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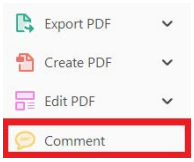

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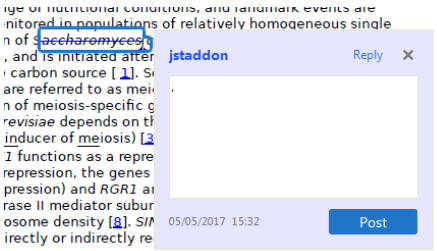

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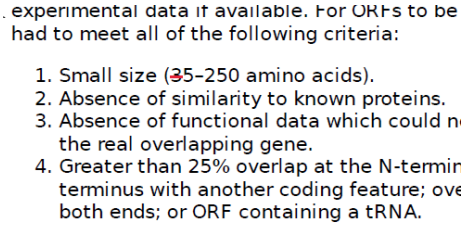

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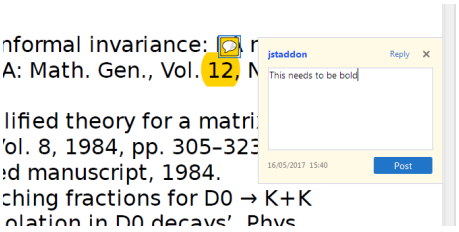

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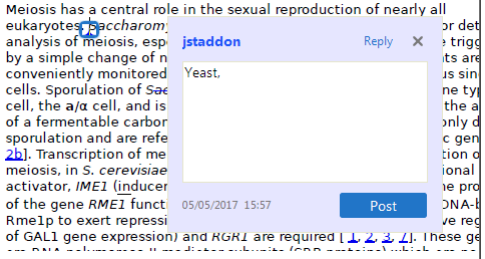

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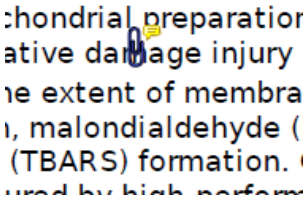

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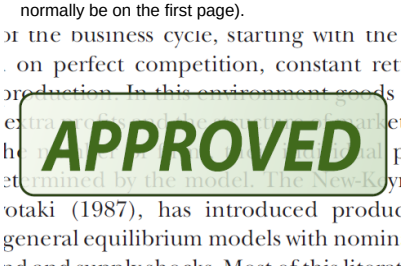

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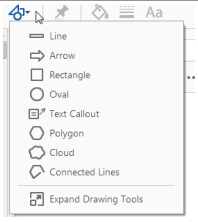

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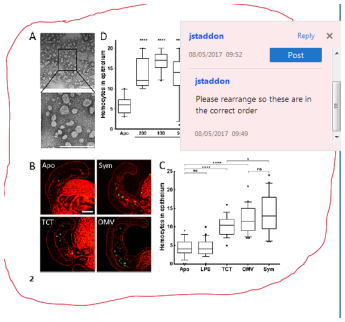

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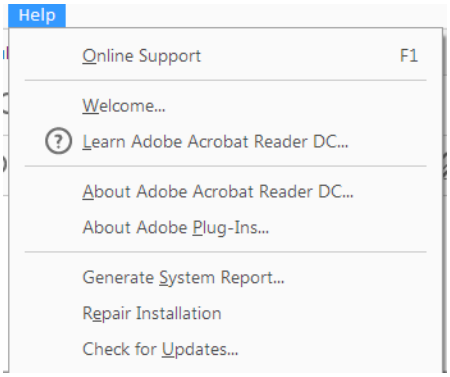

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RESEARCH PAPER

A novel study approach on Scutellaria altissima L. cultivated at the Ghirardi Botanic Garden (Lombardy, Italy)

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Keywords
Essential oil; GC/MS; glandular trichomes; HS-SPME; Hydrodistillation; Scutellaria altissima L.; VOC profile.

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ABSTRACT

- Within an Open Science project, research was carried out to describe to the public of the Ghirardi Botanic Garden (BS, Lombardy, Italy) the *invisible* features of plants. This work is dedicated to *Scutellaria altissima* L. (*Lamiaceae*).
- Micromorphological, histochemical and phytochemical investigations were conducted on the vegetative and reproductive organs to correlate the structures involved in the emission of substances and their unique productivity. This work reports volatile organic compound (VOC) profiles of leaves and flowers and the composition of essential oil (EO) obtained from aerial parts of plants cultivated in Italy that have never been described before.
- Three morphotypes of glandular trichomes were observed: peltate, short-stalked capitate and long-stalked capitate. Peltate trichomes were the main producers of terpenes, short-stalked capitates of polysaccharides and long-stalked capitates of terpenes and polyphenols. The leaf VOC profile showed heterogeneous composition, with non-terpene derivatives as the major chemical class (71.04%), while monoterpene hydrocarbons represented almost the totality of the flower (99.73%). The leaf presented a higher number of total (37 *versus* 11) and exclusive (33 *versus* 7) compounds. (*Z*)-3-Hexenol acetate was most abundant in the leaf and (*E*)-β-ocimene in the flower. Four common compounds were detected: β-pinene, β-caryophyllene, γ-muurolene and germacrene-D. The EO contaied 21 compounds, dominated by β-caryophyllene, linalool and hexahydrofarnesyl acetone.
- This research allowed us to correlate morphotypes of the secretory structures with the production of secondary metabolites, with the aim of providing the public of the Ghirardi Botanic Garden with a dedicated iconographic approach, which accounts for olfactory perception linked to *S. altissima*.

INTRODUCTION

Scutellaria L. is a genus in the *Lamiaceae* and includes approximately 360 species, commonly known as skullcaps (Formisano *et al.*, 2013; Sripathi & Ravi, 2017; Safikhani *et al.*, 2018). *Scutellaria* is widespread primarily in Europe, North America and East Asia (Qin Shu, 1994; Bruno *et al.*, 2002). The species belonging to this genus are mostly perennial herbs and small shrubs, but there are also annual herbs and aquatic plants (Formisano *et al.*, 2013). *Scutellaria altissima* L. is an herbaceous plant, widespread in Europe and in the Mediterranean region; in Italy, it is distributed in Marche, Lazio, Abruzzo and, as adventitious, in Friuli (Richardson, 1972; Pignatti 2003). The stem is erect, quadrangular and pubescent; the leaves are dark green and almost glabrous, ovate in shape with serrate margins; the bract length is less than that of the flowers, the calyx is subglabrous and the corolla is 10–14-mm long and blue-violet in colour.

For thousands of years, species belonging to *Scutellaria* genus have been regularly employed in traditional medicine (Duke 1986). Calming, haemostatic and tonic properties are referred to the infusion of the leaves in East Anatolia (Özçelik *et al.* 1990; Baytop 1999; Kurkcuoglu *et al.*, 2019), as well as anti-inflammatory, antiviral, antithrombotic and antioxidant effects to the tincture alone or with other herbs in East Asia, especially in China, Korea and Japan (Shang *et al.*, 2010; Grzegorczyk-Karolak *et al.*, 2016). *S. altissima* is a well-known species in the Chinese traditional medicine, useful for the treatment of respiratory tract infections, pneumonia, bronchitis, in cases of hypertension (Gao *et al.*, 2017), hepatitis and cancer (Li & Wei, 1994; Malakov & Papanove 1996; Sripathi & Ravi 2017). Other uses are described for *Scutellaria* spp. coming from many other regions of the world (Kokakowska 2017; Irvin *et al.* 2019).

In the *Lamiaceae* family, glandular trichomes are the main sites for the synthesis of natural bioactive compounds, which

play a crucial role in mediating the plant–environment relationships (Giuliani *et al.,* 2017a; Giuliani *et al.,* 2017b; Giuliani *et al.,* 2018; Najar *et al.,* 2018). The literature proposes some morphological studies concerning the secretory structures in *Scutellaria* species (Giuliani & Bini 2008; Dereboylu *et al.,* 2012), but none of them refer to *S. altissima*.

Concerning the current known phytochemical composition, more than 295 compounds have been isolated from *Scutellaria* species, with the majority of the compounds being flavonoids and diterpenes (Josher *et al.,* 2013), while there are few studies on essential oil (EO) characterization for *Scutellaria* species (Skaltsa *et al.,* 2000; Yu *et al.* 2004; Skaltsa *et al.,* 2005; Rosselli *et al.,* 2007; Yilmaz *et al.,* 2019). In detail, there is only one contribution about the EO analysis of *S. altissima*, which comes from Turkey (Kurkcuoglu *et al.,* 2019), while studies on volatile organic compound (VOC) emission profiles are lacking. Moreover, there are no contributions on the existing connection between the production/emission of these secondary metabolites and their ecological role.

Referring to the biological activity, studies on EO are lacking. However, the antioxidant action of the ethanol extract of aerial parts and roots is known (Grzegorczyk-Karolak *et al.,* 2019), together with the inhibitory effect of the methanol extract on the tyrosinase enzyme (Revoltella *et al.,* 2019) and the antifeedant, cytotoxic, chemo-sensitizing and neuroprotective properties of some molecules isolated from the plant (Bozov & Georgieva, 2017; Gao *et al.,* 2017; Jia *et al.,* 2019). In addition, *S. scordifolia* L. methanolic extracts, obtained at room temperature, showed activity against *Candida* spp., *Malassezia furfur* and other dermatophytes (Giordani *et al.,* 2020).

This work is part of an Open Science research project entitled 'Botanic Garden, factory of molecules', recently financed by the Lombardy Region (Italy). The aim is to investigate a selected pool of species preserved at the Ghirardi Botanic Garden (Toscolano Maderno, BS, Italy), including *S. altissima*, towards a new vision of the plant, beyond what is macroscopically visible, in order to: **(i)** describe the morphology and distribution pattern of the glandular trichomes on the vegetative and reproductive organs; **(ii)** characterize the secretion products through histochemical assays; **(iii)** correlate the micromorphological investigations on secretory structures to secondary metabolite biosynthesis through the phytochemical characterization of the VOC profiles spontaneously emitted from leaves and flowers; and (iv) analysis of the EO obtained from the aerial parts. The present investigation was conducted in order to transfer accurate scientific knowledge to the public by means of a new specifically designed iconographic approach.

MATERIAL AND METHODS

Plant material

Scutellaria altissima was cultivated at the Ghirardi Botanic Garden (Toscolano Maderno, BS) of the Department of Pharmaceutical Sciences, University of Milan. The samplings for micro-morphological and phytochemical (VOC and EO) investigations were performed simultaneously on plants in full bloom in June 2019.

Micromorphological analysis

Both vegetative and reproductive organs (stems, leaves, bracts, calyces and corollas) were examined under light (LM) and scanning electron microscopy (SEM). At least ten replicates, similar in size and position, for each of the examined plant parts were evaluated to assess the level of consistency in the overall morphology, distribution pattern and histochemical features of the glandular trichomes. Referring to trichome localization on the examined plant parts, we qualitatively evaluated their distribution using the following symbols: (-) absent, not observed in any replicates; (+) present in all replicates; (±) sporadic in no more than four replicates; (++) abundant in all replicates with distribution on the whole organ surface.

Light microscopy

Fresh and fixed material were used. Fresh samples were frozen and cryo-sectioned; other samples were fixed in FAA solution (formaldehyde:acetic acid:ethanol 70%, 5:5:90) for 5 days, dehydrated in an ascending ethanol series up to absolute, and embedded in Technovit/Historesin. Several histochemical dyes were employed to evidence the different components of the secretion. In detail: Fluoral Yellow-088 for total lipids (Brundett *et al.,* 1991), Nile Red for neutral lipids (Greenspan *et al.,* 1985), Nadi reagent for terpenes (David & Carde, 1964), Ruthenium Red for acid polysaccharides (Jensen, 1962), Alcian Blue for mucopolysaccharides (Beccari & Mazzi, 1966), and Ferric trichloride for polyphenols (Gahan, 1984). Control procedures were carried out for each of the employed histochemical staining techniques. Observations were made with a Leitz DM-RB Fluo optical microscope.

Scanning electron microscopy

Small segments of each plant part were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 6.8 for 7 days, dehydrated in ethanol and ascending series up to absolute, then critical-point dried. The samples, mounted on stubs and coated with gold, were observed with a Philips XL-20 SEM.

Phytochemical investigations

Volatile organic compounds (VOC)

Three leaves and three flowers were cut and immediately inserted into separate glass vials of suitable volume for the analysis.

Head space–Solid phase microextraction sample analysis (HS-SPME)

The headspace sampling conditions were as reported in Ascrizzi *et al.* (2017). Headspace sampling used Supelco SPME (Solid Phase Micro-Extraction) devices, with samples coated with polydimethylsiloxane (PDMS, 100 μm); the same new fibre, preconditioned according to the manufacturer instructions, was employed for all the analyses. To ensure a stable temperature, samplings were conducted in an air-conditioned room at 22 ± 1 °C; this temperature was chosen to avoid thermal damage to plant material and, thus, prevent any artificial-induced volatile release. After 30 min of equilibration, the fibre

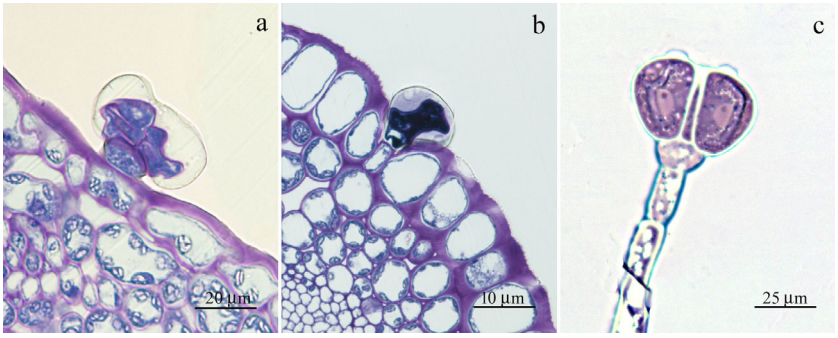

Fig. 1. a–c. Glandular trichome morphotypes in *Scutellaria altissima* L. (Light microscopy). a. Peltate. b. Short capitate. c. Long capitate.

was exposed to sample the headspace for 30 min. Both the equilibration and sampling times were experimentally determined to obtain optimal adsorption of the volatiles, and to avoid both under- and over-saturation of the fibre and of the mass spectrometer ion trap. Once sampling was finished, the fibre was withdrawn into the needle and transferred to the injection port of the GC-MS system. Both the sampling and desorption conditions were identical for all the samples. Furthermore, blanks were performed before each first SPME extraction and randomly repeated during each series. Quantitative comparisons of relative peaks areas were performed between the same compounds in the different samples.

Essential oil (EO)

Plant aerial parts at blooming were air-dried at room temperature in the dark and stored under the same conditions until the hydrodistillation process. The EO hydrodistillation was performed in a standard Clevenger apparatus for 2 h.

Analysis with GC-MS

The GC-MS analyses were performed at the Department of Chemistry, University of Milan, using a TRACE ISQ QD Single Quadrupole GC-MS.

Separation of EO was performed with a capillary column VF-5ms (5% phenyl-methyl-polisiloxane, length 30 m, 0.25 mm i.d., 0.1 µm film thickness). The temperature gradient was: 8 min at 50 °C, then 4 °C min^{-1} to 60 °C, then 6 °C min^{-1} from 60 °C to 160 °C, and finally 20 °C min^{-1} from 160 °C to 280 °C. Injector and detector temperatures were set to 280 °C; carrier gas Helium, flux 1 ml min^{-1}: mass range detected was 50–500 m/z. The EO were analysed in pure or diluted 1:100 with *n*-hexane form, with injection volume of 1 µl.

Mass spectra were analysed using the Wiley Mass spectra Library, NIST Mass Spectral Search Program and NIST Tandem Mass Spectral library 2.3. Compounds were identified by mass fragmentation and retention index, compared with data stored in mass databases (WILEY, NIST18).

RESULTS

Micromorphological investigation

Trichome morphotypes and distribution pattern

The indumentum included both glandular and non-glandular trichomes. The glandular trichomes belonged to three main morphotypes: peltate, short capitate and long capitate (**Fig. 1**). The distribution pattern and abundance on the investigated plant parts are shown in **Table 1** and **Fig. 2**.

Table 1. Distribution pattern of the glandular and non-glandular trichomes on the vegetative and reproductive organs of *Scutellaria altissima* L. Symbols: (–) absent, (±) sporadic, (+) present, (++) abundant.

Trichome type	Stem	Leaf		Bract		Calyx		Corolla	
		adax	abax	adax	abax	adax	abax	adax	abax
peltate	±	–	+	–	+	–	+	–	+
short capitate	+	+	++	+	+	+	+	+	++
long capitate	–	–	–	–	+	–	+	–	+
non-glandular	+	++	++	++	++	–	++	–	++

For symbols details see Material and Methods section.

The peltate trichome consisted of one basal cell, one short unicellular stalk and one multicellular globose head with a wide storage chamber (**Fig. 1a**). The short capitate trichome was composed of one basal cell, one stalk cell and an elliptical two-celled head with a thin subcuticular space (**Fig. 1b**). The long capitate trichome, upright or clinging to the surface, was composed of one basal cell, a stalk of two to three cells and a large multicellular (up to 8 cells) head with a small subcuticular space for each of the secreting cells (**Fig. 1c**).

Non-glandular trichomes were bicellular to multicellular, simple, uniseriate, slightly bent and with a pointed apex (**Fig. 2a–h**). They occurred on all epidermal surfaces; their length varied distinctly from very short hairs on the adaxial leaf side (**Fig. 2a, b**) to moderately long hairs on the abaxial leaf side and on the calyx and corolla (**Fig. 2c–h**).

The stem presented both peltate and short capitate trichomes. The leaf had the same trichome types, uniformly distributed on the abaxial side, whereas on the adaxial side only short capitate trichomes were observed along the veinal system (**Fig. 2a, b**). The bract was characterized by a similar distribution pattern, except for the presence of long capitate trichomes along the edges (**Fig. 2c, d**).

The calyx presented all trichome types on the abaxial side and only short capitate trichomes on the adaxial side (**Fig. 2e, f**). The corolla exhibited abundant peltate and short capitate trichomes on the tube and on the lower and upper lips

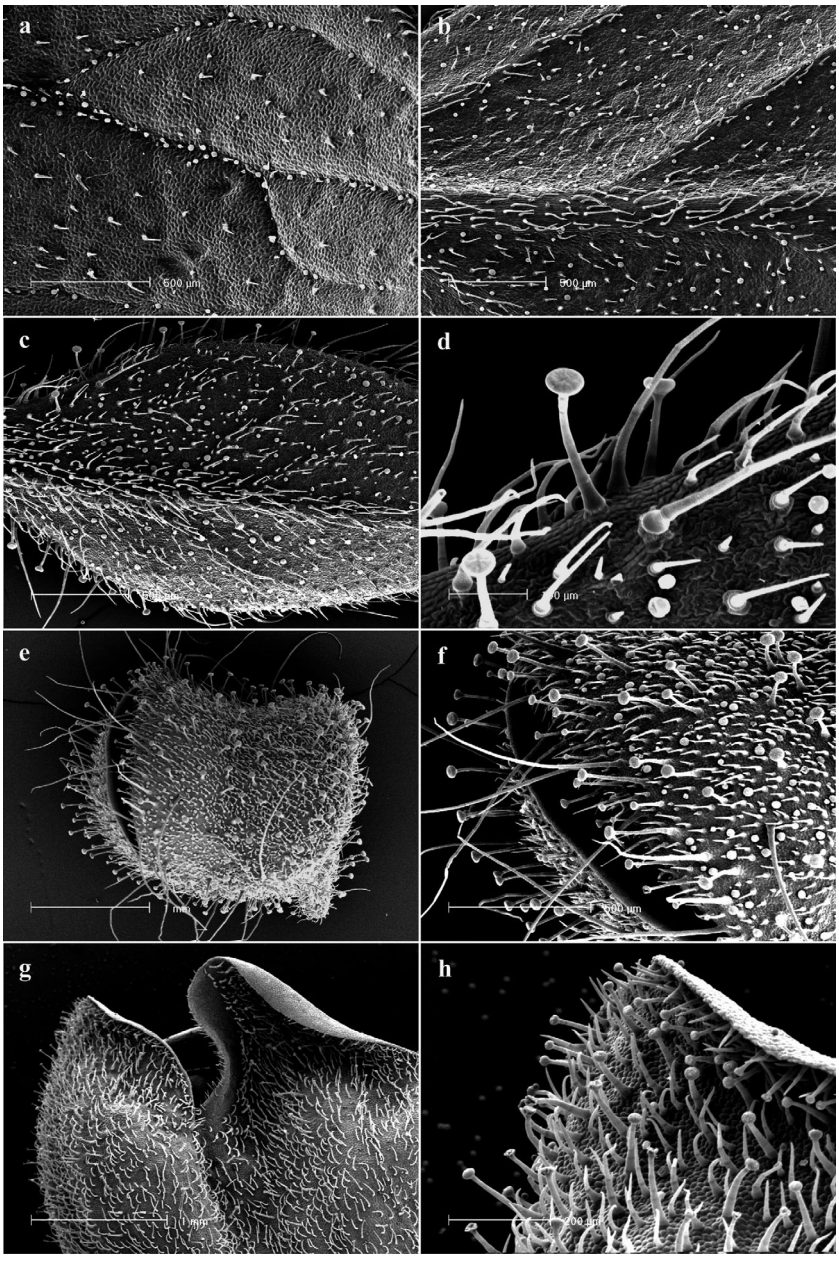

Fig. 2. a–h. Trichome distribution pattern in *Scutellaria altissima* L., (Scanning electron micrographs). a. Leaf adaxial surface with peltate, short capitate and non-glandular trichomes. b. Leaf abaxial surface with short capitate trichomes and non-glandular hairs. c. Bract abaxial surface with the three types of glandular hairs and non-glandular trichomes. d. High resolution of the bract abaxial surface with long capitate trichomes along the edges. e. General view of the calyx with all the types of glandular trichomes. f. High resolution of the distal region of the calyx sides. g. Corolla abaxial surface with peltate, short and long capitate trichomes. h. High resolution of the distal region of upper lip with abundant long capitate trichomes.

(**Fig. 2g**); at the distal region of the lower lip, long capitate trichomes were also observed. The corolla adaxial side presented only short capitate trichomes.

Histochemistry

The results of the histochemical investigation are reported in **Table 2** and **Fig. 3**. Lipophilic dyes gave a positive response in peltate trichomes, in particular Nadi reagent and Fluoral Yellow-088 (**Fig. 3a, b**), indicating the exclusive synthesis of terpenes. In the short capitate trichomes, only muco-polysaccharides were produced, as indicated by the positive response following application of Alcian Blue (**Fig. 3c**). The long capitate trichomes were characterized by a complex secretion due to the occurrence of both terpenoidic and polyphenolic fractions (**Fig. 3d, e**).

Phytochemical investigation

Volatile organic compounds

The VOC emission profiles of *S. altissima* revealed a total of 44 compounds. In particular, 37 compounds were identified in the leaf profile, while only 11 were observed in the floral profile (**Table 3**).

Table 2. Results of histochemical tests on the glandular trichomes of the vegetative and the reproductive organs of *Scutellaria altissima* L. Symbols: (–) negative response; (+) positive response; (++) intensely positive response.

Stainings	Target compounds	peltate	short capitate	long capitate
Fluoral yellow-088	Total lipids	++	–	++
Nile Red	Neutral lipids	+	–	+
Nadi reagent	Terpenoids	++	–	++
Ruthenium Red	Acid polysaccharides	–	–	–
Alcian Blue	Muco-polysaccharides	–	+	–
Ferric tricholoride	Polyphenols	–	–	++

The leaf profile was dominated by non-terpene derivatives (71.04%), followed by sesquiterpene hydrocarbons (9.16%), while oxygenated monoterpenes and apocarotenoids were present in comparable amounts, 7.60% and 7.30%, respectively. Monoterpene hydrocarbons (2.35%) and oxygenated sesquiterpenes (0.72%) were the classes with the lowest relative abundances. The main compound was (*Z*)-3-hexenol acetate (*7*, 44.14%), followed by (*E*)-3-hexen-1-ol (*1*, 9.05%), (*E*)-geranyl acetone (*34*, 7.30%) and decanal (*23*, 6.43%). A total of 33 exclusive compounds were detected, including the above-mentioned most abundant compounds (*7*, *1*, *34*, *23*), followed by 1,8-cineole (*10*, 2.89%), nonanal (*15*, 2.81%), (*Z*)-3-hexenyl isovalerate (*24*, 2.23%) and linalool (*14*, 2.04%). The other exclusive compounds occurred in amounts below 2.0%.

The floral profile was dominated by monoterpene hydrocarbons (99.73%), followed by sesquiterpene hydrocarbons (0.17%), while oxygenated monoterpenes and sesquiterpenes, apocarotenoids and non-terpene derivatives were absent. The main compound was (*E*)-β-ocimene (*12*, 88.67%), followed by (*Z*)-β-ocimene (*11*, 4.78%). Seven exclusive compounds were identified, among which were major compounds (*12*, *11*), followed by *allo*-ocimene (*16*, 1.80%), *neo-allo*-ocimene (*18*, 1.57%) and myrcene (*5*, 1.4%). The remaining exclusive compounds were present in relative percentages below 1.0%.

The profiles revealed four common compounds: β-pinene (*3*) (2.11% leaves, 0.56% flowers), β-caryophyllene (*31*) (1.17% leaves, 0.17% flowers), γ-muurolene (*36*) (3.36% leaves, traces in flowers) and germacrene D (*37*) (0.51% leaves, traces in flowers).

Essential oils

The EO composition is reported in **Table 4**. A total of 21 compounds were identified. Oxygenated monoterpenes were the most abundant chemical class (26.47%), followed by sesquiterpene hydrocarbons (24.63%) and oxygenated sesquiterpenes (13.81%). Oxygenated sesquiterpenes and monoterpene hydrocarbons occurred in comparable amounts, 13.81% and 12.23%, respectively. The main compound was β-caryophyllene (*13*, 19.57%), followed by linalool (*8*, 17.57%), hexahydrofarnesyl acetone (*20*, 11.66%), α-pinene (*1*, 11.02%), caryophyllene oxide (*16*, 10.50%) and 1,8-cineole (*7*, 5.50%). *Trans*-2-decenal (*10*, 2.35%), humulene (*14*, 2.25%) and *n*-heptaeicosane (*21*, 2.06%) showed similar relative percentages. The remaining compounds occurred in amounts below 2.0%.

DISCUSSION

Scutellaria altissima had two main types of glandular trichome, widespread in the *Lamiaceae*: peltate and capitate trichomes (Werker, 2000; Giuliani *et al.*, 2017a; Giuliani

COLOR

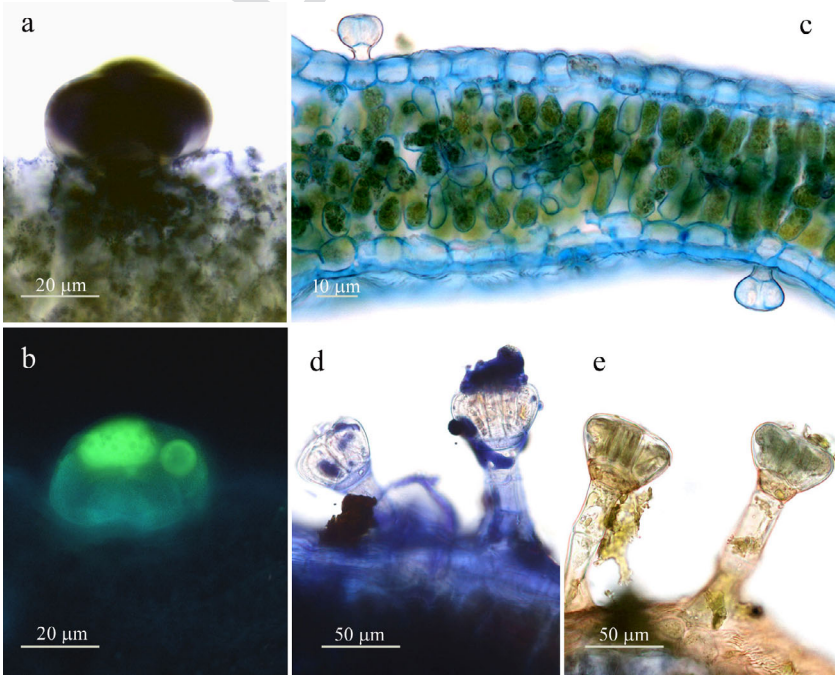

Fig. 3. a–e. Histochemistry of the glandular trichomes in *Scutellaria altissima* L. (light microscopy). a, b. Peltate trichome: Nadi reagent (a), Fluoral Yellow-088 (b); c. Short capitate trichome: Alcian Blue; d, e. Long capitate trichome: Nadi reagent (d), Ferric trichloride (e).

Table 3. Head space–Solid phase microextraction sample analysis (HS-SPME) profiles of leaves and flowers of *Scutellaria altissima* L.

			Relative abundance (%)	
	l.r.i.[a]	Compounds	Leaves	Flowers
1	853	(*E*)-3-hexen-1-ol	9.05	–[b]
2	941	α-pinene	0.24	–
3	982	β-pinene	2.11	0.56
4	985	6-methyl-5-hepten-2-one	0.86	–
5	993	myrcene	–	1.40
6	993	3-octanol	0.44	–
7	1009	(*Z*)-3-hexenol acetate	44.14	–
8	1011	δ-3-carene	–	0.12
9	1032	limonene	–	0.83
10	1034	1.8-cineole	2.89	–
11	1042	(*Z*)-β-ocimene	–	4.78
12	1052	(*E*)-β-ocimene	–	88.67
13	1071	1-octanol	0.38	–
14	1101	linalool	2.04	–
15	1102	nonanal	2.81	–
16	1129	allo-ocimene	–	1.8
17	1143	camphor	0.85	–
18	1145	*neo-allo*-ocimene	–	1.57
19	1173	menthol	0.68	–
20	1187	(*Z*)-3-hexenyl-butyrate	0.75	–
21	1192	methyl salicylate	0.82	–
22	1199	*n*-dodecane	0.17	–
23	1204	decanal	6.43	–
24	1240	(*Z*)-3-hexenyl isovalerate	2.23	–
25	1277	citronellyl formate	1.14	–
26	1306	undecanal	0.73	–
27	1376	α-copaene	0.87	–
28	1384	β-bourbonene	0.54	–
29	1399	*n*-tetradecane	0.38	–
30	1408	dodecanal	0.72	–
31	1420	β-caryophyllene	1.17	0.17
32	1429	β-copaene	0.15	–
33	1441	aromadendrene	1.03	–
34	1453	(*E*)-geranyl acetone	7.30	–
35	1461	*allo*aromadendrene	0.10	–
36	1477	γ-muurolene	3.36	tr[c]
37	1482	germacrene D	0.51	tr
38	1498	α-muurolene	0.76	–
39	1507	(*E,E*)-α-farnesene	0.67	–
40	1570	(*Z*)-3-hexenyl benzoate	0.38	–
41	1574	dendrolasin	0.47	–
42	1600	*n*-hexadecane	0.34	–
43	1683	α-bisabolol	0.25	–
44	1700	*n*-heptadecane	0.41	–
		Monoterpene hydrocarbons	2.35	99.73
		Oxygenated monoterpenes	7.60	–
		Sesquiterpene hydrocarbons	9.16	0.17
		Oxygenated sesquiterpenes	0.72	–
		Apocarotenoids	7.30	–
		Non-terpene derivatives	71.04	–
		Total	98.17%	99.90%

[a]Linear retentions indices on a DB5 capillary column: [b] Not detected; [c]Trace, <0.1%.

Table 4. Composition of the essential oil obtained from the aerial parts of *Scutellaria altissima* L.

	l.r.i	Compounds	Relative abundance (%)
1	928	α-pinene	11.02
2	977	1-octen-3-ol	1.61
3	984	3-(2-methylpropyl)-cyclohexane	1.23
4	1001	α-ocimene	0.12
5	1020	*o*-cymene	0.12
6	1024	limonene	0.96
7	1028	1,8-cineole	5.50
8	1091	linalool	17.57
9	1194	α-terpineol	1.79
10	1264	*(E)*-2-decenal	2.35
11	1376	β-bourbonene	0.84
12	1396	*iso*-caryophyllene	0.87
13	1413	β-caryophyllene	19.57
14	1450	humulene	2.25
15	1476	germacrene-D	1.11
16	1580	caryophyllene oxide	10.50
17	1635	β-acoradienol	0.71
18	1650	β-eudesmol	1.35
19	1660	phytol	1.26
20	1834	hexahydrofarnesyl acetone	11.66
21	2700	*n*-heptaecosane	2.06
		Monoterpene hydrocarbons	12.23
		Oxygenated monoterpenes	26.47
		Sesquiterpene hydrocarbons	24.63
		Oxygenated sesquiterpenes	13.81
		Apocarotenoids	11.66
		Non-terpenic derivatives	5.64
		Total	94.45

et al., 2018; Najar *et al.*, 2018). The former were present on the whole plant surface, as already documented for other *Scutellaria* species (Giuliani & Maleci Bini, 2008; Dereboylu *et al.*, 2012; De Oliveira *et al.*, 2013: Fico G., *personal observation*). The latter were distinguished in two subtypes: short-stalked capitate and long-stalked capitate trichomes, with different distribution patterns. Indeed, the short-stalked trichomes were uniformly distributed both on the vegetative and the reproductive organs and were particularly abundant on the leaf and corolla abaxial sides. In contrast, the long-stalked trichomes were recorded only on the abaxial surfaces of the bract and calyx and on the distal portion of the lower lip of the corolla. These observations on the peltate and short-stalked capitate trichomes are consistent with the literature data (Dereboylu *et al.*, 2012; Giuliani *et al.*, 2017a) and with results for *S. brevibracteata* subsp. *subvelutina* in our research group (Fico G., *personal observation)*. The medium-stalked capitate trichomes were not observed on *S. altissima*, while the long-stalked hairs with a multicellular head were confirmed as exclusive to the reproductive organs. However, the secretory heads exhibited different features; in *S. altissima* each secreting cell displayed a single small subcuticular space, in accordance with results already known for *S. galericulata* (Giuliani & Maleci Bini, 2008). In contrast, in *S. brevibracteata* subsp. *subvelutina* this morphotype has a head with a subcuticular space common to all

the secreting cells and located in a central position (Fico G., *personal observation*).

Besides the morphological investigations, a histochemical survey was carried out for the first time on *S. altissima*. This revealed that the activity of the peltate hairs was characterized by the exclusive production of lipophilic substances, in particular terpenes. The short-stalked capitate trichomes showed a positive response only to hydrophilic dyes, among which was the Alcian Blue assay, specific for mucopolysaccharides. The long-stalked capitate trichomes displayed a more complex secretion, due to the occurrence of both terpenes and polyphenols. The comparison of the histochemical results with the different distribution patterns of the trichomes allowed us to hypothesize the existence of a synergy in terpene production between the peltate and the long-stalked capitate trichomes, only on the abaxial surfaces of the bract, calyx and the distal portion of the lower lip of the corolla, while the peltate trichomes were the main producers of lipophilic substances on the stem, leaf and the remaining corolla surfaces. On this basis, considering both the ubiquitous distribution and the wide storage chamber, the peltate trichomes played a dominant role in EO production in the investigated species, confirming the literature data for other members of the *Lamiaceae* (Hallahan, 2000; Werker, 2000). On the other hand, the short-stalked capitate trichomes were responsible for the biosynthesis of polysaccharides on all examined organs, in particular the leaf and corolla, due to their abundance on these organs. Thaler *et al.* (1992) observed that dictyosomes are abundant in these last trichomes and hence we can link the presence of this organelle with the presence of polysaccharides, and hence to the Alcian blue positive reaction.

Concerning the phytochemical investigation, the characterization of the VOC profiles represents an element of novelty. A high level of variability was recorded between leaves and flowers. First, the leaf profile was much more complex than that of the flowers due to the presence of a higher number of compounds, 37 *versus* 11, respectively. Moreover, the former was characterized by different compound classes, among which non-terpene derivatives dominated (71.04%), followed by sesquiterpene hydrocarbons (9.16%), oxygenated monoterpenes (7.60%) and apocarotenoids (7.30%); while monoterpene hydrocarbons (2.35%) and oxygenated sesquiterpenes (0.72%) occurred in small percentages. Instead, almost all the compounds in the floral profile belonged to the monoterpene hydrocarbons class (99.73%).

These phytochemical results matched the histochemical data. In fact, the dominance of terpene derivatives in the flowers could be related to the synergy of action between peltate and long-stalked capitate trichomes in the production process of these substances, in particular at the bract and calyx level, to which the major secretion of peltate trichomes on the corolla could be added. In contrast, peltate trichomes were the only producers of terpenes on the leaves. Another distinctive element between the two emission profiles was represented by exclusive compounds: 33 in the leaves and seven in the flowers. (*Z*)-3-Hexenol acetate (*7*, 44.14%) dominated among the former, (*E*)-β-ocimene (*12*, 88.67%) among the latter; in both cases they were the major compounds in the whole profile. Four common compound were detected in low amounts or in traces: β-pinene (*3*) (2.11% leaves, 0.56% flowers), β-caryophyllene (*31*) (1.17% leaves, 0.17% flowers), γ-muurolene (*36*) (3.36% leaves, traces in flowers) and germacrene D (*37*) (0.51% leaves, traces in flowers).

In regard to the ecological role of the leaf exclusive compounds, a protective action emerged. Indeed, previous studies underlined that (*Z*)-3-hexenol acetate (*7*, 44.14%) is responsible for the antifeedant action towards insects of the genus *Lygus,* parasites of cotton and other crops in North America (Williams *et al.,* 2008) and aphids (Hedge *et al.,* 2011), as well as for tritrophic interactions – plants–herbivores–parasites (Stevens *et al.,* 2017). (*E*)-Geranyl acetone (*34*) and (*Z*)-3-hexenyl isovalerate (*24*) contribute to the protective role (Heil *et al.,* 2008; Morawo *et al.,* 2016; Pinto-Zevallos *et al.,* 2018). 1,8-Cineole (*10*) showed acaricidal (Hu *et al.,* 2015), fumigant and larvicidal effects (Lucia *et al.,* 2012), reinforced by the deterrent action of linalool (*14*) (Lobo *et al.,* 2019; Stevenson, 2019), for which also an attractive power is already recognized (Stevenson, 2019). On the other hand, specific studies concerning the remaining major exclusive compounds (> 2.0%) are lacking. With regard to the dominant exclusive compounds of the floral profile, (*E*)-β-ocimene (*12*), (*Z*)-β-ocimene (*11*), *allo*-ocimene (*16*) and *neo-allo*-ocimene (*18*) are considered common attractants for pollinators (Jayanthi *et al.,* 2012; Steen *et al.,* 2019). However, (*E*)-β-ocimene (*12*) is also involved in tritrophic protective mechanisms (Ghosh & Venkatesan, 2019), together with myrcene (*5*), showing allelopathic defence functions (Hsiung *et al.,* 2013). Referring to the common compounds, β-pinene (*3*), β-caryophyllene (*31*) and germacrene D (*37*) (Birkett *et al.,* 2008; Zhang 2018; Abraham *et al.,* 2018; Lobo *et al.,* 2019), these may contribute to the overall defence action; moreover, an attractant role is also recognized to β-caryophyllene (*31*) (Abraham *et al.,* 2018). γ-Muurolene (*36*), in turn, is a common repellent compound, as are most sesquiterpene hydrocarbons, a chemical class particularly active in mediating defence mechanisms (Chizzola 2013).

On this basis, it is possible to suggest a clear separation of the ecological roles displayed by the vegetative and reproductive organs. A protective action is primarily ascribed to the leaves, due to the more complex VOC profile, dominated by defensive compounds. In contrast, the noticeable abundance of (*E*)-β-ocimene (*12*), exclusive to the floral profile, underlines the major attractant action of the reproductive organs. The differences in volatile emissions based on the ecological role of the plant organs has also been reported for *Capparis spinosa* L., showing the importance of these compounds in the plant–habitat relationship (Ascrizzi *et al.,* 2016). Moreover, these authors reported (*E*)-β-ocimene as the main compound emitted in the floral headspace analysis, confirming the pollinator–attraction role hypothesized for this volatile in the present study (Ascrizzi *et al,* 2016).

In addition, this study reports the EO characterization of *S. altissima* grown in Italy in different environmental conditions to those previously analysed (Thaler *et al.* 1992; Bruno *et al.* 1996). The comparison with literature data showed that the profile of these samples presented a lower number of compounds with respect to the Turkish samples (Kurkcuoglu *et al.,* 2019). In our samples, oxygenated monoterpenes (26.47%) were the main compound class, followed by sesquiterpene hydrocarbons (24.63%), which were more abundant in the Turkish samples (41.30%). In both profiles, β-caryophyllene was the main constituent and also linalool occurred in high relative percentages. Differences were recorded for the following

major compounds: hexahydrofarnesyl acetone and caryophyll-5-en-12-al were exclusive to these samples and the Turkish samples, respectively. Among the main compounds of the Turkish EO, it is important to note the presence of hexadecanoic acid, which was totally absent in our profile, but emerged for the EO of *S. brevibracteata* subsp. *subvelutina* of Italian origin (Fico G., *personal observation*). However, the comparison with literature data was difficult due to the different geographic origin of the analysed samples.

Concerning the biological activity of the EO in *S. altissima*, previous contributions are lacking. Nevertheless, some evaluations are possible referring to the biological activity ascribed to the major EO compounds. In particular, as an example, some studies report the anti-inflammatory and hypolipidemic properties of β-caryophyllene (*13*) (Baldissera *et al.,* 2017a; Baldissera *et al.,* 2017b), as well as the inhibitory power of its oxidation derivatives, such as caryophyllene oxide (*16*), towards ABC proteins in cases of hepatocellular carcinoma, leading to improved response to anticancer drugs (Di Giacomo *et al.,* 2019). In the case of linalool (*8*), anti-inflammatory and antioxidant actions are documented (Li *et al.,* 2015; Seol *et al.,* 2016); for α-pinene (*1*) the inhibitory potential of metastatic action in breast cancer cases (Kang *et al.,* 2016), antioxidant, antiproliferative and cytotoxic properties have been studied (Aydin *et al.,* 2013). Concerning the biological activity of hexahydrofarnesyl acetone (*20*), specific studies on the pure compound are lacking; Radulović *et al.* (2006), however, reported the *in vitro* antimicrobial activity exerted on Gram-positive and Gram-negative bacterial strains by an *Equisetum arvense* L. EO mainly rich in this apocarotenoid.

This multidisciplinary approach of our investigation, according to the aims of the research project 'Botanic Garden, factory of molecules', allowed us to characterize the target species by combining the morphological description of the glandular indumentum with productivity of volatile molecules. Moreover, the overall information concerning the chemical nature of the emitted volatile substances may finally contribute to make hypotheses on the biotic interactions established for *S. altissima*, thus constituting the basis for future insights into the ecological roles of the secondary metabolites. The characterization of the essential oil profile might be useful to evaluate potential biological activity in *S. altissima*. It is well known that essential oils have antimicrobial and antifungal activity. However, these properties could be ascribed to other compound classes besides terpenes, such as flavones, as demonstrated in *S. scordifolia* (Giordani *et al.,* 2020).

In light of the current Open Science policies, these results will converge in the realization of a new dedicated iconographic approach at the Ghirardi Botanic Garden (Toscolano Maderno, BS, Lombardy, Italy), with the aim of transmitting to visitors the results of the scientific research in the area where it takes place.

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REFERENCES

Abraham A.A., Verghese A., Muthangi S. (2018) Role of colour and volatile in foraging behaviour of honeybee *Apis cerana* on *Jacquemontia pentanthos*. *Journal of Asia-Pacific Entomology*, **21**, 1122–1128.

Ascrizzi R., Cioni P.L., Giusti G., Pistelli L., Flamini G. (2016) Patterns in Volatile Emission of Different Aerial Parts of Caper (*Capparis spinosa* L.). *Chemistry & Biodiversity*, **13**, 904–912.

Ascrizzi R., Cioni P.L., Amadei L., Maccioni S., Flamini G. (2017) Geographical patterns of *in vivo* spontaneously emitted volatile organic compounds in *Salvia* species. *Microchemical Journal*, **133**, 13–21.

Aydin E., Türkez H., Geyikoğlu F. (2013) Antioxidative, anticancer and genotoxic properties of α-pinene on N2a neuroblastoma cells. *Biologia*, **68**, 1004–1009.

Baldissera M.D., Souza C.F., Doleski P.H., Leal D.B., Stefani L.M., Boligon A.A., Monteiro S.G. (2017a) Enzymes that hydrolyze adenine nucleotides in a model of hypercholesterolemia induced by Triton WR-1339: protective effects of β-caryophyllene. *Molecular and Cellular Biochemistry*, **434**, 127–134.

Baldissera M.D., Souza C.F., Grando T.H., Doleski P.H., Boligon A.A., Stefani L.M., Monteiro S.G. (2017b) Hypolipidemic effect of β-caryophyllene to treat hyperlipidemic rats. *Naunyn-Schmiedeberg's Archives of Pharmacology*, **390**, 215–223.

Baytop T. (1999) *Therapy with medicinal plants in Turkey, past and present*2nd edn. Nobel Tip Kitabevleri, Istanbul: 375 pp.

Beccari N., Mazzi V. (1966) *Manuale di tecnica microscopica [Manual of microscopic technique]*. Società Editrice Libraria, Como, Italy.

Birkett M.A., Al Abassi S., Kröber T., Chamberlain K., Hooper A.M., Guerin P.M., Pettersson J., Pickett A.J., Slade R., Wadhams L.J. (2008) Antiectoparasitic activity of the gum resin, gum haggar, from the East African plant, *Commiphora holtziana*. *Phytochemistry*, **69**, 1710–1715.

Bozov P.I., Georgieva Y.P. (2017) Antifeedant Activity of Neo-clerodane Diterpenoids from *Scutellaria altissima* Against Colorado Potato Beetle Larvae. *Natural Product Communications*, **12**(3), 1934578X1701200303.

Brundrett M.C., Kendrick B., Peterson C.A. (1991) Efficient lipid staining in plant material with Sudan Red 7B or Fluoral Yellow 088 in polyethylene glycol-glycerol. *Biotechnic Histochemistry*, **66**, 111–116.

Bruno M., Piozzi F., Rodríguez B., María C., Vassallo N., Servettaz O. (1996) Neo-clerodane diterpenoids from *Scutellaria altissima* and *S. albida*. *Phytochemistry*, **42**, 1059–1064.

Bruno M., Piozzi F., Maggio A.M., Simmonds M.S.J. (2002) Antifeedant activity of neo-clerodanediterpenoids from two Sicilian species of *Scutellaria*. *Biochemical Systematics and Ecology*, **30**, 73.

Chizzola R. (2013) Regular monoterpenes and sesquiterpenes (essential oils). *Natural Products*, **10**, 978–3.

David R., Carde J.P. (1964) Coloration differentielle des inclusions lipidiques et terpeniques des pseudophylles du Pin maritime au moyen du reactif NADI. *Comptes rendu de l'Acadèmie des Sciences, Paris*, **258**, 1338–1340.

De Oliveira A.B., De Mendonça M.S., Meira R.M. (2013) Anatomy of vegetative organs of *Scutellaria agrestis*, a medicinal plant cultivated by riverine populations of the Brazilian Amazon. *Revista Brasileira de Farmacognosia*, **23**, 386–397.

Dereboylu A.E., Sarikahya N.B., Sengonca N., Kirmizigul S., Yasa I., Gucel S., Guvensen A. (2012) Glandular trichome morphology, chemical composition and antimicrobial activity of the essential oil of three endemic *Scutellaria* taxa (Lamiaceae). *Asian Journal of Chemistry*, **24**, 4911–4916.

Di Giacomo S., Briz O., Monte M.J., Sanchez-Vicente L., Abete L., Lozano E., Mazzanti G., Di Sotto A., Marin J.J. (2019) Chemosensitization of hepatocellular carcinoma cells to sorafenib by β-caryophyllene oxide-induced inhibition of ABC export pumps. *Archives of Toxicology*, **93**, 623–634.

Duke A.D. (1986) *Handbook of medicinal herbs*. CRC Press, Boca Raton, FL, USA: 440.

Formisano C., Rigano D., Senatore F., Arnold N.A., Simmonds M.S.J., Rosselli S., Bruno M., Loziene K. (2013) Essential oils of three species of *Scutellaria* and their influence on *Spodoptera littoralis*. *Biochemical Systematics and Ecology*, **48**, 206–210.

Gahan P.B. (1984) *Plant Histochemistry and Cytochemistry: An Introduction*. Academic Press, London, UK.

Gao C., Zhou Y., Jiang Z., Zhao Y., Zhang D., Cong X., Cao R., Li H., Tian W. (2017) Cytotoxic and chemosensitization effects of Scutellarin from traditional Chinese herb *Scutellaria altissima* L. in human prostate cancer cells. *Oncology Reports*, **38**, 1491–1499.

Ghosh E., Venkatesan R. (2019) Plant Volatiles Modulate Immune Responses of *Spodoptera litura*. *Journal of Chemical Ecology*, **45**, 715–724.

Giordani C., Simonetti G., Natsagdorj D., Choijamts G., Ghirga F., Calcaterra A., Quaglio D., De Angelis

G., Toniolo C., Pasqua G. (2020) Antifungal activity of Mongolian medicinal plant extracts. *Natural Product Research*, **34**, 449–455.

Giuliani C., Bini L.M. (2008) Insight into the structure and chemistry of glandular trichomes of Labiatae, with emphasis on subfamily Lamioideae. *Plant Systematics and Evolution*, **276**, 199.

Giuliani C., Ascrizzi R., Tani C., Bottoni M., Maleci Bini L., Flamini G., Fico G. (2017a) *Salvia uliginosa* Benth: glandular trichomes as bio-factories of volatiles and essential oil. *Flora*, **233**, 12–21.

Giuliani C., Ascrizzi R., Corrà S., Bini L.M., Flamini G., Fico G. (2017b) Ultrastructural insight into terpene-producing trichomes and essential oil profile in *Salvia greggii* A. *Gray. Flora*, **236**, 107–114.

Giuliani C., Ascrizzi R., Lupi D., Tassera G., Santagostini L., Giovanetti M., Flamini G., Fico G. (2018) *Salvia verticillata*: Linking glandular trichomes, volatiles and pollinators. *Phytochemistry*, **155**, 53–60.

Greenspan P., Mayer E.P., Fowler S.D. (1985) Nile red: a selective fluorescent stain for intracellular lipids droplets. *Journal of Cell Biology*, **100**, 965–973.

Grzegorczyk-Karolak Izabela, Gołąb Krzysztof, Gburek Jakub, Wysokińska Halina, Matkowski Adam (2016) Inhibition of Advanced Glycation End-Product Formation and Antioxidant Activity by Extracts and Polyphenols from Scutellaria alpina L. and S. altissima L. *Molecules*, **21**(6), 739.

Grzegorczyk-Karolak I., Kontek B., Kontek R., Wysokińska H., Olas B. (2019) Evaluation of antioxidant activity of extracts from the roots and shoots of *Scutellaria alpina* L. and *S. altissima* L. in selected blood cells. *Advances in Clinical and Experimental Medicine*, **28**, 453–460.

Hallahan D.L. (2000) Monoterpenoid biosynthesis in glandular trichomes of Labiatae plants. In: Hallahan, D.L., Gray, J.C. (Eds.) *Advances in Botanical Research. Plant Trichomes*. Academic Press, New York, USA, vol. **31**, 77–120 pp.

Hegde M., Oliveira J.N., Da Costa J.G., Bleicher E., Santana A.E., Bruce T.J., Caulfield J., Dewhirst S.Y., Woodcock C.M., Pickett J.A., Birkett M.A. (2011) Identification of semiochemicals released by cotton, *Gossypium hirsutum*, upon infestation by the cotton aphid, *Aphis gossypii*. *Journal of Chemical Ecology*, **37**, 741–750.

Heil M., Lion U., Boland W. (2008) Defense-inducing volatiles: in search of the active motif. *Journal of Chemical Ecology*, **34**, 601–604.

Hsiung Y.C., Chen Y.A., Chen S.Y., Chi W.C., Lee R.H., Chiang T.Y., Huang H.J. (2013) Volatilized myrcene inhibits growth and activates defense responses in rice roots. *Acta Physiologiae Plantarum*, **35**, 2475–2482.

Hu Z., Chen Z., Yin Z., Jia R., Song X., Li L., Zou Y., Liang X., Li L., He C., Yin L., Lv C., Zhao L., Su G., Ye G., Shi F. (2015) In vitro acaricidal activity of 1, 8-cineole against *Sarcoptes scabiei* var. *cuniculi* and regulating effects on enzyme activity. *Parasitology Research*, **114**, 2959–2967.

Irvin L., Jackson C., Hill A.L., Bajaj R., Mahmoudi C., Brajesh N.V., Joshee N. (2019) Skullcaps *(Scutellaria spp.):* Ethnobotany and Current Research. In: Joshee N., Dhekney S., Parajuli P. (Eds), *Medicinal Plants*. Switzerland, Springer, Cham, pp 141–168.

Jayanthi P.D.K., Woodcock C.M., Caulfield J., Birkett M.A., Bruce T.J. (2012) Isolation and identification of host cues from mango, *Mangifera indica*, that attract gravid female oriental fruit fly, *Bactrocera dorsalis*. *Journal of Chemical Ecology*, **38**, 361–369.

Jensen W.A. (1962) *Botanical histochemistry: principles and practice*. WH Freeman, San Francisco, CA, USA.

Jia X., Jia M., Yang Y., Wang D., Zhou F., Zhang W., Huang X., Guo W., Cai D., Chen H., Qi J., Zhou S., Ren H., Xu B., Ma T., Wang P., Qi J. (2019) Synthesis of Novel Baicalein Amino Acid Derivatives and Biological Evaluation as Neuroprotective Agents. *Molecules*, **24**, 3647.

Josher N., Tascan A., Medina-Bolivar F., Parajuli P., Rimando A.M., Shannon D.A., Adelberg J.W. (2013) *Scutellaria*: biotechnology, phytochemistry and its potential as a commercial medicinal crop. *Biotechnology for medicinal plants*. Springer, Berlin, Germany, pp 69–99.

Kang E., Jung Y.J., Shin S.Y., Koh D., Lee Y.H. (2016) α-Pinene inhibits tumor invasion through downregulation of nuclear factor (NF)-κB-regulated matrix metalloproteinase-9 gene expression in MDA-MB-231 human breast cancer cells. *Applied Biological Chemistry*, **59**, 511–516.

Kosakowska O. (2017) Intrapopulation variability of flavonoid content in roots of Baikal skullcap (*Scutellaria baicalensis* Georgi). *Herba Polonica*, **63**, 20–31.

Kurkcuoglu M., Yildiz G., Kose Y.B. (2019) Essential oil composition of two *Scutellaria* from Tokat province of Turkey. *Journal of the Turkish Chemical Society*, **6**, 115–118.

Li Z.P., Wei H.Q. (1994) Chemical compounds of the genus *Scutellaria*. *Phytomedicines*, **9**, 47.

Li Y., Lv O., Zhou F., Li Q., Wu Z., Zheng Y. (2015) Linalool inhibits LPS-induced inflammation in BV2 microglia cells by activating Nrf2. *Neurochemical Research*, **40**, 1520–1525.

Lobo A.P., Da Camara C.A.G., De Melo J.P.R., De Moraes M.M. (2019) Chemical composition and repellent activity of essential oils from the leaves of *Cinnamomum zeylanicum* and *Eugenia uniflora* against *Diaphania hyalinata* L. (Lepidoptera: Crambidae). *Journal of Plant Diseases and Protection*, **126**, 79–87.

Lucia A., Juan L.W., Zerba E.N., Harrand L., Marcó M., Masuh H.M. (2012) Validation of models to estimate the fumigant and larvicidal activity of Eucalyptus essential oils against *Aedes aegypti* (Diptera: Culicidae). *Parasitology Research*, **110**, 1675–1686.

Malakov P.Y., Papanove G.Y. (1996) A clerodanediterpenoid from *Scutellaria altissima*. *Phytochemistry*, **41**, 855.

Morawo T., Burrows M., Fadamiro H. (2016) Electroantennogram response of the parasitoid, Microplitis croceipes to host-related odors: The discrepancy between relative abundance and level of antennal responses to volatile compound. *F1000Research*, **5**, 2725.

Najar B., Pistelli L., Cervelli C., Fico G., Giuliani C. (2018) *Salvia broussonetii* Benth.: aroma profile and micromorphological analysis. *Natural Product Research*, **32**, 1660–1668.

Özçelik H., Ay G., Öztürk M. (1990) Some traditional plants of East and Southern Anatolia. Proceedings of the 10th National Symposium on Biology, Atatürk University, Erzurum, Turkey. 1-10.

Pignatti S. (2003) *Scutellaria* L. In: Flora d'Italia. Volume secondo, Edeagricole, Italy.

Pinto-Zevallos D.M., Bezerra R.H., Souza S.R., Ambrogi B.G. (2018) Species- and density-dependent induction of volatile organic compounds by three mite species in cassava and their role in the attraction of a natural enemy. *Experimental and Applied Acarology*, **74**, 261–274.

Qin Shu H (1994) Flora of China.,**17**, 75–103.

Radulović N., Stojanović G., Palić R. (2006) Composition and antimicrobial activity of *Equisetum arvense* L. essential oil. *Phytotherapy Research*, **20**, 85–88.

Revoltella S., Rainer B., Waltenberger B., Pagitz K., Schwaiger S., Stuppner H. (2019) HPTLC Autography Based Screening and Isolation of Mushroom Tyrosinase Inhibitors of European Plant Species. *Chemistry & Biodiversity*, **16**(3), e1800541.

Richardson I.B.K. (1972) Scutellaria. In: L. Tutin, T. G., Heywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walters, S. M., Webb, D. A. (Eds), *Flora Europaea*. Vol. **3**, Cambridge University Press, Cambridge, UK, 135–136 pp.

Rosselli S., Bruno M., Simmonds M.S.J., Senatore F., Rigano D., Formisano C. (2007) Volatile constituents of *Scutellaria rubicunda* Hornem subsp. *linnaeana* (Caruel) Rech. (Lamiaceae) endemic in Sicily. *Biochemical Systematics and Ecology*, **35**, 797–800.

Safikhani K., Jamzad Z., Saeidi H. (2018) Phylogenetic relationships in Iranian *Scutellaria* (Lamiaceae) based on nuclear ribosomal ITS and chloroplast *trnL-F* DNA data. *Plant Systematics and Evolution*, **304**, 1077–1089.

Seol G.H., Kang P., Lee H.S., Seol G.H. (2016) Antioxidant activity of linalool in patients with carpal tunnel syndrome. *BMC Neurology*, **16**, 17.

Shang X., He X., He X., Li M., Zhang R., Fan P., Zhang Q., Jia Z. (2010) The genus *Scutellaria* an ethnopharmacological and phytochemical review. *Journal of Ethnopharmacology*, **128**, 279–313.

Skaltsa H.D., Lazari D.M., Mavromati A.S., Tiligada E.A., Constantinidis T.A. (2000) Composition and antimicrobial activity of the essential oil of *Scutellaria albida* ssp. *albida* from Greece. *Planta Medica*, **66**, 672–674.

Skaltsa H.D., Lazari D.M., Kyriazopoulos P., Golegou S., Triantaphyllidis S., Sokovic M., Kypriotakis Z. (2005) Composition and antimicrobial activity of the essential oils of *Scutellaria sieberia* Benth. and *Scutellaria rupestris* Boiss. et Heldr. ssp. *adenotricha* (Boiss. et. Heldr.) Greuter et Burdet from Greece. *Journal of Essential Oil Research*, **17**, 232–235.

Sripathi R., Ravi S. (2017) Ethnopharmacology, phytoconstituents, essential oil composition and biological activities of the genus *Scutellaria*. *Journal of Pharmaceutical Sciences and Research*, **9**, 275–287.

Steen R., Norli H.R., Thöming G. (2019) Volatiles composition and timing of emissions in a moth-pollinated orchid in relation to hawkmoth (Lepidoptera: Sphingidae) activity. *Arthropod-Plant Interactions*, **13**, 581–592.

Stevens M.M., Faulder R.J., Mo J., Mudford E.M., Morris S.G. (2017) Attraction of *Parastethorus nigripes* and other insect species to methyl salicylate and (Z)-3-hexenyl acetate dispensers in a citrus grove and vineyard in south-eastern Australia. *Phytoparasitica*, **45**, 639–649.

Stevenson P.C. (2019) For antagonists and mutualists: the paradox of insect toxic secondary metabolites in nectar and pollen. *Phytochemistry Reviews*, **1–12**, https://doi.org/10.1007/s11101-019-09642-y.

Thaler I., Gailhofer M., Pfeifhofer H.W. (1992) Proteinkörper in Drüsenhaaren von *Scutellaria altissima* (Lamiaceae). *Phyton*, **31**, 263–280.

Werker E. (2000) Trichome diversity and development. In: Hallahan D. L., Gray J. C. (Eds), *Advances in Botanical Research. Plant Trichomes*. Academic Press, New York, USA, pp 1–35.

Williams L., Rodriguez-Saona C., Castle S.C., Zhu S. (2008) EAG-active herbivore-induced plant volatiles modify behavioral responses and host attack by an egg parasitoid. *Journal of Chemical Ecology*, **34**, 1190–1201.

Yilmaz G., Ciçek M., Demirci B., Başer H.C. (2019) Essential oil compositions of subspecies of *Scutellaria brevibracteata* Stapf from Turkey. *Journal of Essential Oil Research*, **31**, 255–262.

Yu J., Lei J., Yu H., Cai X., Zou G. (2004) Chemical composition and antimicrobial activity of the essential oil of *Scutellaria barbata*. *Phytochemistry*, **65**, 881–884.

Zhang X.M. (2018) Floral volatile sesquiterpenes of *Elsholtzia rugulosa* (Lamiaceae) selectively attract Asian honey bees. *Journal of Applied Entomology*, **142**, 359–362.

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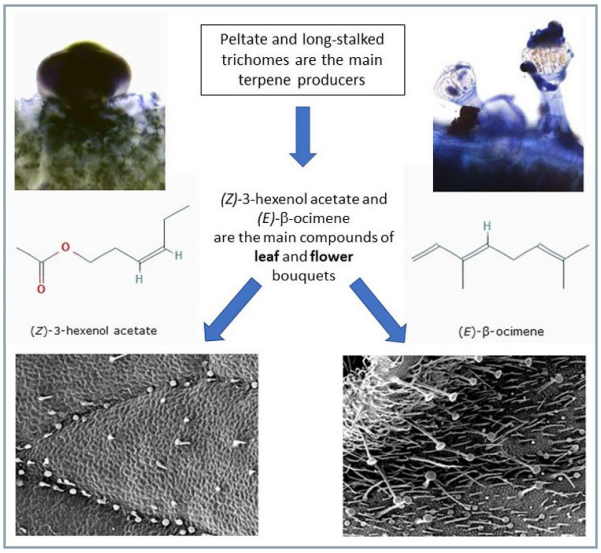

Two trichomes morphotypes, peltate and long-stalked capitate, are responsible for the production of the most abundant terpenes, i.e. (Z)-3-Hexenol acetate in the foliar, (E)-β-ocimene in the floral VOC profile.