

# Flora

## Scutellaria caucasica A. Ham.: morphological features and headspace characterization

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<b>Abstract:</b>	<p>In the context of a wide research project, a micromorphological and phytochemical characterization was performed on the vegetative and reproductive organs of <i>Scutellaria caucasica</i> A. Ham. (Lamiaceae), preserved at the Ghirardi Botanic Garden (Toscolano Maderno, BS, Lombardy, Italy). The morphological survey revealed the presence of both non-glandular and glandular trichomes. The latter belonged to three different morphotypes: peltate, short-stalked and long-stalked capitate. Histochemical assays demonstrated that the terpenes biosynthesis mainly took place in the peltates, while short-stalked capitates secreted only polysaccharides; the long-stalked ones mainly produced polysaccharides, coupled with terpene and polyphenolic fractions. An element of novelty was represented by the characterization of the VOC emission profile. Leaves and flowers showed differences in their emissions: the floral profile had a higher number of compounds than that of the leaves (37 vs 29), with a higher heterogeneity. The almost totality of the leaf profile is characterized by sesquiterpene hydrocarbons (98.76%), while the flowers presented a more varied composition, with sesquiterpene hydrocarbons (87.19%), monoterpenes (10.39% oxygenated, 1.82% hydrocarbons) and non-terpenes derivatives (0.58%). The most abundant compounds were <math>\gamma</math>-muurolene (42.57%) and <math>\beta</math>-caryophyllene (34.97%) in the leaves and in the flowers, respectively. In the flower headspace, 16 exclusive compounds were identified, among which germacrene D (31.65%) dominated; leaves had 8 exclusive compounds, with valencene (1.82%) as the most represented one. 21 common compounds were revealed: <math>\beta</math>-caryophyllene (34.12% leaves; 34.97% flowers), <math>\alpha</math>-humulene (3.01% leaves; 3.08% flowers), alloaromadendrene (2.43% leaves; 1.04% flowers), <math>\alpha</math>-copaene (2.10% leaves; 2.72% flowers) and <math>\beta</math>-copaene (2.17% leaves; 1.52% flowers) were the most abundant ones. <math>\gamma</math>-Muurolene relative abundances (42.57% leaves; 0.65% flowers) were very different between the two profiles. Overall, this work represented the first multidisciplinary study on <i>S. caucasica</i>, combining a scientific research approach with the policies of the Open Science.</p>
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## Highlights

- A multidisciplinary study approach was adopted for *S. caucasica* A. Ham.
- Morphological, histochemical and phytochemical investigations were performed.
- Three trichome morphotypes formed the glandular *indumentum* of leaves and flowers.
- The VOC profiles of leaves and flowers were characterized for the first time.

1 ***Scutellaria caucasica* A. Ham.: morphological features and headspace**  
2 **characterization**

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36 **Abstract**

37 In the context of a wide research project, a micromorphological and phytochemical  
38 characterization was performed on the vegetative and reproductive organs of *Scutellaria*  
39 *caucasica* A. Ham. (Lamiaceae), preserved at the Ghirardi Botanic Garden (Toscolano Maderno,  
40 BS, Lombardy, Italy). The morphological survey revealed the presence of both non-glandular  
41 and glandular trichomes. The latter belonged to three different morphotypes: peltate, short-  
42 stalked and long-stalked capitate. Histochemical assays demonstrated that the terpenes  
43 biosynthesis mainly took place in the peltates, while short-stalked capitates secreted only  
44 polysaccharides; the long-stalked ones mainly produced polysaccharides, coupled with terpene  
45 and polyphenolic fractions. An element of novelty was represented by the characterization of  
46 the VOC emission profile. Leaves and flowers showed differences in their emissions: the floral  
47 profile had a higher number of compounds than that of the leaves (37 vs 29), with a higher  
48 heterogeneity. The almost totality of the leaf profile is characterized by sesquiterpene  
49 hydrocarbons (98.76%), while the flowers presented a more varied composition, with  
50 sesquiterpene hydrocarbons (87.19%), monoterpenes (10.39% oxygenated, 1.82%  
51 hydrocarbons) and non-terpenes derivatives (0.58%). The most abundant compounds were  $\gamma$ -  
52 muurolene (42.57%) and  $\beta$ -caryophyllene (34.97%) in the leaves and in the flowers,  
53 respectively. In the flower headspace, 16 exclusive compounds were identified, among which  
54 germacrene D (31.65%) dominated; leaves had 8 exclusive compounds, with valencene  
55 (1.82%) as the most represented one. 21 common compounds were revealed:  $\beta$ -caryophyllene  
56 (34.12% leaves; 34.97% flowers),  $\alpha$ -humulene (3.01% leaves; 3.08% flowers),  
57 *alloaromadendrene* (2.43% leaves; 1.04% flowers),  $\alpha$ -copaene (2.10% leaves; 2.72% flowers)  
58 and  $\beta$ -copaene (2.17% leaves; 1.52% flowers) were the most abundant ones.  $\gamma$ -Muurolene  
59 relative abundances (42.57% leaves; 0.65% flowers) were very different between the two  
60 profiles.

61 Overall, this work represented the first multidisciplinary study on *S. caucasica*, combining a  
62 scientific research approach with the policies of the *Open Science*.

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64 **Keywords**

65 *Scutellaria caucasica* A. Ham., Glandular trichomes, Microscopy, VOC profile, HS-SPME.

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## 71 **1. Introduction**

72 The family Lamiaceae is widespread worldwide and includes 252 genera and 6800 species  
73 (Judd et al., 2009; De Oliveira et al., 2013). Due to their pleasant fragrances, produced in the  
74 glandular trichomes, many species are used in the herbal, food and cosmetic sectors (Akçin et  
75 al., 2011) and are widely employed in the folk medicine and as ornamentals (Baytop, 1999;  
76 Özdemir and Şenel, 2001).

77 *Scutellaria* L., or *Skullcap*, is a genus comprising about 350 species with a cosmopolitan  
78 distribution (Paton, 1990; Pool, 2006) and mainly found in the temperate areas. An important  
79 centre of diversity is represented by the Eurasian region (Minareci and Pekönür, 2017). The  
80 term *Skullcap* refers to a peculiar scale-shaped appendage of the calyx, called *scutellum*, which  
81 is formed by the folding of the upper lip and which comes off before fruiting (Paton, 1990;  
82 Minareci and Pekönür, 2017).

83 *Scutellaria caucasica* A. Ham. is an herbaceous plant native to the North Caucasus. It has  
84 sturdy stems, slightly curved, 10-32 cm long; green leaves, 0.8-3 cm long and 0.3-1.5 cm  
85 wide, ovate-oblong in shape with dentate margins; sturdy petiole, 0.2-2 cm long. It blooms in  
86 May-August and shows inflorescences from 3.5 cm to 6 cm long, elongated-ovoid or conical  
87 and dense; large and ovate bracts with entire margins; calyx 3 mm long; large corolla, from 3  
88 to 3.5 cm long and 8 mm wide at throat, yellow in colour, with lips approximately equal in  
89 length. The whole plant surface is characterized by the presence of trichomes (Komarov,  
90 1976).

91 In Asia, Europe and America, many species of *Scutellaria* are used as remedies in traditional  
92 medicine (Shang et al., 2010; Sripathi et al., 2017). For example, *S. orientalis* L. is used in  
93 case of constipation, as haemostatic, tonic and in wounds treatment in Anatolia (Yilmaz et al.,  
94 2019), *S. baicalensis* Georgi, thanks to the beneficial properties of its root, has been included  
95 in the Chinese, Japanese, Korean and European Pharmacopeia (Kosakowska, 2017) and *S.*  
96 *altissima* L. is a well-known plant in the Traditional Chinese Medicine, useful for the treatment  
97 of respiratory infections, pneumonia, bronchitis and in cases of hypertension (Bozov and Coll,  
98 2015; Grzegorzczak-Karolak et al., 2016; Gao et al., 2017;), hepatitis and cancer (Li and Wei,  
99 1994; Malakov and Papanove 1996; Sripathi and Ravi 2017). *S. caucasica* A. Ham. is known in  
100 the traditional American medicine against viral infections (Li et al., 2000). In addition, different  
101 uses are described for *Scutellaria* species coming from other regions of the World (Kosakowska  
102 2017; Sripathi et al., 2017; Irvin et al. 2019).

103 The commercial interest of the Lamiaceae is mainly related to the presence of glandular  
104 trichomes (Werker, 2006), responsible for the synthesis of natural bioactive compounds that  
105 display a crucial ecological role (Maffei, 2010; Giuliani et al., 2017a; Giuliani et al., 2017b;  
106 Giuliani et al., 2018). The literature proposed several morphological studies focused on the  
107 glandular *indumentum* of species belonging to *Scutellaria* (Giuliani and Maleci Bini, 2008;

108 Dereboylu et al., 2012; De Oliveira et al., 2013; Cali, 2017a, 2017b; Giuliani et al.,  
109 *Unpublished results* (a), (b)), however none of them referred to *S. caucasica*.  
110 Concerning the phytochemical state of the art, previous works on congeneric species reported  
111 the analysis of the essential oil composition (Rosselli et al., 2007; Cicek et al., 2011;  
112 Formisano et al., 2013; Kurkcuoglu et al., 2019; Yilmaz et al., 2019) and the characterization  
113 of the profiles of the volatile organic compounds (VOCs) (Takeoka et al., 2008, 2009; Giuliani  
114 et al., *Unpublished results* (a), (b)). Nevertheless, similar studies are lacking for *S. caucasica*,  
115 since the existing phytochemical literature only reported the isolation and NMR characterization  
116 of diterpenes (De La Torre et al., 1997; Bruno et al., 2000) and the isolation of typical  
117 flavonoids (Bandyukova and Boikova, 1969).  
118 Regarding the biological activity, no published study is reported about this species. On the  
119 contrary, studies were conducted on the pharmacological activity of some compounds isolated  
120 from congeneric species (Irvin et al., 2019).  
121 This work is part of a wider project entitled "Botanic Garden, factory of molecules", recently  
122 financed by the Lombardy Region (Italy). The primary goal of the project is to investigate a  
123 selected pool of species preserved at the Ghirardi Botanic Garden (Toscolano Maderno, BS,  
124 Lombardy, Italy), including *S. caucasica*, in order to: **1.** describe the morphological features  
125 and the distribution pattern of the glandular trichomes observed on the vegetative and  
126 reproductive organs by means of light and scanning electron microscopy; **2.** characterize the  
127 secretion products through histochemical tests; **3.** correlate the micromorphological  
128 investigation of the secreting structures with the productivity in secondary metabolites through  
129 the phytochemical characterization of VOCs spontaneously emitted by leaves and flowers.  
130 These results, along with those obtained by our research group in previous investigations on  
131 congeneric species, *i.e.* *S. brevibracteata* subsp. *subvelutina* (Giuliani et al., *Unpublished*  
132 *results* (a)) and *S. altissima* (Giuliani et al., *Unpublished results* (b)), will flow into the  
133 realization of novel iconographic devices devoted to the visitors of the Garden. In this way, the  
134 generic public will be able to learn updated details of the scientific research in an *Open Science*  
135 contest.

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## 144 **2. Materials and Methods**

### 145 **2.1 Plant material**

146 *Scutellaria caucasica* A. Ham. was cultivated at the Ghirardi Botanic Garden (Toscolano  
147 Maderno, BS, Lombardy, Italy) of the Department of Pharmaceutical Sciences of the University  
148 of Milan. Samplings were performed in June 2019. The samples were used for both the  
149 morphological and the phytochemical surveys on the vegetative and the reproductive organs.  
150 Voucher specimens were deposited in the Herbarium of the Ghirardi Botanic Garden under the  
151 identification codes GBG2019/016 and GBG2019/017.

### 152 **2.2 Micromorphological survey**

153 This survey was carried out in order to characterize the glandular and non-glandular  
154 *indumentum*, the distribution pattern of the trichomes and to evaluate the chemical nature of  
155 the secreted material using scanning electron microscopy (SEM) and light microscopy (LM).  
156 Various histochemical techniques were used to better locate the sites of synthesis and storage  
157 of the secondary metabolites, with special focus on volatiles.

#### 158 **2.2.1 Scanning Electron Microscopy (SEM)**

159 Plant material was firstly hand-prepared, by fixing it in 2.5 % glutaraldehyde in phosphate  
160 buffer (0.1 M, pH 6.8). Then, it was dehydrated in an ascending ethanol series up to absolute  
161 and then dried using a critical point dryer apparatus. The samples, previously mounting on  
162 aluminium stubs, were coated with gold and examined with a Philips XL 20 SEM operating at  
163 10 kV.

#### 164 **2.2.2 Light Microscopy (LM)**

165 Fresh and fixed samples were prepared. Fresh material was frozen and cryo-sectioned; other  
166 samples were fixed in FAA solution (formaldehyde:acetic acid:ethanol 70% = 5:5:90) for 7  
167 days, dehydrated in ascending ethanol series up to absolute, embedded in  
168 Technovit/Historesin and sectioned with an ultramicrotome. The following histochemical  
169 stainings were employed: Fluoral Yellow-088 for total lipids (Brundett et al., 1991), Nile Red  
170 for neutral lipids (Greenspan et al., 1985), Nadi reagent for terpenes (David and Carde, 1964),  
171 Ruthenium Red for acid polysaccharides (Jensen, 1962), Alcian Blue for mucopolysaccharides  
172 (Beccari and Mazzi, 1966), and Ferric Trichloride for polyphenols (Gahan, 1984). Control tests  
173 were performed at the same time. Observations were made with a Leitz DM-RB Fluo optical  
174 microscope.

### 175 **2.3 Phytochemistry**

#### 176 **2.3.1 Volatile Organic Compounds (VOCs)**

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

179 *HS-SPME Sample analysis* – The headspace sampling conditions were as reported in Ascrizzi et  
180 al. (2017). For the headspace samplings, Supelco SPME (Solid Phase Micro-Extraction) devices,



181 coated with polydimethylsiloxane (PDMS, 100  $\mu\text{m}$ ) were used; the same new fibre,  
182 preconditioned according to the manufacturer instructions, was employed for all the analyses.  
183 To ensure a stable temperature, samplings were conducted in an air-conditioned room at  $22 \pm$   
184  $1^\circ\text{C}$ ; this temperature was chosen to avoid the thermal damage of the plant material and,  
185 thus, any artificial-induced volatiles release. After 30 min of equilibration, the fibre was  
186 exposed to sample the headspace for 30 min. Both the equilibration and sampling times were  
187 experimentally determined to obtain an optimal adsorption of the volatiles, and to avoid both  
188 under- and over-saturation of the fibre and of the mass spectrometer ion trap. Once sampling  
189 was finished, the fibre was withdrawn into the needle and transferred to the injection port of  
190 the GC-MS system. Both the sampling and desorption conditions were identical for all the  
191 samples. Furthermore, blanks were performed before each first SPME extraction and randomly  
192 repeated during each series. Quantitative comparisons of relative peaks areas were performed  
193 between the same compounds in the different samples.

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212 **3. Results**

213 **3.1 Micromorphological investigation**

214 **3.1.1 Trichomes morphotypes and distribution pattern**

215 The *indumentum* of the vegetative and reproductive organs of *S. caucasica* showed both  
216 glandular and non-glandular trichomes on the overall epidermal surfaces (**Table 1, Figures 1**  
217 **a-l**). The glandular ones belonged to three main morphotypes: peltate, short capitate and long  
218 capitate (**Fig. 1**). The distribution pattern and abundance on the investigated plant parts are  
219 shown in **Table 1**.

220 The peltate trichome (**Figure 1 a**) occurred on both the leaf surfaces (**Table 1, Figures 1 d-**  
221 **f**) and on the abaxial sides of the bracts, calyx and corolla (**Table 1, Figures 1 g, i, k**). This  
222 morphotype consisted of a basal cell, a neck cell and a multicellular head surrounded by a wide  
223 storing chamber. Two types of capitate trichomes were observed: short-stalked and long-  
224 stalked capitate (**Figures 1 a-c**). The former were very abundant and scattered on the whole  
225 plant (**Table 1, Figure 1 f, g, i, k**) and consisted of a basal cell, a stalk cell and a 2-4 celled  
226 head.

227 The long capitates, only present on the inflorescences, particularly on the abaxial surfaces of  
228 sepals and petals (**Table 1, Figures 1 g-i, l**), possessed a multicellular head (5-6 cells) with a  
229 median, small subcuticular space (**Figures 1 b, c, h**); the length of the stalk resulted variable.  
230 The secretion was firstly accumulated in the subcuticular spaces and then flowed out along the  
231 stalk. The non-glandular hairs were multicellular, uniseriate, with acute apices; they were  
232 ubiquitous and their length was variable ranging from 1-2 cells on stems, leaves and bracts up  
233 to 5-7 cells on the abaxial side of calyx and corolla (**Figure 1 a-l**).

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235 **3.1.2 Histochemistry of the glandular trichomes**

236 The results of the histochemical investigation are reported in **Table 2** and **Figure 2**. The  
237 peltates secretion proved positive only to stainings specific for lipophilic substances, showing in  
238 particular an intense positive response to Nadi reagent (**Table 2, Figure 2 a**), indicating the  
239 peculiar secretion of terpenes. The histochemical stainings indicated the exclusive production of  
240 acid polysaccharides (**Table 2, Figure 2 b**) for the short-stalked capitate morphotype. The  
241 histochemical assays on the long-stalked capitate showed mainly polysaccharidic with terpenic  
242 and polyphenolic fractions (**Table 2, Figures 2 c-e**).

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## 3.2 Phytochemical investigation

### 3.2.1 VOCs emission profile

The VOC emission profile of *S. caucasica* revealed a total of 45 different compounds. 29 compounds were identified in the foliar profile, while 37 in the floral one (**Table 3**).

The sesquiterpene hydrocarbons accounted for almost the totality of the leaf profile (98.76%), followed by the oxygenated sesquiterpenes (0.90%). Monoterpenes and non-terpene derivatives were not detected.  $\gamma$ -Muurolene (33, 42.57%) dominated, followed by  $\beta$ -caryophyllene (22, 34.12%). Among all the other compounds, only  $\alpha$ -humulene (29, 3.01%), *alloaromadendrene* (31, 2.43%),  $\beta$ -copaene (23, 2.17%) and  $\alpha$ -copaene (17, 2.10%) exhibited relative concentrations higher than 2%. Eight exclusive compounds were characterized, among which valencene (36, 1.82%) was the most abundant one. The other exclusive compounds were present in percentages lower than 1.0% or in traces.

The floral profile was dominated by the sesquiterpene hydrocarbons (87.19%), followed by oxygenated monoterpenes (10.39%), monoterpene hydrocarbons (1.82%) and non-terpene derivatives (0.58%). Oxygenated sesquiterpenes were not detected. The most abundant compound was  $\beta$ -caryophyllene (22, 34.97%), followed by germacrene D (34, 31.65%), 1,8-cineole (3, 8.00%),  $\alpha$ -humulene (29, 3.08%), bicylogermacrene (37, 2.89%) and  $\alpha$ -copaene (17, 2.72%). The other compounds were present with relative amounts lower than 2.0%. 16 exclusive compounds were identified, among which the above-mentioned major compounds (34, 3, 37). The others occurred with relative abundances lower than 2.0%.

21 common compounds were detected. The compounds found in higher relative contents in both the organs were  $\beta$ -caryophyllene (22, 34.12% leaves; 34.97% flowers), followed by  $\alpha$ -humulene (29, 3.01% leaves; 3.08% flowers), *alloaromadendrene* (31, 2.43% leaves; 1.04% flowers),  $\alpha$ -copaene (17, 2.10% leaves; 2.72% flowers) and  $\beta$ -copaene (23, 2.17% leaves; 1.52% flowers).  $\gamma$ -Muurolene (33) displayed a higher relative abundance in the leaves (42.57%) than in the flowers (0.65%). A similar pattern, with less noticeable differences in relative abundance, was found for  $\beta$ -cubebene (19, 1.28% leaves; 0.66% flowers) and *cis*-muurolo-4(14),5-diene (32, 1.31% leaves; 0.91% flowers). The remaining common compounds showed comparable percentages in the two profiles (<2.0%).

#### 282 4. Discussion

283 The *indumentum* of the vegetative and reproductive organs of *S. caucasica* showed a high  
284 level of consistency for both morphology and distribution pattern in all the examined  
285 replicates. The glandular trichomes were numerous and belonged to the two main types  
286 occurring in the family Lamiaceae: peltate and capitate (Werker, 2006; Giuliani et al., 2017a,  
287 Giuliani et al., 2018). The peltate ones occurred on both the vegetative and reproductive  
288 organs, as documented in other *Scutellaria* species (Giuliani and Maleci Bini, 2008; Dereboylu  
289 et al., 2012; De Oliveira et al., 2013; Giuliani et al., *Unpublished results* (a), (b)). The capitate  
290 morphotype was distinguished in short-stalked and long-stalked capitate, already described in  
291 other works on congeneric species (Giuliani and Maleci Bini, 2008; Giuliani et al., *Unpublished*  
292 *results* (a), (b)) and presented a different distribution pattern. The short-stalked capitates,  
293 widespread in all the members of the Lamiaceae family (Hallahan, 2000), were evenly  
294 distributed on the entire epidermal surface of the plant and were particularly abundant on the  
295 abaxial surfaces of the leaves and corolla. On the contrary, the long-stalked capitates were  
296 typical of the reproductive organs, as described in several members of the Lamioideae  
297 subfamily (Giuliani and Maleci Bini, 2008) and as already documented by our research group in  
298 *S. brevibracteata* subsp. *subvelutina* and *S. altissima* (Giuliani et al., *Unpublished results* (a),  
299 (b)). The histochemical tests were performed to localize *in situ* the main compound classes of  
300 metabolites present in plant secretions and were widely used to accurately describe the  
301 glandular trichomes of many Lamiaceae species (Giuliani and Maleci Bini, 2008; Giuliani et al.,  
302 *Unpublished results* (a), (b)). As regards to the composition of the secreted material, each  
303 trichome type was generally characterized by a single or by a prevailing kind of secretion, as  
304 already reported in devoted reviews by Hallahan (2000) and Werker (2000) (see literature  
305 therein). Peltate trichomes are generally considered typical producers of terpenes; capitate  
306 trichomes produce generally a more complex secretion of both hydrophilic and lipophilic  
307 fractions, in which polysaccharides prevail. In *S. caucasica*, the composition of the secreted  
308 material was clearly related to the trichome type. Indeed, the histochemical tests revealed that  
309 the peltate hairs were the main sites of production and accumulation of terpenes, in  
310 consistency with the results on *S. altissima* (Giuliani et al., *Unpublished results* (b)). On the  
311 contrary, in *S. brevibracteata* subsp. *subvelutina* the secretion product of the peltates  
312 appeared more complex because of the contemporary synthesis of polyphenols and flavonoids  
313 (Giuliani et al., *Unpublished results* (a)). The short capitates were exclusive polysaccharide  
314 producers, as well as in *S. altissima* and *S. brevibracteata* subsp. *subvelutina* (Giuliani et al.,  
315 *Unpublished results* (a), (b)). The long capitate exhibited, as in the two congeneric species, a  
316 more complex secretion of both hydrophilic and lipophilic substances, in which terpenes  
317 represent a minor fraction. Therefore, it can be postulated that in *S. caucasica* the productivity  
318 in volatile substances depended exclusively on the peltates on stems, leaves and bracts and on  
319 the synergistic action of peltates and long capitates on calyces and corollas. However, given

320 the greater productivity of the peltates compared to that of the long-stalked capitates, a  
321 feature that can be directly correlated to the presence of a wider storing chamber in the  
322 former, the peltates were confirmed as the main producers of terpenes on the reproductive  
323 organs.

324 Concerning the phytochemical survey, the characterization of the VOC emission profiles  
325 represents an element of novelty for *S. caucasica*. A high level of variability was recorded  
326 between leaves and flowers. In fact, the latter presented a higher number of compounds  
327 compared to the former (37 vs 29) and exhibited greater heterogeneity in the compound  
328 classes. Indeed, the floral profile showed the presence of sesquiterpenes (hydrocarbons  
329 87.19%), monoterpenes (oxygenated 10.39%; hydrocarbons 1.82%) and non-terpene  
330 substances (0.58%). On the contrary, the leaves only emitted sesquiterpenes, with a clear  
331 preponderance of hydrocarbons (98.76%) over oxygenated derivatives (0.90%). Another  
332 distinctive element was represented by the principal compounds: the foliar profile presented  $\gamma$ -  
333 muurolene (33, 42.57%) and  $\beta$ -caryophyllene (22, 34.12%) as the most abundant  
334 compounds, while the floral profile had  $\beta$ -caryophyllene (22, 34.97%) and germacrene D (34,  
335 31.65%) as the most represented ones. Germacrene D (34) and  $\gamma$ -muurolene (33) can be  
336 considered the representative compounds of the two profiles: the former was absent in the  
337 leaves, while the latter showed scarce abundance in the flowers (0.65%). Moreover, the  
338 flowers had a number of exclusive compounds twice as many those of the leaves (16 vs 8).  
339 Among the former, germacrene D (34, 31.65%), 1,8-cineole (3, 8.00%) and  
340 bicyclogermacrene (37, 2.89%) dominated, among the latter valencene (36, 1.82%). Twenty-  
341 one common compounds were identified:  $\beta$ -caryophyllene (22, 34.12% leaves; 34.97%  
342 flowers) was the most abundant one, followed by  $\alpha$ -humulene (29, 3.01% leaves; 3.08%  
343 flowers), *alloaromadendrene* (31, 2.43% leaves; 1.04% flowers),  $\alpha$ -copaene (17, 2.10%  
344 leaves; 2.72% flowers) and  $\beta$ -copaene (23, 2.17% leaves; 1.52% flowers). Making a  
345 comparison with previous investigations, a higher degree of homogeneity was shown  
346 compared to the *S. brevibracteata* subsp. *subvelutina* volatile emission profiles: the floral  
347 profile was more complex than the foliar one; sesquiterpenes hydrocarbons was the  
348 representative compound class in both profiles;  $\beta$ -caryophyllene was the most abundant  
349 compound both in leaves and flowers. Conversely, concerning these aspects, *S. altissima*  
350 presented a foliar profile more complex than the floral one: the former was dominated by non-  
351 terpene substances, the latter was almost totally constituted by monoterpenes hydrocarbons.  
352 In addition, the main compounds were different: (*Z*)-3-hexenol acetate in the leaves, (*E*)- $\beta$ -  
353 ocimene in the flowers; however,  $\beta$ -caryophyllene appeared among the common compounds  
354 (Giuliani et al., *Unpublished results* (a), (b)).

355 Regarding the ecological role of the most abundant exclusive compounds of the floral profile,  
356 they all contribute to a defensive action. In particular, germacrene D (34) develops a  
357 protective and fly-killing role (Kiran and Devi, 2007; Birkett et al., 2008); 1,8-cineole (3)  
358 shows an acaricidal (Hu et al., 2015), fumigant and larvicidal effect (Lucia et al., 2012); for

359 bicyclogermacrene (37), a larvicidal activity towards *Aedes aegypti* L. larvae, developed  
360 through a synergistic action with germacrene D (34) and  $\beta$ -caryophyllene (22) is reported  
361 (Dória et al., 2010). However, germacrene D (34) and 1,8-cineole (3) also exert an attractive  
362 role, together with  $\beta$ -caryophyllene (22) and  $\alpha$ -humulene (29) (Cha et al., 2008; Nelson and  
363 Jackson, 2013). The major exclusive compounds of the foliar profile express a protective  
364 action. Indeed, this type of activity is ascribed to the sesquiterpene hydrocarbons, to which  
365 valencene (36) and  $\gamma$ -muurolene (33) belong to (Chizzola 2013). In particular, pesticide  
366 activity is recognized in valencene derivatives (Panella et al., 2005). Referring to the ecological  
367 role of the common compounds, the promiscuous action of  $\beta$ -caryophyllene (22) dominates. In  
368 fact, several studies assign to this compound an attraction function towards pollinators,  
369 sometimes realized in synergy with  $\alpha$ -humulene (29) (Abraham et al., 2018; Zhang 2018) and  
370 germacrene D (34) (Cha et al., 2008), as well as a defensive role against parasites and  
371 herbivores (Curtois et al., 2012; Köllner et al., 2013; Feng et al., 2017). Finally,  
372 *alloaromadendrene* (31), together with  $\beta$ -caryophyllene (22) and  $\alpha$ -copaene (17), display  
373 larvicidal action (Senthilkumar et al., 2008; Costa et al., 2011). On these bases, it is possible  
374 to affirm that in *S. caucasica* a protective action is predominantly associated to the leaves,  
375 thanks to the dominant presence of sesquiterpene hydrocarbons in their profile. On the  
376 contrary, an attractive role is assigned to the flowers due to the exclusive and abundant  
377 percentage of germacrene D. Nevertheless, given the co-occurrence of  $\beta$ -caryophyllene in both  
378 the vegetative and reproductive emissions, both leaves and flowers act together to play a  
379 protective and attractive role. The differentiation of the volatile emission profiles based on the  
380 organ function is, indeed, reported in the literature (Ascrizzi et al, 2016). If we examine the  
381 other main chemical classes occurring in the secretory products of the glandular trichomes,  
382 previous works revealed that the presence of superficial hydrophilic secretions on plant  
383 epidermis appeared to have the function of protecting the organs against desiccation,  
384 maintaining a balanced water status, especially at early stages of expansion (Ascensão, et al.,  
385 1999; Huang et al., 2008). Considering the polyphenols, these molecules are known as  
386 powerful antioxidants and protein complexing agents (Romani et al., 2014), with this latter  
387 activity particularly expressed in plant-herbivorous interactions (Haslam, 1988). Indeed, the  
388 presence of polyphenols in plant exudates is related to the main role of increasing the  
389 resistance to herbivores, by repelling or poisoning the phytophagy (Werker, 2000).

390

#### 391 **4.1 Conclusions**

392 This multidisciplinary approach represents a further step in the study of species belonging to  
393 *Scutellaria* genus and, overall, in the characterization of the species collected at the G. E.  
394 Ghirardi Botanic Garden (Toscolano Maderno, Lombardy, Italy), totally dedicated to medicinal  
395 plants. The set of information concerning the chemical nature of the emitted volatile  
396 substances may finally contribute to make hypothesis on the biotic interactions established by

397 the examined species, thus constituting the basis for future insights on the ecological roles of  
398 the secondary metabolites.

399

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402

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404

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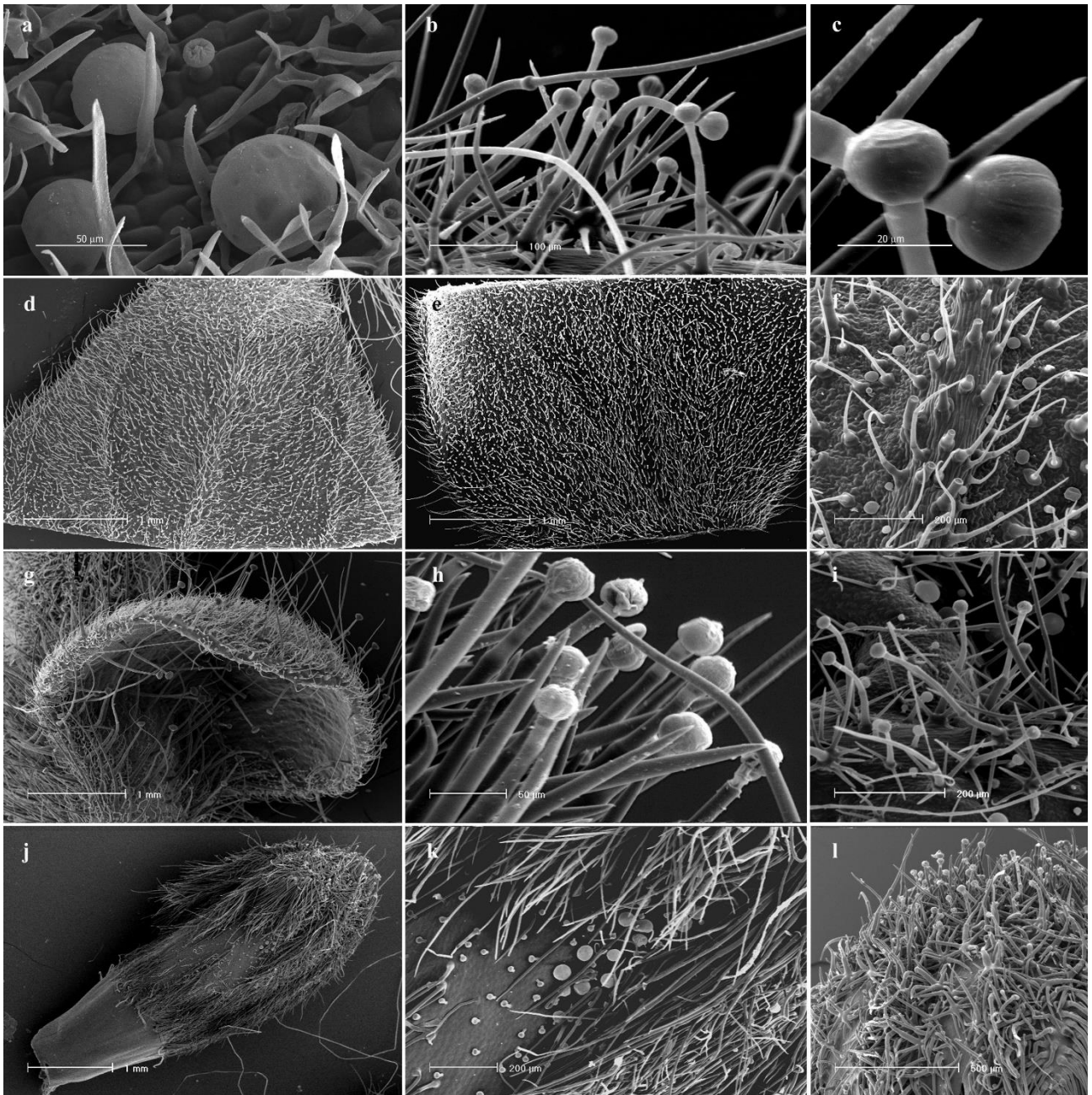
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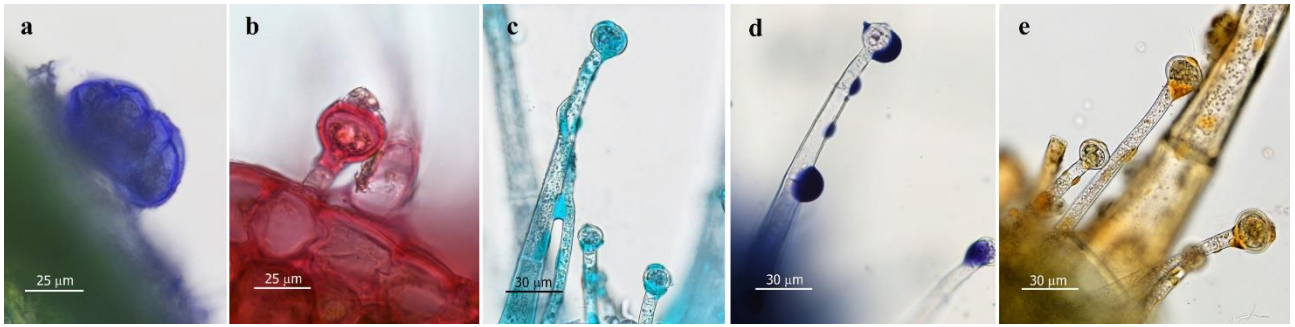
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600 **Figure 2.**



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628 **Table 1.** Distribution pattern of the glandular trichomes in *Scutellaria caucasica* A. Ham.

Trichome type	Stem	Leaf		Bract		Calyx		Corolla	
		adax	abax	adax	abax	adax	abax	adax	abax
<b>peltate</b>	±	+	+	-	+	-	+	-	+
<b>short capitate</b>	+	+	++	+	+	+	+	+	++
<b>long capitate</b>	-	-	-	-	+	-	+	-	+

629 Symbols: (-) missing, (±) sporadic, (+) present, (++) abundant

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658 **Table 2.** Results of the histochemical tests on the glandular trichomes in *Scutellaria caucasica*  
 659 A. Ham.

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

660 Symbols: (–) negative response; (+) positive response; (++) intently positive response

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689**Table 3.** HS-SPME profiles of the leaves and flowers of *Scutellaria caucasica* A. Ham.

	I.r.i. <sup>a</sup>	Compounds	Relative Abundance (%)	
			Leaves	Flowers
1	993	myrcene	- <sup>b</sup>	0.33
2	1032	limonene	-	tr <sup>c</sup>
3	1034	1,8-cineole	-	8.00
4	1052	( <i>E</i> )- $\beta$ -ocimene	-	1.49
5	1140	nopinone	-	0.15
6	1143	camphor	-	0.12
7	1178	4-terpineol	-	0.56
8	1187	( <i>Z</i> )-3-hexenyl-butyrate	-	0.43
9	1204	decanal	-	0.15
10	1241	methyl carvacrol	-	1.40
11	1259	linalool acetate	-	0.16
12	1340	$\delta$ -elemene	0.24	0.59
13	1351	$\alpha$ -cubebene	0.27	0.24
14	1368	cyclosativene	0.13	-
15	1372	$\alpha$ -ylangene	tr	-
16	1373	longicyclene	-	0.14
17	1376	$\alpha$ -copaene	2.10	2.72
18	1384	$\beta$ -bourbonene	0.83	0.72
19	1390	$\beta$ -cubebene	1.28	0.66
20	1410	$\alpha$ -gurjunene	0.42	0.19
21	1416	<i>cis</i> - $\alpha$ -bergamotene	0.37	tr
22	1420	$\beta$ -caryophyllene	34.12	34.97
23	1429	$\beta$ -copaene	2.17	1.52
24	1432	$\beta$ -gurjunene	0.40	0.48
25	1439	$\alpha$ -guaiene	-	0.47
26	1441	aromadendrene	0.60	-
27	1447	<i>cis</i> -muurola-3,5-diene	0.20	0.16
28	1454	<i>trans</i> -muurola-3,5-diene	0.11	-
29	1456	$\alpha$ -humulene	3.01	3.08
30	1460	sesquisabinene	-	tr
31	1461	<i>allo</i> aromadendrene	2.43	1.04
32	1462	<i>cis</i> -muurola-4(14),5-diene	1.31	0.91
33	1477	$\gamma$ -muurolene	42.57	0.65
34	1482	germacrene D	-	31.65
35	1491	<i>trans</i> -muurola-4(14),5-diene	0.38	0.33
36	1492	valencene	1.82	-
37	1495	bicyclogermacrene	-	2.89
38	1498	$\alpha$ -muurolene	0.45	0.44
39	1507	( <i>E,E</i> )- $\alpha$ -farnesene	1.04	1.52
40	1513	<i>trans</i> - $\gamma$ -cadinene	0.86	0.61
41	1524	$\delta$ -cadinene	1.11	1.03
42	1534	cadina-1,4-diene	0.25	-
43	1538	$\alpha$ -cadinene	0.29	0.18
44	1575	germacrene D-4-ol	0.64	-
45	1581	caryophyllene oxide	0.26	-
		<b>Monoterpene hydrocarbons</b>	-	<b>1.82</b>
		<b>Oxygenated monoterpenes</b>	-	<b>10.39</b>
		<b>Sesquiterpene hydrocarbons</b>	<b>98.76</b>	<b>87.19</b>
		<b>Oxygenated sesquiterpenes</b>	<b>0.90</b>	-
		<b>Non-terpenes derivatives</b>	-	<b>0.58</b>
		<b>Total</b>	<b>99.66%</b>	<b>99.98%</b>

<sup>a</sup> Linear retention indices on a DB-5 capillary column; <sup>b</sup> Not detected; <sup>c</sup> Traces, <0.1%.

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692 **Captions to Figures**

693 **Figure 1.** SEM micrographs showing distribution and types of trichomes of *Scutellaria*  
694 *caucasica* A. Ham. (a). Peltate and short-stalked capitate trichomes. (b) Long-stalked capitate  
695 trichomes. (c) Particular of the multicellular head of the long-stalked capitate trichome. (d, e)  
696 Leaf abaxial (d) and adaxial (e) surfaces with simple non-glandular hairs, peltate and short-  
697 stalked capitate trichomes. (f) Particular of the leaf abaxial surface with peltate, short-capitate  
698 and simple non-glandular trichomes. (g) Particular of the calyx at the skullcup. (h) Details the  
699 long capitates; notice the secreted material on the head surfaces. (i) Particular of the calyx  
700 abaxial surface exhibiting scanty peltates, short capitates and abundant long capitates. (j)  
701 General view of a floral bud. (k) Particular of the corolla abaxial surface at the median region  
702 with abundant long simple trichomes, peltates and short capitates. (l) Particular of the corolla  
703 abaxial surface at the distal region with abundant long simple trichomes and long capitates.

704 **Figure 2.** Histochemistry of the glandular trichomes of the vegetative and reproductive organs  
705 of *Scutellaria caucasica* A. Ham. (a) Peltate trichome: Nadi reagent. (b) Short-stalked capitate  
706 trichome: Ruthenium Red. (c-e) Long-stalked capitate trichomes: capitate Trichome: Alcian  
707 Blue (c), Nadi reagent (d),  $\text{FeCl}_3$  (e).

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