A new study approach in the context of Open Science: The case of *Scutellaria altissima*L.

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A new study approach in the context of Open Science: The case of *Scutellaria altissima* L.

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Scutellaria altissima: An Open Science study approach.

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Keywords

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One-sentence summary

Micromorphological and phytochemical investigations were conducted on *Scutellaria altissima* L. within a multidisciplinary Open Science project.

Abstract

- Within an Open Science project, a 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 peculiar productivity. This work reported the volatile organic compound (VOC) profiles of leaves and flowers and the composition of the essential oil (EO) obtained from the aerial parts of plants cultivated in Italy, never described before.
- Three morphotypes of glandular trichomes were observed: peltates, short-stalked capitates, long-stalked capitates. Peltates were the main producers of terpenes, short-stalked capitates of polysaccharides, long-stalked capitates of terpenes and polyphenols. The leaf VOC profile showed a 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*vs*11) and exclusive compounds (33*vs*7). (*Z*)-3-Hexenol acetate was the most abundant in the leaf, (*E*)-β-ocimene in the flower. Four common compounds were detected: β-pinene, β-caryophyllene, γ-muurolene, germacrene-D. The EO showed 21 compounds, dominated by β-caryophyllene, linalool and hexahydrofarnesyl acetone.
- This research allowed us to correlate the morphotypes of the secreting structures with the production of secondary metabolites, with the aim to propose to the public of the Ghirardi Botanic Garden a dedicated iconographic apparatus, which accounts for the olfactory perception linked to *S. altissima*.

Introduction

Scutellaria L. is a genus belonging to the Lamiaceae family and it includes approximately 300 species, commonly known as skullcaps (Formisano et al., 2013; Sripathi and Ravi, 2017). *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 bracts length is lower than those of the flowers, the calyx is sub-glabrous and the corolla is 10-14 mm long, blue-violet in colour.

For thousands of years, species belonging to *Scutellaria* genus were largely employed in traditional medicine (Duke 1986). Calming, haemostatic and tonic properties are referred to the infusion of the leaves in East Anatolia (Özçelik and Öztürk, 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 and Wei, 1994; Malakov and Papanove 1996; Sripathi and 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, that play a crucial role in mediating the plant-environment relationships (Giuliani et al., 2018; Najar et al., 2018; Giuliani et al., 2017a; Giuliani et al., 2017b). The literature proposes some morphological studies concerning the secretory structures in *Scutellaria* species (Giuliani and Maleci Bini, 2008; Dereboylu et al., 2012), none of them referring to *S. altissima*.

Concerning the phytochemical state of the art, there are few works on the essential oil (EO) characterization of *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* coming from Turkey (Kurkcuoglu et al., 2019), while studies on the volatile organic compound (VOC) emission profiles are lacking.

Moreover, there are no contributions about the existing connection between the production/emission of these secondary metabolites and their ecological role.

Referring to the biological activity, works on the EOs are lacking. However, the antioxidant action of the ethanol extract of the aerial parts and roots is known (Grzegorczyk-Karolak et al., 2019), along 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 and Georgieva, 2017; Gao et al., 2017; Jia et al., 2019).

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*, under a new vision of the plant, beyond what it is macroscopically visible, in order to: **1.** describe the morphology and the distribution pattern of the glandular trichomes on the vegetative and reproductive organs; **2.** characterize the secretion products through some histochemical assays; **3.** correlate the micromorphological investigation on the secretory structures to the secondary metabolites biosynthesis through the phytochemical characterization of the VOC profiles spontaneously emitted from leaves and flowers and the analysis of the EO obtained from the aerial parts. The present investigation was conducted in order to transfer a correct scientific knowledge to the public by means of a new specifically designed iconographic apparatus.

Materials and methods

Plant material

Scutellaria altissima L. was cultivated at the Ghirardi Botanic Garden (Toscolano Maderno, BS) of the Department of Pharmaceutical Sciences of the University of Milan. The samplings for the micro-morphological and phytochemical (VOCs 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 microscopy (LM) and scanning electron microscopy (SEM). At least ten replicates, similar for 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.

LM - Fresh and fixed material was 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 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 and Carde, 1964), Ruthenium Red for acid polysaccharides (Jensen, 1962), Alcian Blue for mucopolysaccharides (Beccari and Mazzi, 1966), and Ferric Trichloride for polyphenols (Gahan, 1984). Control procedures were carried out for each of the employed histochemical staining. Observations were made with a Leitz DM-RB Fluo optic microscope.

SEM - 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 in ascending grades up to absolute and then critical-point dried. The samples, mounted on stubs and coated with gold, were observed with a Philips XL-20 SEM.

Phytochemical investigation

Volatile Organic Compounds (VOCs)

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

HS-SPME Sample analysis – The headspace sampling conditions were as reported in Ascrizzi et al. (2017). For the headspace samplings, Supelco SPME (Solid Phase Micro-Extraction) devices, coated with polydimethylsiloxane (PDMS, 100 μm) were used; 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 the thermal damage of the plant material and, thus, any artificial-induced volatiles release. After 30 min of equilibration, the fibre was exposed to sample the headspace for 30 min. Both the equilibration and sampling times were experimentally determined to obtain an 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.

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

EO separation was performed by 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 till 60 °C, then 6 °C/min from 60 °C to 160 °C and finally 20 °C/min from 160 °C to 280 °C. Injector and detector temperatures were set to 280°C; carrier gas He, flux 1 ml/min: the mass range detected was 50-500 m/z. EO were analyzed pure or diluted 1:100 with *n*-hexane, with injection volume of 1 μl.

Mass spectra were analyzed by Wiley Mass spectra Library, NIST Mass Spectral Search Program e 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 ones 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**.

The peltate trichome consisted of one basal cell, one short unicellular stalk, and one multicellular globose head with a wide storing chamber (**Fig. 1a**). The short capitate trichome was composed by one basal cell, one stalk cell and by an elliptical 2-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-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. 2 a-h**). They occurred on all the 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 peltates and short capitates. The leaf showed the same trichome types uniformly distributed on the abaxial side, whereas on the adaxial one only short capitates were observed along the veinal system (**Fig. 2a, b**). The bract was characterized by a similar distribution pattern, except for the presence of long capitates along the edges (**Fig. 2c, d**).

The calyx presented all the trichome types on the abaxial side and only short capitate on the adaxial one (**Fig. 2e, f**). The corolla exhibited abundant peltate and short capitate trichomes on the tube and on the lower and upper lips (**Fig. 2g**); at the distal region of the lower lip, long capitate were observed, as well. The corolla adaxial presented only short capitate.

Histochemistry - The results of the histochemical investigation are reported in **Table 2** and **Figure 3**. Lipophilic dyes gave 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 capitates, only muco-polysaccharides were produced, as indicated by the positive response following the application of Alcian Blue (**Fig. 3 c**). The long capitates were characterized by a complex secretion due to the occurrence of both terpenoidic and polyphenolic fractions (**Fig. 3d, e**).

Phytochemical investigation

VOCs - 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 one (**Table 3**).

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%). 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 lower than 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%). 7 exclusive compounds were identified, among which there were the 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 lower than 1.0%.

The profiles revealed 4 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).

***EO* -** The EO composition is reported **Table 4**: 21 total 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 lower than 2.0%.

Discussion

S. altissima showed the two main types of glandular trichomes widespread in the Lamiaceae family: peltates and capitates (Werker, 2000; Giuliani et al., 2017a; Giuliani et al., 2018; Najar et al., 2018). The former were present on the whole plant surface, in accordance with what was already documented for other *Scutellaria* species (Giuliani and 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 capitates and long-stalked capitates, with a different distribution pattern. 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. On the contrary, the long-stalked trichomes were recorded only on the abaxial surfaces of the bract and the calyx and on the distal portion of the lower lip of the corolla. These observations on the peltates and short-stalked capitates are consistent with the literature data (Dereboylu et al., 2012; Giuliani et al., 2017a) and with the results on *S. brevibracteata* subsp. *subvelutina* by our research group (Fico G., *personal observation)*; the medium-stalked capitates were not observed on *S. altissima*, while the long-stalked hairs with a multicellular head were confirmed as exclusive of the reproductive organs. However, the secretory heads exhibited different features; indeed, in *S. altissima* each secreting cell displayed a single small subcuticular space, in accordance with what was already known for *S. galericulata* (Giuliani and Maleci Bini, 2008). On the contrary, in *S. brevibracteata* subsp. *subvelutina* this morphotype showed a head with a subcuticular space common to all the secreting cells and located in central position (Fico G., *personal observation*).

Besides the morphological investigation, a histochemical survey was carried out for the first time on *S. altissima*. It revealed that the activity of the peltate hairs was characterized by the exclusive production of lipophilic substances, in particular terpenes. The short-stalked capitates showed a positive response only to the hydrophilic dyes, among which the Alcian Blue assay, specific for mucopolysaccharides. The long-stalked capitates displayed a more complex secretion, due to the occurrence of both terpenes and polyphenols. The comparison of the histochemical results with the different distribution pattern of the trichomes allowed us to hypothesize the existence of a synergy in terpene production between the peltates and the long-stalked capitates, only on the abaxial surfaces of the bract, calyx and the distal portion of the lower lip of the corolla, while the peltates were the main producers of lipophilic substances on the stem, leaf and the remaining corolla surfaces. On these bases, considering both the ubiquitous distribution and the wide storing chamber, the peltates played a dominant role in the EO production in the investigated species, confirming the literature data concerning different members of the Lamiaceae family (Hallahan, 2000; Werker, 2000). On the other hand, the short-stalked capitates were responsible for the biosynthesis of polysaccharides on all the examined organs, with particular regard to 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 positivity.

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. Firstly, the leaf profile resulted much more complex than that of the flowers due to the presence of a higher number of compounds, 37 and 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%); 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 with the histochemical data. In fact, the dominance of terpene derivatives in the flowers could be related to the synergy of action between peltates and long-stalked capitates in the productivity process of these substances, in particular at the bract and calyx level, to which the major secretion of peltates on the corolla could be added. On the contrary, peltates were the only producers of terpenes on the leaves. Another distinctive element between the two emission profiles was represented by the exclusive compounds: 33 in the leaves and 7 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 of 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).

As 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 insect 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 plant-herbivores-parasites (Stevens et al., 2017). (*E*)-Geranyl acetone (*34*) and (*Z*)-3-hexenyl isovalerate (*24*) contribute to the protective role (Pinto-Zevallos et al., 2018; Morawo et al., 2016; Heil et al., 2008). 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), to which also an attractive power is recognized (Stevenson, 2019). On the other hand, specific studies concerning the remaining major exclusive compounds (> 2.0%) are lacking. With regards 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 attractors for pollinators (Steen et al., 2019; Jayanthi et al., 2012). However, (*E*)-β-ocimene (*12*) is also involved in tritrophic protective mechanisms (Ghosh and Venkatesan, 2019) together with myrcene (*5*), showing an allelopathic defence functions (Hsiung et al., 2013). Referring to the common compounds, β-pinene (*3*), β-caryophyllene (*31*) and germacrene D (*37*) (Lobo et al., 2019; Zhang 2018; Abraham et al., 2018; Birkett et al., 2008) may contribute to the overall defence action; anyway, an attractive role is also recognized to β-caryophyllene (*31*) (Abraham et al., 2018). γ-Muurolene (*36*), in turn, is a common repellent compound as well as most sesquiterpenes hydrocarbons, a chemical class particularly active in mediating defence mechanisms (Chizzola 2013).

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

In addition, this work reported the EO characterization of plants grown in Italy in different environmental conditions than 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 ones (Kurkcuoglu et al., 2019). In our samples, oxygenated monoterpenes (26.47%) resulted the main compound class, followed by sesquiterpene hydrocarbons (24.63%), that 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 for these samples and the Turkish ones, respectively. Among the main compounds of the Turkish EO, it is important to note the presence of hexadecanoic acid, totally absent in our profile, as it 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 geographical origin of the analysed samples.

Concerning the biological activity of the EO, previous contributions are lacking. Nevertheless, some evaluations are possible referring to the biological activity ascribed to the EO major compounds. In particular, as an example, some works reported 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, with improved response to anticancer drugs (Di Giacomo et al., 2019). In the case of linalool (*8*), anti-inflammatory and antioxidant actions were 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 were 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 + and Gram – bacterial strains by an *Equisetum arvense* L. EO mainly rich in this apocarotenoid.

This multidisciplinary approach of investigation, according to the aim 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 the productivity in volatile molecules. Moreover, the overall set of information concerning the chemical nature of the emitted volatile substances may finally contribute to make hypothesis on the biotic interactions established by the examined species, thus constituting the basis for future insights on the ecological roles of the secondary metabolites. The characterization of the essential oil profile might be useful to evaluate its potential biological activity.

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

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Figure Legend

Figure 1 a-c. Glandular trichome morphotypes in *Scutellaria altissima* L., LM. a. Peltate. b. Short capitate. c. Long capitate.

Figure 2 a-h. Trichomes distribution pattern in *Scutellaria altissima* L., SEM. a. Leaf adaxial surface with peltates, short capitates and non-glandular trichomes. b. Leaf abaxial surface with short capitate and non-glandular hairs. c. Bract abaxial surface with the three types of glandular hairs and non-glandular trichomes. d. Particular of the bract abaxial surface with long capitates along the edges. e. General view of the calyx with all the types of glandular trichomes. f. Particular of the distal region of the calyx sides. g. Corolla abaxial surface with peltates, short and long capitates. h. Particular of the distal region of upper lip with abundant long capitate trichomes.

Figure 3. **a-e**. Histochemistry of the glandular trichomes in *Scutellaria altissima* L.*,* LM. 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 1. Distribution pattern of the glandular and non-glandular trichomes in *Scutellaria altissima* L. Symbols: (-) missing, (±) 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	+	++	++	++	++	-	++	-	++

Table 2. Results of the histochemical tests on the glandular trichomes in *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	–	–	++

Table 3. HS-SPME profiles of the leaves and flowers of *Scutellaria altissima* L.

	l.r.i.[a]	Compounds	Relative abundance (%)	
			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-terpenes derivatives	**71.04**	**-**
		Total	**98.17%**	**99.90%**

[a] Linear retentions indices on a DB5 capillary column: [b] Not detected; [c] Traces, <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**
		Oxigenated monoterpenes	**26.47**
		Sesquiterpene hydrocarbons	**24.63**
		Oxigenated sesquiterpenes	**13.81**
		Apocarotenoids	**11.66**
		Non-terpenic derivatives	**5.64**
		Total	**94.45**

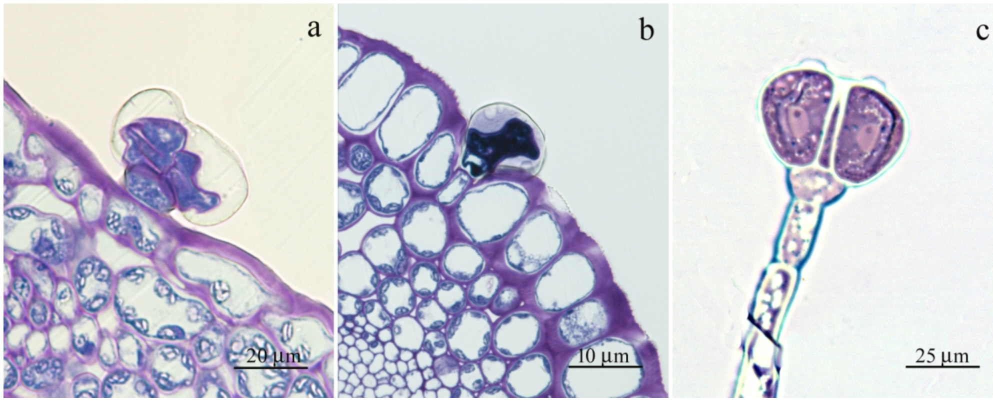

Figure 1 a-c. Glandular trichome morphotypes in Scutellaria altissima L., LM. a. Peltate. b. Short capitate. c. Long capitate.

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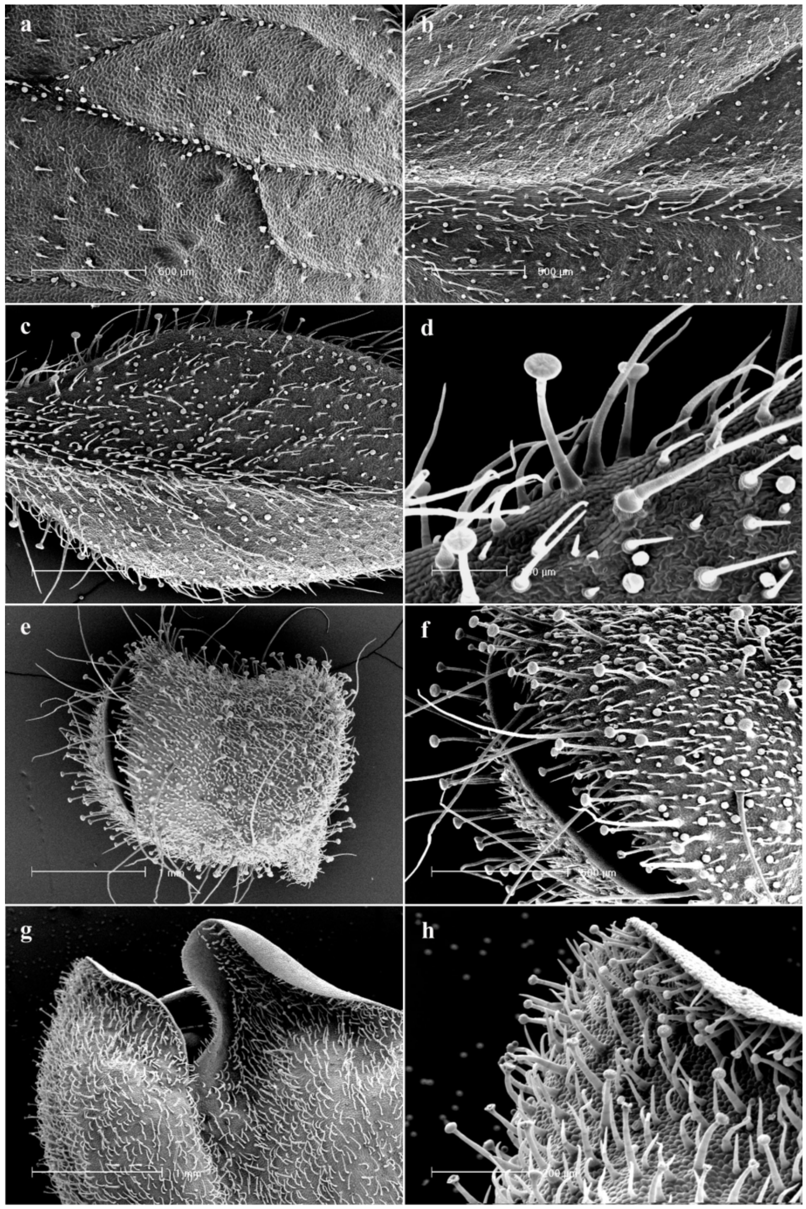

Figure 2 a-h. Trichomes distribution pattern in Scutellaria altissima L., SEM. a. Leaf adaxial surface with peltates, short capitates and non-glandular trichomes. b. Leaf abaxial surface with short capitate and non-glandular hairs. c. Bract abaxial surface with the three types of glandular hairs and non-glandular trichomes. d. Particular of the bract abaxial surface with long capitates along the edges. e. General view of the calyx with all the types of glandular trichomes. f. Particular of the distal region of the calyx sides. g. Corolla abaxial surface with peltates, short and long capitates. h. Particular of the distal region of upper lip with abundant long capitate trichomes.

160x241mm (300 x 300 DPI)

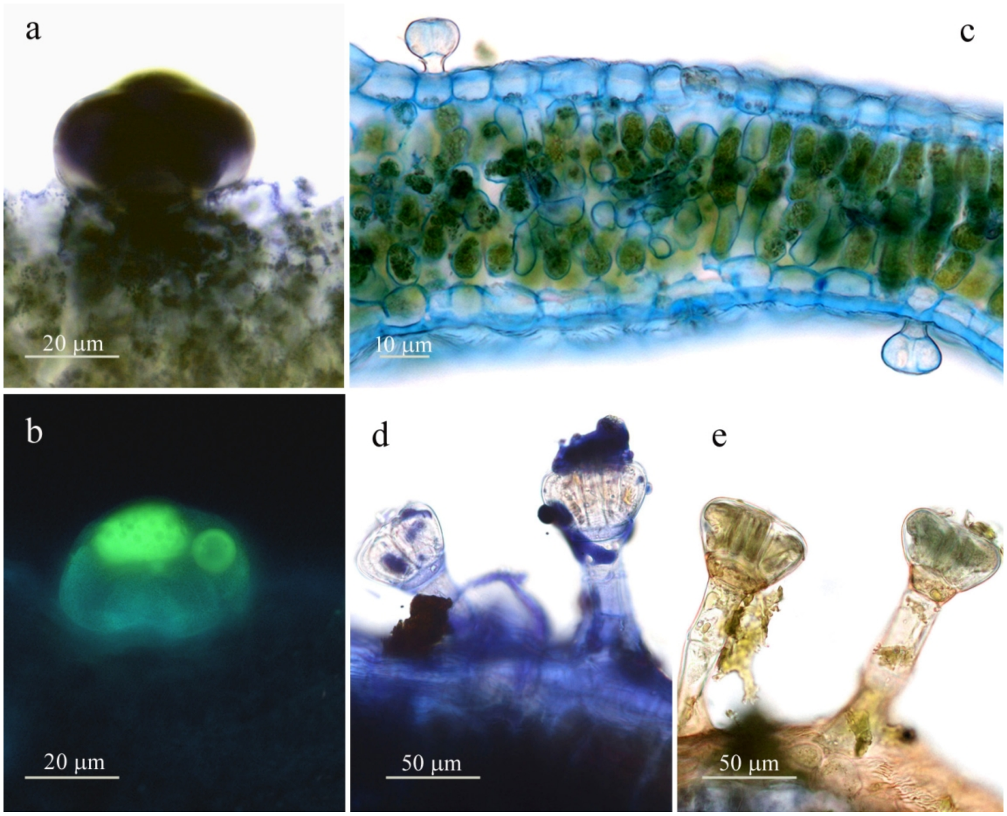

Figure 3. a-e. Histochemistry of the glandular trichomes in Scutellaria altissima L., LM. a-b. Peltate trichome: Nadi reagent (a), Fluoral Yellow-088 (b). c. Short capitate trichome: Alcian Blue. d-e. Long capitate trichome: Nadi reagent (d), Ferric Trichloride (e).

136x110mm (300 x 300 DPI)