal). 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 (Figures 7-13). *Stachys megalodonta* subsp. *mardinensis* (ESSE 15295). Cross-section of herbaceous stem. A diagrammatic, B detail from, c: cuticle, ch: covering hair, e: epidermis, en:endodermis, cl: collenchyma, cp: cortex parenchyma, gh: glandular hair, oc: oil cells; x: xylem, ps: pericyclic scleranchyma, ph: phloem, pr: pith ray, pi: pith.



Figures 7-10: ***Stachys megalodonta*** **subsp.** ***mardinensis*** **(ESSE 15295). Cross-section of the leaf: A diagram of the midrib, B detail from intervessel area; surface views of epidermis: C upper epidermis, D lower epidermis in LM, respectively, ue: upper epidermis, m: mesophyll, le: lower epidermis, 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.**



Figures 11-13: Covering and glandular hairs of ***Stachys megalodonta*** **subsp.** ***mardinensis.***

We hope that these results can be useful for chemotaxonomic classification of *Stachys* species. *"Presented at the* XII. International Symposium on the Actual Problems of Chemistry, Biology and Technology of Natural Compounds,7-8 September 2017, Tashkent.

4. Conclusion

Stachys species possess remarkable sedative, antispasmodic, stomachic and diuretic properties in tea preparations. In the present work, the aerial parts of *Stachysme galodonta* subsp. *mardinensis* were obtained essential oil. The main constituents were identified as α-pinene, germacrene D, 1,8-cineole and α-caryophyllene. It's anatomical properties are described. To the best of our knowledge, this is the first report of the essential oil composition and anatomical properties.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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