Micromorphological and phytochemical insights on *Phlomis fruticosa* L. cultivated at the G.E. Ghirardi Botanical Garden (Lombardy, Northern Italy)

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ABSTRACT

A multi-level study was performed on the vegetative and reproductive organs of *Phlomis fruticosa* L. (Lamiaceae), cultivated at the G.E. Ghirardi Botanical Garden (Toscolano Maderno, Brescia, Northen Italy). This work is part of the project *Ghirardi Botanical Garden, factory of molecules…work in progress*, intended to preserve and enhance the plant heritage hosted at the study site. The multidisciplinary research combined four approaches: I) micromorphological, to describe the structures responsible for the productivity of secondary metabolites; II) histochemical, to define the chemical nature of the secretory products by Light Microscopy, Fluorescence Microscopy, and Scanning Electron Microscopy; III) phytochemical, to characterize the Essential Oil obtained from the blooming aerial parts by hydrodistillation with a Clevenger-type apparatus, consequently analysed by Gas Chromatography-Mass Spectrometry; IV) biological, to assess the potential biological activity of the most abundant EO components based on literature data. Overall, *P. fruticosa* presented non-glandular and glandular trichomes. The former were multicellular stellate or simple uniseriate, the latter

capitate belonging to three morphotypes: branched stalked with a one-celled head, simple short-stalked with a one(two)-celled head, simple medium-stalked with a four-celled head. For the first time, the histochemical survey reported digital images showing a predominant terpenes secretion by the branched-stalked and simple medium capitates, while the simple short hairs were responsible for the secretion of mucopolysaccharides and acid polysaccharides. The EO profile revealed 50 compounds and was dominated by sesquiterpene hydrocarbons (51.1%) and oxygenated sesquiterpenes (33.6%), with *ar*-curcumene (24.3%), caryophyllene oxide (22.5%) and α-cedrene (12.8%) as most representative compounds. Finally, based on literature data, antimicrobial, antioxidant, and anti-inflammatory properties were hypothesized.
In the context of Open Science, an original iconographic apparatus was drafted based on these results to make them accessible to the visitors of the G.E. Ghirardi BG, as an opportunity to discover the plant heritage from an unusual perspective.

KEYWORDS

Microscopy, glandular trichomes, hydrodistillation, essential oil, Open Science

1. INTRODUCTION

The genus *Phlomis* L. (Lamiaceae) includes over 100 species native to Europe, Asia, and North Africa. Typical botanical traits are represented by entire, opposite, and decussate leaves, with yellow-pink flowers arranged in whorls around squared stems, with a tomentose appearance (Amor et al., 2009; Pignatti et al., 2017). Traditionally, the infusion of their flowered parts is used to treat gastrointestinal disorders and has a protective action on the liver, kidney, bone, and cardiovascular system (Amor et al., 2009).
Phlomis fruticosa L., commonly known as "Yellow Sage" or "Jerusalem Sage", is a subshrub native to the European southern coasts, from Spain to Greece, reaching up to 1 m in height. It grows wild on cliffs and limestone garrigues up to 900 m a.s.l., showing woody stems at the base, erect and branched at the distal portion, covered with dense yellowish hairs; rounded-cuneate basal leaves, sessile and ovate cauline leaves, with a long petiole: the adaxial surface is green with sparse stellate trichomes, the abaxial white-tomentose with dense stellate hairs. Flowers are grouped in cymose inflorescences; calyx with subcylindrical tube; bright yellow bilabiate corolla, 25 mm long; the fruit is a hairless brownish tetrachenium (Pignatti et al., 2017). Since ancient time, the warm juice of *P. fruticosa* has been drunk to treat malaria. Nowadays, the species is still used in Turkish and Greek folk medicine to treat gastric ulcers or as a tonic beverage, whereas in Italy for its anticough properties and in case of wound healing (Adams et al., 2011; Aligiannis et al., 2004; Amor et al., 2009).

Regarding the micromorphological characterization, the literature proposes three investigations, focused only on the vegetative organs of samples of Greek origin (Christodoulakis, 1989; Nikolakaki and Christodoulakis, 2007; Psaras and Sofroniou, 2004). The morphoanatomy of roots, stems and leaves was explored in relation to the seasonal variability, mainly discussing the dimorphism connected to the adaptation of the species to the natural environment (Christodoulakis, 1989; Psaras and Sofroniou, 2004). Leaves were also microscopically investigated, highlighting the presence of non-glandular and glandular capitate trichomes on the epidermal surfaces, jointly with a histochemical survey on their secretory products without the proposal of digital images (Nikolakaki and Christodoulakis, 2007). Concerning the phytochemical point of view, the genus *Phlomis* has been widely investigated, exploring the chemical composition of essential oils (EOs) and hydroalcoholic extracts of plants from the native range. Based on the EO composition, *Phlomis* species have been classified in four chemotypes: sesquiterpene profile (I); monoterpene and sesquiterpene profile (II); fatty acids, aliphatic and alcohol compounds-based profile (III); third chemotype added in terpenes (IV) (Amor et al., 2009). In detail, previous works on *P. fruticosa* focused on the characterization of hydrophilic and hydrophobic extracts (*i.e.,* methanol, ethanol, hydroalcoholic, distilled water, *n*-hexane, ethyl acetate) often associated with evaluations of their potential biological properties (*i.e.,* antimicrobial, anti-enzymatic, cytotoxic, antioxidant, anti-inflammatory, apoptotic) (Ferrante et al., 2019; Stojković et al., 2022, 2021; Tarhan et al., 2022). Literature data also report several studies about the EO composition of aerial parts, leaves, and flowers of *P. fruticosa* from Greece and Montenegro, highlighting the potential antimicrobial and antimutagenic activity (Aligiannis et al., 2004; Georgescu et al., 2016; Soković et al., 2002b, 2002a; Tsitsimi et al., 2000), but none referred to Italian samples. With these premises, the work aims to increase the micromorphological and phytochemical knowledge on *P. fruticosa* cultivated at the G.E. Ghirardi Botanical Garden of the University of Milan (Toscolano Maderno, Brescia, Northen Italy) through a four-step complementary research approach. The goals are: 1. description of the glandular and non-glandular *indumenta* on the vegetative and reproductive organs by means of Light Microscopy (LM), Fluorescence Microscopy (FM) and Scanning Electron Microscopy (SEM); 2. proposal of digital images resulting from the histochemical protocols performed to identify the main compound classes secreted and released by the glandular trichomes; 3. characterization, for the first time, of the essential oil (EO) distilled from the flowering aerial parts of the Italian species, comparing it with the chemical components previously identified in Greek and Montenegrin species; and 4. correlation of the EO composition with available literature data referring to the potential biological activity of the most abundant compounds. Lastly, this work is part of a wider project entitled *Ghirardi Botanical Garden, factory of molecules…work in progress*, aimed at studying and enhancing a selection of species preserved at the G.E. Ghirardi Botanical Garden under a multi-level research proposal. The correlation between the micromorphological and phytochemical data, with the ecological and biological potential of the plant derivatives, will

converge in a wide-spectrum dissemination plan, starting from the realization of new pictorial labels.

2. MATERIALS AND METHODS

2.1. PLANT MATERIAL

Phlomis fruticosa L. is cultivated at the G.E. Ghirardi Botanical Garden (Northern Italy, Lombardy, Toscolano Maderno, BS) of the Department of Pharmaceutical Sciences of the University of Milan. Aerial parts at blooming stage were sampled in May 2022. The samples were used for both the micromorphological and the phytochemical surveys. Voucher specimens were deposited in the Herbarium of the G.E. Ghirardi Botanical Garden under the identification codes GBG2022/087 and GBG2022/088.

2.2. MICROMORPHOLOGICAL INVESTIGATION

The micromorphology and distribution pattern of the non-glandular and glandular *indumenta* on the vegetative and reproductive organs (stem, leaves, calyx, and corolla) of *P. fruticosa,* along with the histochemical profile of the glandular trichomes, were investigated by means of Light Microscopy (LM), Fluorescence Microscopy (FM), and Scanning Electron Microscopy (SEM). At least ten replicates for each plant part were considered to evaluate the variability level of the microscopic features. Overall, the adopted protocols followed those reported in Bottoni et al., 2022 (Bottoni et al., 2022).

2.2.1. LIGHT MICROSCOPY (LM) AND FLUORESCENCE MICROSCOPY (FM)

The micromorphological investigation was carried out on both fresh samples and fixed material included in historesin (Technovit® 7100). For the fresh samples, 30 µm-thick sections were cut using a vibratome. Samples were also fixed in F.A.A. solution (Formaldehyde:Acetic Acid:Ethanol 70% = 5:5:90) for 10 days at 4 °C; afterwards, these samples underwent a subsequent passage in 70% ethanol for 12 h and were then subjected to a progressive dehydration process in ascending ethanol series, up to absolute. Pre-inclusion was then operated initially with ethanol and historesin in 1:1 ratio for one night, then with a 1:2 ratio for 2 h, and in pure historesin for 3 h. Finally, the inclusion was completed in a polypropylene capsule with the addition of a hardener with a ratio of 1:15 of basic resin (Giuliani and Maleci Bini, 2008). The historesin samples were cut in 2 µm-thick sections using an ultramicrotome. The following histochemical stainings were applied (Giuliani and Maleci Bini, 2008): Fluoral Yellow-88 for total lipids (Brundrett et al., 1991); Nile Red for neutral lipids (Greenspan et al., 1985); Nadi reagent for terpenes(David et al., 1964); Alcian Blue for mucopolysaccharides (Beccari and Mazzi, 1966); Ruthenium Red for pectins (Jensen, 1962); Ferric Trichloride for polyphenols (Gahan, 1984); Aluminum Trichloride for flavonoids (Guerin et al., 1971). Control staining procedures were concurrently performed. Observations were performed with a Leitz

DM-RB Fluo (Oberkochen, Germany) optical microscope equipped with a Nikon DS-L1 digital camera.

2.2.2. SCANNING ELECTRON MICROSCOPY (SEM)

For SEM observations, small-sized segments of the studied plant parts were fixed in F.A.A. solution for 7 days, subjected to a dehydration process with ascending ethanol series up to absolute, critical-point dried, mounted on aluminium stubs, and carbon gold-coated. Observations were carried out under a Zeiss® EVO MA15 SEM (Oberkochen, Germany) at the Interdepartmental Center for Electron Microscopy and Microanalysis Services (M.E.M.A.) of the University of Florence (Florence, Italy).

2.3. PHYTOCHEMICAL INVESTIGATION

2.3.1. ESSENTIAL OIL DISTILLATION

The flowered aerial parts of *P. fruticosa* were air-dried and stored at room temperature, in the dark. For the hydrodistillation, 76 g of plant samples were grounded, moved in a 4 L flask containing 2.0 L of water and subjected to distillation in a Clevenger-type apparatus for 3 h. The distillation process was performed in triplicate and oils were separately analysed by Gas Chromatography-Mass Spectrometry (GC/MS). Overall, the adopted protocols followed those reported in Giuliani et al., 2023 (Giuliani et al., 2023).

2.3.2. GC/MS CHARACTERIZATION

The phytochemical characterization was performed by GC/MS analyses, according to the procedure used in Pieracci et al., 2023 (Pieracci et al., 2023). The collected EOs were diluted to 5% in HPLC-grade *n*-hexane prior to injection in the GC-MS apparatus. The analyses on EOs were performed in triplicate.

2.4. ICONOGRAPHIC APPARATUS

Finally, micromorphological and phytochemical results were addressed to the dissemination plan of the G.E. Ghirardi Botanical Garden. Scientific information was selected to realize a novel iconographic apparatus and didactic labelling suitable for presenting *P. fruticosa* to the visitors of the Botanical Garden.

3. RESULTS AND DISCUSSION

3.1. MICROMORPHOLOGICAL OBSERVATIONS

The vegetative and reproductive organs of *P. fruticosa* displayed an *indumentum* of both non-glandular and glandular trichomes (**Table 1**, **Figures 1 and 2**). Two different morphotypes of non-glandular trichomes were detected: multicellular stellate, which appear branched at the tip of the stalk cell to form a star-shaped structure, with secondary branching with acute apices

and a smooth cuticular surface (**Figure 1 a-e**), and multicellular simple and uniseriate, presenting variable length, acute apices, and a striated cuticle (**Figure 1 g**). The former covered all the leaf epidermises and the abaxial surfaces of calyx and corolla (**Figure 2 a-f**), while the latter were distributed only on the terminal portion of the adaxial surfaces of calyx and corolla (**Figure 2 e-f**). On leaves and calyces, the multicellular dendritic hairs showed straight secondary branching, whereas on corollas they were generally curved.

As to the glandular *indumentum,* the capitate trichome was the only morphotype observed. Based on the stalk features and on the number of secreting cells, three main subtypes were distinguished: branched stalked with a one-celled head, sporadic on leaves and flowers; simple short-stalked with a one(two)-celled head, ubiquitous on the whole epidermises; simple medium-stalked with a four-celled head, exclusive of the reproductive organs (**Table 1**, **Figure 1**). The branched stalked capitate showed a basal cell and a multicellular branched stalk with several secondary branching with a pointed apex. The terminal branching supported the glandular head, presenting a single secretory cell with a large subcuticular space, responsible for their typical spherical shape (**Figure 1 b-c**). Other than a secretory activity, these trichomes contributed to a protective function on the leaves, constituting a compact and uniform coverage jointly with the multicellular stellate trichomes (**Figure 2 a-e**). The short simple-stalked capitate showed one basal cell, one stalk cell, and one-(two) secreting cells with a very thin subcuticular space, protruding from epidermal depressions (**Figure 1 f, Figure 2 d-h**). The medium simple-stalked capitate had a protruding epidermal basal cell, an elongated stalk cell, and four secreting cells, and represented the sole secreting structure of both the upper and lower lip of calyxes and corollas (**Figure 1 g-h, Figure 2 f-h**).

This micromorphological survey on *P. fruticosa* represents an element of novelty as it simultaneously proposes a comprehensive description of both the non-glandular and glandular *indumenta* on both the vegetative and reproductive organs, whereas previous contributions were focused only on leaves from samples of Greek origin (Nikolakaki and Christodoulakis, 2007). The secreting structures defined herein were congruent with the observations performed by previous authors (Nikolakaki and Christodoulakis, 2007), both in terms of structure and distribution of the trichomes.

As to the non-glandular *indumentum,* the stellate multicellular morphotypes were common in the genus *Phlomis,* and were documented in some species of *Ballota*, representing in the latter features of taxonomic value for the recognition of some groups. The simple uniseriate multicellular trichomes were ubiquitous in the whole family, even if they exhibited a high level of variability in terms of total length, density, and localization on the plant organs.

In the target species, we only observed the capitate morphotype, as the peltate type was completely absent on the whole plant epidermises, as already reported for some other Lamiaceae species belonging to the genus *Stachys, i.e., S. sylvatica, S. heraclea, S. plumosa, S. recta* (Giuliani and Maleci Bini, 2008). Peltates were instead abundant in most members of the Lamiaceae family, as observed in many species of the genus *Teucrium*, *Ballota*, *Lavandula*,

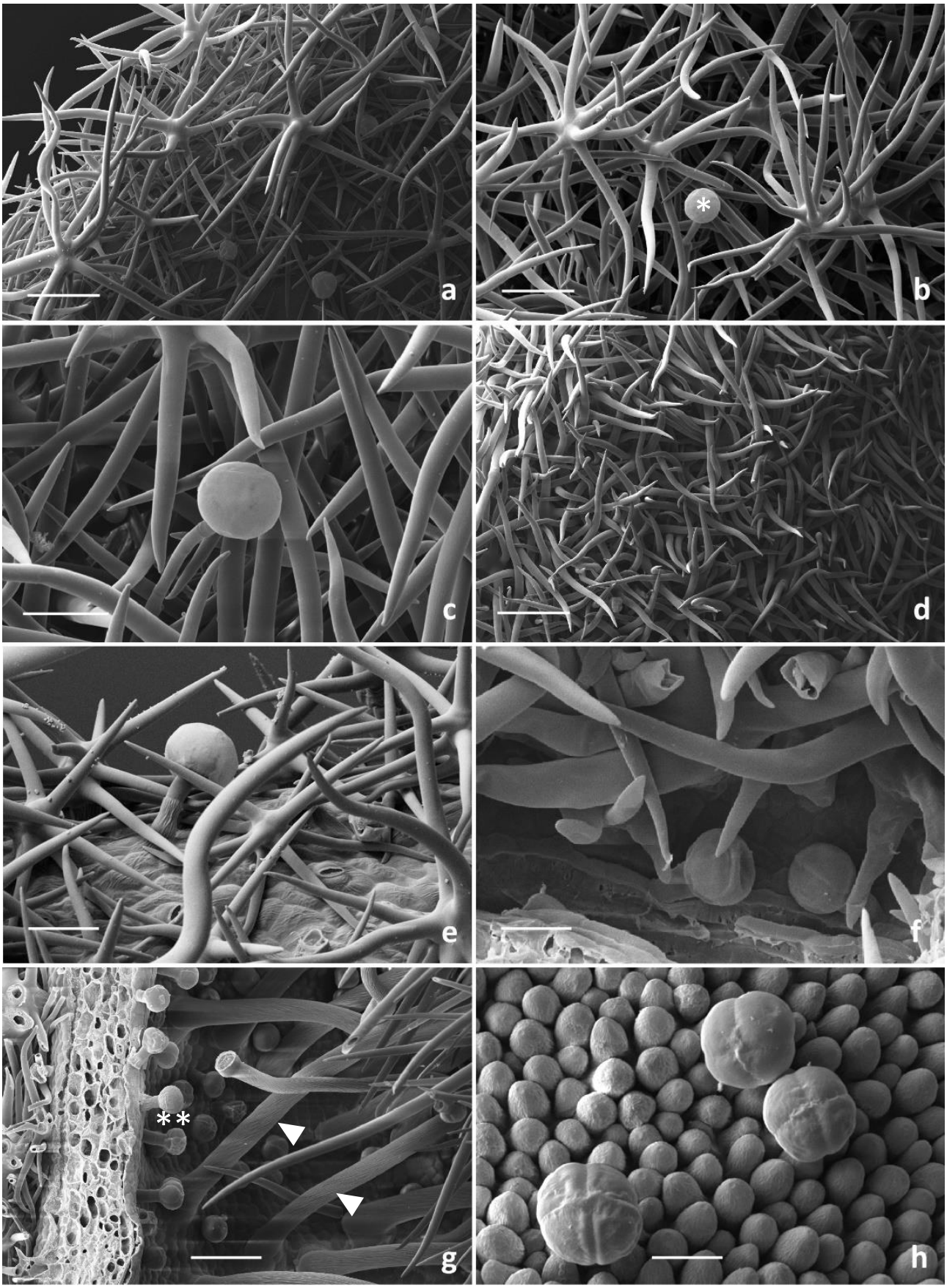
and *Scutellaria* hosted at the G.E. Ghirardi Botanical Garden (Giuliani et al., 2021; Giuliani et al., 2021a, 2020b, 2020a). The stellate capitates, even if only occasionally observed, were described in *P. fruticosa* for the first time, while the short simple capitates with a one(two)-celled head, were ubiquitous throughout the family, occurring generally on both the vegetative and the reproductive organs. This type of hairs was also detected in *Teucrium chamaedrys*, *T. fruticans*, *Scutellaria brevibracteata*, *S. altissima*, *S. caucasica*, and *Ballota acetabulosa* preserved at the G.E. Ghirardi Botanical Garden. On the contrary, the simple medium-stalked capitate occurred solely on the reproductive organs of the target species. The exclusive distribution on calices and corollas of this capitate morphotype has also been recorded in *T. fruticans*, *S. brevibracteata*, and *B. acetabulosa*, (Giuliani et al., 2021, 2020; Giuliani et al., 2021a, 2021b, 2020a; Giuliani et al., 2023), along with long-stalked capitate morphotypes, not detected here.

Table 1. Distribution pattern of the non-glandular and glandular trichomes of *P. fruticosa*

Trichomes	**Stem**	**Leaf**		**Calyx**		**Corolla**	
		adax	abax	adax	abax	adax	abax
Non-glandular							
multicellular stellate	++	++	++	-	++	-	++
multicellular simple	-	-	-	++	-	++	-
Capitate							
branched stalked	±	±	±	±	±	±	±
simple short stalked	+	+	+	+	+	+	+
simple medium stalked	-	-	-	++	++	++	++

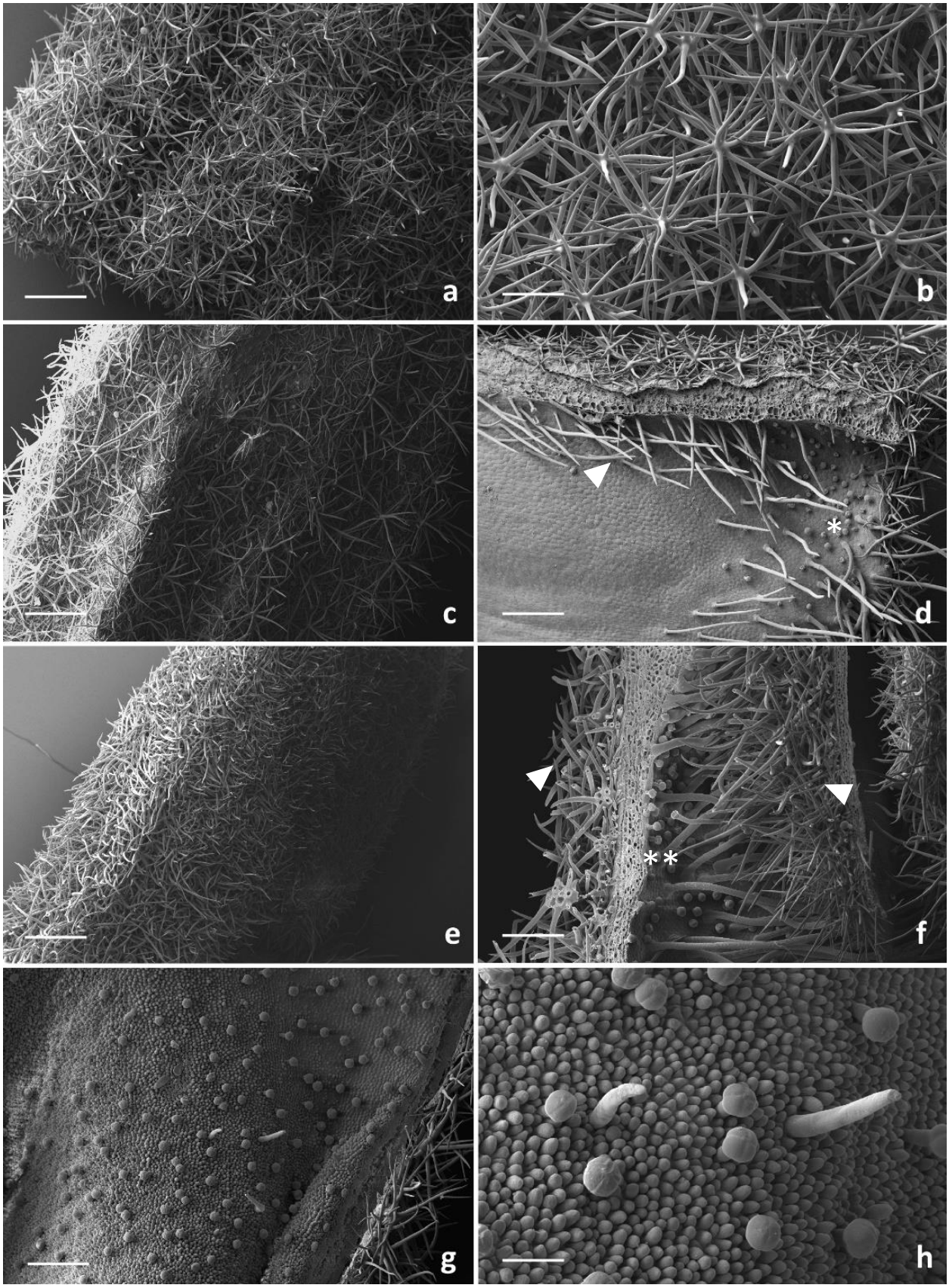
- missing, ± sporadic, + ubiquitous, ++ abundant

233 **FIGURE 1**



234

FIGURE 2



3.2. HISTOCHEMISTRY

The results of the histochemical investigation are reported in **Table 2** and **Figure 3**. Branched-stalked and medium-stalked capitates showed positive responses to lipophilic dyes and to ferric and aluminium trichloride, suggesting the synthesis and accumulation of terpenes, polyphenols, and flavonoids (**Table 2, Figure 3 a-b, e**). On the contrary, the application of the histochemical dyes on the short-stalked capitates exhibited positive responses only to hydrophilic dyes revealing the synthesis of acid polysaccharides and mucopolysaccharides stored in the sub-cuticular space (**Table 2, Figure 3 c-d**). These results highlighted that the terpenes secretion was attributable only to the branched-stalked and medium-stalked capitates. However, based on the different density rates and distribution pattern, the medium-stalked capitates resulted as the main site responsible for the productivity of these secondary metabolites at both foliar and floral levels. As a matter of fact, the proposal of digital images and the application of dyes specific for terpenes represented further elements of novelty in comparison to the previous contribution (Nikolakaki and Christodoulakis, 2007), thus finalizing the histochemical profile of the glandular *indumentum* at foliar and floral level. Indeed, the previous authors have only proposed schematic results, employing different staining techniques for the detection of only mucopolysaccharides and phenols without defining the trichome morphotype involved in the secretion process of such substances; therefore, the comparative evaluation of the results cannot be easily carried out and the consistency emerged only for the synthesis and accumulation of mucopolysaccharides and phenols at the foliar level (Nikolakaki and Christodoulakis, 2007).

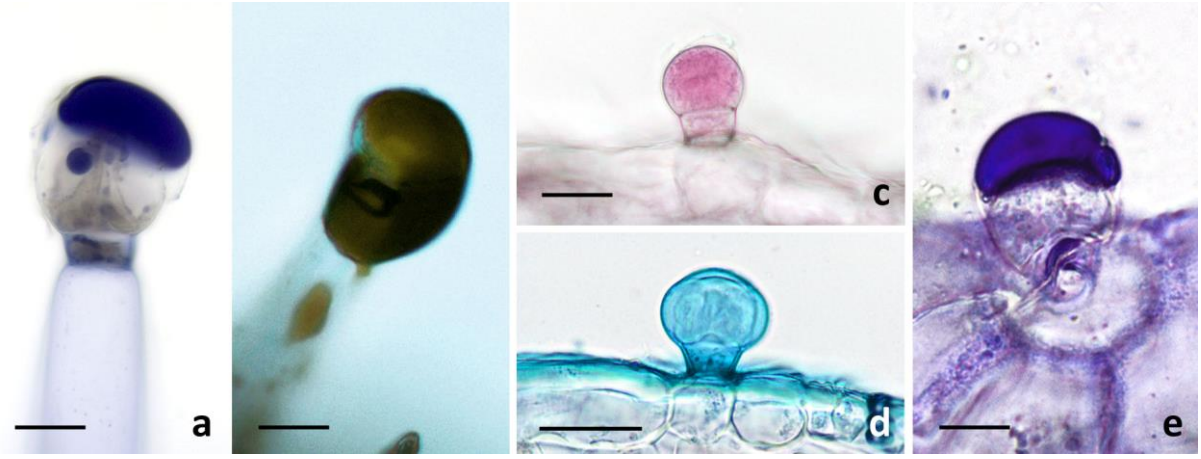
On the contrary, our previous works performed on other members of the Lamiaceae family supported the histochemical profile evidenced herein for the simple short- and stalked capitates: simple-short capitates are responsible for the hydrophilic secretion also for species belonging to the genera *Teucrium*, *Scutellaria*, and *Ballota* while the simple-medium morphotype contributes to the production of terpene, sometimes showing the simultaneous synthesis of polyphenols and flavonoids both at foliar and floral levels in *T. fruticans*, *S. brevibracteata* and *B. acetabulosa* (Giuliani et al., 2021; Giuliani et al., 2021b; Giuliani et al., 2023).

Table 2. Histochemical results on the secretory products of the glandular trichomes of *Phlomis fruticosa* L.

Staining	Target-compounds	branched capitate	short capitate	medium capitate
Fluoral Yellow-088	Total lipids	++	–	++
Nile Red	Neutral lipids	+	–	+
Nadi Reagent	Terpenoids	++	–	++
Ruthenium Red	Acid polysaccharides	–	+	–
Alcian Blue	Mucopolysaccharides	–	+	–
Ferric Trichloride	Polyphenols	+	–	+
Aluminium Trichloride	Flavonoids	+	–	++

Symbols: (–) negative response; (+) positive response; (++) intensely positive response

FIGURE 3



3.3. PHYTOCHEMICAL CHARACTERIZATION

The chemical composition of the EO obtained from the flowering aerial parts of *P. fruticosa* is reported in **Table 3**. The essential oil yield was 0.043%. The GC-MS analysis revealed the presence of 50 compounds, representing 96.6% of the total. Sesquiterpene hydrocarbons dominated (51.1%), followed by oxygenated sesquiterpene (33.6%), while monoterpene compounds were present in lower amounts, *i.e.,* 4.5% for hydrocarbons and 4.3% for oxygenated derivatives. Apocarotenoids and non-terpene derivatives showed the lowest relative abundances, with percentages of 1.9% and 1.2%, respectively. *Ar*-curcumene (*38,* 24.3%), caryophyllene oxide (*46,* 22.5%) and α-cedrene (*28,* 12.8%) were the most abundant compounds, followed by *trans*-sesquisabinene hydrate (*45,* 3.9%), α-pinene (*2*, 3.6%), alloaromadendrene (*35*, 3.1%), β-cedrene (*30*, 3.0%), humulene epoxide II (*47*, 2.7%), *trans*-longipinecarveol (*49*, 2.2%) and β-caryophyllene (*29*, 2.0%). Six compounds were determined with relative abundance in the range 1.0%-2.0%: hexahydrofarnesylacetone (*50*, 1.8%), β-bisabolene (*39*, 1.5%), guaia-6,9-diene (*32*, 1.3%), *trans*-verbenol (*15*, 1.1%), *cis*-α-copaene-8-ol (*48*, 1.1%), and elemol (*44*, 1.0%). The remaining compounds were recorded in lesser amounts, accounting for less than 1.0%.

Based on literature data, the presence of a moderate amount of α-pinene (*2*) in the profile, although accompanied by a clear prevalence of sesquiterpenes both among the dominant and minor components, allows us to assign the target-species to the second chemotype (II) (Amor et al., 2009). This assignment is not influenced by the low occurrence of non-terpene compounds, as already observed in previous studies(Aligiannis et al., 2004; Soković et al., 2002a).

Regarding the detailed analysis of the overall chemical composition, a qualitative-quantitative variability was recorded compared to previous EO profiles, due to the different geographical origin of the samples, the investigated plant parts and the collection times (Aligiannis et al., 2004; Georgescu et al., 2016; Soković et al., 2002b, 2002a; Tsitsimi et al., 2000). Overall, the total number of compounds found here was comparable to that of the Montenegrin plants (50 vs. 48), also showing the clear predominance of the sesquiterpenes (Soković et al., 2002a, 2002b). With regards to the major compounds, a high variability emerged: the investigated EO profile was characterized by *ar*-curcumene (*38*), caryophyllene oxide (*46*), and α-cedrene (*28*) as the most representative components, which were detected in lower amounts (3.1%, 8.1%, and 0.6%, respectively) in the Montenegrin EO profile. Conversely, Montenegrin EO was dominated by *(E)*-methyl-isoeugenol and α-asarone, which instead were not found in our work (Soković et al., 2002a). Contrasting differences were also detected for several minor compounds such as α-pinene (*2*, 3.6% *vs* 6.6%), alloaromadendrene (*35*, 3.1% *vs* 5.1%) and β-caryophyllene (*29*, 2.0% *vs* 12.0%). Nevertheless, the two profiles retained 23 common compounds, in some cases with significantly different abundances.

To assess the biological potential of the EO investigated here, a literature survey on the biological activity of the most abundant compounds was performed. In detail, anti-inflammatory, antimicrobial, antioxidant, anti-enzymatic, apoptotic, analgesic, hypolipidemic, and cytotoxic potential was documented. With regards to *ar*-curcumene (*38*), the literature reports a high antimicrobial activity against Gram-positive, Gram-negative bacteria and yeasts, linked to its pronounced hydrophobicity, which leads to the interaction with the cell membrane of the species analysed (Narjara Santos da Silva et al., 2015). On the other hand, caryophyllene oxide (*46*) is involved in numerous processes of biological interest, since it has been shown to interact with P450 systems linked to xenobiotic metabolism and with cannabinoid receptors, both in animal systems and in humans (Lněničková et al., 2018). In addition, it has shown significant pro-apoptotic activity against several tumour cell lines and concomitant analgesic activity (Gyrdymova and Rubtsova, 2022). Finally, α-cedrene (*28*) has been recognised as having significant antimicrobial activity against anaerobic bacteria and yeasts (Johnston et al., 2001) and a marked anti-obesity activity in animals, leading to its evaluation as potential anti-obesity drug (Kim et al., 2015). Among the minor constituents, for which a general antibacterial activity has been reported, previous studies have documented an anti-inflammatory and hypolipidemic action for β-caryophyllene (*29*) (Baldissera et al., 2017b, 2017a), while α-pinene (*2*) has shown inhibitory activity towards the metastatic potential of breast cancer (Kang et al., 2016), as well as antioxidant, antiproliferative and cytotoxic effects (Aydin et al., 2013). In addition, β-cedrene (*30*) has been shown to possess antiseptic, anti-inflammatory, and antifungal effects, as well as a marked inhibitory effect on liver cytochromes P450. However, considering the chemical heterogeneity of the investigated phytocomplex, the different biological activities explored for single compounds may be intended as a development of synergistic actions by all the detected components.

Table 3. GC-MS profile obtained from the aerial parts at blooming of *Phlomis fruticosa* L.

	LRI[1]	Class	Constituents	Relative abundance (%)
1	933	MH	α-thujene	0.1
2	941	MH	α-pinene	**3.6**
3	959	MH	thuja-2,4(10)-diene	0.1
4	982	MH	β-pinene	0.2
5	992	NH	2-pentyl furan	0.1
6	1028	MH	*p*-cymene	0.2
7	1032	MH	limonene	0.2
8	1034	OM	1,8-cineole	0.1
9	1042	MH	*(Z)*-β-ocimene	0.1
10	1101	OM	linalool	0.7
11	1102	NH	nonanal	0.2
12	1126	OM	α-campholenal	0.7

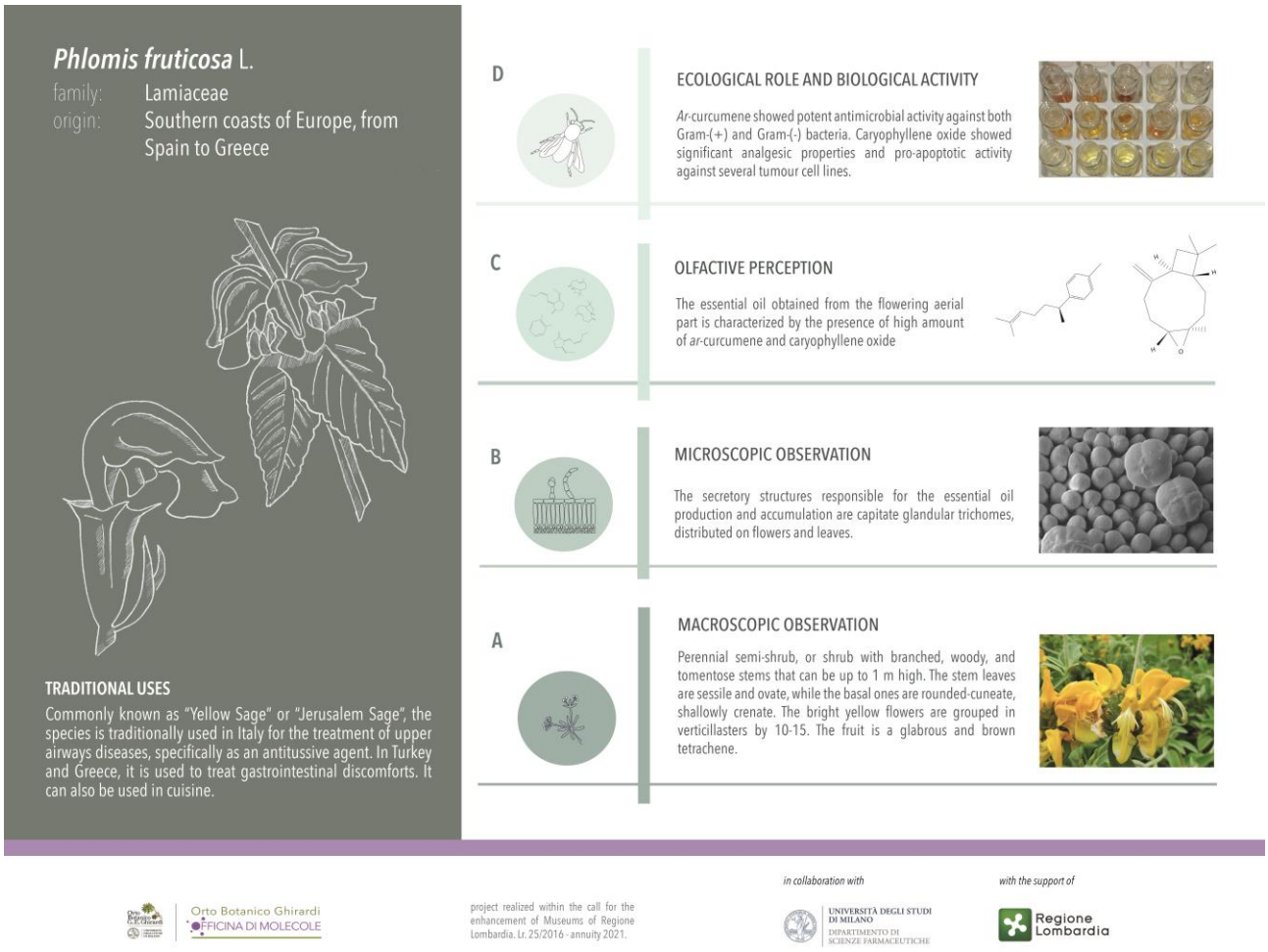
13	1141	OM	*trans*-pinocarveol	0.2
14	1142	OM	*cis*-sabinol	0.3
15	1143	OM	*trans*-verbenol	**1.1**
16	1163	OM	pinocarvone	0.2
17	1168	OM	*p*-mentha-1,5-dien-8-ol	0.1
18	1185	OM	*p*-cymen-8-ol	0.1
19	1191	OM	α-terpineol	0.1
20	1194	OM	myrtenal	0.3
21	1205	OM	verbenone	0.1
22	1220	OM	*trans*-carveol	0.2
23	1222	AC	β-cyclocitral	0.1
24	1257	OM	geraniol	0.1
25	1377	SH	α-copaene	0.1
26	1386	SH	β-bourbonene	0.1
27	1406	SH	*(Z)*-caryophyllene	0.1
28	1411	SH	α-cedrene	**12.8**
29	1419	SH	β-caryophyllene	**2.0**
30	1420	SH	β-cedrene	**3.0**
31	1437	SH	*trans*-α-bergamotene	0.6
32	1444	SH	guaia-6,9-diene	**1.3**
33	1455	SH	α-humulene	0.4
34	1459	SH	*(E)*-β-farnesene	0.3
35	1462	SH	alloaromadendrene	**3.1**
36	1463	NH	2-methyltetradecane	0.9
37	1482	SH	germacrene D	0.3
38	1483	SH	*ar*-curcumene	**24.3**
39	1508	SH	β-bisabolene	**1.5**
40	1513	SH	β-curcumene	0.8
41	1515	SH	*cis*-γ-bisabolene	0.2
42	1524	SH	β-sesquiphellandrene	0.2
43	1545	OS	*cis*-sesquisabinene hydrate	0.2
44	1550	OS	elemol	**1.0**
45	1580	OS	*trans*-sesquisabinene hydrate	**3.9**
46	1581	OS	caryophyllene oxide	**22.5**
47	1607	OS	humulene epoxide II	**2.7**
48	1611	OS	*cis*-α-copaene-8-ol	**1.1**
49	1618	OS	*trans*-longipinecarveol	**2.2**
50	1845	AC	hexahydrofarnesylacetone	**1.8**
			Monoterpene hydrocarbons (MH)	**4.5**
			Oxygenated monoterpenes (OM)	**4.3**
			Sesquiterpene hydrocarbons (SH)	**51.1**
			Oxygenated sesquiterpenes (OS)	**33.6**
			Apocarotenes (AC)	**1.9**
			Non-terpene derivatives (NH)	**1.2**
			Total identified	**96.6**

352 [1] Linear retention index on a HP 5-MS capillary column.

3.4. SCIENTIFIC DISSEMINATION

Within the framework of a wider dissemination plan, the scientific results reported in the "Micromorphological observations", "Histochemistry", and "Phytochemical characterization" sections represented the starting point for the realization of the textual content of the new iconographic apparatus for *Phlomis fruticosa* L. hosted at the G.E. Ghirardi Botanical Garden (Toscolano Maderno, BS, Italy) (**Figure 4**). The traditional uses, together with the macroscopic and microscopic features of the plant have been enriched with the presentation of the main chemical components of the EO, along with information on their potential biological activity. Finally, the textual content was adorned with an original line botanical drawing and photographic images.

FIGURE 4



4. CONCLUSIONS

This work focused on a multidisciplinary research approach on *P. fruticosa*, representing a new way to preserve, enhance and disseminate the plant heritage maintained at the G.E. Ghirardi

Botanical Garden of the University of Milan (Toscolano Maderno, BS, Italy) in an Open Science context. For the first time, samples of the target species from Italy were investigated. The micromorphological observations on the vegetative and reproductive organs revealed the presence of multicellular stellate and simple uniseriate non-glandular trichomes, as well as three capitate morphotypes with a peculiar distribution pattern on the epidermal surfaces. The proposal of digital images referring to the histochemical results represented an element of novelty for the target species, underling the pivotal role of the branched-stalked and medium-stalked capitates for terpenes production. As confirmed by the phytochemical survey, the mono- and sesquiterpene based-profile defined the chemotype of the Italian species. The EO profile was dominated by sesquiterpene hydrocarbons and oxygenated sesquiterpenes, whereas monoterpenes were present in a small amount; the main EO constituents detected were *ar*-curcumene, caryophyllene oxide and α-cedrene. Moreover, a comparison with literature data on the documented biological activity of the most abundant compounds, allowed us to hypothesize a potential antioxidant, antimicrobial, anti-inflammatory, cytotoxic, pro-apoptotic, anti-enzymatic, analgesic and hypolipidemic activity of the phytocomplex. However, based on this evidence and considering the synergistic actions among all the chemical constituents, further studies will be needed. Finally, within the framework of a Third Mission plan at the G.E. Ghirardi Botanical Garden, the research results will be available to visitors through the proposal of a new interpretative apparatus, making them participants in the progress of the scientific research.

CAPTIONS TO FIGURES

FIGURE 1 a-h, SEM. Non-glandular and glandular trichome morphotypes observed on the vegetative and reproductive organs of *Phlomis fruticosa*. **a.** Non-glandular multicellular stellate trichomes showing straight secondary branching. **b.** Abundant non-glandular stellate trichomes and sporadic branched-stalked capitates (asterisk). **c.** Branched-stalked capitate, a detail. **d-e.** Non-glandular multicellular stellate trichomes with curved arms. **f.** Simple short-stalked capitates. **g.** Non-glandular simple uniseriate trichomes (arrowheads) and simple medium-stalked capitates (double asterisk). **h.** Simple medium-stalked capitates. *Scale bars: 200 μm (a, b, d); 50 μm (c, e, g); 25 μm (f, h).*

FIGURE 2 a-h, SEM. Distribution pattern of the trichomes in *Phlomis fruticosa*. **a.** Leaf abaxial surface with non-glandular stellate trichomes. **b.** Leaf abaxial surface, a detail. **c.** Calyx abaxial surface with non-glandular stellate trichomes. **d.** Calyx adaxial surface with non-glandular simple uniseriate trichomes (arrowhead) and simple short-stalked capitates (asterisk). **e.** Corolla abaxial surface with non-glandular stellate trichomes. **f.** Adaxial surface of the corolla upper lip with non-glandular uniseriate trichomes (arrowheads) and simple short- and

medium-stalked capitates (double asterisk). **g, h.** Adaxial surface of the corolla lower lip with medium-stalked capitates. *Scale bars: 200 μm (a, c-g); 100 μm (b); 50 μm (h).*

FIGURE 3 a-e, LM. Histochemistry of the glandular trichomes in *Phlomis fruticosa*. **a-b.** Branched stalked capitate, details of the main glandular arm: Nadi reagent (a), Ferric Trichloride (b). **c-d.** Simple short-stalked capitate: Ruthenium Red (c), Alcian Blue (d). **e**. Simple medium-stalked capitates: Nadi reagent.

FIGURE 4. New interpretative apparatus of *Phlomis fruticosa* L. at the Ghirardi Botanical Garden (Department of Pharmaceutical Sciences, University of Milan, Toscolano Maderno, Brescia, Italy).

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Claudia Giuliani: Methodology, Investigation, Writing - review & editing. **Martina Bottoni:** Investigation, Writing - original draft. **Alberto Spada:** Writing - review & editing. **Sara Falsini:** Writing - review & editing. **Laura Santagostini:** Methodology, Investigation, Writing - review & editing. **Ylenia Pieracci:** Investigation, Writing - review & editing. **Guido Flamini:** Investigation, Writing - review & editing. **Fabrizia Milani:** Investigation, Writing - review & editing. **Gelsomina Fico:** Funding acquisition, Conceptualization, Supervision, Writing - review & editing. All authors have read and approved the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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