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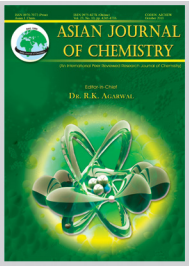

Glandular Trichomes Morphology, Chemical Composition and Antimicrobial Activity of the Essential Oil of Three Endemic *Scutellaria* Taxa (Lamiaceae)

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The glandular trichomes morphology, volatile composition and antimicrobial activity of the aerial parts of three endemic *Scutellaria* taxa [*S. cypria* var. *cypria* Rechinger, *S. cypria* var. *elatior* Meikle and *S. sipthorpii* (Benth.) Hal.] from Lamiaceae were investigated. Examined species posses two morphologically distinct types of glandular trichomes. Capitate trichomes were observed in all three taxa. Peltate glandular trichomes had a large secretory head forming 1 central and 3-8 peripheral cells. Peltate trichomes were observed only in *S. cypria* var. *elatior's* petiole. The volatiles and the essential oil mixtures of three taxa were obtained by steam distillation in a Clevenger-type apparatus with a 0.26-0.47 % (v/w) yield. Their analyses were performed by GC-FID and GC-MS techniques. Identification of these compounds was exhibited by comparison of their mass spectra, relative retention indices and some literature records. In total, 23 components were identified. While, total volatile percentages were detected as 99.99 %, the essential oil yield ranged between 34.64 and 92.25 % for 3 species. *Trans*-caryophyllene (22.58 %) and germacren-D (42.01 %) were detected as major components for *S. sibthorpii* Eugenol (23.05 %) and palmitic acid (27.00 %) were the main components for *S. cypria* var. *cypria* Additionally, *S. cypria* var. *elatior* has a considerable amount of linalool and palmitic acid as 10.92 % and 46.76 %, respectively. The MIC values of the volatiles ranged as 10-20 mg/mL against Gram negative and Gram positive bacteria. *C. albicans* was found as the most resistant yeast-like fungus with a MIC values higher than 20 mg/mL.

Key Words: *Scutellaria*, Lamiaceae, Trichomes, Essential oil, GC-FID, GC-MS, Antimicrobial activity.

INTRODUCTION

Lamiaceae consist of 240 genera and 3000-3200 species with a cosmopolitan distribution of some taxa in the mediterranean region[1]. The morphological characteristics of the hairs are to separate plants at subgeneric or subspecific level[2,3]. Many taxa belonging to this family mostly include different types of chemical components due to the presence of external glandular structures that produce essential oil[2]. The essential oils produced by glandular trichomes are one of the characteristic features of Lamiaceae[4].

There are some studies related to the morphology of glandular hairs of species belonging to Lamiaceae[3,5-7]. However, there are not many studies conducted on glandular hairs in the species of the genus *Scutellaria*.

Scutellaria is a unique widespread genus of the subfamily Scutellarioideae belonging to Lamiaceae. About 360 species are distributed throughout the world and in different climatic areas[8]. The genus *Scutellaria* is represented by 3 taxa in Cyprus, including 1 species [*S. sipthorpii* (Benth.) Hal.] and 2 varieties (*S. cypria* var. *cypria* Rechinger and *S. cypria* var. *elatior* Meikle)[9]. In different regions of the world, many taxa of *Scutellaria* genus are used as traditional remedies against hypertension, arteriosclerosis, hepatitis, allergy, cancer and inflammotory diseases. At the same time, these plants have also sedative, antioxidant, antithrombotic, cytotoxic, antispasmodic, antimicrobial and antiviral properties[10-13]. The volatile components exhibiting the above-mentioned effects are secreted by the glandular hairs, which are known one of the family characteristics. To our best of knowledge, it has been reported the essential oils were investigated in only a few *Scutellaria* species[14-18].

In this study, chemical constituents of the essential oils of three endemic *Scutellaria* taxa were determined by GC-FID, GC-MS techniques and their antimicrobial potentials were examined by MIC method taking into consideration the results of their glandular trichomes morphology for the first time.

EXPERIMENTAL

Plant materials and glandular trichomes: The aerial parts of all investigated plant materials were collected in Cyprus

(*Scutellaria cypria* var. *cypria*: Mylikouri village, 900 m., herbarium no. 6615; *S. cypria* var. *elatior*: around Amiandos mining area, 1600 m., herbarium no. 6616; *S. sibthorpii*: Alevkaya, 870 m., herbarium no. 898) during the flowering stage in April-July 2010. Collection and identification of the plants were established by herbarium of near East University. A voucher specimen is deposited at Herbarium of the Environmental Sciences Institute, near East University. The collected plant samples were fixed with 70 % alcohol, stem, petiol and leaf cross-sections were stained with the Sartur reagent[19]. Photographs were taken with JENA NF-binocular microscope and Olympus BX51-Altra 20 Soft Imaging System camera[20,21].

Preparation of the essential oils: The air-dried plant materials (20 g each) were cut into small pieces and were hydrodistilled for 6 h using a Clevenger-type apparatus. The steam distillates were extracted with *n*-hexane and dried over anhydrous granule sodium sulphate. The essential oil yields are shown in Table-2.

GC and GC-MS analysis: The dried volatiles were solved in HPLC grade *n*-hexane (Merck 1.04391) and analyzed by GC-FID and GC-MS. The analyses were performed on an Agilent 6890 GC system and GC coupled to an Agilent 5973 mass selective detector (MSD) which worked in EI ionization mode (70 eV). The GC analyses were accomplished with a GC-6890 Agilent instrument equipped with HP-Innowax (19091N-216) capillary column (60 m × 0.32 mm, 0.50 μm film thickness). The oven temperature was programmed to increase from 70 to 210 ºC at 7 ºC/min and finally held at 210 ºC for 10 min. The injector and detector temperatures were kept at 70 ºC and 250 ºC, respectively. Detector duol was FID. The carrier gas was helium with a flow rate of 0.7 mL/min and the injection volume was 1 μL. The examples were studied in airless conditions and the pressure was 2.26 psi. The split ratio and split flow were 1/50 and 34.8 mL/min, respectively. The average linear velocity was 22 cm/sec.

Identification of the compounds: The identification of the components was based on the comparison of their relative retention times with *n*-alkane standards (Supelco 49452-U) in gas chromatographs. The identity of the peaks as well as relative retention indices values were achieved by mass spectra of the components using with Nist-Wiley (Wiley 7.n-2005) and Arge-Far essential oil (ucucu-2001 library) data base of the GC-MS system together with literature findings[22].

Antimicrobial activity: The antimicrobial activities of the volatile compounds of *S. sibthorpii*, *S. cypria* var. *cypria* and *S. cypria* var. *elatior* were evaluated *in vitro* against bacteria and one fungus. Gentamycin and clotrimazole were used as standard antibacterial and antifungal agents respectively. All test microorganisms were obtained from Ege University Faculty of Science, Basic and Industrial Microbiology Department, Izmir-Turkey. Test organisms included: *Staphylococcus aureus* ATCC6538-P and *Bacillus subtilis* ATCC 6633 as Gram-positive bacteria, *Salmonella typhimurium* CCM 5445, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 12228, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* CCM 2318 as Gram-negative bacteria and *Candida albicans* ATCC 10239 as yeast-like fungus. Test microorganisms were grown in Mueller- Hinton Agar (MHA) at 37 ºC and maintained on at 4 ºC. The minimum inhibitory concentrations (MICs) of the volatiles of *S. sibthorpis*, *S. cypria* var. cypri, *S. cypria var. elatior* and reference antibiotics were determined by microdilution techniques (MHB) for bacteria and *C. albicans*[23]. Briefly, the volatiles were firstly dissolved at a concentration of 250 mg/mL in 10 % dimethyl sulfoxide (DMSO). Reference antibiotics were initially tested using a concentration of 0.40 mg/mL for gentamycin in distilled water and 0.50 mg/mL for clotrimazole in ethanol. Then serial dilutions of each sample were performed in 10 % DMSO. Inocula for assays were prepared from activated cultures in MHB by dilution to give a final viable cell count of 4.0-5.5 × 10^5 CFU/mL. Each sample solution (25 μL) and inoculum of test microorganism (25 μL) were added into each well of a flat-bottom, 96-well microtiter plate prefilled with 200 μL of MHB to give a total volume of 250 μL. Microtiter plates were incubated at 37 ºC for 24 h for bacteria and 48-72 h for *C. albicans*. The solvents, 10 % DMSO and ethanol, were used as the negative controls for all experiments. After incubation, the MIC value was detected by adding 50 μL of 0.5 % TTC (triphenyltetrazolium chloride, Merck) aqueous solution[24,25]. MIC was defined as the lowest concentration of extract that inhibited visible growth as indicating by the TTC reduction. In the presence of bacterial growth by reduction reactions, TTC changes the colour of microbial cells from colourless to red. This provides clearly defined and easily readable endpoints. All tests were repeated three times to confirm the results.

RESULTS AND DISCUSSION

Trichomes morphology: In the literature, only peltat and capitate glandular hairs have been observed from *Scutellaria orientalis* ssp. *bicolour, S. orientalis* ssp. *santolinoides* and *Salvia argentea* species in Lamiaceae[5,26]. Because of the economic values of the essential oils secreted by the glandular hairs, their hair morphology was studied in detail[3,5]. However, there are few studies on the properties of glandular hairs of species belonging to the genus *Scutellaria*. In present results, different capitate hairs were observed in two subspecies (*S. cypria* var. *cypria* and *S. cypria* var. *elatior*) and the capitate hairs were classified according to their head cell morphology and secretory types. In type I, the head cells are round and the secretory material was secreted from the cell without fragmenting the cell walls[26]. On the other hand, in type II, the secretory material was secreted out after breaking down the cell wall and head cells were pear-shaped. However, type III has a cup-like head cells. The same study also reported that peltate hairs had multicellular head cells and contained central and peripheral cells in *Scutellaria* sub-species.

Our morphological findings revealed 3-5 peripheral peltate hair cells in the stem, leaf and petiole of *S. cypria var. cypria* and 3-8 peripheral peltate hair cells in the stem, leaf and petiole of *S. sipthorpii*. However, there are 3-5 peripheral peltate cells only in the stem and leaf of *S. cypria var. elatior*. Moreover, all taxa contained 1 central cell.

Type I capitate trichomes were revealed in all the organs of the three studied taxa, but Type II capitate trichomes were only observed in *S. cypria* var. *cypria*'s leaves. While type III capitate trichomes were observed in all the organs of *S. cypria* var. *cypria*, they were only detected in the stem and leaves of *S. cypria* var. *elatior*.

TABLE-1
CELL NUMBERS OF PARTS OF CAPITETE TRICHOMES FOUND ON ORGANS OF *Scutellaria* TAXA

Taxa	Aerial parts	Capitate trichomes									Peltate trichomes	
		Type I			Type II			Type III				
		Base	Stalk	Head	Base	Stalk	Head	Base	Stalk	Head	Centre	Periphery
S.cypria var. *cypria*	Stem	1	1-3	1-2	-	-	-	1	3-4	1	1	3-4
	Petiol	1-2	1-4	1-2	-	-	-	1-2	3-4	1-2	1	3-5
	Leaf	1	1-4	1-2	1	2-4	1	1	3-4	1-2	1	4
S.cypria var. *elatior*	Stem	1-2	1-4	1-2	-	-	-	1	3	1	1	4-5
	Petiol	1-2	1-2	1-2	-	-	-	-	-	-	-	
	Leaf	1	1	1-2	-	-	-	1	1	1	1	3-4
S. sipthorpii	Stem	1	1	2	-	-	-	-	-	-	1	3-4
	Petiol	1	1-2	1-2	-	-	-	-	-	-	1	3-6
	Leaf	1	1	1-2	-	-	-	-	-	-	1	4-8

Type I capitate hairs have 1-2 bases, 1-4 stalks and 1-2 head cells. Type II has 1-celled base, 2-4 celled stalks and 1 celled head, type III has 1-2 cells at the base, 3-4 cells at the stalk and 1-2 cells at the head feathers. Type I and type III capitate hairs (except petiol) were observed on *S. cypria* var. *elatior*; however, type II capitate hairs were not observed in any organ. In addition, type I capitate hairs were observed in a low density on *S. sipthorpii*, whereas type II and type III capitate hairs were not detected in this taxa (Table-1).

In the literature, three types of capitate hairs on *S. orientalis* ssp. *santolinoides* stem, leaf and bract were reported[26], but we observed type I capitate hairs on the stem, leaf and petiol, intensely. However, type II capitates trichomes were determined only in the leaves of *S. cypria* var. *cypria* (Fig. 1).

Chemical composition of the volatiles: The basic components of the essential oils of *S. baicalensis* were reported as 4-phenyl-3-buten-2-one, 1-phenyl-1,3-butanedione, palmitic acid and oleic acid by Fukuhara *et al.*[14]. Another study about the volatile oil of *S. rubicunda* ssp. *linnaeana* consisted mainly of terpenoids and the main fraction of the oil was represented by the oxygenated monoterpenes (44.5 %) such as linalool (27.8 %) and nerol (4.2 %). In this study, only *p*-cymene (0.2 %) was present as monoterpene hydrocarbon. While sesquiterpenes were found as the second most abundant group of components (39.0 %), caryophyllene (28.7 %), caryophyllene oxide (4.2 %) and α-cedrol (2.3 %) were found as the main constituents. In another study, the phenolics (5.1 %) and 4-vinylguaiacol (3.1 %) were reported as the most abundant components[18]. According to Ghannadi and Mehregan[27], the essential oil composition of *S. pinnatifida* ssp. *alpina* was chemically examined and their structures were determined as germacrene-D (39.7 %), β-caryophyllene (15 %), δ-cadinene (5.3 %) and α-copaene (5.0 %). The essential oil components of *S. albida* ssp. *albida* were characterized by the presence of high amounts of linalool (52.6 %) and *trans*-nerolidol (9.0 %)[15]. Two *Scutellaria* taxa, *S. sieberi* and *S. rupestris* ssp. *adenotricha* also contained high amount of linalool with a value of 22.7 % and 3.8 %, respectively[28]. The main report about the volatiles obtained from the flowers of *S. californica* contained β-caryophyllene (56.6 %), germacrene-D (6.9 %), methyl 2-methylbutyrate (4.9 %), β-bourbonene (4.5 %), α-humulene (2.8 %), methyl butyrate (2.7 %) and α-copaene (1.5 %) as major components[29]. The same researchers also reported the major components of volatile oil of *S. baicalensis* Georgi flowers as β-caryophyllene (22.3- 41.5 %), germacrene D (12.4-27.5 %), δ-cadinene (3.1-5.4 %), γ-muurolene (1.9-3.4 %), γ-cadinene (1.6-3.1 %), α-humulene (1.6-2.6 %), α-copaene (1.4-2.3 %), α-muurolene (1.0-2.6 %), bicyclogermacrene (1.1-2.1 %) and 3-octanone (0.9-3.0 %)[30]. In another study on *Scutellaria* taxa, it was reported that linalool (20-29 %), hexadecanoic acid (13 %) and β-caryophyllene (20 %) were the main components for *S. albida* ssp. *albida, S. albida* ssp. *colchica* and *S. albida* ssp. velenovskyi, respectively[31].

In present study, 23 volatile compounds were identified and classified into four main chemical classes: (1) Oxygenated monoterpenes (7.69-36.09 %), (2) Sesquiterpene hydrocarbons (4.20-72.74 %), (3) Oxygenated sesquiterpenes (2.60-11.82 %), (4) Carboxylic acids and alkanes successively (aliphatic components) (Table-2).

The results have shown that *trans*-caryophyllene (22.58 %) and germacren-D (42.01 %) were detected as major components in *S. sibthorpii*. Eugenol (23.05 %) and palmitic acid (27.00 %) were the main components in *S. cypria* var. *cypria*. While linalool was determined as a major component (10.92 %) for *S. cypria* var. *elatior*, palmitic acid (46.76 %) was the highest amount of volatile in this species. The volatile percentages were detected as 99.99 %, the total essential oil percentage ranged between 34.64-92.25 % and oil yielded as 0.19-0.47 %. The essential oil fractions of *Scutellaria* species consisted mainly of linalool, eugenol, germacren-D and geraniol, in addition to the aliphatic components. Sesquiterpene hydrocarbons are shown to be the main group of terpenoids and oxygenated monoterpenes are presented in almost considerable percentage in all studied *Scutellaria* taxa. While *S. cypria* var. *cypria* was the richest species in terms of various types of components, *S. sibthorpii* has the highest amount of essential oil content in all plants. Because of the contents and volatile percentages of these studied *Scutellaria* taxa, they could be inserted in the first proposed group of Lamiaceae.

The main and the extraordinary results, which are different from the literature findings, were the detection of eugenol in two *S. cypria* taxa especially in *S. cypria* var. *cypria* (23.05 %). This result may be due to the content of type II and type III capitate trichomes density mainly in *S. cypria* var. *cypria* (Table-2).

Antimicrobial activity: The MIC results of the volatiles from these three *Scutellaria* species gave almost the similar values ranging between 10 and 20 mg/mL against the tested Gram-negative and Gram-positive bacteria (Table-3). While

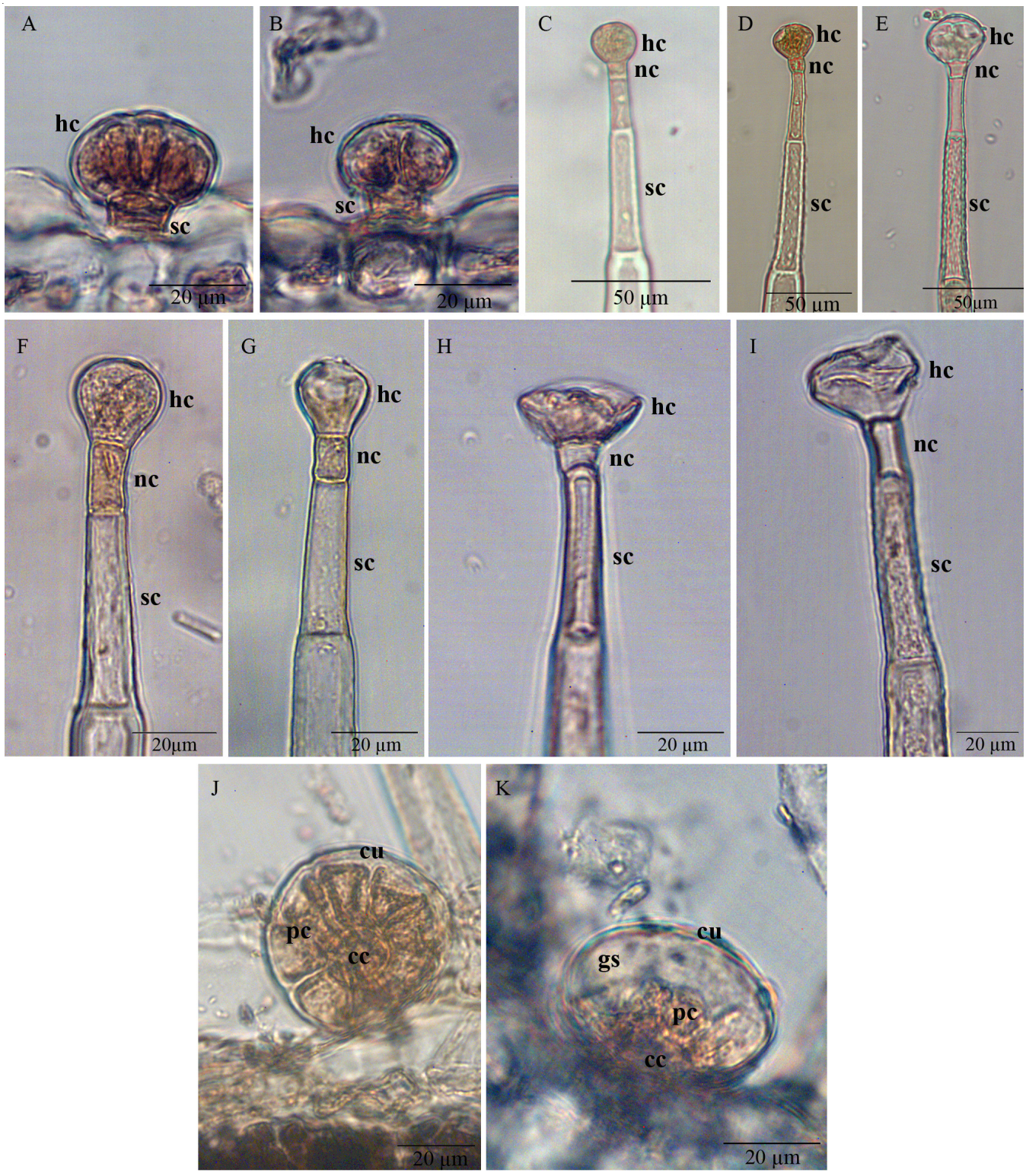

Fig. 1. Photograps of glandular trichomes of *S. sipthorpii, S. cypria* var. *elatior, S. cypria* var. *cypria*. A-E: Type I capitat glandular trichomes; F-G: Type II capitat glandular trichomes; H-I: Type III capitat glandular trichomes; J-K: Peltat trichomes. hc: head cell, nc: neck cell, sc: stalk cell, cu: cuticle, cc: central cell, pc: periphery cells, gs: glandular space

the *S. aureus* was found the most sensitive microorganisms against all *Scutellaria* species with the MIC value changing 10-15 mg/mL, *C. albicans* was found to be the most resistant unicellular fungus with the MIC value higher than 20 mg/mL. Essential oils of *S. barbata* displayed a broad antimicrobial spectrum and exerted a much stronger bactericidal effect against Gram-positive bacteria[17]. In the light of this report, the antimicrobial activity of the volatiles could be raised especially from the menthol, long chain alcohols, linalool components in addition to the minor ones such as thymol and α-terpineol. Therefore, our results were almost the same with this other findings in the literature.

TABLE-2
COMPOSITION (%) OF THE VOLATILE COMPOUNDS OF THREE ENDEMIC *Scutellaria species*[a]

Peak No	RRI[b]	Compounds	1	2	3	Identification methods[c]
		Oxygenated monoterpenes				
1	1442	1-Octen-3-ol	1.85	1.06	-	GC-FID, GC-MS
2	1542	Linalool	6.38	10.92	7.69	GC-FID, GC-MS
3	1701	α-Terpineol	2.02	3.91	-	GC-FID, GC-MS
4	1794	Nerol	-	1.31	-	GC-FID, GC-MS
5	1838	Geraniol	2.79	5.26	-	GC-FID, GC-MS
6	2003	Perilla alcohol	-	1.97	-	GC-FID, GC-MS
7	2160	Eugenol	23.05	3.41	-	GC-FID, GC-MS
		Sesquiterpene hydrocarbons				
8	1614	Trans-caryophyllene	4.97	2.45	22.58	GC-FID, GC-MS
9	1685	α-Humulene	-	-	3.89	GC-FID, GC-MS
10	1723	Germacren-D	2.81	1.75	42.01	GC-FID, GC-MS
11	1747	Bicyclogermacren	-	-	4.26	GC-FID, GC-MS
		Oxygenated sesquiterpenes				
12	2008	Caryophyllene oxide	2.62	-	5.24	GC-FID, GC-MS
13	2032	*Z*-Nerolidol	2.25	2.60	-	GC-FID, GC-MS
14	2035	*E*-Nerolidol	-	-	6.58	GC-FID, GC-MS
15	2188	*trans*-Muurolol	4.85	-	-	GC-FID, GC-MS
		Aliphatic components				
16	2043	Octanoic acid	1.86	-	-	GC-FID, GC-MS
17	2128	2-Pentadecanone	2.34	6.93	-	GC-FID, GC-MS
18	2149	*n*-Nonanoic acid	4.14	2.50	-	GC-FID, GC-MS
19	2252	Capric acid	2.15	-	-	GC-FID, GC-MS
20	2299	Eicosane	-	2.79	7.74	GC-FID, GC-MS
21	2365	2(4H)-Benzofuranone	-	3.89	-	GC-FID, GC-MS
22	2389	Palmitic acid	27.00	46.76	-	GC-FID, GC-MS
23	2416	nd	2.33	2.48	-	-
24	2451	Hexatriacontane	3.65	-	-	GC-FID, GC-MS
25	2466	nd	2.93	-	-	-
Essential oil yield (%)			0.26	0.19	0.47	
Total essential oil			53.59	34.64	92.25	
Total			99.99	99.99	99.99	

[a]1. *S. cypria* var. *cypria,* 2. *S. cypria* var. *elatior*, 3. *S. sibthorpii*; [b]Relative retention indices were calculated against *n*-alkanes on the HP-innovax capillary column, [c]GC and GC-MS identifications based on the basis of computer matching of the mass spectra of the peaks with the Nist-Wiley and Arge-Far essential oil libraries and those reported by Adams, 2001. % values were calculated from FID data

TABLE-3
MIC VALUES (mg/mL) OF THE VOLATILES OF THREE SCUTELLARIA SPECIES

Test Microorganisms	*S. cypria var. cypria*	*S. cypria var. cypria*	*S. sibthorpis*	Gentamycine* (µg/mL)	Clotrimazole* (µg/mL)
E. coli ATCC 12228	20	20	20	1.25	n.t.
K. pneumoniae CCM 2318	15	15	20	2.5	n.t.
E. faecalis ATCC 29212	20	20	>20	1.25	n.t.
S. thyphimirium CCM 5445	15	15	20	1.25	n.t.
P. aeruginosa ATCC 27853	20	20	>20	2.5	n.t.
S. aureus ATCC 6538	15	15	10	1.25	n.t.
B. subtilis ATCC 6633	15	15	20	1.25	n.t.
C. albicans ATCC 10239	>20	>20	>20	n.t.	0.78

*Reference antibiotics; n.t: not tested

Conclusion

Various factors, both endogenous and exogenous, can affect the composition of the essential oil and hairiness of our species. In our opinion, the flowering time and geographical and climatic factors are very important for the essential oil contents and capitate glandular hairs. It is known that variations in the essential oil composition induced by environmental and physiological factors can induce the changes in biosynthesis, accumulation or metabolism of the essential oil profile[22,32,33].

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