Insight into micromorphology and phytochemistry of *Lavandula angustifolia* Mill. from Italy

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Abstract

This work combined a micromorphological and phytochemical study approach on *Lavandula angustifolia* Mill. cultivated at the Ghirardi Botanic Garden (Toscolano Maderno, BS, Italy). The micromorphological observations on leaves and flowers revealed the occurrence of three trichome morphotypes: peltate, short- and medium-stalked capitate. The histochemical dyes were evidence of a huge production of terpenes by peltate and medium-stalked capitates, while short-stalked capitates were responsible for hydrophilic secretions. The phytochemical survey concerned the characterization of volatile organic compounds (VOCs) emitted by leaves and flowers, along with the analysis of the composition of the essential oil (OE) from flowering aerial parts. Oxygenated monoterpenes dominated both the leaf and flower profiles (65.66% *vs* 45.97%). The main compounds emitted by leaves were 1,8-cineole, *p*-cymene and borneol; linalool acetate, β-caryophyllene and linalool by flowers. 1,8-Cineole was also the dominant exclusive compound in leaves, while linalool acetate in flowers. The EO was characterized by linalool, linalool acetate, 4-terpineol, lavandulol acetate and β-caryophyllene. Finally, evaluations about the VOC ecology, the EO biological activity and sensory qualities were proposed based on literature.

This set of information was made available to the visitors of the Ghirardi Botanic Garden through the realization of a dedicated labelling, coupling the scientific research to the educational missions in an *Open Science* context.

Keywords English lavender, glandular *indumentum,* volatile organic compounds, essential oil.

1. Introduction

Lavandula angustifolia Mill. (syn. *Lavandula officinalis* L., *Lavandula vera* DC.), known as English lavender, is an evergreen shrub reaching 1 m of height, with woody and upright branches. Leaves are opposite, sessile, linear, with upturned edges. Its purplish-violet flowers are in groups of two to four at the axil of membranous bracts and are arranged in pedunculated spikes of 3-8 cm. The calyx is tubular with five apical teeth. The corolla is bilabiate, with a bilobed upper lip and a trilobe lower lip. Stamens are four, with an oval-shaped anther, while the ovary is bicarpellary and tetralocular. Fruits are four brown achenes. This species has a Western Steno-Mediterranean distribution and prefers dry soils, up to 1,800 m.a.s.l.

Recent studies referred to traditional uses based on anti-inflammatory (Hajhashemi et al., 2003), diuretic, and sedative properties, as well as for the treatment of cough, spasms, and flatulence (Naghibi et al., 2005).

This species has a high commercial value, mainly related to the presence of glandular trichomes, responsible for the production of an amazing diversity of volatile substances (Giuliani et al., 2018, 2017a, 2017b; Tardugno et al., 2019). Essential oils are widely used in several different fields, e.g. pharmaceutical, dietary, cosmetic, perfumes, and aromatherapy (Aprotosoaie et al., 2017; Costea et al., 2019).

Scientific literature offered some works concerning micromorphological surveys on the *indumentum* of congeneric species (Huang et al., 2008; Küçük et al., 2019), while there were only two studies on *L. angustifolia* (Costea et al., 2019; Blažeković et al., 2012).

With regards to phytochemistry, in the last decade literature presented some contributions about the characterization of VOCs spontaneously emitted by samples of different origin (Demasi et al., 2018; Łyczko et al., 2019; Pistelli et al., 2013; Stierlin et al., 2020).

Concerning the analyses of the composition of EO, there were several studies on samples from all over the world (Chen et al., 2020; Küçük et al., 2018; Li et al., 2019). As for Europe, contributions referred to samples from Romania (Jianu et al., 2013; Oroian et al., 2019), Hungary (Détár et al., 2020), Greece (Hassiotis et al., 2014, 2010), Serbia (Stanojević et al., 2011), Poland (Łyczko et al., 2019; Wesolowska et al., 2010), Spain (Carrasco et al., 2015), and Italy (Binello et al., 2014; Conti et al., 2010; Da Porto et al., 2009; Demasi et al., 2018; Maietti et al., 2013; Pistelli et al., 2017; Tardugno et al., 2019).

With regards to the ecological role of plant derivatives of *L. angustifolia*, a study concerning the relationships between the composition of the bacterial communities of the phyllosphere and the leaf EO profiles was done (Karamanoli et al., 2000). Another survey was dedicated to the link between gene regulation of the volatile terpenoid metabolism at different stages of flowering and types of attracted pollinators (Li et al., 2019). Moreover, antibacterial, antimycotic, and antioxidant properties were ascribed to EO (Carrasco et al., 2015; Jianu et al., 2013; Pistelli et al., 2017; Rostami et al., 2012; Tardugno et al., 2019). It is also used as natural pesticide in the control of parasites and insects (Conti et al., 2010; Wells et al., 2018).

Noteworthy biological activities for human health have also been ascribed to *L. angustifolia* EO, e.g. analgesic, anti-inflammatory (Chen et al., 2020; Donatello et al., 2020; Wells et al., 2018) and cytotoxic (Maietti et al., 2013). Furthermore, aroma-therapeutic applications, regarding its activity on improving sleep quality, anxiety, and depression, are reported, as well as potential neuroprotective properties (Nasiri Lari et al., 2020; Wells et al., 2018). Essential oils are also used as preservatives in the cosmetic industry (Muyima et al., 2002; Wells et al., 2018).

In this framework, the current study combined, for the first time, a novel multidisciplinary survey on samples of *L. angustifolia* cultivated in Italy, with the purpose of increasing knowledge concerning:

1. the micromorphology of the glandular trichomes responsible for volatile production of leaves and flowers by means of Light Microscopy (LM) and Scanning Electron Microscopy (SEM);
2. the evaluation of the histochemical features to define the main classes of secondary metabolites present in the secretions;
3. the characterization of the VOC emission profiles from leaves and flowers and the analyses of EO composition from its flowering aerial parts.

All the collected information is related, after consulting the literature, to the potential ecological role and biological activity.

This study is part of a wider project called *Botanic Garden, factory of molecules*, aimed at enhancing the plant heritage of the Ghirardi Botanic Garden (Toscolano Maderno, BS, Italy) through the combination of research work and dissemination activities in the context of the current *Open Science* policies.

2. Materials and Methods

2.1 Plant material

Lavandula angustifolia was cultivated at the Ghirardi Botanic Garden (Toscolano Maderno, BS, Lombardy, Italy) of the Department of Pharmaceutical Sciences of the State University of Milan. The samples for the morphological and the phytochemical analyses were simultaneously collected in June 2019. Voucher specimens were deposited in the Herbarium of the Ghirardi Botanic Garden under the code labels GBG2019/046 and GBG2019/047.

2.2 Micromorphological investigation

We described the micromorphology, the distribution pattern and the histochemistry of trichomes on the vegetative and reproductive organs by means of SEM and LM. At least ten replicates for each plant part were examined to assess the variability of the micromorphological features.

2.2.1 Scanning Electron Microscopy

Leaves, calyces, and corollas were hand-prepared, fixed in FAA solution (formaldehyde:acetic acid:ethanol 70% = 5:5:90) for five days, dehydrated in an ascending ethanol series up to absolute and critical-point dried. The samples were mounted on aluminium stubs, gold-coated, and observed under a Philips XL 20 SEM operating at 10 kV.

2.2.2 Light Microscopy

Fresh and FAA-fixed samples were prepared. The fresh material was frozen and cryo-sectioned; the fixed samples, following the dehydration process in ascending ethanol series up to absolute, were embedded in Technovit/Historesin and sectioned with a microtome. The following histochemical stains were employed: Toluidine Blue as a general dye (Beccari and Mazzi, 1966); Sudan III/IV (Beccari and Mazzi, 1966) and Fluoral Yellow-088 for total lipids (Brundrett et al., 1991), Nile Red for neutral lipids (Greenspan et al., 1985), Nadi reagent for terpenes (David et al., 1964), Periodic Acid-Schiff (PAS) reagent for total polysaccharides (Jensen, 1962), 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 concurrently. Observations were made with a Leitz DM-RB Fluo optical microscope equipped with a Nikon digital camera.

2.3 Phytochemical investigation

2.3.1 Volatile Organic Compounds

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

Headspace-Solid Phase Microextraction (HS-SPME) analyses – The headspace sampling conditions were as reported in Ascrizzi et al. (2017). For headspace samplings, Supelco SPME (Solid Phase Micro-Extraction) devices (Supelco, St. Louis, MO, USA), coated with polydimethylsiloxane (PDMS, 100 μm), were used; the same new fibre, preconditioned according to manufacturer’s instructions, was employed for all 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 equilibration and sampling times were experimentally determined to obtain an optimal adsorption of 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 Gas Chromatography–Mass Spectrometry (GC-MS) system. Both the sampling and desorption conditions were identical for all samples. Furthermore, blanks were performed before each first SPME extraction and randomly repeated during each series. Quantitative comparisons of relative peak areas were performed between the same compounds in different samples.

2.3.2 Essential oil

Fresh *L. angustifolia* aerial parts gathered at blooming (220 g) phase were hydrodistilled in a Clevenger-type apparatus. The hydrodistillation was prolonged until no further increase in the EO volume was obtained, for a total of 2 hours. The EO was diluted at 5% in HPLC grade n-hexane prior to GC-MS injection.

2.3.3 GC–MS analyses and peaks identification

Gas chromatography–electron impact mass spectrometry (GC–EI-MS) analyses were performed with a Varian CP-3800 (Agilent Technologies Inc., Santa Clara, CA, USA) gas chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm; film thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector (Agilent Technologies Inc., Santa Clara, CA, USA). Analytical conditions were as follows: injector and transfer line temperatures 220 and 240 °C, respectively; oven temperature programmed from 60 to 240 °C at 3 °C min^{-1}; carrier gas helium at 1 ml/min; splitless injection. The identification of constituents was based on a comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons. Computer matching was also used against commercial (NIST 14 and ADAMS) and laboratory-developed library mass spectra built up from pure substances and components of commercial essential oils of known composition and MS literature data (Adams, 1995; Davies, 1990; Jennings and Shibamoto, 1982; Masada, 1976; Stenhagen et al., 1974).

2.4 Scientific dissemination

Finally, special information derived from the scientific results obtained in the micromorphological, histochemical and phytochemical investigations were selected and included in the textual content of novel iconographic and didactic labelling of *Lavandula angustifolia* at the Ghirardi Botanic Garden.

Dr. Patrizia Berera supported us for the design, graphical set-up and realization of the original interpretative apparatus.

3. Results

3.1 Micromorphological investigation

3.1.1 Trichomes morphotypes, distribution pattern and histochemistry

Non-glandular and glandular trichomes were observed on the vegetative and reproductive organs (**Figure 1**). Non-glandular ones were ubiquitous, with the exception of the calyx adaxial surface (**Table 1**), multicellular, and dendritic: they were characterized by a main axis from which numerous branches arose; each arm was uniseriate with an acute apex (**Figure 1 C**).

Three morphotypes of glandular trichomes were distinguished: peltate, short-stalked capitate and medium-stalked capitate (**Figures 1, 2 A-C)**. The peltate ones were made up by a basal cell, a neck cell and a broad multicellular secretory head (**Figure 2 A**). They occurred on both the leaf sides and on the abaxial surfaces of the calyces and the corollas (**Figure 1 A-L**; **Table 1**). The secreted material reacted positively only to the lipophilic stains, being filled with terpenes (**Figure 2 D**; **Table 2**).

The short capitates were formed by a basal cell, a subcylindrical stalk cell and by a multicellular (2 cells) globular head (**Figure 2 B**). They may be sunken or protruding from the epidermis and were observed on the whole plant epidermis, with the exception of the petal adaxial side (**Figure 1 A-B**, **H-L**; **Table 1**). These hairs produced and released only muco-polysaccharides, as indicated by the exclusive positive responses to the Alcian Blue dye (**Figure 2 E**; **Table 2**).

The medium capitates consisted of an elongated basal epidermal cell, which protruded from the level of the adjacent epidermal cells, a subcylindrical stalk cell and a unicellular globular head (**Figure 2 C**). They occasionally occurred on the calyx abaxial side and were uniformly distributed on the petal adaxial surface (**Figure 1 L**; **Table 1**). The secreted material was stained exclusively by the lipophilic dyes and, in particular, by the Nadi reagent (**Figure 2 F**; **Table 2**).

3.2 Phytochemical investigation

3.2.1 Emission profiles of the volatile organic compounds

The VOC emission profile of *L. angustifolia* revealed a total of 76 different compounds. 49 compounds were identified in the leaf profile, while 35 in the flower one (**Table 3**).

The vegetative profile was dominated by oxygenated monoterpenes (65.66%), followed by monoterpene hydrocarbons (19.63%), oxygenated sesquiterpenes (6.47%), and sesquiterpene hydrocarbons (2.55%). Non-terpene derivatives were present in low relative quantities (0.98%). 1,8-Cineole (**13**, 42.17%) dominated the profile, followed by *p*-cymene (**11**, 14.05%), borneol (**32**, 6.32%), caryophyllene oxide (**71**, 4.41%), and *p*-cymen-8-ol (**34**, 4.38%). Camphene (**3**), camphor (**28**), and *trans*-γ-cadinene (**68**) occurred in lower quantities: 3.11%, 2.32%, and 1.70%, respectively. The remaining compounds accounted for relative percentages lower than 1.0%. 40 compounds were exclusively identified in the leaf, including the major compound 1,8-cineole (**13**, 42.17%). Borneol (**32**), *p*-cymen-8-ol (**34**), and camphene (**3**) followed with percentages of 6.32%, 4.38%, and 3.11%, respectively. All other leaf-exclusive compounds showed relative abundances lower than 1.0%.

The floral *bouquet* was dominated by oxygenated monoterpenes (45.97%), followed by sesquiterpene hydrocarbons (34.90%) and monoterpene hydrocarbons (17.98%). Oxygenated sesquiterpenes and non-terpene derivatives showed low relative quantities: 0.48% and 0.11%, respectively. Linalool acetate (**45**, 32.76%) was the major compound, followed by β-caryophyllene (**57**, 21.76%), linalool (**20**, 9.54%), (*E*)-β-ocimene (**15**, 8.65%), and (*E*)-β-farnesene (**65**, 7.63%). Limonene (**12**, 3.31%), myrcene (**7**, 3.10%), γ-muurolene (**66**, 2.10%), (*Z*)-β-ocimene (**14**, 1.50%), and geranyl acetate (**52**, 1.49%) occurred in percentages between 4.0% and 1.0%. All remaining compounds showed relative abundances lower than 1.0%.

26 flower-exclusive compounds were identified, including the most abundant ones (**45**, **57**, **20**, **15**, **65**) and those with relative abundances between 1.0% and 4.0% (**12**, **7**, **66**, **14**, **52**). All remaining compounds showed percentages lower than 1.0%.

Nine compounds were common to both organs, all of them found at higher percentages in the leaf profile, with the exception of δ-3-carene (**10**, 0.14% leaves; 0.24% flowers) and 4-terpineol (**33**, 0.22% leaves; 0.85% flowers). The common compound exhibiting the highest relative abundance was *p*-cymene (**11**, 14.05% leaves; 0.66% flowers). The remaining ones were caryophyllene oxide (**71**, 4.41% leaves; 0.48% flowers), camphor (**28**, 2.32%

leaves; 0.35% flowers), and *trans*-γ-cadinene (**68**, 1.70% leaves; 0.10% flowers). β-Pinene (**5**), α-pinene (**2**), and α-santalene (**58**) showed percentages lower than 1.0% in both profiles.

3.2.2 Essential oils

The EO composition is reported in **Table 4**. A total of 69 compounds were identified, accounting for 97.39% of the total oil.

Oxygenated monoterpenes (70.42%) represented the most abundant class, followed by sesquiterpene hydrocarbons (8.87%), oxygenated sesquiterpenes (6.55%), monoterpene hydrocarbons (6.03%), and non-terpene derivatives (5.35%). Apocarotenoids were present only in limited quantities (0.17%). The most abundant compound was linalool (**23**, 27.70%), followed by its acetic ester (**39**, 17.99%), 4-terpineol (**30**, 5.30%), lavandulol acetate (**42**, 4.29%), β-caryophyllene (**46**, 3.80%), and caryophyllene oxide (**61**, 3.45%). Borneol (**29**), cryptone (**31**), α-terpineol (**32**), cumin aldehyde (**37**), and (*E*)-β-farnesene (**49**) showed relative abundances between 3.0% and 2.0%. Limonene (**15**), 1,8-cineole (**16**), 1-octen-3-yl acetate (**24**), geranyl acetate (**45**), (*E*)-γ-bisabolene (**54**), and *epi*-α-cadinol (**64**) accounted for percentages between 2.0% and 1.0%. All remaining compounds were in percentages lower than 1.0% or in traces (<0.1%).

3.3 Scientific dissemination

The outcomes of the scientific research reported in the “Micromorphological investigation” and “Phytochemical investigation” sections were useful for the processing of the textual content of a new labelling for *Lavandula angustifolia*

Mill. (**Figure 3**) at the Ghirardi Botanic Garden (Toscolano Maderno, BS, Italy). In addition to the macroscopic characteristics, it emphasizes the microscopic features, the main compounds of the volatile profiles, and information on their ecological significance and biological activity, along with data on the plant traditional uses. The textual content was enriched with an original line botanical drawing and photographic images.

4. Discussion

The non-glandular and glandular *indumenta* displayed a high level of consistency among trichome morphotypes, distribution and histochemical features in all examined replicates.

We observed only one type of non-glandular dendritic trichome covering the whole plant surface, except for the adaxial side of the calyx. Blažeković et al. (2012) described several types of non-glandular trichomes on samples from Croatia: two to three branched trichomes on the vegetative and reproductive organs, beside long uniseriate hairs, multicellular papillae and multi-branched hairs on corollas.

The glandular trichomes belonged to the two main types, peltate and capitate, occurring in the family Lamiaceae (Giuliani et al., 2018, 2017b) and described in several congeneric species (Huang et al., 2008; Giuliani et al., unpublished). Peltate trichomes covered the plant epidermis uniformly, with the exception of the adaxial sides of the sepals and petals. On the contrary, Blažeković et al.

(2012) observed peltate trichomes on these surfaces, but not on the abaxial side.

The capitate trichomes were distinguished into two morphotypes, based on the length of the stalk and head features: short- and medium-stalked. Blažeković et al. (2012) described, instead, four different morphotypes of capitates (Types I, II, III and IV). The short-stalked hair observed on our samples corresponded to the Type II, but its distribution pattern was different by being limited to the leaf surfaces and to the inflorescence axis, while it resulted ubiquitous in our samples, lacking on the petal adaxial sides. The medium-stalked hair was consistent to Type III, exclusive of the calyx in the Croatian samples, and detected on all examined reproductive organs herein. Types I and IV were not detected in our samples.

Consistent histochemical results were obtained for each trichome type, independently of their localization on plant organs. Peltate and medium-stalked trichomes were responsible for the production of lipophilic substances, in particular terpenes, as confirmed in congeneric species such as *L.* × *intermedia* (Blažeković et al., 2012) and *L. dentata* (Giuliani et al., 2020). The short-stalked capitates were the exclusive producers of hydrophilic compounds, as it was usually observed in most of Lamiaceae species (Giuliani and Maleci Bini, 2008). We did not detect polyphenol production, while such metabolites were produced in the medium stalked hairs of *L. dentata* (Giuliani et al., 2020), all types of glandular trichomes described by Blažeković et al. (2012) in *L. x intermedia* produced flavonoids.

Concerning the phytochemical survey, HS-SPME analyses were evidence of a high variability between vegetative and floral profiles, mainly due to the higher number of the total compounds: 49 in the leaves *vs.* 35 in the flowers. Oxygenated monoterpenes dominated both the profiles (65.66% leaves, 45.97% flowers), followed by monoterpene hydrocarbons in leaves (19.63%) and by sesquiterpene hydrocarbons in flowers (34.90%). The vegetative profile was dominated by 1,8-cineole (**13**), *p*-cymene (**11**), borneol (**32**), caryophyllene oxide (**71**) and *p*-cymen-8-ol (**34**). The floral profile was dominated by linalool acetate (**45**), β-caryophyllene (**57**), linalool (**20**), (*E*)-β-ocimene (**15**) and (*E*)-β-farnesene (**65**). Furthermore, the leaf profile showed a higher number of exclusive compounds than flowers (40 *vs* 26), with 1,8-cineole and linalool acetate being the most represented in leaves and flowers, respectively. There were nine common compounds, with *p*-cymene as the most abundant (**11**, 14.05% leaves; 0.66% flowers). All of the exclusive compounds were more abundant in leaves than flowers, with the exception for δ-3-carene (**10**) and 4-terpineol (**33**).

There were four literature contributions concerning the characterization of VOC profiles of *L. angustifolia*, and referred to different geographical areas and starting plant material: leaves from samples cultivated in Poland (Łyczko et al., 2019), flowering aerial parts from samples cultivated in France (Stierlin et al., 2020), flowering aerial parts from populations located at different latitudes and altitudes in Northern Italy (Demasi et al., 2018), and aerial parts from Central Italy (Pistelli et al., 2013). In previous studies, oxygenated monoterpenes and monoterpene hydrocarbons represented the major classes of compounds, as is the leaf profile analyzed herein. A higher abundance of sesquiterpenes over monoterpene hydrocarbons, as have been detected in the floral *bouquet* of this study, was found in only a few profiles obtained at certain high-altitude accessions (Demasi et al., 2018).

A general qualitative consistency emerged from the comparison, due to the occurrence of the same major compounds, *i.e.* linalool acetate (**45**), linalool (**20**), 1,8-cineole (**13**), *p*-cymene (**11**), borneol (**32**), camphor (**28**), (*E*)-β-ocimene (**15**), β-caryophyllene (**57**), (*E*)-β-farnesene (**65**), α-pinene (**2**), and δ-3-carene (**10**), though showing very different relative abundances in the above-mentioned studies.

Concerning the ecological roles of the major exclusive compounds of leaves, 1,8-cineole (**13**), for which antifungal, anti-ochratoxigenic and antibacterial activity was widely confirmed, generally enhanced by the synergistic action with camphor (**28**), which was present in minor amounts in our samples (Dammak et al., 2019). In addition, it seemed that the antibacterial activity of 1,8-cineole was strengthened by α-terpineol (**36**) and α-pinene, present herein in low percentages, especially against Gram+ bacteria (Karamanoli et al., 2000). 1,8-Cineole, and borneol (**32**) also showed larvicide properties towards different species of mosquitoes (Dris et al., 2017). Other studies attributed to 1,8-cineole an attractive role toward pollinators (Stevenson, 2019). Literature data regarding the minor exclusive compounds of the leaves are lacking.

With regards to the major exclusive volatiles from flowers, the antifungal activity of linalool (**20**) against phytopathogens of the genera *Botrytis*, *Pythium*, and *Magnaporthe* was known (Maietti et al., 2013), as well as the direct proportionality between the relative abundances of this compound and the degree of its activity against *B. cinerea* (Karamanoli et al., 2000). β-Caryophyllene (**57**) was considered a common attractor, but also a defensive role against pests and herbivores was acknowledged (Abraham et al., 2018; Cha et al., 2008). For linalool acetate (**45**), both attractive and defensive roles were confirmed (Usano-Alemany and Herraiz-Peñalver, 2016).

Though most of the studies ascribed its antibacterial properties solely to linalool acetate and linalool, Jianu et al. (2013) detected similar activities even when these two compounds were absent, due to caryophyllene (**46**), 4-terpineol (**30**), borneol and α-pinene (**2**). These findings, thus, suggested a potential synergistic action among other compounds.

Linalool and (*E*)-β-ocimene (**15**) are other common attractors for pollinators (Steen et al., 2019; Stevenson, 2019), particularly bees, butterflies, and moths (Demasi et al., 2018), though (*E*)-β-ocimene was also considered responsible for tritrophic defensive mechanisms (Ghosh and Venkatesan, 2019), as well as (*E*)-β-farnesene (**65**), especially when both molecular isomers were involved (Wu et al., 2019).

With regards to the major common compounds, *p*-cymene would exert fumigant toxic activity and reproductive inhibition on *Acanthoscelides obtectus* (Say, 1831), a bruchid of kidney bean (Regnault-Roger and C. Hamraoui, 1995). Available literature data showed that caryophyllene oxide has allelopathic effects (Sánchez-Muñoz et al., 2012).

Thus, considering the abundance of 1,8-cineole in the vegetative profile of *L. angustifolia*, it is reasonable to suggest the activation of defensive mechanisms in leaves. Linalool acetate and linalool, abundant in the flower profile and in synergy with β-caryophyllene and (*E*)-β-ocimene, could be mainly involved in attractive roles. However, the importance of minor compounds that could act, along with the major ones, in synergic mechanisms of attraction or repulsion cannot be excluded. Previous studies showed the peculiarity of plant organs volatile emissions based on the ecological role of those parts in the very same specimen (Ascrizzi et al., 2016).

Another possible function of compounds with bactericidal activity such as 1,8-cineole, may be the modulation of the endophyte community in *L. angustifolia.* The endophytes composition is known to vary in different parts of the plant (Emiliani et al., 2014). These authors also suggested that the EO composition itself may be modulated by the endophytes, hence leaving open the question if the plant-produced compounds modulate endophytes or *vice versa.*

To complete the phytochemical survey, the EO from flowering aerial parts was also analyzed. The most represented compound classes were oxygenated monoterpenes (70.42%), sesquiterpene hydrocarbons (8.87%) and oxygenated sesquiterpenes (6.55%). 69 total compounds were detected, of which the most abundant were linalool (**23**), linalool acetate (**39**), 4-terpineol (**30**), lavandulol acetate (**42**), β-caryophyllene (**46**), and caryophyllene oxide (**61**).

The EO of *L. angustifolia* was largely studied for its commercial applications (Da Porto et al., 2009), and previous work highlighted a great degree of intraspecific variability, probably due to the different geographical origin of samples and their different methods of cultivation, conservation, drying process, along with analytical procedures (**Table 5**). Most of the contributions referred to flowering aerial parts, apart from the South-African, Brazilian and Iranian samples (Hassanpouraghdam et al., 2011; Mantovani et al., 2013; Muyima et al., 2002) and the cultivar was indicated only for the samples from Hungary, Romania and central Italy (Détár et al., 2020; Oroian et al., 2019; Pistelli et al., 2017).

Oxygenated monoterpenes invariably dominated all the EO profiles from literature. Linalool (**23**) and linalyl acetate (**39**), as in the samples analyzed herein, dominated the EO profiles reported in the previous contribution from Italy referring to samples from the northern (Binello et al., 2014; Da Porto and Decorti, 2008; Demasi et al., 2018; Maietti et al., 2013; Tardugno et al., 2019) and central regions (Pistelli et al., 2017). However, Binello et al. (2014) compared different distillation techniques and did not detected linalool acetate among the main compounds of the EO obtained from hydrodistillation. On the contrary, the low concentration of camphor (**26**) related to a higher percentage of β-caryophyllene (**46**), was confirmed (Aprotosoaie et al., 2017). However, a quite different composition of *L. angustifolia* EO, obtained by hydrodistillation, was also reported in Conti et al. (2010) for an Italian specimen, as it was mainly rich in fenchone, camphor, and camphene (Conti et al., 2010). Linalool (**23**) and linalyl acetate were also detected as the main compounds in *L. angustifolia* EOs from different geographical areas referred to both extra-European [China (Chen et al., 2020; Li et al., 2019), Turkey (Küçük et al., 2018) and European countries: Romania (Oroian et al., 2019), Hungary (Détár et al., 2020), Poland (Łyczko et al., 2019; Wesolowska et al., 2010), Serbia (Stanojević et al., 2011), Greece (Hassiotis et al., 2014, 2010) and Spain (Carrasco et al., 2015).

A high level of variability was found, however, concerning the other main compounds, with the evidence of notable quantitative differences. For instance, the presence of 4-terpineol (**30**) was detected in samples from China and Spain (Carrasco et al., 2015; Chen et al., 2020) and in some Italian accessions from Friuli Venezia Giulia and Emilia Romagna (Da Porto et al., 2009; Maietti et al., 2013), while it was not found in other Italian samples (Binello et al., 2014; Pistelli et al., 2017). The same consideration arises for α-terpineol (**32**), detected in higher concentration in samples from Tuscany (Pistelli et al., 2017) compared to our work as well as in other national surveys (Binello et al., 2014; Da Porto et al., 2009; Maietti et al., 2013). Finally, as opposed to our profile, in some of the Italian samples coumarins were detected (Binello et al., 2014): typical of the leaves, their occurrence may be justified by the starting plant material, or by the adopted extraction technique (Aprotosoaie et al., 2017). Among the other extra-European investigated samples, 1,8-cineole, present at percentage slightly higher than 1.0% in our samples, was among the most abundant compounds in Iranian, Brazilian and South-African plants (Hassanpouraghdam et al., 2011; Mantovani et al., 2013; Muyima et al., 2002). Furthermore, in contrast to the results reported herein, borneol (**29**) turned out to be a dominant compound in Brazilian and Iranian samples (Hassanpouraghdam et al., 2011; Mantovani et al., 2013). Its abundance seem to be linked to the processed plant material, in particular to young leaves and floral buds (Hassiotis et al., 2014), while aerial parts in full bloom were examined herein.

With regards to monoterpene hydrocarbons, limonene occurred in amounts higher than 1.0%, while other compounds accounted for lower amounts.

Among the sesquiterpenes, β-caryophyllene (**46**), caryophyllene oxide (**61**), β-farnesene (**49**), and (*E*)-γ-bisabolene (**54**) were detected. These compounds were considered among the most frequent sesquiterpenes of this species in literature (Aprotosoaie et al., 2017).

Biological activities of *L. angustifolia* EO were widely investigated for their wide applications in medicine, food and cosmetic industry, as natural preservatives, as well as in agriculture, against phytopathogens (Maietti et al., 2013; Muyima et al., 2002; Tardugno et al., 2019; Wells et al., 2018).

Lavandula species seem to have lower antioxidant properties than other plants, because of their low content of flavonoids and phenols. Nevertheless, *L. angustifolia* EO showed antioxidant activity against lipid peroxidation (Carrasco et al., 2015; Wells et al., 2018). The heterogeneous data present in literature could be explained as a result of different extraction procedures, which influence the final derivatives (Pistelli et al., 2017; Wells et al., 2018).

A dose-dependent anti-inflammatory action was recognized in *L. angustifolia* EO, in particular to the compound linalool (**23**) and linalool acetate (**39**) (Wells et al., 2018) that showed strong analgesic and anti-inflammatory properties.

In particular, due to the abundance of these molecules, *L. angustifolia* EO displayed cardio-protective effects related to their anti-inflammatory, radical scavenging, and anti-oxidant properties (Wells et al., 2018). A recent *in vivo* study demonstrated an anti-inflammatory action of *L. angustifolia* EO higher than ibuprofen due to its high content of linalool, linalool acetate, borneol (**29**), and 1,8-cineole (**16**) (Chen et al., 2020). Other important evidences came from an *in vivo* study on chronic pain (inflammatory and neuropathic): a significant reduction of the hyperalgesia followed the inhalation of lavender EO, relatable to its high concentrations of linalool, linalool acetate, lavandulyl acetate, α-terpineol (**32**), geranyl acetate (**45**), caryophyllene oxide (**61**), and 1,8-cineole (Donatello et al., 2020). Other inflammatory-based conditions, such as allergies and asthma, seemed to be improved after inhalation or topical application of lavender EO. Through these two dosage forms, EOs can be easily absorbed, so much so that linalool and linalool acetate could be traced in the blood stream. These pharmacokinetic aspects were widely investigated in aromatherapy and it seems that the anxiolytic effects recorded in this field could be attributed to the presence of these two compounds in the blood stream (Wells et al., 2018).

In the light of all these evidences, the traditional uses of the target species based on its anti-inflammatory (Hajhashemi et al., 2003) and sedative (Naghibi et al., 2005) properties appear to be validated by the scientific literature regarding the biological activity. In addition, based on the composition of the EO analysed herein, it is reasonable to suggest that the investigated EO may possess properties similar to those here discussed.

Therefore,

Concerning sensory attributes, the typical scent of the genus *Lavandula* is due to monoterpenes. The high concentrations of linalool and linalool acetate seem to improve sensory qualities and pharmaceutical properties of EOs (Aprotosoaie et al., 2017). Indeed, the most appreciated EOs in cosmetic and perfume industries are distinguished by their high content of these two compounds, and the low concentration of camphor. However, the abundance of camphor is valued for EOs used in aromatherapy and phytotherapy. Linalool (**23**) is responsible for fresh, floral, sweeter, and lemon notes, while linalool acetate (**39**) for floral, herbaceous, woody, and bergamot-reminiscent features (Aprotosoaie et al., 2017). Therefore, these sensory notes may be attributed to the samples of *L. angustifolia* investigated herein.

Finally, the studied samples meet the quality parameters for *L. angustifolia* essential oil (ISO 3515:2002) regarding the main compounds linalool (**23**), 1,8-cineole (**16**), and 4-terpineol (**30**) (Aprotosoaie et al., 2017).

Concerning the dissemination actions, the outcomes of this multidisciplinary investigation converged in the realization of a novel interpretative apparatus for the target species at the Ghirardi Botanic Garden. In this way, the scientific research becomes more transparent, and accountable for visitors.

1. Conclusions

In this work, for the first time, a combined morphological and phytochemical approach of study on both the vegetative and reproductive organs of *Lavandula angustifolia* was reported. The primary goal was to establish a link between the glandular *indumentum* and the production and emission of volatiles. In addition, the VOC profiles from leaves and flowers and the composition of the EO from the flowering aerial parts were characterized.

The peltate and the medium-stalked capitates were responsible for the synthesis of terpenes, resulting in the emission of VOCs and in the production of EOs. From the phytochemical perspective, the vegetative *bouquet* resulted more complex than the floral one due to the higher number of both the total and the leaf-exclusive compounds. The dominant compounds corresponded to the main exclusive compounds, *i.e.* 1,8-cineole in leaves and linalool acetate and linalool in flowers. Nine common compounds, including *p*-cymene, camphor, δ-3-carene, and 4-terpineol were detected in both profiles. Literature data concerning the ecological roles of the exclusive compounds and of the major common compounds emphasized the dominance of repulsive mechanisms taking place at leaf-level and of attractive strategies towards pollinators played by flowers.

The EO profile was dominated by linalool and linalool acetate, both conferring pleasant sensory qualities as floral, sweet and citrus-like notes; those compounds can also grant pharmaceutical properties.

These obtained results will also enrich the Ghirardi Botanic Garden with new information, and a dedicated iconographic and didactic labelling was developed, thus combining the scientific research to the educational missions in the context of the *Open Science*. What we propose is an immediate relationship between the scientific research and the fruition by the general public, creating a cycle that has the same starting and ending points, the plant heritage of the Ghirardi Botanic Garden.

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2. References

Abraham, A.A., Verghese, A., Muthangi, S., 2018. Role of colour and volatile in foraging behaviour of honeybee *Apis cerana* on *Jacquemontia pentanthos*. J. Asia. Pac. Entomol. 21, 1122–1128.

Adams, R.P., 1995. Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy. Allured Publishing Corporation, Illinois, USA.

Al-Sarar, A.S., Hussein, H.I., Abobakr, Y., Bayoumi, A.E., Al-Otaibi, M.T., 2014. Fumigant toxicity and antiacetylcholinesterase activity of Saudi Mentha longifolia and *Lavandula dentata* species against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Turkiye Entomoloji Derg. 38, 11–18. https://doi.org/10.16970/ted.10646

Aprotosoaie, A.C., Gille, E., Trifan, A., Luca, V.S., Miron, A., 2017. Essential oils of *Lavandula* genus: a systematic review of their chemistry. Phytochem. Rev. 16, 761–799. https://doi.org/10.1007/s11101-017-9517-

1

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

Ascrizzi, R., Cioni, P.L., Giusti, G., Pistelli, L., Flamini, G., 2016. Patterns in Volatile Emission of Different Aerial Parts of Caper (*Capparis spinosa* L.). Chem. Biodivers. 13, 904–912. https://doi.org/10.1002/cbdv.201500292

Beccari, N., Mazzi, V., 1966. Manuale di tecnica microscopica.

Bedini, S., Flamini, G., Ascrizzi, R., Venturi, F., Ferroni, G., Bader, A., Girardi, J., Conti, B., 2018. Essential oils sensory quality and their bioactivity against the mosquito *Aedes albopictus*. Sci. Rep. 8. https://doi.org/10.1038/s41598-018-36158-w

Binello, A., Orio, L., Pignata, G., Nicola, S., Chemat, F., Cravotto, G., 2014. Effect of microwaves on the *in situ* hydrodistillation of four different Lamiaceae. Comptes Rendus Chim. 17, 181–186. https://doi.org/10.1016/j.crci.2013.11.007

Blažeković, B., Stabentheiner, E., Brantner, A., Vladimir-Knežević, S., 2012. A Comparative study on glandular trichomes of *Lavandula* x *intermedia*, Budrovka and *L. angustifolia*. Phyt. - Ann. Rei Bot. 52, 227–244.

Bouazama, S., Harhar, H., Costa, J., Desjobert, J.M., Talbaoui, A., Tabyaoui, M., 2017. Chemical composition and antibacterial activity of the essential oils of *Lavandula pedunculata* and *Lavandula dentata*. J. Mater. Environ.

Sci. 8, 2154–2160.

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

Carrasco, A., Martinez-Gutierrez, R., Tomas, V., Tudela, J., 2015. *Lavandula angustifolia* and *Lavandula latifolia* Essential Oils from Spain: Aromatic Profile and Bioactivities. Planta Med. 82, 163–170. https://doi.org/10.1055/s-0035-1558095

Cha, D.H., Nojima, S., Hesler, S.P., Zhang, A., Linn, C.E., Roelofs, W.L., Loeb, G.M., 2008. Identification and field evaluation of grape shoot volatiles attractive to female grape berry moth (*Paralobesia viteana*). J. Chem. Ecol. 34, 1180–1189.

Chen, X., Zhang, L., Qian, C., Du, Z., Xu, P., Xiang, Z., 2020. Chemical compositions of essential oil extracted from *Lavandula angustifolia* and its prevention of TPA-induced inflammation. Microchem. J. 153. https://doi.org/10.1016/j.microc.2019.104458

Conti, B., Canale, A., Cioni, P.L., Flamini, G., 2010. Repellence of essential oils from tropical and Mediterranean Lamiaceae against *Sitophilus zeamais*. Bull. Insectology 63, 197–202.

Costea, T., Străinu, A.-M., Gîrd, C.E., 2019. Botanical characterization, chemical composition and antioxidant activity of romanian lavender (*Lavandula Angustifolia* mill.) flowers. Stud. Univ. Vasile Goldis Arad, Ser. Stiint. Vietii 29, 151–159.

Da Porto, C., Decorti, D., 2008. Analysis of the volatile compounds of flowers and essential oils from *Lavandula angustifolia* cultivated in northeastern Italy by headspace solid-phase microextraction coupled to gas chromatography-mass spectrometry. Planta Med. 74, 182–187. https://doi.org/10.1055/s-2008-1034295

Da Porto, C., Decorti, D., Kikic, I., 2009. Flavour compounds of *Lavandula angustifolia* L. to use in food manufacturing: Comparison of three different extraction methods. Food Chem. 112, 1072–1078.

Dammak, I., Hamdi, Z., Kammoun El Euch, S., Zemni, H., Mliki, A., Hassouna, M., Lasram, S., 2019. Evaluation of antifungal and anti-ochratoxigenic activities of *Salvia officinalis*, *Lavandula dentata* and *Laurus nobilis* essential oils and a major monoterpene constituent 1,8-cineole against *Aspergillus carbonarius*. Ind. Crops Prod. 128, 85–93. https://doi.org/10.1016/j.indcrop.2018.11.006

David, R., Carde, J.P., David, R., 1964. Histochimie–coloration differentielle des inclusions lipidiques et terpeniques des pseudophylles du pin maritime au moyen du reactif NADI. C. R. Biol. 258, 1338–1340.

Davies, N.W., 1990. Gas chromatographic retention indices of monoterpenes and sesquiterpenes on Methyl Silicon and Carbowax 20M phases. J. Chromatogr. A 503, 1–24.

Demasi, S., Caser, M., Lonati, M., Cioni, P.L., Pistelli, L., Najar, B., Scariot, V., 2018. Latitude and altitude influence secondary metabolite production in peripheral alpine populations of the mediterranean species *Lavandula* *angustifolia* mill. Front. Plant Sci. 9. https://doi.org/10.3389/fpls.2018.00983

Détár, E., Németh, É.Z., Gosztola, B., Demján, I., Pluhár, Z., 2020. Effects of variety and growth year on the essential oil properties of lavender (*Lavandula angustifolia* Mill.) and lavandin (*Lavandula* x *intermedia* Emeric ex Loisel.). Biochem. Syst. Ecol. 90. https://doi.org/10.1016/j.bse.2020.104020

Donatello, N.N., Emer, A.A., Salm, D.C., Ludtke, D.D., Bordignon, S.A.S.R., Ferreira, J.K., Salgado, A.S.I., Venzke, D., Bretanha, L.C., Micke, G.A., Martins, D.F., 2020. *Lavandula angustifolia* essential oil inhalation reduces mechanical hyperalgesia in a model of inflammatory and neuropathic pain: The involvement of opioid and cannabinoid receptors. J. Neuroimmunol. 340. https://doi.org/10.1016/j.jneuroim.2020.577145

Dris, D., Tine-Djebbar, F., Soltani, N., 2017. *Lavandula dentata* Essential Oils: Chemical Composition and Larvicidal Activity Against *Culiseta longiareolata* and *Culex pipiens* (Diptera: Culicidae). African Entomol. 25, 387–394. https://doi.org/10.4001/003.025.0387

Emiliani, G., Mengoni, A., Maida, I., Perrin, E., Chiellini, C., Fondi, M., Gallo, E., Gori, L., Maggini, V., Vannacci, A., Biffi, S., Firenzuoli, F. and Fani, R., 2014. Linking bacterial endophytic communities to essential oils: Clues from *Lavandula angustifolia* Mill. Evid. Based Complement. Altern. Med. ID 650905. https://doi.org/10.1155/2014/650905.

Gahan, P.B., 1984. Plant histochemistry and cytochemistry. Academic Press, London.

Ghosh, E., Venkatesan, R., 2019. Plant Volatiles Modulate Immune Responses of Spodoptera litura. J. Chem. Ecol. 45, 715–724.

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

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

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

Giuliani, C., Bottoni, M., Ascrizzi, R., Milani, F., Papini, A., Flamini, G., Fico, G., 2020. *Lavandula dentata* from Italy: analysis of trichomes and volatiles. Chemistry & Biodiversity, 17, p.e2000532.

Giuliani, C., Maleci Bini, L., 2008. Insight into the structure and chemistry of glandular trichomes of Labiatae, with emphasis on subfamily Lamioideae. Plant Syst. Evol. 276. https://doi.org/https://doi.org/10.1007/s00606-008-0085-0

Greenspan, P., Mayer, E.P., Fowler, S.D., 1985. Nile red: a selective fluorescent stain for intracellular lipid droplets. J. Cell Biol. 100, 965–973.

Hajhashemi, V., Ghannadi, A., Sharif, B., 2003. Anti-inflammatory and analgesic properties of the leaf extracts and essential oil of *Lavandula angustifolia* Mill. J. Ethnopharmacol. 89, 67–71.

Hassanpouraghdam, M.B., Hassani, A., Vojodi, L., Asl, B.H., Rostami, A., 2011. Essential oil constituents of *Lavandula officinalis* Chaix. from Northwest Iran. chemija 22, 167–171.

Hassiotis, C.N., Lazari, D.M., Vlachonasios, K.E., 2010. The effects of habitat type and diurnal harvest on essential oil yield and composition of *Lavandula angustifolia* Mill. Fresenius Environ. Bull. 19, 1491–1498.

Hassiotis, C.N., Ntana, F., Lazari, D.M., Poulios, S., Vlachonasios, K.E., 2014. Environmental and developmental factors affect essential oil production and quality of *Lavandula angustifolia* during flowering period. Ind. Crops Prod. 62, 359–366.

Huang, S.S., Kirchoff, B.K., Liao, J.P., 2008. The capitate and peltate glandular trichomes of *Lavandula pinnata* L. (Lamiaceae): Histochemistry, ultrastructure, and secretion1. J. Torrey Bot. Soc. 135, 155–167. https://doi.org/10.3159/07-RA-045.1

Jennings, W., Shibamoto, T., 1982. Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography, Food / Nahrung. Academic Press, New York, London, Sydney, Toronto, San Francisco.

Jensen, W.A., 1962. Botanical histochemistry: principles and practice. San Francisco.

Jianu, C., Pop, G., TGruia, A., Horhat, F.G., 2013. Chemical composition and antimicrobial activity of essential oils of lavender (*Lavandula angustifolia*) and lavandin (*Lavandula* x *intermedia*) grown in Western Romania. Int. J. Agric. Biol. 15.

Karamanoli, K., Vokou, D., Menkissoglu, U., Constantinidou, H.-I., 2000. Bacterial colonization of phyllosphere of mediterranean aromatic plants. J. Chem. Ecol. 26, 2035–2048. https://doi.org/10.1023/A:1005556013314

Kim, N.-S., Lee, D.-S., 2002. Comparison of different extraction methods for the analysis of fragrances from *Lavandula* species by gas chromatography-mass spectrometry. J. Chromatogr. A 982, 31–47. https://doi.org/10.1016/S0021-9673(02)01445-0

Küçük, S., Altıntaş, A., Demirci, B., Koca, F., Başer, K.H.C., 2019. Morphological, anatomical and phytochemical characterizations of *Lavandula stoechas* l. subsp. *stoechas* growing in Turkey. Nat. Volatiles Essent. Oils 6, 9–19.

Küçük, S., Çetintaş, E., Kürkçüoğlu, M., 2018. Volatile compounds of the *Lavandula angustifolia* Mill. (Lamiaceae) species cultured in Turkey. J. Turkish Chem. Soc. Sect. A Chem. 5, 1303–1308. https://doi.org/10.18596/jotcsa.463689

Li, H., Li, J., Dong, Y., Hao, H., Ling, Z., Bai, H., Wang, H., Cui, H., Shi, L., 2019. Time-series transcriptome provides insights into the gene regulation network involved in the volatile terpenoid metabolism during the flower development of lavender. BMC Plant Biol. 19, 1–17.

https://doi.org/10.1186/s12870-019-1908-6

Łyczko, J., Jałoszyński, K., Surma, M., Masztalerz, K., Szumny, A., 2019. Hs-spme analysis of true lavender (*Lavandula angustifolia* Mill.) leaves treated by various drying methods. Molecules 24. https://doi.org/10.3390/molecules24040764

Maietti, S., Rossi, D., Guerrini, A., Useli, C., Romagnoli, C., Poli, F., Bruni, R., Sacchetti, G., 2013. A multivariate analysis approach to the study of chemical and functional properties of chemo-diverse plant derivatives: Lavender essential oils. Flavour Fragr. J. 28, 144–154. https://doi.org/10.1002/ffj.3145

Mantovani, A.L.L., Vieira, G.P.G., Cunha, W.R., Groppo, M., Santos, R.A., Rodrigues, V., Magalhães, L.G., Crotti, A.E.M., 2013. Chemical composition, antischistosomal and cytotoxic effects of the essential oil of Lavandula angustifolia grown in Southeastern Brazil. Rev. Bras. Farmacogn. 23, 877–884.

Martins, R.P., Gomes, R.A.D.S., Malpass, A.C.G., Okura, M.H., 2019. Chemical characterization of lavandula dentata l. Essential oils grown in uberaba-mg. Cienc. Rural 49. https://doi.org/10.1590/0103-8478cr20180964

Masada, Y., 1976. Analysis of essential oils by gas chromatography and mass spectrometry. New York, NY.

Muyima, N.Y.O., Zulu, G., Bhengu, T., Popplewell, D., 2002. The potential application of some novel essential oils as natural cosmetic preservatives in an aqueous cream formulation. Flavour Fragr. J. 17, 258–266.

Naghibi, F., Mosadegh, M., Mohammadi, M.S., Ghorbani, A.B., 2005. Labiatae family in folk medicine in Iran: from ethnobotany to pharmacology.

Nasiri Lari, Z., Hajimonfarednejad, M., Riasatian, M., Abolhassanzadeh, Z., Iraji, A., Vojoud, M., Heydari, M., Shams, M., 2020. Efficacy of inhaled *Lavandula angustifolia* Mill. Essential oil on sleep quality, quality of life and metabolic control in patients with diabetes mellitus type II and insomnia. J. Ethnopharmacol. 251, 112560. https://doi.org/10.1016/j.jep.2020.112560

Oroian, C., Odagiu, A., Racz, C.P., Oroian, I., Mureşan, I.C., Duda, M., Ilea, M., Braşovean, I., Iederan, C., Marchiş, Z., 2019. Composition of *Lavandula angustifolia* L. cultivated in Transylvania, Romania. Not. Bot. Horti Agrobot. Cluj-Napoca 47, 643–650. https://doi.org/10.15835/nbha47311565

Pistelli, L., Najar, B., Giovanelli, S., Lorenzini, L., Tavarini, S., Angelini, L.G., 2017. Agronomic and phytochemical evaluation of lavandin and lavender cultivars cultivated in the Tyrrhenian area of Tuscany (Italy). Ind. Crops Prod. 109, 37–44. https://doi.org/10.1016/j.indcrop.2017.07.041

Pistelli, L., Noccioli, C., D'Angiolillo, F., Pistelli, L., 2013. Composition of volatile in micropropagated and field grown aromatic plants from tuscany islands. Acta Biochim. Pol. 60, 43–50.

Qneibi, M., Jaradat, N., Hawash, M., Zaid, A.N., Natsheh, A.-R., Yousef, R., AbuHasan, Q., 2019. The neuroprotective role of *Origanum syriacum* L. and Lavandula den*tata* L. Essential oils through their effects on AMPA receptors. Biomed Res. Int. 2019. https://doi.org/10.1155/2019/5640173

Regnault-Roger, C. Hamraoui, A., 1995. Fumigant toxic activity and reproductive inhibition induced by monoterpenes on *Acanthoscelides obtectus* (Say)(Coleoptera), a bruchid of kidney bean (*Phaseolus vulgaris* L.). J. Stored Prod. Res. 31, 291–299.

Rostami, H., Kazemi, M., Shafiei, S., 2012. Antibacterial activity of lavandula officinalis and *Melissa officinalis* against some human pathogenic bacteria. Asian J. Biochem. 7, 133–142. https://doi.org/10.3923/ajb.2012.133.142

Saeidnia, S., Gohari, A.R., Haddadi, A., Amin, G., Nikan, M., Hadjiakhoondi, A., 2014. Presence of monoterpene synthase in four Labiatae species and Solid-Phase Microextraction- Gas chromatography-Mass Spectroscopy analysis of their aroma profiles. Pharmacognosy Res. 6, 138–142. https://doi.org/10.4103/0974-8490.129033

Sánchez-Muñoz, B.A., Aguilar, M.I., King-Díaz, B., Rivero, J.F., Lotina-Hennsen, B., 2012. The sesquiterpenes β-caryophyllene and caryophyllene oxide isolated from *Senecio salignus* act as phytogrowth and photosynthesis inhibitors. Molecules 17, 1437–1447.

Stanojević, L., Stanković, M., Cakić, M., Nikolić, V., Nikolić, L., Ilić, D., Radulović, N., 2011. The effect of hydrodistillation techniques on yield, kinetics, composition and antimicrobial activity of essential oils from flowers of *Lavandula officinalis* L. Hem. Ind. 65, 455–463.

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

Stenhagen, E., Abrahamsson, S., McLafferty, F.W., 1974. Registry of Mass spectral data. New York, NY.

Stevenson, P.C., 2019. For antagonists and mutualists: the paradox of insect toxic secondary metabolites in nectar and pollen. Phytochem. Rev. 1–12.

Stierlin, É., Michel, T., Fernandez, X., 2020. Field analyses of lavender volatile organic compounds: performance evaluation of a portable gas chromatography–mass spectrometry device. Phytochem. Anal. 1–8. https://doi.org/10.1002/pca.2942

Tardugno, R., Serio, A., Pellati, F., D'Amato, S., Chaves López, C., Bellardi, M.G., Di Vito, M., Savini, V., Paparella, A., Benvenuti, S., 2019. Lavandula x intermedia and *Lavandula angustifolia* essential oils: phytochemical composition and antimicrobial activity against foodborne pathogens. Nat. Prod. Res. 33, 3330–3335. https://doi.org/10.1080/14786419.2018.1475377

Usano-Alemany, J., Herraiz-Peñalver, D., 2016. Ecological Aspects of the Essential Oils from Different *Salvia lavandulifolia* Vahl Aerial Parts. J. Essent. Oil-Bearing Plants 19, 1000–1005. https://doi.org/10.1080/0972060X.2014.886165

Wells, R., Truong, F., Adal, A.M., Sarker, L.S., Mahmoud, S.S., 2018. *Lavandula* essential oils: A current review of applications in medicinal, food, and cosmetic industries of lavender. Nat. Prod. Commun. 13, 1403–1417.

Wesolowska, A., Jadczak, D., Grzeszczuk, M., 2010. Influence of distillation time on the content and composition of essential oil isolated from lavender (*Lavandula angustifolia* Mill.). Herba Pol. 56.

Wu, H., Li, R.-T., Dong, J.-F., Jiang, N.-J., Huang, L.-Q., Wang, C.-Z., 2019. An odorant receptor and glomerulus responding to farnesene in *Helicoverpa assulta* (Lepidoptera: Noctuidae). Insect Biochem. Mol. Biol. 115, 103106.

Table 1. Distribution pattern of trichomes on the examined vegetative and reproductive organs of *Lavandula angustifolia* Mill.

Trichome type	**Leaf**		**Calyx**		**Corolla**	
	adax	abax	adax	abax	adax	abax
peltate	+	+	-	+	-	+
short capitate	+	+	+	+	-	±
medium capitate	-	-	-	±	+	-
dendritic	++	++	-	+	+	+

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

Table 2. Results of the histochemical tests on the glandular trichomes on vegetative and reproductive organs of *Lavandula angustifolia* Mill.

Stainings	**Target-compounds**	**peltate**	**short capitate**	**medium capitate**
Fluoral Yellow-088	Total lipids	+	−	++
Nile Red	Neutral lipids	+	−	+
Nadi reagent	Terpenoids	++	−	++
PAS reagent	Total polysaccharides	−	+	−
Ruthenium Red	Acid polysaccharides	−	−	−
Alcian Blue	Muco-polysaccharides	−	+	−
Ferric Tricholoride	Polyphenols	−	−	−

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

Table 3. HS-SPME profiles of leaves and flowers of *Lavandula angustifolia* Mill. The main compounds are indicated in bold.

	l.r.i.	**Compounds**	**Relative Abundance (%)**	
			Leaves	**Flowers**
1	931	α-thujene	0.29	-
2	941	α-pinene	0.52	0.11
3	954	camphene	3.11	-
4	976	sabinene	0.12	-
5	982	β-pinene	0.85	0.09
6	992	2,3-dehydro-1,8-cineole	0.70	-
7	993	myrcene	-	3.10
8	1009	(*Z*)-3-hexenol acetate	0.25	-
9	1010	hexyl acetate	-	-
10	1011	δ-3-carene	0.14	0.24
11	**1027**	***p*-cymene**	**14.05**	0.66
12	1032	limonene	-	3.31
13	**1034**	**1,8-cineole**	**42.17**	-
14	1042	(*Z*)-β-ocimene	-	1.50
15	**1052**	**(*E*)-β-ocimene**	-	**8.65**
16	1062	γ-terpinene	0.12	-
17	1076	*trans*-linalool oxide (furanoid)	0.16	-
18	1089	*p*-cymenene	0.43	-
19	1095	*trans*-sabinene hydrate	0.15	-
20	**1101**	**linalool**	-	**9.54**
21	1104	α-thujone	0.21	-
22	1111	1-octen-3-yl acetate	0.73	-
23	1118	β-thujone	0.28	-
24	1122	*cis*-*p*-mentha-2,8-dien-1-ol	0.2	-
25	1123	*cis*-*p*-menth-2-en-1-ol	0.69	-
26	1129	*allo*ocimene	-	0.32
27	1140	nopinone	0.30	-
28	1143	camphor	2.32	0.35
29	1144	*trans*-verbenol	-	0.12
30	1145	*neo*-*iso*pulegol	0.36	-
31	1154	menthone	0.82	-
32	**1167**	**borneol**	**6.32**	-
33	1178	4-terpineol	0.22	0.85
34	**1183**	***p*-cymen-8-ol**	**4.38**	-
35	1187	cryptone	-	0.11
36	1189	α-terpineol	0.44	-
37	1192	dihydro carveol	0.26	-
38	1205	verbenone	0.88	-
39	1218	*trans*-carveol	0.33	-
40	1226	*neo*-*iso*-dihydrocarveol	0.26	-
41	1232	*iso*bornyl formate	0.44	-
42	1240	cumin aldehyde	0.62	-
43	1244	carvone	0.69	-
44	1252	piperitone	0.82	-
45	**1259**	**linalool acetate**	-	**32.76**
46	1265	*cis*-carvone oxide	0.31	-
47	1285	*iso*bornyl acetate	0.36	-
48	1289	*p*-cymen-7-ol	0.97	-
49	1352	α-terpinyl acetate	-	0.10
50	1366	neryl acetate	-	0.76
51	1384	β-bourbonene	0.17	-
52	1385	geranyl acetate	-	1.49
53	1391	7-*epi*-sesquithujene	-	0.40
54	1402	italicene	-	0.16
55	1409	α-cedrene	0.12	-

56	1416	*cis*-α-bergamotene	-	0.59
57	**1420**	**β-caryophyllene**	-	**21.76**
58	1421	α-santalene	0.45	tr
59	1433	γ-elemene	-	0.14
60	1438	*trans*-α-bergamotene	-	0.52
61	1441	aromadendrene	-	0.68
62	1445	(*Z*)-β-farnesene	-	tr
63	1449	*epi*-β-santalene	-	0.29
64	1456	α-humulene	-	0.31
65	**1460**	**(*E*)-β-farnesene**	-	**7.63**
66	1477	γ-muurolene	-	2.10
67	1507	(*E*,*E*)-α-farnesene	-	0.12
68	1513	*trans*-γ-cadinene	1.70	0.10
69	1524	β-sesquiphellandrene	-	0.10
70	1531	(*E*)-α-bisabolene	0.11	-
71	**1581**	**caryophyllene oxide**	**4.41**	0.48
72	1614	1,10-*di*-*epi*-cubenol	0.42	-
73	1630	5-cedranone	0.11	-
74	1636	caryophylla-4(14),8(15)-dien-5-ol	0.21	-
75	1640	*epi*-α-cadinol	0.98	-
76	1682	*cis*-14-*nor*-muurol-5-en-4-one	0.34	-
		Monoterpene hydrocarbons	**19.63**	**17.98**
		Oxygenated monoterpenes	**65.66**	**45.97**
		Sesquiterpene hydrocarbons	**2.55**	**34.90**
		Oxygenated sesquiterpenes	**6.47**	**0.48**
		Non-terpene derivatives	**0.98**	**0.11**
		Total identified	**95.29%**	**99.44%**

Table 4. Composition of the essential oil obtained from aerial parts of *Lavandula angustifolia* Mill. The main compounds are indicated in bold.

	l.r.i.	Compounds	Relative Abundance (%)
1	931	α-thujene	tr[a]
2	941	α-pinene	0.16
3	954	camphene	0.13
4	976	sabinene	0.10
5	980	1-octen-3-ol	0.41
6	982	β-pinene	0.17
7	987	3-octanone	0.33
8	993	myrcene	0.46
9	1005	α-phellandrene	tr
10	1010	hexyl acetate	0.25
11	1011	δ-3-carene	0.12
12	1018	α-terpinene	tr
13	1024	*o*-cymene	0.18
14	1027	*p*-cymene	0.60
15	1032	limonene	1.49
16	1034	1,8-cineole	1.38
17	1042	(*Z*)-β-ocimene	0.84
18	1052	(*E*)-β-ocimene	0.99
19	1062	γ-terpinene	0.17
20	1070	*cis*-sabinene hydrate	0.34
21	1076	*trans*-linalool oxide (furanoid)	0.56
22	1088	terpinolene	0.62
23	**1101**	**linalool**	**27.7**
24	1111	1-octen-3-yl acetate	1.21
25	1123	*cis*-*p*-menth-2-en-1-ol	0.58
26	1143	camphor	0.40
27	1152	hexyl isobutyrate	0.40
28	1163	pinocarvone	tr
29	1167	borneol	2.63
30	**1178**	**4-terpineol**	**5.30**
31	1187	cryptone	2.75
32	1189	α-terpineol	2.08
33	1205	verbenone	0.50
34	1218	*trans*-carveol	0.13
35	1230	nerol	0.28
36	1232	*cis*-*p*-mentha-1(7),8-dien-2-ol	0.48
37	1240	cumin aldehyde	2.50
38	1244	carvone	0.95
39	**1259**	**linalool acetate**	**17.99**
40	1272	phellandral	tr
41	1285	*iso*bornyl acetate	0.16
42	**1288**	**lavandulol acetate**	**4.29**
43	1289	*p*-cymen-7-ol	0.12
44	1366	neryl acetate	0.66
45	1385	geranyl acetate	1.39
46	**1420**	**β-caryophyllene**	**3.80**
47	1438	*trans*-α-bergamotene	0.31
48	1449	*epi*-β-santalene	0.12
49	1460	(*E*)-β-farnesene	2.08
50	1461	*allo*aromadendrene	0.17
51	1477	γ-muurolene	0.51
52	1509	β-bisabolene	tr
53	1513	*trans*-γ-cadinene	0.28
54	1535	(*E*)-γ-bisabolene	1.14
55	1545	*cis*-sesquisabinene hydrate	0.23
56	1556	germacrene B	0.46

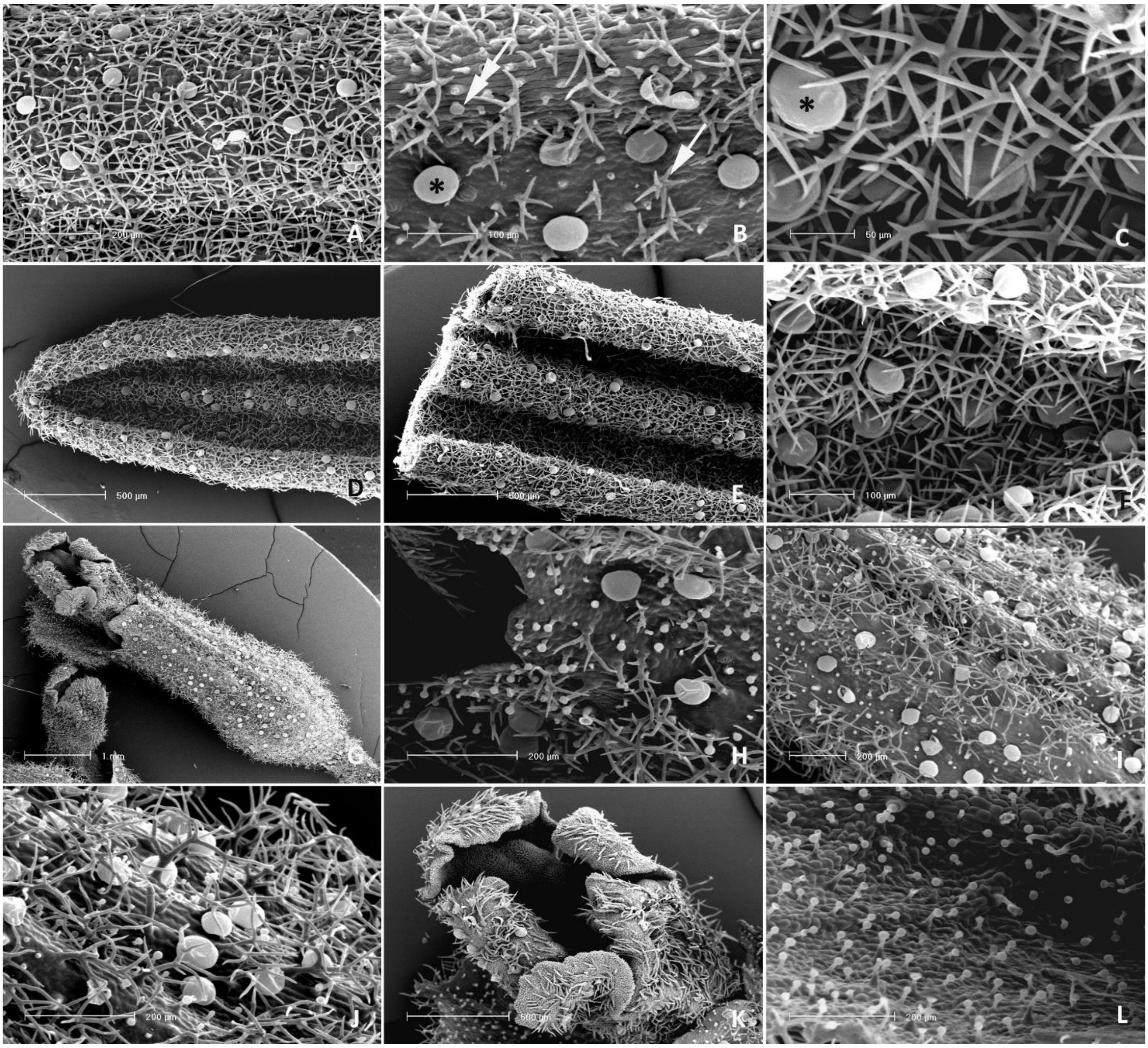
57	1565	(*E*)-nerolidol	tr
58	1574	dendrolasin	tr
59	1576	spathulenol	0.13
60	1579	*trans*-sesquisabinene hydrate	0.12
61	**1581**	**caryophyllene oxide**	**3.45**
62	1606	humulene epoxide II	0.13
63	1614	1,10-*di*-*epi*-cubenol	0.23
64	1640	*epi*-α-cadinol	1.34
65	1660	patchouli alcohol	0.23
66	1672	β-bisabolol	0.19
67	1673	aromadendrene epoxide I	0.15
68	1689	muurol-5-en-4-one	0.35
69	1845	hexahydrofarnesyl acetone	0.17
		Monoterpene hydrocarbons	**6.03**
		Oxygenated monoterpenes	**70.42**
		Sesquiterpene hydrocarbons	**8.87**
		Oxygenated sesquiterpenes	**6.55**
		Apocarotenoids	**0.17**
		Non-terpene derivatives	**5.35**
		Total identified	**97.39%**

[a] Traces, <0.1%.

Table 5. Overview of the intra-specific variation of *Lavandula angustifolia* essential oil composition in accessions from different geographical areas.

Geographical origin	**Main compounds**	**Reference**
South Africa	1,8-cineole, camphor, 2-bornanol	(Muyima et al., 2002)
Brazil	Borneol, epi-D-muurolol, α-bisabolol	(Mantovani et al., 2013)
China	Linalyl acetate, linalool	(Chen et al., 2020)
China	linalool, linalyl acetate, lavandulyl acetate	(Li et al., 2019)
Iran	Inflorescences: linalool, 1,8-cineole, borneol Leaves: 1,8-cineole, borneol, camphor	(Hassanpouraghdam et al., 2011)
Turkey	Linalool, linalyl acetate	(Küçük et al., 2018)
Hungary	Different cultivars: Linalool, linalyl acetate	(Détár et al., 2020)
Romania	*Mailette* variety: Linalool, linalyl acetate *Vera* variety*:* linalyl acetate, linalool , trans-β-ocimene	(Oroian et al., 2019)
Romania	Caryophyllene, beta-phellandrene, eucalyptol	(Jianu et al., 2013)
Serbia	Linalool, camphor, linalyl acetate, 1,8-cineole	(Stanojević et al., 2011)
Poland	Linalool, γ-cadinene, linalyl acetate	(Łyczko et al., 2019)
Poland	Linalool, linalyl acetate, α-terpineol	(Wesolowska et al., 2010)
Greece	Linalool, linalyl acetate	(Hassiotis et al., 2014)
Greece	Linalyl acetate, linalool, 1,8-cineole	(Hassiotis et al., 2010)
Spain	Linalool, linalyl acetate	(Carrasco et al., 2015)
Italy (Emilia Romagna)	Linalool, linalyl acetate, terpinen-4-ol	(Maietti et al., 2013)
Italy (Emilia Romagna)	Linalyl acetate, linalool	(Tardugno et al., 2019)
Italy (Piedmont)	Coumarin, borneol, linalool	(Binello et al., 2014)
Italy (Piedmont)	Linalool, linalyl acetate	(Demasi et al., 2018)
Italy (Tuscany)	*Mailette* variety: Linalool, linalyl acetate	(Pistelli et al., 2017)
Italy (Friuli Venezia Giulia)	Linalool, linalyl acetate, 1,8-cineole	(Da Porto and Decorti, 2008)
Italy (unspecified location)	Fenchone, camphor, camphene	(Conti et al., 2010)

Figure 1



A
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C
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Figure 2

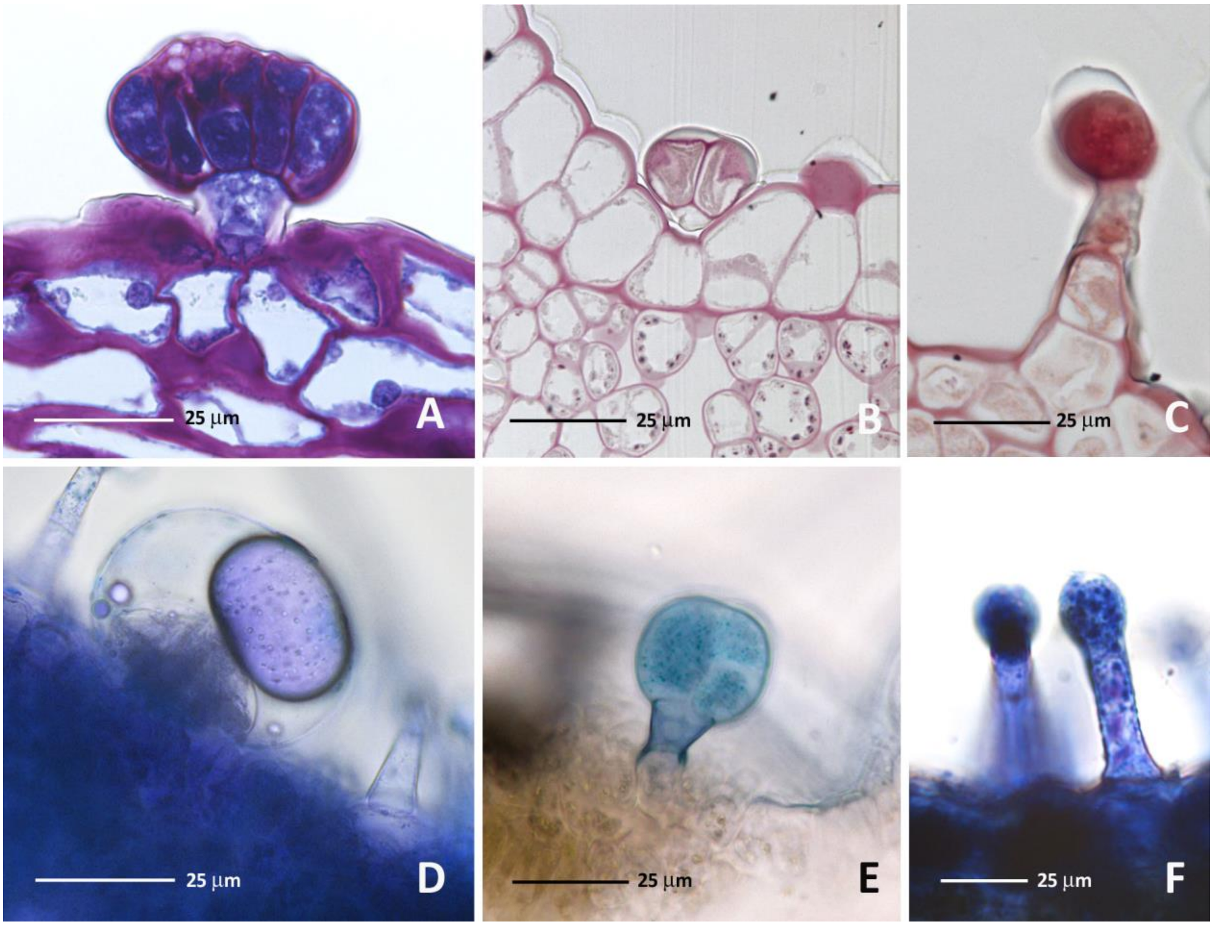
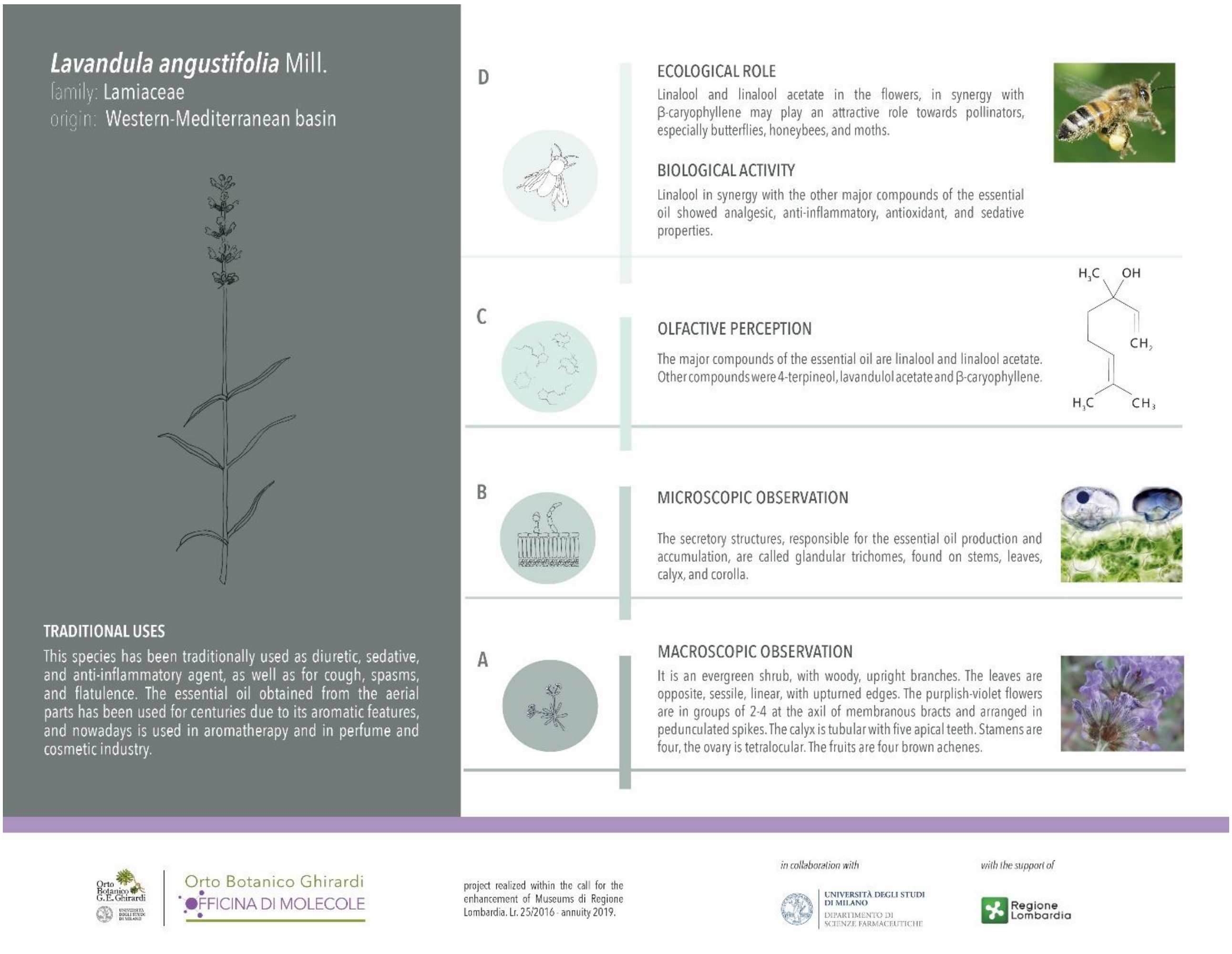


Figure 3



Lavandula angustifolia Mill.
family: Lamiaceae
origin: Western-Mediterranean basin

TRADITIONAL USES

This species has been traditionally used as diuretic, sedative, and anti-inflammatory agent, as well as for cough, spasms, and flatulence. The essential oil obtained from the aerial parts has been used for centuries due to its aromatic features, and nowadays is used in aromatherapy and in perfume and cosmetic industry.

ECOLOGICAL ROLE

Linalool and linalool acetate in the flowers, in synergy with β-caryophyllene may play an attractive role towards pollinators, especially butterflies, honeybees, and moths.

BIOLOGICAL ACTIVITY

Linalool in synergy with the other major compounds of the essential oil showed analgesic, anti-inflammatory, antioxidant, and sedative properties.

OLFACTIVE PERCEPTION

The major compounds of the essential oil are linalool and linalool acetate. Other compounds were 4-terpineol, lavandulol acetate and β-caryophyllene.

MICROSCOPIC OBSERVATION

The secretory structures, responsible for the essential oil production and accumulation, are called glandular trichomes, found on stems, leaves, calyx, and corolla.

MACROSCOPIC OBSERVATION

It is an evergreen shrub, with woody, upright branches. The leaves are opposite, sessile, linear, with upturned edges. The purplish-violet flowers are in groups of 2-4 at the axil of membranous bracts and arranged in pedunculated spikes. The calyx is tubular with five apical teeth. Stamens are four, the ovary is tetralocular. The fruits are four brown achenes.

Orto Botanico G.E. Ghirardi
Orto Botanico Ghirardi
OFFICINA DI MOLECOLE

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in collaboration with
UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI SCIENZE FARMACEUTICHE

with the support of
Regione Lombardia

Captions to Figures

Figure 1. SEM micrographs showing distribution pattern and trichome morphotypes in *Lavandula angustifolia* Mill*.* (A-C) Leaf adaxial surface: general view (A) and particulars (B, C) with peltates (asterisks), short-stalked capitates (double arrow) and dendritic non-glandular (arrows) trichomes. (D-F) Leaf abaxial surface: general views at the apical (D) and median portion (E) and particular (F) with peltates and dendritic non-glandular trichomes. (G) General view of a floral bud. (H-J) Calyx abaxial surfaces: dorsal region (H) and ventral region (I, J) with dendritic non-glandular hairs, peltates and short-stalked capitates. (K) Corolla abaxial surface at the apical region with peltates and non-glandular trichomes. (L) Corolla adaxial surface at the upper lip with medium-stalked capitates.

Figure 2. LM micrographs showing the morphotypes (A-C) and the histochemistry (D-F) of the glandular trichomes observed on the vegetative and reproductive organs of *Lavandula angustifolia* Mill. (A) Peltate trichome. (B) Short-stalked capitate trichome. (C) Medium-stalked capitate trichome. (D) Peltate trichome: Nadi reagent. (E) Short-stalked capitate trichome: Alcian Blue. (F) Medium-stalked capitate trichome: Nadi reagent.

Figure 3. New labelling of *Lavandula angustifolia* Mill. (Lamiaceae) at the Ghirardi Botanic Garden (Toscolano Maderno, Brescia, Italy)