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FULL PAPER

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Lavandula dentata from Italy: Analysis of Trichomes and Volatiles

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This study presented a micromorphological and phytochemical survey on *Lavandula dentata* L. cultivated at the Ghirardi Botanic Garden (Toscolano Maderno, BS, Italy). The morphological investigation revealed the presence of peltate, short- and medium-stalked capitate trichomes. The histochemical survey showed terpene production by peltates and medium-stalked capitates, hydrophilic secretions by short-stalked capitates. The phytochemical survey was developed on leaf and flower volatile organic compounds (VOCs) and on the essential oil (EO) from the flowering aerial parts. The VOC profiles represented an element of novelty and were dominated by oxygenated monoterpenes, among which 1,8-cineole and β-pinene were the most abundant (77.40%, 7.11% leaves; 81.08%, 10.46% flowers). The EO of *L. dentata* was dominated by oxygenated monoterpenes with a high percentage of 1,8-cineole (69.08%), followed by β-pinene, *trans*-pinocarveol and myrtenal. Evaluations about the ecological role, the potential biological activity and the sensory attributes were proposed, based on literature contributions.

Keywords: *Lavandula dentata*, fringed lavender, glandular *indumentum*, terpenoids, VOC profile, essential oil, fragrances.

Introduction

Lavandula dentata L. (Lamiaceae), known as fringed lavender, is a highly aromatic shrub with upright branches, woody at the base, which produces long floral stems. Its leaves are pinnate with toothed margins, greyish-green in color and sticky. Its violet-blue flowers are grouped in pedunculate spikes 2.5–5 cm long.[1] It is native to southern and eastern Spain, north-western Africa, Ethiopia, Eritrea, Israel, Jordan, and the Arabian Peninsula; it is naturalized elsewhere around the Mediterranean.[2] It has a long history of cultivation, being known in the Arab world since time immemorial; nowadays, it is grown in gardens all over the world because of its extended winter-flowering season. As with other lavenders, its flowers attract bees, making *L. dentata* a useful addition to wildlife-friendly gardens. Its essential oils are used in aromatherapy and as scents in cosmetic creams.

In the native range, the folk medicine refers to antidiabetic, antihypertensive, antiprotozoal, antiseptic and anti-inflammatory properties, as well as uses in the case of wounds, rheumatisms, urine retention, kidney stones and digestive troubles.[3]

Its commercial value is mainly linked to the presence of glandular trichomes, responsible for the production of an amazing diversity of volatile substances.[4–7] The literature proposed two previous micromorphological studies on leaves and flowers, both focused on the characterization of anatomical markers, including trichomes.[3,8] In the phytochemical field, works about the characterization of volatile

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organic compounds (VOCs) emitted by leaves and flowers are lacking. Only Chinese authors reported the analysis of aromatic volatiles emitted by the whole plant with the aim of evaluating its potential antioxidant activity on mice kept in conditions of oxygen deficiency.[9] On the contrary, several contributions focused on the composition of the essential oils (EOs) obtained from samples coming from different regions in the world.[3,10–15] However, there are no studies on plants cultivated in Italy.

Concerning the ecological role, different authors reported the antifungal and anti-ochratoxigenic activity of the EO of *L. dentata* against *Aspergillus carbonarius*, its deterrence ability towards *Aedes albopictus* oviposition, along with larvicidal, insecticidal, fumigant and antimicrobic activities.[3,11,16–18] Regarding the EO biological activity, antioxidant properties, cytotoxic effects towards tumor cell lines, as well as neuroprotective, sedative, antidepressant and carminative abilities were documented.[3,14,17,19,20]

In this framework, the work presented hereafter proposes a multidisciplinary study on *L. dentata* grown in Italy, combining a morphological and phytochemical study approach in order to: 1) describe the trichome morphotypes on leaves and flowers by means of Light Microscopy and Scanning Electron Microscopy; 2) evaluate the histochemical features to localize *in situ* the main compound classes of metabolites present in their secretions; 3) characterize the leaf and flower VOC emission profiles and analyze the composition of the EO obtained from the aerial parts at blooming. These results were related to hypothesis on the ecological role and the biological activity of the volatile substances based on assessments focused on the literature data.

These findings will be useful for enhancing knowledge about a species of high horticultural interest. At the same time, they will contribute to strengthen the value of the plant heritage preserved at the Ghirardi Botanic Garden (Toscolano Maderno, BS, Lombardy, Italy).

Results and Discussion

Micromorphological Investigation

Trichomes morphotypes and distribution pattern. The *indumentum* of the vegetative and reproductive organs of *L. dentata* included both glandular and non-glandular trichomes (*Figures 1–3*). There were two main types of glandular trichomes, peltate and capitate.

Peltate ones were made up by a basal cell, a short neck cell and a broad globular head (50–70 μm in diameter), with several secretory cells, up to 8, arranged in one layer (*Figure 1A*). Capitate trichomes included a basal cell, a stalk cell and a head of one to 2–4 cells, with the length of the stalk more than half the height of the head. Two capitate subtypes were recognized: short-stalked and medium-stalked (*Figure 1B*, *C*). The first were formed by a basal cell, a subcylindrical stalk cell, with a longitudinal axis of 15–

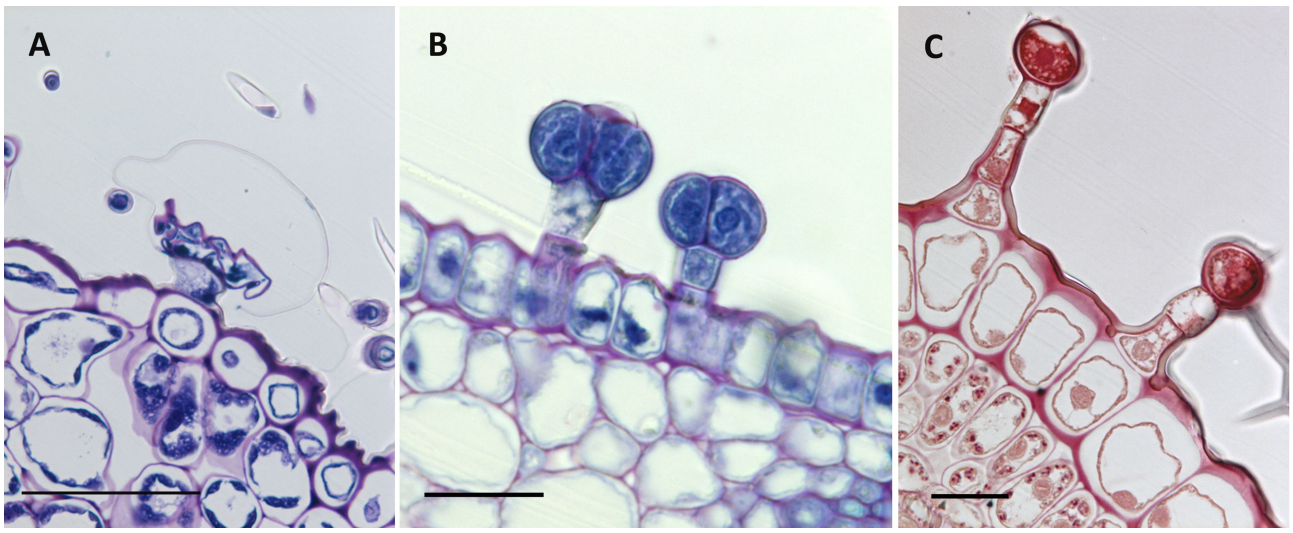

Figure 1. LM micrographs showing the morphotypes of glandular trichomes observed on the vegetative and reproductive organs of *Lavandula dentata* L. Peltate (A), short-stalked capitate (B), and medium-stalked capitate (C). Fixed samples. Scale Bar (B–C) = 20 μm; (A) = 50 μm.

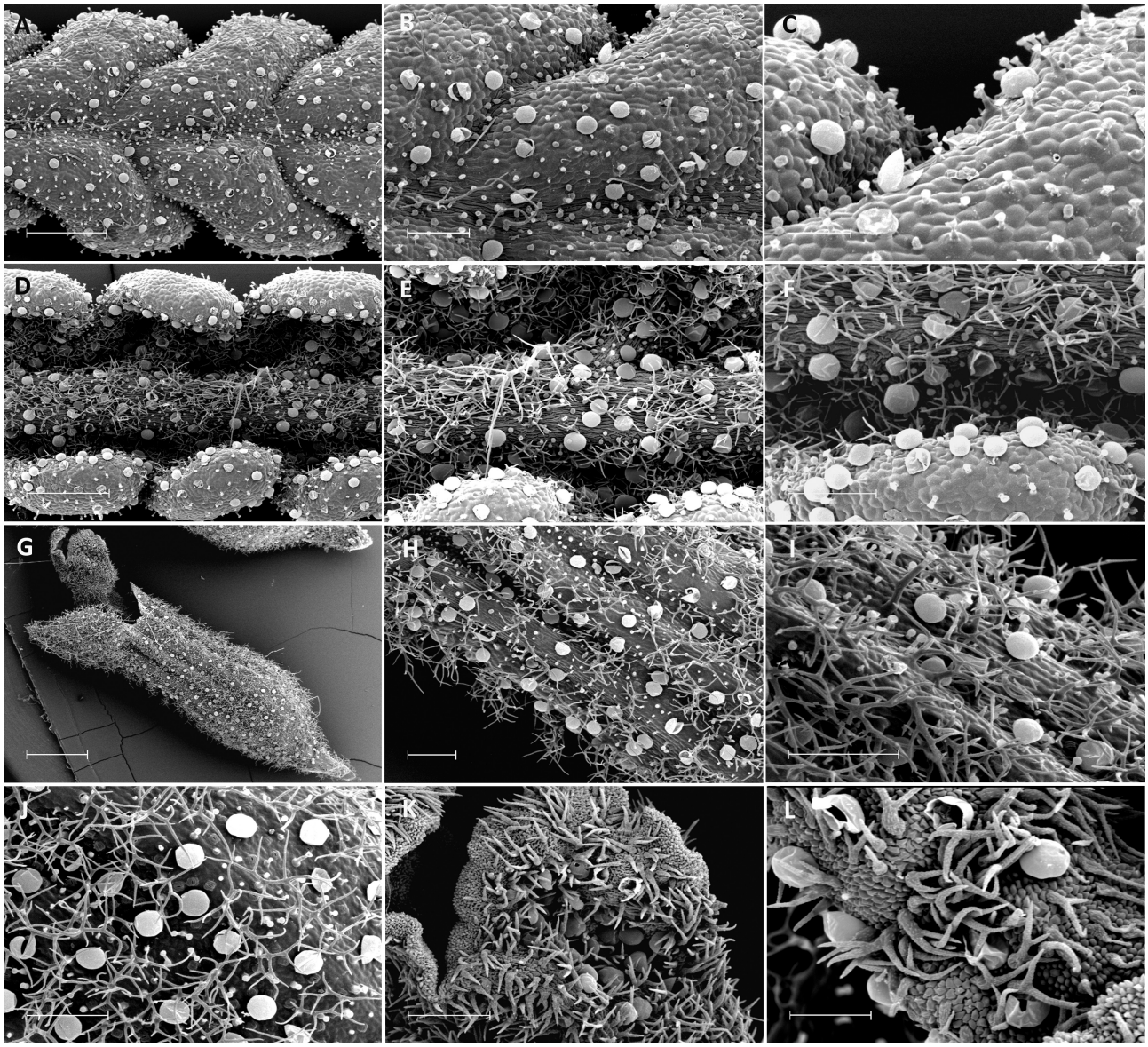

Figure 2. SEM micrographs showing distribution pattern and trichome morphotypes in *Lavandula dentata* L. Leaf adaxial surface: general view (A) and details (B, C) with peltate, short-stalked and medium-stalked capitate trichomes. (D–F) Leaf abaxial surface: general view (D) and details (E, F) with peltates, short-stalked capitates 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, peltate, short-stalked and medium-stalked capitate trichomes. (K, L) Corolla abaxial surface: upper lip (K), lower lip (L) with peltates and non-glandular trichomes. Scale Bar (C, L) = 100 μm; (B, E, F, H–K) = 200 μm; (A, D) = 500 μm; (G) = 1 mm.

20 μm, and a 2–4 celled globular head, up to 25 μm in diameter (*Figure 1B*). Medium-stalked capitates consisted of an elongated basal epidermal cell which protruded from the level of the adjacent epidermal cells, 1–2 subcylindrical stalk cells, with a longitudinal axis of 20–25 μm, and a 1-celled globular head, 20 μm in diameter (*Figure 1C*).

Non-glandular hairs were multicellular dendritic trichomes: they had 4–6 arms branching off from the basal region (*Figures 2* and *3*); each arm was uniseriate with acute apices. These hairs were ubiquitous (*Table 1*) and their length was variable, being shorter on stems and leaves and longer on the reproductive structures. The main longitudinal axis was generally perpendicular to the plant surfaces, however, on corollas these hairs were appressed to the epidermis and the single arms were characterized by a greater length than on the other plant parts.

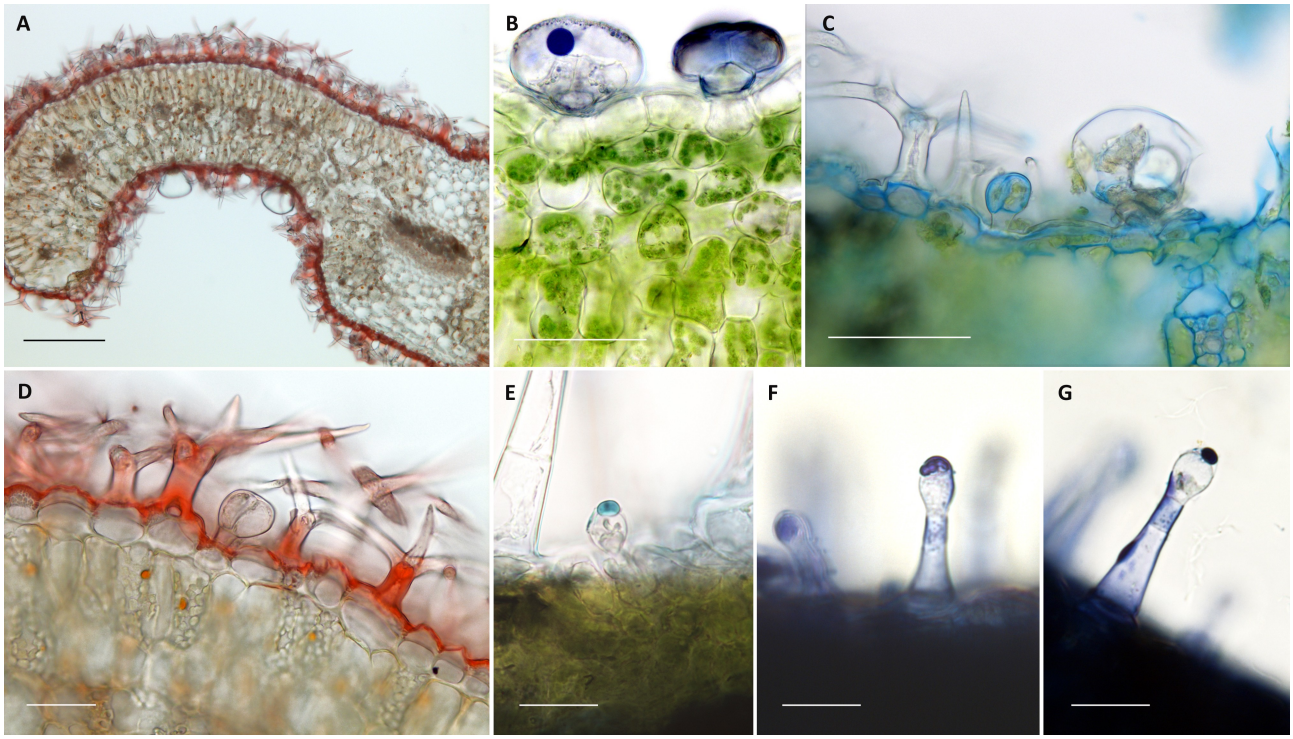

Figure 3. LM micrographs showing the histochemistry of the glandular trichomes of the vegetative and reproductive organs of *Lavandula dentata* L. (A–G). (A) Cross-section of the calyx; notice the abundant dendritic trichomes and the peltates, Sudan III/IV. (B) Peltate trichome: Nadi reagent. (C) Peltate and short-stalked capitate trichomes: Alcian Blue. (D, E) Short-stalked capitate trichomes: Sudan III/IV (D), Alcian Blue (E). (F, G) Medium-stalked capitate trichome: Nadi reagent in leaf (F) and flower (G). Fresh samples. Scale Bar (B–G) = 50 μm; (A) = 100 μm.

Table 1. Distribution pattern of the glandular trichomes in *Lavandula dentata* L. For symbol details, see the Experimental section.[a]

Species	Trichome type	Stem	Leaf		Calyx		Corolla	
			adax	abax	adax	abax	adax	abax
L. dentata	peltate	+	+	++	−	+	−	+
	short capitate	+	+	++	+	+	−	±
	medium capitate	−	±	−	−	±	−	−
	dendritic	+	±	++	−	++	+	+

[a] Symbols: (−) missing, (±) sporadic, (+) present, (++) abundant.

Stems presented peltate and short capitate uniformly distributed on their surface (not shown). Leaves presented peltates and short capitates evenly distributed on the adaxial lamina and sporadic medium capitates located along the revolute margins; non-glandular trichomes were occasional and distributed at the middle region (*Table 1*, *Figure 2 A–C*). On the contrary, on the abaxial lamina, the massive presence of dendritic hairs often hid the glandular ones; however, peltate trichomes were visible (*Figure 2 D–F*), whereas short capitates were easily observed only in leaf cross sections. Observations on the calyx abaxial surfaces evidenced the presence of numerous peltate trichomes with a balloon shape and a smooth head, mainly localized along the ribs, intermingled with short capitates (*Figure 2 G–J*). Both peltate and capitate trichomes usually showed a deeply wrinkled surface, but evident cuticle ruptures were rare. Aggregates of small roundish bodies, corresponding in size and shape to the lipid droplets observed in light microscope specimens, were also present on the outermost trichome cuticle (not shown). Dendritic trichomes were also observed. The *indumentum* of the corolla abaxial surface was characterized by peltates and short capitates randomly distributed on the upper

and lower lips (*Figure 2 K–L*), while the adaxial surface was hairless.

Histochemistry

The results of the histochemical survey on the same trichome morphotype were consistent in all the analyzed plant parts (*Table 2* and *Figure 3*). The application of the Sudan III/IV dyes stained only the cuticular layer covering the plant epidermis, without evidencing the secretion of the glandular trichomes (*Figure 3 A*, *D*). In mature peltate trichomes, a secretion vesicle was developed in the cavity between the cuticle and the outermost layer of the secretory cell walls. This vesicle reacted positively only to the lipophilic stainings, being filled with terpenes (*Figure 3 B*, *C*). Generally, however, several secretion droplets coalesced to form a large mass. In short capitates, only the Alcian Blue dye specific for muco-polysaccharides gave positive responses evidencing a tiny droplet in the thin storing chamber at the apex of the secretory head (*Figure 3 E*). In the medium-stalked capitates, the secreted material was stained exclusively following the application of the lipophilic dyes, in particular to the Nadi reagent (*Figure 3 F*, *G*). Secreted terpenes were localized in the subcuticular space, but usually did not aggregate to form large drops, as in peltate hairs. However, some of them displayed numerous droplets of lipidic material, lined over the outer cuticle or trapped among the surrounding dendritic trichomes.

Phytochemical Investigation

VOCs. VOC emission profiles revealed a total of 51 different compounds. 42 and 30 compounds were identified in the leaf and in the flower profiles, respectively (*Table 3*).

The foliar emission profile was dominated by oxygenated monoterpenes (83.57%), followed by monoterpene hydrocarbons (9.84%), and sesquiterpene hydrocarbons (6.41%). Non-terpene derivatives occurred in small quantities (0.13%), while oxygenated sesquiterpenes were absent. 1,8-Cineole (10, 77.40%) dominated the profile, followed by β-pinene (6, 7.11%). Myrcene (7), α-terpineol (26), *cis*-muurola-3,5-diene (39), δ-terpineol (22), and linalool (14) accounted for relative percentages between 1.0% and 2.0%. The remaining compounds were present with relative abundances lower than 1.0% or in traces (<0.1%). 21 exclusive compounds were detected only in the leaf sample, among which myrcene (7, 1.55%) was the most abundant one, followed by *cis*-muurola-3,5-diene (39, 1.26%) and δ-terpineol (22, 1.07%). The other exclusive compounds occurred in percentages lower than 1.0% or in traces.

In the floral profile, oxygenated monoterpenes (84.06%) were the most abundant compound class, followed by monoterpene (13.63%) and sesquiterpene (1.90%) hydrocarbons. Oxygenated sesquiterpenes occurred in negligible percentages (0.20%), while non-terpene derivatives were absent. 1,8-Cineole (10, 81.08%) was the main compound, followed by β-pinene (6, 10.46%) and α-pinene (3, 2.77%). The remaining VOCs were detected in abundances lower than 1.0%. Nine exclusive compounds were identified, all in amounts lower than 0.20%.

The total common compounds were 21, including the two main ones, 1,8-cineole (10, 77.40% leaves, 81.08% flowers) and β-pinene (6, 7.11% leaves; 10.46% flowers). α-Pinene (3) was present in higher relative percentages in flowers (0.91% leaves; 2.77% flowers), while α-terpineol (26, 1.44% leaves; 0.10% flowers) and linalool (14, 1.04% leaves; traces in flowers) were more abundant in leaves. The other common compounds were recorded in comparable relative percentages lower than 1.0% in both profiles.

Table 2. Results of the histochemical tests on the glandular trichomes in *Lavandula dentata* L.[a]

Staining	Target compounds	*L. dentata* peltate	short capitate	medium capitate
Sudan III/IV	Total lipids	−	−	−
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 Trichloride	Polyphenols	−	−	−

[a] Symbols: (−) negative response; (+) positive response; (++) intensely positive response.

Table 3. HS-SPME profiles of the leaves and flowers of *Lavandula dentata* L.

	L.r.i.[a]	Compounds	Relative abundance (%) Leaf	Flower
1	928	tricyclene	_[b]	Tr[c]
2	931	α-thujene	tr	–
3	941	α-pinene*	0.91	2.77
4	954	camphene*	tr	0.40
5	976	sabinene*	tr	tr
6	982	β-pinene*	7.11	10.46
7	993	myrcene*	1.55	–
8	993	1,8-dehydrocineole	–	0.14
9	1027	*p*-cymene*	tr	–
10	1034	1,8-cineole*	77.40	81.08
11	1062	γ-terpinene*	0.10	tr
12	1070	*cis*-sabinene hydrate	0.63	0.13
13	1088	*p*-mentha-2,4(8)-diene	0.17	–
14	1101	linalool*	1.04	tr
15	1104	α-thujone*	tr	–
16	1125	α-campholenal	tr	–
17	1139	*trans*-pinocarveol*	0.63	0.68
18	1143	camphor*	0.53	0.74
19	1162	*trans*-pinocamphone	–	tr
20	1163	pinocarvone	0.38	tr
21	1167	borneol*	–	0.10
22	1170	δ-terpineol	1.07	–
23	1175	*cis*-pinocamphone	tr	0.60
24	1178	4-terpineol*	tr	0.12
25	1187	(*Z*)-3-hexenyl butyrate	0.13	–
26	1189	α-terpineol*	1.44	0.10
27	1193	myrtenol	–	0.17
28	1195	myrtenal	0.45	–
29	1205	verbenone*	tr	0.20
30	1351	α-cubebene	tr	–
31	1390	β-cubebene	0.17	–
32	1409	α-cedrene*	–	tr
33	1416	*cis*-α-bergamotene	0.30	–
34	1420	β-caryophyllene*	0.49	0.24
35	1421	α-santalene	–	0.11
36	1433	γ-elemene	0.13	–
37	1438	*trans*-α-bergamotene	0.62	0.30
38	1445	(*Z*)-β-farnesene	0.16	0.12
39	1447	*cis*-muurola-3,5-diene	1.26	–
40	1460	(*E*)-β-farnesene	0.26	–
41	1462	*cis*-muurola-4(14),5-diene	0.89	–
42	1471	β-acoradiene	tr	–
43	1485	β-selinene	0.83	0.84
44	1493	viridiflorene	tr	–
45	1504	(*Z*)-α-bisabolene	tr	–
46	1509	β-bisabolene	0.35	0.19
47	1513	*trans*-γ-cadinene	0.23	0.10
48	1514	lavandulyl isovalerate	–	tr
49	1531	(*E*)-α-bisabolene	0.72	–
50	1538	α-cadinene	tr	–
51	1581	caryophyllene oxide	–	0.20
		Monoterpene hydrocarbons	9.84	13.63
		Oxygenated monoterpenes	83.57	84.06
		Sesquiterpene hydrocarbons	6.41	1.90
		Oxygenated sesquiterpenes	–	0.20
		Non-terpene derivatives	0.13	–
		Total identified	99.95 %	99.79 %

[a] Linear retention indices on a DB5 capillary column; [b] Not detected; [c] Traces, < 0.1 %; * Injection of analytical standard for comparison.

EOs

The EO composition is reported in *Table 4*. A total of 39 compounds were identified, accounting for 100.00% of the oil.

Table 4. Composition of the essential oil obtained from the aerial parts of *Lavandula dentata* L.

	L.r.i.[a]	Compounds	Relative abundance (%)
1	928	tricyclene	Tr[b]
2	941	α-pinene*	1.56
3	954	camphene*	0.23
4	959	thuja-2,4(10)-diene	tr
5	976	sabinene*	0.37
6	982	β-pinene*	4.84
7	993	2,3-dehydro-1,8-cineole	0.21
8	1018	α-terpinene*	tr
9	1027	*p*-cymene*	0.19
10	1034	1,8-cineole*	69.08
11	1062	γ-terpinene*	0.19
12	1070	*cis*-sabinene hydrate	0.57
13	1076	*trans*-linalool oxide (furanoid)	0.40
14	1090	*cis*-linalool oxide (furanoid)	0.48
15	1101	linalool*	0.89
16	1123	*cis*-*p*-menth-2-en-1-ol	tr
17	1125	α-campholenal	0.58
18	1139	*trans*-pinocarveol*	3.40
19	1140	nopinone*	0.60
20	1143	*cis*-verbenol*	1.41
21	1144	*trans*-verbenol	0.17
22	1158	sabina ketone	0.18
23	1163	pinocarvone	2.62
24	1170	δ-terpineol	2.56
25	1178	4-terpineol*	0.77
26	1183	*p*-cymen-8-ol	0.16
27	1189	α-terpineol*	tr
28	1193	myrtenol	1.91
29	1195	myrtenal	3.38
30	1205	verbenone*	0.29
31	1218	*trans*-carveol	0.28
32	1240	cuminaldehyde	0.12
33	1244	carvone*	0.18
34	1295	perilla alcohol*	0.15
35	1495	α-selinene	0.39
36	1581	caryophyllene oxide	0.47
37	1650	β-eudesmol*	0.95
38	1674	cadalene	tr
39	1682	*cis*-14-*nor*-muurol-5-en-4-one	0.43
		Monoterpene hydrocarbons	7.38
		Oxygenated monoterpenes	90.39
		Sesquiterpene hydrocarbons	0.39
		Oxygenated sesquiterpenes	1.85
		Total identified	100.00%

[a] Linear retention indices on a DB5 column; [b] Traces, < 0.1%; * Injection of analytical standard for comparison.

Oxygenated monoterpenes (90.39%) dominated, followed by monoterpene hydrocarbons (7.38%), while sesquiterpenes occurred in percentages equal to 1.85% and 0.39%, for oxygenated and hydrocarbon compounds, respectively. 1,8-Cineole (10, 69.08%) was the most abundant compound, followed by β-pinene (6, 4.84%), *trans*-pinocarveol (18, 3.40%) and myrtenal (29, 3.38%). Pinocarvone (23, 2.62%) and δ-terpineol (24, 2.56%) were detected in relative percentages between 2.0–3.0%, while myrtenol (28, 1.91%), α-pinene (2, 1.56%), and *cis*-verbenol (20, 1.41%) in the range 1.0–2.0%. The remaining compounds showed abundances lower than 1.0% or were present in traces (< 0.1%).

Discussion

The glandular and non-glandular *indumenta* showed a high level of consistency for what concerns both morphology and distribution pattern in all the examined replicates. The observed glandular trichomes belonged to the two main types, peltate and capitate, already described in previous studies on *L. dentata*.[3,8] Peltate ones were uniformly distributed on the whole epidermal surfaces, with the exception of the adaxial side of sepals and petals; they were very abundant on the leaf abaxial surface, as previously reported.[8] Capitates were distinguished in short-stalked and medium-stalked, while published studies referred to the capitate trichomes in a generic way, without describing the subtypes.[3,8] These trichomes presented a different distribution pattern. Short-stalked capitates were ubiquitous, except for the petal adaxial surface. On the contrary, medium-stalked capitates were occasionally observed along the leaf adaxial margins and on the sepal abaxial surface. Concerning the non-glandular *indumentum*, previous contributions also reported the presence of dendritic trichomes,[3,8] however, these authors did not describe their distribution pattern.

In the histochemical survey, consistent results were obtained for the observed morphotypes, independently of their localization on the plant organs. Peltates and medium-stalked capitates were responsible for the secretion of lipophilic substances, in consistency with literature.[3,8] For the first time, we reported the terpenoid nature of the secreted lipophilic materials. The short-stalked capitates represented the exclusive producers of hydrophilic substances, as already observed in other Lamiaceae.[21] In particular, they synthesized mucopolysaccharides and this productiv-

ity could explain the presence of sticky substances on the foliar surfaces.

Concerning the phytochemical investigation, the HS-SPME analysis showed a more complex profile in leaves compared to flowers, already evident in the presence of a higher number of total compounds (42 vs. 30). However, leaves and flowers shared the most abundant chemical classes, oxygenated monoterpenes (83.57% *vs.* 84.06%) and monoterpene hydrocarbons (9.84% *vs.* 13.63%), as well as the two dominant compounds, 1,8-cineole (10, 77.40% *vs.* 81.08%) and β-pinene (6, 7.11% *vs.* 10.46%). In leaves, other major compounds showed relative percentages between 2.0% and 1.0%, e.g., myrcene (7, 1.55%), α-terpineol (26, 1.44%), *cis*-muurola-3,5-diene (39, 1.26%), δ-terpineol (22, 1.07%) and linalool (14, 1.04%). In the floral bouquet, α-pinene (3, 2.77%) was the third compound in order of relative abundance, while the remaining ones occurred in relative concentrations lower than 1.0%. Moreover, leaves showed a higher number of exclusive compounds compared to flowers (21 *vs.* 9): myrcene (7, 1.55%) was the most abundant one in the former, while in the latter these compounds occurred in negligible percentages or in traces (< 0.1%). Leaves and flowers had 21 common compounds, including the major ones, 1,8-cineole (10 77.40% leaves; 81.08% flowers), β-pinene (6, 7.11% leaves; 10.46% flowers), and α-pinene (3, 0.91% leaves; 2.77% flowers), with higher relative percentages in flowers, while α-terpineol (26, 1.44% leaves; 0.10% flowers), and linalool (14, 1.04% leaves; traces in flowers) were more abundant in leaves. A critical comparison with our results was not possible, since only a single contribution referred to the characterization of the VOC profile of Chinese samples through a different analytical technique.[9] Anyway, it is interesting to evidence that 1,8-cineole (82.82%) was confirmed as the most abundant compound. Due to its high relative abundance, the authors stated that this compounds was responsible for the antioxidant activity documented *in vivo*.[9]

To complete the phytochemical survey, the EO profile of the aerial parts at blooming was analyzed. The dominant compound classes were oxygenated monoterpenes (90.39%), followed by monoterpene hydrocarbons (7.38%). 1,8-Cineole (10, 69.08%), β-pinene (6, 4.84%), *trans*-pinocarveol (18, 3.40%) and myrtenal (29, 3.38%) were the most abundant compounds.

From the comparison with literature data (see *Table 5*), a high level of quali-quantitative variability in the EO compositions emerged, probably due to the different areas of origin of the samples, the heterogeneity of the employed plant material and the different extraction methods.[18] In most studies, the dominance of the oxygenated monoterpenes was confirmed,[10,16,17,22] but both the total number of compounds and the major compounds varied. According to this, different chemotypes were recognized in *L. dentata*, i.e., i) 1,8-cineole/β-pinene (like the one in this study) in samples from Algeria[23,24] and Mexico;[25] ii) linalool/linalyl acetate in specimens from Palestine[14] and one accession from Tunisia;[13] iii) 1,8-cineole/camphor/fenchone in almost all published EO compositions for Brazilian specimens[12,26,27] and Tunisia.[11,15] Linalool and its acetic ester were commonly reported as main constituents in *L. angustifolia* Mill. EO,[18] although some published studies reported compositions mainly rich in fenchone and camphor,[28] confirming the high level of intra-specific variation on the EO composition for this genus.

Our profile was one of the richest among those present in literature[11,29] and 1,8-cineole (10) was confirmed as the main compound in samples coming from Morocco,[19] Tunisia,[11,15] Algeria,[30] and

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

Geographical origin	Main compounds	Source
Algeria	1,8-cineole, β-pinene, *trans*-pinocarveol	[23]
	1,8-cineole, β-pinene, *trans*-pinocarveol	[24]
	1,8-cineole, *cis*-verbenol, *p*-cymen-8-ol	[30]
	α-terpinolene, camphor	[31]
Brazil	1,8-cineole, camphor, linalool oxide	[29]
	1,8-cineole, camphor, fenchone	[26]
	1,8-cineole, *iso*-limonene, thuj-3-en-10-al	[3]
	1,8-cineole, camphor, fenchone	[12]
	1,8-cineole, camphor, fenchone	[27]
Italy	1,8-cineole, β-pinene, *trans*-pinocarveol	Present study
Mexico	1,8-cineole, β-pinene	[25]
Morocco	1,8-cineole, sabinene, myrtenal	[46]
	camphor, *trans*-pinocarveol, β-eudesmol	[16]
	β-pinene, *trans*-pinocarveol, myrtenal	[19]
Palestine	linalyl acetate, linalool	[14]
Saudi Arabia	camphor, fenchone	[10]
Tunisia	1,8-cineole, camphor, fenchone	[11]
	linalool, linalyl acetate	[13]
	1,8-cineole, camphor, fenchone	[15]
Yemen	camphor, *trans*-pinocarveol, α-guaiol	[47]

Brazil.[3,12] β-Pinene (6) was well represented, in association with its isomer α-pinene (2), in samples of different origin.[22] In other profiles, among the dominant compounds, there were camphor,[10,13,16,17,31] fenchone,[10–12] linalyl acetate, and linalool (15),[13,14] all accounting for low amounts or lacking in our samples. Among the other identified compounds, *trans*-pinocarveol (18) was detected in samples from Brazil,[3] Morocco[16], and Yemen,[17] and myrtenal (29) from Morocco.[19] On these bases, our EO profile can be assigned to the 1,8-cineole/β-pinene (69.08/4.84%) chemotype, already described for Algerian (48.0%/6.1%, respectively)[22] and Mexican (68.59%/11.53%, respectively)[25] samples.

The variability connected to the presence of different main components can certainly explain the different sensory profile and the diverse biological activities ascribed to EOs of *L. dentata* coming from different regions of the world.[22] Concerning the sensory attributes of the EO of our samples, the high relative amount of 1,8-cineole (10) would be responsible of fresh mint-like attributes, whereas β-pinene (6) would give drier, more resinous and spicy notes.[22] Other dominant compounds, e.g., camphor and fenchone, found in several of the EO profiles reported by literature, would give more camphoraceous, sweet and intense notes, while linalool e linalyl acetate would bring more citrus, fresh, floral, herbaceous, and woody features.[22]

Regarding the ecological role of the dominant compound, 1,8-cineole (10), the literature highlighted its antifungal and anti-ochratoxigenic activity against *Aspergillus carbonarius*, enhanced by the synergy of action of minor compounds, such as camphor (18),[11] here present in low amount. The synergy of action of these two compounds was also demonstrated for the antibacterial effect[11] that is increased, in association with α-terpineol (26) and α-pinene (3), towards Gram$^+$ bacteria.[32] In addition, the larvicidal activity of 1,8-cineole (10) and 4-terpineol (24),[31] occurring in very low percentage in our samples, was reported. Defensive roles were ascribed to the second most abundant compound of both profiles, β-pinene (6);[33] an allelopathic action in association with 1,8-cineole (10) was also documented.[34] Furthermore, linalool (14) showed antifungal activity towards phytopathogens of the genera *Botrytis*, *Pythium*, and *Magnaporthe*.[35] However, attractive roles towards pollinators were also recognized to β-pinene (6), linalool (14), and 1,8-cineole (10), often in association with β-caryophyllene (34), occurring with relative abundances lower than 0.50% in our profiles.[36–38] Among the exclusive foliar compounds, myrcene (7) possessed allelopathic effects[39] and inhibited fungal proliferation together with α- and β-pinene (3, 6).[40] Concerning δ-terpineol (22), specific information was not found in literature, all referring to terpineol, which was recognized as common antifungal and antibacterial agent.[32,41] Regarding the exclusive floral compounds, all with abundances lower than 0.20% in our profile, a noteworthy example was represented by the defensive role of several phytocomplexes containing caryophyllene oxide (51).[42] In this context, considering the dominance of 1,8-cineole and β-pinene, we may hypothesize a simultaneous protective and attractive role for both the vegetative and reproductive bouquets. Defensive mechanisms would be enhanced at the leaf level due to the major percentages of linalool and to the occurrence of the exclusive compounds myrcene and α-terpineol. On the contrary, due to the negligible relative abundances of the exclusive compounds in the flowers, it was challenging to make hypothesis about the dominant ecological role. Anyway, based on literature data, it is noteworthy that we cannot exclude the minor compounds from these assessments, since their relevance would result in the expression of synergistic mechanisms underlying the repulsive or attractive roles of the most abundant compounds.

Concerning the evaluation of the biological activity, a recent study documented the repellent effect of the EO rich in camphor and fenchone against *Aedes albopictus*.[10] A neuroprotective action deriving from the inhibition of the NMDA glutamate receptors was demonstrated for EOs rich in linalool,[14] while a cytotoxic effect towards some human tumor cell lines was documented for EOs rich in 1,8-cineole.[19,20] This compound, in association with conventional therapies, is considered promising in the treatment of chronic respiratory, cardiovascular and neurodegenerative diseases; indeed it possessed recognized anti-inflammatory and antioxidant activities,[43] as well as analgesic effects demonstrated in animal models.[44] The literature also proposed evaluations concerning its genotoxicity and cytotoxicity.[45]

Conclusions

This multidisciplinary survey on *L. dentata* represents an element of novelty, since we combined for the first time a morphological and phytochemical survey on leaves and flowers with the aim to depict a link between their productivity in volatiles and their glandular trichomes. We attributed the synthesis of

the terpenic component, finally resulting in the emission of VOCs and in the production of essential oil, to the active biosynthetic pathways in peltate hairs and in medium-stalked capitates. These trichomes occurred both on the aerial vegetative and reproductive parts, with peltates being ubiquitous and capitates being sporadic on leaves and calices. We described, for the first time, the distribution pattern of dendritic trichomes.

From the phytochemical perspective, the vegetative bouquet resulted more complex than the floral one; however, general affinity emerged due to the occurrence of dominant common compounds, i.e., 1,8-cineole, β-pinene. The literature evidences on the ecological role of the major and exclusive compounds are consistent with dominant repulsion strategies played by leaves and with the synergistic repulsion/attraction mechanism established by flowers. We analyzed, for the first time, the EO composition of specimens cultivated in Italy. These samples belonged to the 1,8-cineole/β-pinene chemotype, since these compounds resulted as the main EO components. Their high abundances affects the sensory attributes of their EO, to which they confer fresher, spicier, and resinous terpene-like notes.

Finally, this set of information contributed to increasing the knowledge on an important horticultural species, constituting the basis for future insights on the ecological roles of the secondary metabolites and their promising applications, also in the framework of the enhancing program on the plant heritage preserved at the Ghirardi Botanic Garden.

Experimental Section

Plant Material

Lavandula dentata was cultivated at the Ghirardi Botanic Garden (Toscolano Maderno, BS, Lombardy, Italy) of the Department of Pharmaceutical Sciences of the University of Milan. The samples were collected in June 2019 and were used for the morphological and the phytochemical analyses on the vegetative and the reproductive organs. Voucher specimens of *L. dentata* were deposited in the Herbarium of the Ghirardi Botanic Garden under the code label GBG2019/045. The samples were identified by G. Fico.

Micromorphological Analysis

We described the distribution pattern of the glandular and non-glandular *indumenta* on stem, leaves, calyces and corollas by means of Scanning Electron Microscopy (SEM). We also evidenced the histochemical nature of the secreted material of the glandular trichomes on all the analyzed plant parts by means of Light Microscopy (LM). At least ten samples for each examined plant parts were analyzed to evaluate the level of variability of the micromorphological features. Referring to the trichomes localization on the examined plant parts, we qualitatively evaluated the distribution using the following symbols: (−) missing, not observed in any of the replicates; (±) sporadic in no more than four replicates; (+) present in all the replicates; (++) abundant in all the replicates with a distribution on the whole organ surface.

Scanning Electron Microscopy (SEM)

Plant material (stem, leaf, calyx, corolla) was firstly hand-prepared, fixed in 2.5% glutaraldehyde in phosphate buffer (0.1 M, pH 6.8), dehydrated in an ascending ethanol series up to absolute and then, dried using a critical point dryer apparatus. The samples, mounted on aluminum stubs, were coated with gold and observed with a Philips XL 20 SEM operating at 10 kV.

Light Microscopy

Fresh material was frozen and cryo-cut obtaining sections 20–30 μm thick or hand-cut obtaining sections 60–70 μm thick. Several samples were also fixed in FAA solution (formaldehyde/acetic acid/ethanol 70% = 5:5:90) for 7 days, dehydrated in ascending ethanol series up to absolute, embedded in Technovit/Historesin and sectioned with an ultramicrotome. The following histochemical staining was employed: Toluidine Blue as a general dye;[48] Sudan III/IV[48] and Fluoral Yellow-088 for total lipids,[49] Nile Red for neutral lipids,[50] Nadi reagent for terpenes,[51] Periodic Acid-Schiff (PAS) reagent for total polysaccharides,[52][48] Ruthenium Red for acid polysaccharides,[52] Alcian Blue for mucopolysaccharides,[48] and Ferric Trichloride for polyphenols.[53] Control tests were carried out simultaneously. Observations were made with a Leitz DM-RB Fluo optical microscope equipped with a Nikon digital camera.

Phytochemistry

Volatile Organic Compounds (VOCs). Three leaves and three flowers were cut and immediately inserted into separate glass vials of suitable volume for the analysis.

HS-SPME Sample analysis. The headspace sampling conditions were as reported in Ascrizzi et al., 2017.[54] For the headspace samplings, Supelco SPME (Solid Phase Micro-Extraction) devices, coated with polydimethylsiloxane (PDMS, 100 μm) were used; the same new fiber, preconditioned according to the manufacturer instructions, was employed for all the analyses. To ensure a stable temperature, samplings were conducted in an air-conditioned room at 22 ± 1 °C; this temperature was chosen to avoid the thermal damage of the plant material and, thus, any artificial-induced volatiles release. After 30 min of equilibration, the fiber was exposed to sample the headspace for 30 min. Both the equilibration and sampling times were experimentally determined to obtain an optimal adsorption of the volatiles, and to avoid both under- and over-saturation of the fiber and of the mass spectrometer ion trap. Once sampling was finished, the fiber was withdrawn into the needle and transferred to the injection port of the GC/MS system. Both the sampling and desorption conditions were identical for all the samples. Furthermore, blanks were performed before each first SPME extraction and randomly repeated during each series. Quantitative comparisons of relative peaks areas were performed between the same compounds in the different samples.

Essential Oil (EO) Hydrodistillation

Fresh *L. dentata* aerial parts gathered in the blooming 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 h. The EO was diluted at 5% in HPLC grade hexane prior to GC/MS injection.

GC/MS Analyses and Peaks Identification

Gas chromatography-electron impact mass spectrometry (GC-EI-MS) analyses were performed with a Varian CP-3800 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. 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. Identification of the constituents was based on a comparison of the retention times with those of the 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.[55–59]

Analytical Standards for GC/MS Analysis

The comparison between mass spectra of pure analytical standards and most abundantly detected compounds in VOC and EO analyses are reported in *Figures S1–S9* of the *Supporting Information*. All analytical standards used for confirmation and comparison in the GC/MS analysis were purchased from Sigma-Aldrich Inc. (MO, USA).

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Author Contribution Statement

Claudia Giuliani: methodology, investigation, writing–review and editing. Martina Bottoni: writing-original draft preparation. Roberta Ascrizzi: methodology, investigation, writing-review and editing. Fabrizia Milani: writing-review and editing. Alessio Papini: writing-review and editing. Guido Flamini: methodology, investigation, writing-review and editing. Gelsomina Fico: funding acquisition, conceptualization, supervision, writing-review and editing.

References

[1] S. Pignatti, 'Flora d'Italia', Bologna, Edagricole, 1982.
[2] T. Upson, S. Andrews, 'The Genus Lavandula', Royal Botanic Gardens, Kew, 2004.
[3] B. Justus, V. P. de Almeida, M. M. Gonçalves, D. P. da Silva Fardin de Assunção, D. M. Borsato, A. F. M. Arana, B. H. L. N. S. Maia, J. D. F. P. de Paula, J. M. Budel, P. V. Farago, 'Chemical composition and biological activities of the essential oil and anatomical markers of *Lavandula dentata* L. Cultivated in Brazil', *Braz. Arch. Biol. Technol.* **2018**, *61*, DOI 10.1590/1678-4324–2018180111.
[4] C. Giuliani, R. Ascrizzi, D. Lupi, G. Tassera, L. Santagostini, M. Giovanetti, G. Flamini, G. Fico, '*Salvia verticillata*: Linking glandular trichomes, volatiles and pollinators', *Phytochemistry* **2018**, *155*, 53–60.

[5] C. Giuliani, R. Ascrizzi, S. Corrà, L. M. Bini, G. Flamini, G. Fico, 'Ultrastructural insight into terpene-producing trichomes and essential oil profile in *Salvia greggii* A.Gray', *Flora* **2017**, *236*, 107–114.
[6] C. Giuliani, R. Ascrizzi, C. Tani, M. Bottoni, L. M. Bini, G. Flamini, G. Fico, '*Salvia uliginosa* Benth.: glandular trichomes as bio-factories of volatiles and essential oil', *Flora* **2017**, *233*, 12–21.
[7] R. Tardugno, A. Serio, F. Pellati, S. D'Amato, C. Chaves López, M. G. Bellardi, M. Di Vito, V. Savini, A. Paparella, S. Benvenuti, '*Lavandula*×*intermedia* and *Lavandula angustifolia* essential oils: phytochemical composition and antimicrobial activity against foodborne pathogens', *Nat. Prod. Res.* **2019**, *33*, 3330–3335.
[8] M. do Rocio Duarte, D. Carvalho de Souza, 'Microscopic characters of the leaf and stem of *Lavandula dentata* L. (Lamiaceae)', *Microsc. Res. Tech.* **2014**, *77*, 647–652.
[9] Z. Hu, C. Wang, H. Shen, K. Zhang, P. Leng, 'Antioxidant effect of aromatic volatiles emitted by *Lavandula dentata*, *Mentha spicata*, and *M. piperita* on mouse subjected to low oxygen condition', *Biosci. Biotechnol. Biochem.* **2017**, *81*, 2386–2395.
[10] S. Bedini, G. Flamini, R. Ascrizzi, F. Venturi, G. Ferroni, A. Bader, J. Girardi, B. Conti, 'Essential oils sensory quality and their bioactivity against the mosquito *Aedes albopictus*', *Sci. Rep.* **2018**, *8*, DOI 10.1038/s41598-018-36158-w.
[11] I. Dammak, Z. Hamdi, S. Kammoun El Euch, H. Zemni, A. Mliki, M. Hassouna, S. Lasram, '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.* **2019**, *128*, 85–93.
[12] R. de P. Martins, R. A. da S. Gomes, A. C. G. Malpass, M. H. Okura, 'Chemical characterization of *Lavandula dentata* L. essential oils grown in Uberaba-MG', *Ciência Rural* **2019**, *49*, DOI 10.1590/0103-8478cr20180964.
[13] K. Msaada, N. Salem, S. Tammar, M. Hammami, M. Jamal Saharkhiz, N. Debiche, F. Limam, B. Marzouk, 'Essential Oil Composition of *Lavandula dentata*, *L. stoechas* and *L. multifida* Cultivated in Tunisia', *J. Essent. Oil Bear. Plants* **2012**, *15*, 1030–1039.
[14] M. Qneibi, N. Jaradat, M. Hawash, A. N. Zaid, A.-R. Natsheh, R. Yousef, Q. AbuHasan, 'The neuroprotective role of *Origanum syriacum* L. and *Lavandula dentata* L. Essential oils through their effects on AMPA receptors', *BioMed Res. Int.* **2019**, *2019*, DOI 10. 1155/2019/5640173.
[15] B. Touati, H. Chograni, I. Hassen, M. Boussaïd, L. Toumi, N. B. Brahim, 'Chemical composition of the leaf and flower essential oils of Tunisian *Lavandula dentata* L. (Lamiaceae)', *Chem. Biodiversity* **2011**, *8*, 1560–1569.
[16] S. Bouazama, H. Harhar, J. Costa, J. M. Desjobert, A. Talbaoui, M. Tabyaoui, 'Chemical composition and antibacterial activity of the essential oils of *Lavandula pedunculata* and *Lavandula dentata*', *J. Mater. Environ. Sci.* **2017**, *8*, 2154–2160.
[17] R. A. Mothana, M. S. Alsaid, S. S. Hasoon, N. M. Al-Mosaiyb, A. J. Al-Rehaily, M. A. Al-Yahya, 'Antimicrobial and antioxidant activities and gas chromatography mass spectrometry (GC/MS) analysis of the essential oils of *Ajuga bracteosa* Wall. ex Benth. and *Lavandula dentata* L. growing wild in Yemen', *J. Med. Plants Res.* **2012**, *6*, 3066–3071.
[18] R. Wells, F. Truong, A. M. Adal, L. S. Sarker, S. S. Mahmoud, '*Lavandula* essential oils: A current review of applications in medicinal, food, and cosmetic industries of lavender', *Nat. Prod. Commun.* **2018**, *13*, 1403–1417.
[19] B. Imelouane, A. El Bachiri, J.-P. Wathelet, J. Dubois, H. Amhamdi, 'Chemical Composition, Cytotoxic and Antioxidant Activity of The Essential Oil of *Lavandula dentata*', *World J. Chem.* **2010**, *5*, 103–110.
[20] B. Justus, C. C. Kanunfre, J. M. Budel, M. F. de Faria, V. Raman, J. P. de Paula, P. V. Farago, 'New insights into the mechanisms of French lavender essential oil on non-small-cell lung cancer cell growth', *Ind. Crops Prod.* **2019**, *136*, 28–36.
[21] C. Giuliani, L. Maleci Bini, 'Insight into the structure and chemistry of glandular trichomes of Labiatae, with emphasis on subfamily Lamioideae', *Plant Syst. Evol.* **2008**, *276*, DOI https://doi.org/10.1007/s00606-008-0085–0.
[22] A. C. Aprotosoaie, E. Gille, A. Trifan, V. S. Luca, A. Miron, 'Essential oils of *Lavandula* genus: a systematic review of their chemistry', *Phytochem. Rev.* **2017**, *16*, 761–799.
[23] L. Bousmaha, J. B. Boti, F. A. Bekkara, V. Castola, J. Casanova, 'Infraspecific chemical variability of the essential oil of *Lavandula dentata* L. from Algeria', *Flavour Frag. J.* **2006**, *21*, 368–372.
[24] L. Bousmaha, F. A. Bekkara, F. Tomi, J. Casanova, 'Advances in the chemical composition of *Lavandula dentata* L. essential oil from Algeria', *J. Essent. Oil Res.* **2005**, *17*, 292–295.
[25] P. G. Silva-Flores, L. A. Pérez-López, V. M. Rivas-Galindo, D. Paniagua-Vega, S. A. Galindo-Rodríguez, R. Álvarez-Román, 'Simultaneous GC-FID Quantification of Main Components of *Rosmarinus officinalis* L. and *Lavandula dentata* Essential Oils in Polymeric Nanocapsules for Antioxidant Application', *J. Anal. Methods Chem.* **2019**, *2019*, 1–9.
[26] J. M. Q. Luz, S. M. Silva, G. R. Marquez, P. A. M. Nogueira, P. B. Alves, M. F. Alves, I. L. Matos, 'Agronomic production and essential yield of *Lavandula dentata* L. in different systems and fertilization', *Acta Hortic.* **2016**, *1125*, 113–120.
[27] M. A. M. Masetto, C. Deschamps, A. F. Mógor, H. R. Bizzo, 'Teor e composição do óleo essencial de inflorescências e folhas de *Lavandula dentata* L. em diferentes estádios de desenvolvimento floral e épocas de colheita', *Rev. Bras. Plant. Med.* **2011**, *13*, 413–421.
[28] B. Conti, A. Canale, P. L. Cioni, G. Flamini, 'Repellence of essential oils from tropical and Mediterranean Lamiaceae against *Sitophilus zeamais*', *Bull. Insectol.* **2010**, *63*, 197–202.
[29] L. F. Cossetin, E. M. T. Santi, J. F. Cossetin, J. B. Dillmann, M. D. Baldissera, Q. I. Garlet, T. P. De Souza, L. Loebens, B. M. Heinzmann, M. M. Machado, S. G. Monteiro, 'In vitro safety and efficacy of lavender essential oil (lamiales: Lamiaceae) as an insecticide against houseflies (diptera: Muscidae) and blowflies (diptera: Calliphoridae)', *J. Econ. Entomol.* **2018**, *111*, 1974–1982.
[30] T. Dob, D. Dahmane, B. Tayeb, C. Chelghoum, 'Chemical composition of the essential oil of *Lavandula dentata* L. from Algeria', *Int. J. Aromather.* **2005**, *15*, 110–114.
[31] D. Dris, F. Tine-Djebbar, N. Soltani, '*Lavandula dentata* Essential Oils: Chemical Composition and Larvicidal Activity

Against *Culiseta longiareolata* and *Culex pipiens* (Diptera: Culicidae)', *Afr. Entomol.* **2017**, *25*, 387–394.
[32] K. Karamanoli, D. Vokou, U. Menkissoglu, H.-I. Constantinidou, 'Bacterial colonization of phyllosphere of Mediterranean aromatic plants', *J. Chem. Ecol.* **2000**, *26*, 2035–2048.
[33] A. A. Abraham, A. Verghese, S. Muthangi, 'Role of colour and volatile in foraging behaviour of honeybee *Apis cerana* on *Jacquemontia pentanthos*', *J. Asia-Pac. Entomol.* **2018**, *21*, 1122–1128.
[34] N. Nishida, S. Tamotsu, N. Nagata, C. Saito, A. Sakai, 'Allelopathic effects of volatile monoterpenoids produced by *Salvia leucophylla*: inhibition of cell proliferation and DNA synthesis in the root apical meristem of *Brassica campestris* seedlings', *J. Chem. Ecol.* **2005**, *31*, 1187–1203.
[35] S. Maietti, D. Rossi, A. Guerrini, C. Useli, C. Romagnoli, F. Poli, R. Bruni, G. Sacchetti, 'A multivariate analysis approach to the study of chemical and functional properties of chemo-diverse plant derivatives: Lavender essential oils', *Flavour Fragrance J.* **2013**, *28*, 144–154.
[36] C. Chen, Q. Song, 'Responses of the pollinating wasp *Ceratosolen solmsi marchali* to odor variation between two floral stages of *Ficus hispida*', *J. Chem. Ecol.* **2008**, *34*, 1536–1544.
[37] R. Steen, H. R. Norli, G. Thöming, 'Volatiles composition and timing of emissions in a moth-pollinated orchid in relation to hawkmoth (Lepidoptera: Sphingidae) activity', *Arthropod. Plant. Interact.* **2019**, *13*, 581–592.
[38] P. C. Stevenson, 'For antagonists and mutualists: the paradox of insect toxic secondary metabolites in nectar and pollen', *Phytochem. Rev.* **2019**, 1–12.
[39] Y.-C. Hsiung, Y.-A. Chen, S.-Y. Chen, W.-C. Chi, R.-H. Lee, T.-Y. Chiang, H.-J. Huang, 'Volatilized myrcene inhibits growth and activates defense responses in rice roots', *Acta Physiol. Plant.* **2013**, *35*, 2475–2482.
[40] L. Zamponi, M. Michelozzi, P. Capretti, 'Effects of four monoterpenes on the growth in vitro of some *Heterobasidion* spp. and two *Leptographium* species', *J. Plant Dis. Prot.* **2006**, *113*, 164–167.
[41] G. X. Jing, N. G. Tao, L. Jia, H. E. Zhou, 'Influence of α-terpineol on the growth and morphogenesis of *Penicillium digitatum*', *Bot. Stud.* **2015**, *56*, 35.
[42] A. Rahman, M. A. Hossain, S. C. Kang, 'Control of phytopathogenic fungi by the essential oil and methanolic extracts of *Erigeron ramosus* (Walt.) BSP', *Eur. J. Plant Pathol.* **2010**, *128*, 211–219.
[43] G. H. Seol, K. Y. Kim, in 'Drug Discov. from Mother Nat.', Springer, 2016, pp. 389–398.
[44] M. de B. M. V. Damasceno, S. A. A. R. Santos, T. M. Barbosa, J. R. C. Araújo, A. E. Vieira-Neto, D. V. T. Wong, R. C. P. Lima-Júnior, A. R. Campos, 'Acute and neuropathic orofacial antinociceptive effect of eucalyptol', *Inflammopharmacology* **2017**, *25*, 247–254.
[45] B. Dörsam, C.-F. Wu, T. Efferth, B. Kaina, J. Fahrer, 'The eucalyptus oil ingredient 1,8-cineol induces oxidative DNA damage', *Arch. Toxicol.* **2015**, *89*, 797–805.
[46] T. Ainane, M. Elkouali, A. Ainane, M. Talbi, 'Moroccan traditional fragrance based essential oils: Preparation, composition and chemical identification', *Pharm. Chem.* **2014**, *6*, 84–89.
[47] R. A. Mothana, 'Antimicrobial and antioxidant activities and gas chromatography mass spectrometry (GC/MS) analysis of the essential oils of *Ajuga bracteosa* Wall. ex Benth. and *Lavandula dentata* L. growing wild in Yemen', *J. Med. Plants Res.* **2012**, *6*, 3066–3071.
[48] N. Beccari, V. Mazzi, Manuale Di Tecnica Microscopica, Società Editrice Libraria, Como, Italy, 1966.
[49] M. C. Brundrett, B. Kendrick, C. A. Peterson, 'Efficient lipid staining in plant material with Sudan Red 7B or Fluoral Yellow 088 in polyethylene glycol-glycerol', *Biotech. Histochem.* **1991**, *66*, 111–116.
[50] P. Greenspan, E. P. Mayer, S. D. Fowler, 'Nile red: a selective fluorescent stain for intracellular lipid droplets', *J. Cell Biol.* **1985**, *100*, 965–973.
[51] R. David, J. P. Carde, R. David, 'Histochimie-coloration differentielle des inclusions lipidiques et terpeniques des pseudophylles du pin maritime au moyen du reactif NADI', *C. R. Biol.* **1964**, *258*, 1338–1340.
[52] W. A. Jensen, 'Botanical Histochemistry: Principles and Practice', San Francisco, 1962.
[53] P. B. Gahan, 'Plant Histochemistry and Cytochemistry', Academic Press, London, 1984.
[54] R. Ascrizzi, P. L. Cioni, L. Amadei, S. Maccioni, G. Flamini, 'Geographical patterns of in vivo spontaneously emitted volatile organic compounds in *Salvia* species', *Microchem. J.* **2017**, *133*, 13–21.
[55] R. P. Adams, 'Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy', Allured Publishing Corporation, Illinois, USA, 1995.
[56] N. W. Davies, 'Gas chromatographic retention indices of monoterpenes and sesquiterpenes on Methyl Silicon and Carbowax 20 M phases', *J. Chromatogr. A* **1990**, *503*, 1–24.
[57] W. Jennings, T. Shibamoto, 'Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography', Academic Press, New York, London, Sydney, Toronto, San Francisco, 1982.
[58] Y. Masada, 'Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry', New York, NY, 1976.
[59] E. Stenhagen, S. Abrahamsson, F. W. McLafferty, 'Registry of Mass Spectral Data', Wiley & Sons, New York, NY, 1974.

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