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Abstract: The profile of volatile organic compounds (VOC), the composition of the essential oils (EOs) and the micromorphology of the leaf trichomes were investigated in two rosemary hybrids, Salvia × mendizabalii (Sagredo ex Rosúa) Roma-Marzio & Galasso (S×m) and Salvia × lavandulacea (de Noé) Roma-Marzio & Galasso (S×l). The phytochemical analysis highlighted the dominance of monoterpene hydrocarbons in both aroma profiles (65.8% in S×m and 77.9% in S×l, respectively), although they displayed different main compounds except for α-pinene (28.2% in S×m and 24.5% in S×l). In S×m the EOs extracted from both fresh and dried leaves exhibited camphor (29.7% and 25.9%, respectively), α-pinene (21.0% and 18.2%, respectively) and 1,8-cineole (11.1% and 13.6%, respectively) as major compounds. Noteworthy is the occurrence of β-pinene (2.6%) in the EO dried samples, whereas sabinene and limonene were exclusive of the fresh samples. In S×l the EOs from the fresh and dried leaves shared the same main compounds: camphor (24.0% and 27.5%, respectively), myrcene (14.9% and 14.8%, respectively) and α-pinene (13.1% and 12.2%, respectively).
The micro-morphological observations on leaves proved the occurrence of non-glandular dendritic hairs with smooth cuticle in both hybrids. The glandular trichomes include three main morphotypes: peltate, short capitate and medium-long capitate. The peltate and the short capitates are common to both hybrids, however in the peltate the number of the head secreting cells is different, eight in S×m and sixteen in S×l. The medium-long capitate occurs exclusively on the leaf adaxial side of S×m.

UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI
SCIENZE FARMACEUTICHE

Prof. John Patrick Bower

Editor of Scientia Horticulturae

We are very grateful to you for the possibility to revise our manuscript () entitled "*Volatilome and micromorphological analysis of two Rosemary hybrids*" by B. Najar, L. Pistelli, C. Cervelli, M.C. Salvatici, G. Fico, and C. Giuliani, according to the reviewer's indications. We also wish to thank you for the kind attention towards our work.
All the scientific modifications are highlighted in yellow color in the revised version of the manuscript. Please find hereafter the answers to each of the comments raised by the reviewers.

Reviewer 3
This work is aimed to characterize two rosemary hybrids based on their VOC profile, EO composition and micromorphology of leaf trichomes. Due to the high importance of the whole genus, recently merged within the genus Salvia, this is a significant work as for novelty and for broader taxonomical implications. The work falls within the scope of the Journal, English form is fine, overall description is rather well-written, interpretation and conclusions are sound and supported by results; hence, in my opinion, it deserves to be published after several minor amendments.
A: We thank the Reviewer 3 for this comment.

My observations are reported in the following list:
- keywords, abstract, graphical abstract and elsewhere in the text: I greatly appreciate the choice of the Authors to use the newest botanical classification of rosemary. Anyway, the Latin term following the name "*Salvia*" must be accorded to feminine gender; hence, the denomination *Salvia x lavandulaceus* must be modified to *Salvia x lavandulacea*. For further information, check on Italian Botanist 7: 31-33 (2019) (doi: 10.3897/italianbotanist.7.34379), that you correctly already inserted along cited references.
A: We modified the denomination throughout the manuscript, as asuggested.

- Page 2, line 35: eliminate the first "the" (are common to both hybrids). The same in the abstract, at page 5, line 112, page 15, lines 254 and 260, page 20, line 376 and elsewhere in the text.
A: Done

- Page 3, lines 53-54: slightly modificate the sentence (the color of flowers is not due to the presence of volatile oils).
A: We changed the sentence as follows: "Salvia rosmarinus is a perennial evergreen shrub with needle-like leaves and light blue-lilac flowers (Yeddes et al., 2019); it displays a peculiar aroma due to the volatile oil accumulated in typical peltate and capitate glandular trichomes (Marin et al., 2006; Díaz-Maroto et al., 2007)".
The previous sentence was confusing.

- Page 3, line 55: the reference Díaz-Maroto et al., 2007 is not reported in the reference list.
A: Done

- Page 3, line 56: "This species differs…" maybe should be better.
*A: We changed the sentence as follows: "*Several features distinguish this species from *S. rosmarinus*, *e.g.* the prostrate habit, the smaller leaves and the more densely hairy flowers".

- Page 3, line 59: correct "costs" to "coasts".
A: Done

- Page 3, line 60: are you sure*? Salvia granatensis* is not reported in the IUCN list at https://www.iucnredlist.org/search; check and add reference.
A: We deleted the information, erroneously reported.

- Page 4, line 73: correct "Andrare" to "Andrade".
- Page 4, line 78: correct "Bendelaid" to "Benbelaïd"
A: Done.

- Page 5, line 92: since the paper is addressed to an international audience, it would be advisable to add some further reference for the cultivation site (geographical coordinates or, at least, the abbreviation of province).
A: We added the abbreviation of the province.

- Page 5, lines 92-97: additional details about the sampling methods are necessary. Due to the high variability of rosemary according to the age of plants and the used part of plant, the following points should be addressed: how old were the plants? Which part of plant was used for sampling? (of course, they were leaves, but it should be better if you specify, for example, that they were collected from "young twigs", or "1-year-old twigs", or whatever). When they were collected? Rosemary has many flowering flushes, hence it is not enough talking about "full blooming"; specify the month of collection, or at least the season.
A: We agree with the Reviewer 3 and accordingly added the requested information: "….The climate of this site is Mediterranean-type, with mild winters, very light and rare (if any) frosts, hot and sunny summers. The plants were propagated by cutting and potted in 9-liter plastic containers using a peat-based substrate containing about 10% of pumex 7-12 mm (Hochmoor Vulcan invernale, Terflor, Brescia, Italy), added with 4 g/l of a slow release fertilizer (Osmocote Exact Standard 5-6M, 15-9-12+2MgO+ME, ICL Corporate). Plants were grown for one year in open air under uniform experimental conditions, providing water during all growth period by a drip irrigation system. The samplings for the phytochemical and micromorphological investigations were performed simultaneously from 1-year-old, 30 cm tall plants in full blooming in October 2018. For the VOC analysis and the morphological survey, similar leaves for whole size and position were selected from 1-year-old twigs of the same individual. For the EO analysis, both fresh and dried samples were used".

Finally, how was the drying process performed? Were the samples air-dried (if so, how long?) to obtain herbal product, or artificially dried (if so, at which temperature/time conditions?) to obtain dry matter?
*A: We added this sentence: "*the air-drying process was carried out at room temperature, in the dark, and in controlled humidity conditions for one week".

- Page 5, line 103: how much time passed between leaves collection and VOC analyses? In my experience, VOC results by HS-SPME are deeply different according to this time span.
A: We clarified this point, adding the following sentence: "cut from the same individual and immediately put in glass vial for 3 min before analysis (equilibration time)".

- Page 5, lines 105-107: as it is presently written, it seems that distillation was performed on individual samples of 33 g each, that are rather few for usual hydrodistillation process; if this is not your meaning, check the opportunity to rephrase the sentence.
A: Concerning the fresh samples we had only 100 g available; so we divided them in 3 equal aliquots each distilled in a Clevenger-microdistillation apparatus. For the dried plant material we used 100 g for each distillation.

After the statement about European Pharmacopoeia, add the reference.
A: we added the reference as suggested, along with the reference in extenso *in the Reference List.*

- Page 5, line 112: insert the reference for the PAST software (within the text: Hammer et al., 2001; in the reference list; Hammer Ø., Harper D.A.T., Ryan P.D. 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 4(1): 9pp.)
A: Done.

- **Page 6, line 123: I am not sure that "consistency" is the most proper term. Do you mean maybe the "occurrence" or the "morphology" or the "structure" of glandular hairs?**
A: *We replaced the term "consistency" with "level of variability".*

- Page 7, line 134: insert "w/w" after the essential oil yield percentages.
A: Done.

- Pages 8-9 (table 1): add to the table caption, after "Average of triplicate analyses", "± standard deviation" (or what else your dispersion measurement is).
A: Done.

- Page 10, line 165: Figure 1 does not refer to PCA analysis. Probably figures 1 and 2 should be inverted.
A: Done

- Page 13, Figure 1: a dendrogram should not be drawn in an empty space; please insert the X axis (distance) with graduations; in central position, write "distance", or "similarity", or whatever is the output from your statistical package.
A: We inserted the X axis (distance) with graduation as recommended in the Figure.

- Page 13, captions to figures 1 and 2: substitute "represented" with "represent" (it is the present figure, there is no reason to refer it to the past).
A: Done.

- Page 17, lines 286-288: check the opportunity to substitute the entire sentence with: "The total number of the isolated compounds, the dominant terpenoidic classes, and the major components of aroma profiles, although present in relatively variable percentages, resulted consistent between the two hybrids."
A: Done.

- Page 17, line 289: substitute "are" with "were".
A: Done.

- Page 17, line 290: check the opportunity to rephrase as: "…being δ-3-carene and β-pinene the major ones, respectively." or "…with a prevailing presence of δ-3-carene and β-pinene in the two hybrids, respectively."
A: Done.

- Page 17, lines 292-293: when discussing VOCs in rosemary, please consider the opportunity to insert the following reference: Carrubba et al., 2020, Characterization of Sicilian rosemary (Rosmarinus officinalis L.) germplasm through a multidisciplinary approach, Planta, Vol 251, Issue 2, Article number 37. doi: 10.1007/s00425-019-03327-8
A: We added the reference as suggested.

- Page 17, line 296: "…showed a similar α–pinene amount"
A: Done.

- Page 17, line 297: where was it lower?
A: We added "in our samples"

- Page 18, line 313: "Among the main exclusive compounds…"
- Page 18, line 315: correct to "Hetherington-Rauth and Ramırez, 2016"
- Page 19, line 343: "A more recent study"
- Page 19, lines 343-344: "half of the identified fraction" (remove "the").
A: Done.

- Page 19, line 346: check the opportunity to substitute "the comparison with the literature" with "the literature survey" (there is nothing to compare with)
A: Done.

- Page 19, lines 349-350: check the opportunity to substitute "a part from the different relative abundance" with: "although represented in comparatively different amounts"
A: Done.

- Page 19, line 351: correct "As regards to" to "as regards", or "in regard to" or "concerning". The same at page 20, line 381.
A: Done.

- Page 19, lines 353-354: check the opportunity to modify the sentence as, for example, "showed antimicrobial, insecticidal and repellent, cytotoxic and anti-inflammatory activities" (adjectives cannot stand alone).
- Page 19, line 365: correct "the formers" to "the former" or "the former authors".
A: Done.

- Page 20, lines 358-359: check the opportunity to rephrase the sentence as, for example: "In both hybrids, the drying process implied the increase or the decline of several volatiles, including the major ones".
A: Done.

- References, page 22, line 430: the reference "Bendif et al, 2016" although inserted in the reference list, is not quoted within the text. Please check.
A: This citation was erroneously inserted in the text. The reference in extenso *was deleted from the Reference List.*

- References, page 25, line 509: correct "Mishara" to "Mishra".
A: Done

- An additional advice would be to check carefully the text (above all the last part, pages 20-22), to ensure that verb tense consistency is maintained within sentences and paragraphs, since somewhere it happens that tenses are changed without any reason. I suggest to take a look to the following web page: https://webapps.towson.edu/ows/tenseconsistency.htm
A: We carefully checked the text and corrected the verb tense as recommended.

Reviewer 4

In the present manuscript, the leaf aroma profile (VOC), the composition of the essential oils (EOs) and the micromorphology of the leaf trichomes of two rosemary hybrids were investigated.
Overall, the manuscript is well presented and deserves to be published in Scientia Horticulturae journal. However some issues have to be addressed first.

Specific comments
Highlights have to be shortened to 85 characters per bullet. Provide also a highlight related with EO composition.
A: Done

Line 18: The abbreviation of VOC should be related with abbreviated term e.g. volatile organic compounds instead of leaf aroma profile.
A: Done

Line 92: Use the full names when first cited in the M&M section.
A: Done

More details about the growing conditions, the experimental material and the statistical design are needed.
A: Done, as also suggested by the Reviewer 3.

Line 166: explain SPME in Table 1 legend.
A: We deleted SPME adding VOC in Table 1 legend and in the Table 1 itself.

Lines 183-184: The increase in EO yield is 50% and not 30%.
A: Done.

Check the values for cumulative % of peaks 2 to 4 in Table 3. Check also statistical differences for peak 13
A: Done. There was a mistake in the cumulative percentage.

Figure legends are being put after the figure.
Line 297: correct to "Noteworthy is the lack".
A: Done.

Lines 351-357 are irrelevant to the study.

A: We thank Reviewer 4 for this comment, however we prefer to maintain the paragraph. Even if it does not contain information directly related to the purpose of our work, it does present literature data on the potential applications of interest to humans and this could indicate future research perspective on both hybrids in order to enhance the plant heritage of CREA.

Editor:

Overall, the manuscript is acceptable for publication. For revision, please see the comments above.

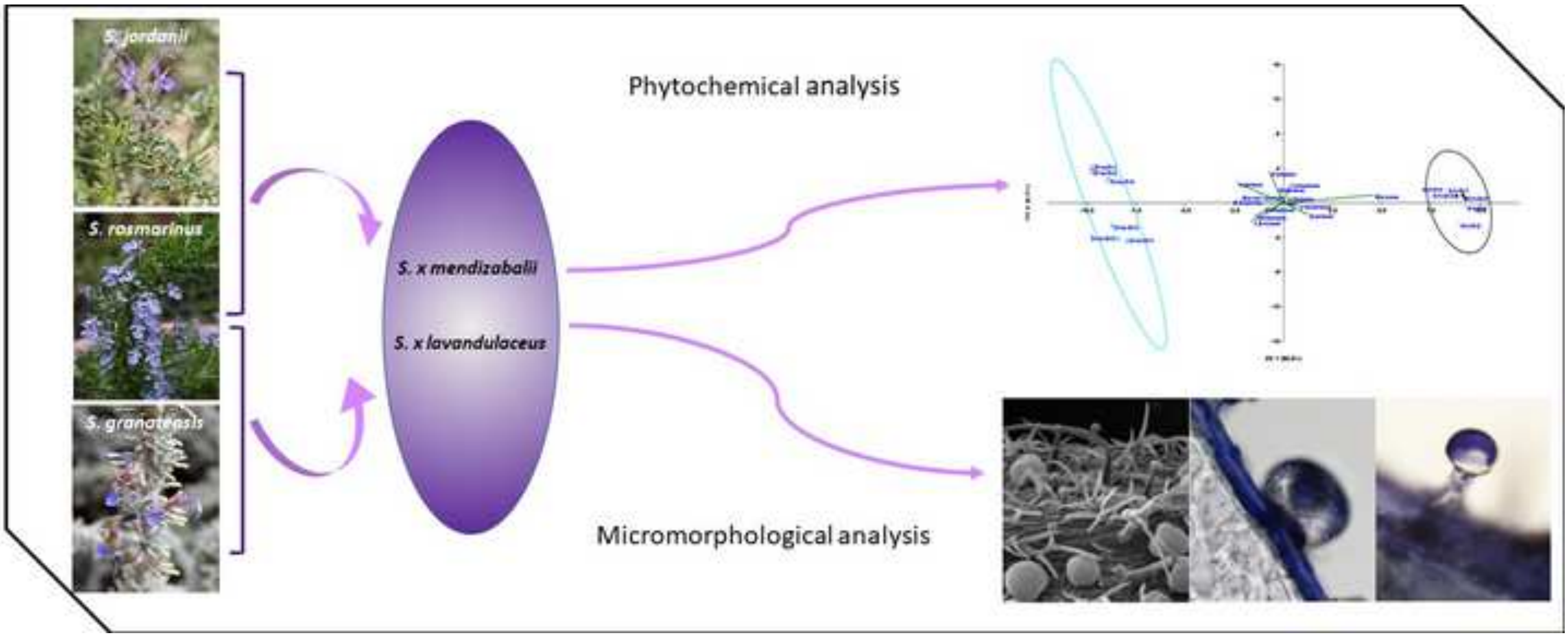
I hope that our manuscript in the present form may be of interest for you.

I remain truly yours,

Milano, 12th February 2020

Prof. Gelsomina Fico

Graphical Abstract



Highlights

- Phytochemical and morphological surveys were combined in two Rosemary hybrids.
- The leaf aroma profiles characterized specifically each hybrid.
- The leaf essential oils varied in fresh and dried samples in both hybrids.
- The leaf glandular trichomes resulted useful for the discrimination of the hybrids.

RESEARCH PAPER

Volatilome and micromorphological analysis of two Rosemary hybrids

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Abstract

The profile of volatile organic compounds (VOC), the composition of the essential oils (EOs) and the micromorphology of the leaf trichomes were investigated in two rosemary hybrids, *Salvia × mendizabalii* (Sagredo ex Rosúa) Roma-Marzio & Galasso (*S*×*m*) and *Salvia × lavandulacea* (de Noé) Roma-Marzio & Galasso (*S*×*l*). The phytochemical analysis highlighted the dominance of monoterpene hydrocarbons in both aroma profiles (65.8% in *S*×*m* and 77.9% in *S*×*l*, respectively), although they displayed different main compounds except for α-pinene (28.2% in *S*×*m* and 24.5% in *S*×*l*). In *S*×*m* the EOs extracted from both fresh and dried leaves exhibited camphor (29.7% and 25.9%, respectively), α-pinene (21.0% and 18.2%, respectively) and 1,8-cineole (11.1% and 13.6%, respectively) as major compounds. Noteworthy is the occurrence of β-pinene (2.6%) in the EO dried samples, whereas sabinene and limonene were exclusive of the fresh samples. In *S*×*l* the EOs from the fresh and dried leaves shared the same main compounds: camphor (24.0% and 27.5%, respectively), myrcene (14.9% and 14.8%, respectively) and α-pinene (13.1% and 12.2%, respectively).

The micro-morphological observations on leaves proved the occurrence of non-glandular dendritic hairs with smooth cuticle in both hybrids. The glandular trichomes include three main morphotypes: peltate, short capitate and medium-long capitate. The peltate and the short capitates are common to both hybrids, however in the peltate the number of the head secreting cells is different, eight in *S*×*m* and sixteen in *S*×*l*. The medium-long capitate occurs exclusively on the leaf adaxial side of *S*×*m*.

Keywords

Salvia × *mendizabalii*, *Salvia* × *lavandulacea*, *Rosmarinus*, VOCs, essential oil, glandular trichomes.

1. Introduction

Rosmarinus L. (rosemary, Lamiaceae) has been recently included within the genus *Salvia* L. on the base of the phylogenetic results by Drew *et al*. (2017). In this updated circumscription, *Salvia* accounts for almost 1000 species native to Asia, Africa, America and Europe, with the Mediterranean basin being an important centre of diversity (Kasmaei *et al*., 2019). *Salvia* uses vary from medicinal, culinary and cosmetic sectors to ornamental purposes and this can explain its worldwide cultivation (de Mesquita *et al.*, 2019).

Within the rosemary complex, three species native to the Mediterranean region are recognized (Bendif *et al*., 2018): *Salvia rosmarinus* Schleid (ex *Rosmarinus officinalis* L), *Salvia jordanii* J.B.Walker (ex *Rosmarinus eryocalix* Jord. & Fourr.) and *Salvia granatensis* B.T.Drew (ex *Rosmarinus tomentosus* Hub.-Mor. & Maire). *Salvia rosmarinus* is a perennial evergreen shrub with needle-like leaves and light blue-lilac flowers (Yeddes *et al*., 2019); it displays a peculiar aroma due to the volatile oil accumulated in typical peltate and capitate glandular trichomes (Marin *et al.*, 2006; Díaz-Maroto *et al.*, 2007). *Salvia jordanii* is endemic to North Africa (Dobignard and Chatelain, 2010-2013) and Spain (Fadel *et al*., 2011). Several features distinguish this species from *S. rosmarinus*, *e.g.* the prostrate habit, the smaller leaves and the more densely hairy flowers (Fadel *et al*., 2011; Benbelaïd *et al*., 2016). *Salvia granatensis* is endemic to South-Eastern Spain (Málaga and Granada), where it grows along the coasts.

The introgression of the latter two species with *S. rosmarinus* originate two hybrids (Roma-Marzio and Galasso, 2019): *Salvia x lavandulacea* (de Noé) Roma-Marzio & Galasso (*S. jordanii* x *S. rosmarinus*; ≡ *Rosmarinus* × *lavandulaceus* Noë, ≡ *R. eriocalyx x R. officinalis*) and *Salvia x mendizabalii* (Sagredo ex Rosúa) Roma-Marzio & Galasso (*S. granatensis* × *S. rosmarinus*; ≡ *Rosmarinus* × *mendizabalii* Sagredo ex Rosa, ≡ *R. officinalis* × *R. tomentosum*). These hybrids display variable morphological characteristics especially with regards to inflorescences and flowers (in particular, the calyx), and this caused problems in the recognition from the parental species. Diagnostic traits are represented by the features of the leaf adaxial surface, defined cano-tomentose in *S. x mendizabalii* and almost hairless in *S. x lavandulacea* (Rosuà, 1981).

Among the parental species, *S. rosmarinus* is the most exploited and studied one with an average number of 120 papers focused on phytochemistry and biological activity per year since 2010 (Andrade *et al*., 2018). It is cultivated all over the world mainly due to its richness in essential oils with high commercial value (Borges *et al*., 2019). About S. *jordanii* and *S. granatensis*, previous phytochemical investigations were focused on the essential oil composition of Spanish natural populations (Cano and Sánchez, 1993), while the former was also analysed for both spontaneous volatiles and essential oils from Algerian samples (Benbelaïd *et al*., 2016; Bendif *et al*., 2017). Concerning the hybrids, the literature reports information on the essential oil composition of plants grown in Spain (Cano and Sánchez, 1993), while a detailed description of the *indumentum* is lacking, apart from general indications on the occurrence of trichomes on leaves and calyces.

In this framework, the primary objective was to investigate the volatilome (volatile organic compounds (VOCs) and essential oils (EOs)) obtained from both fresh and dried leaves of *S. x mendizabalii* (*S*×*m*) and *S. x lavandulacea* (*S*×*l*), collected at Centro di Ricerca Orticoltura e Florovivaismo (CREA-OF, Sanremo Italy) as part of their collection addressed to maintain and improve plant species for ornamental purposes. In addition, for the first time, we combined the production of these secondary metabolites to the micromorphology of the leaf *indumentum*.

2. Materials and methods

2.1 Plant material

The plants of *Salvia x menzizabalii* and *Salvia x lavandulacea* used in this study were cultivated in Sanremo (Imperia, Italy) at the Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CREA). The climate of this site is Mediterranean-type, with mild winters, very light and rare (if any) frosts, hot and sunny summers. The plants were propagated by cutting and potted in 9-liter plastic containers using a peat-based substrate containing about 10% of pumex 7-12 mm (Hochmoor Vulcan invernale, Terflor, Brescia, Italy), added with 4 g/l of a slow release fertilizer (Osmocote Exact Standard 5-6M, 15-9-12+2MgO+ME, ICL Corporate). Plants were grown for one year in open air under uniform experimental conditions, providing water during all growth period by a drip irrigation system. The samplings for the phytochemical and micromorphological investigations were performed simultaneously from 1-year-old, 30 cm tall plants in full blooming in October 2018. For the VOC analysis and the morphological survey, similar leaves for whole size and position were selected from 1-year-old twigs of the same individual. For the EO analysis, both fresh and dried samples were used.

2.2 Phytochemical analysis

2.2.1 VOC analysis and EO extraction

The VOC analysis was carried on at least three fresh leaves cut from the same individual and immediately put in glass vial for 3 min before analysis (equilibration time). The SPME was performed by Supelco SPME devise coated with polydimethylsiloxane (PDMS, 100 μm) following the procedure described by Najar *et al*. (2017).

EOs were obtained from plant material collected from at least 5 individuals per hybrid. Fresh and air-dried samples (each 100 g) were used for each hybrid; the air-drying process was carried out at room temperature, in the dark, and in controlled humidity conditions for one week. The whole plant material was divided into three equal aliquots that were separately subjected to hydrodistillation for 2h using a Clevenger-type apparatus according to the European Pharmacopeia (EMA, 2015). The obtained oils were preserved at 4°C until the time of the analysis by GC-MS.

2.2.2 Statistical analyses

The multivariate statistical analyses were carried out on all the detected EO compounds for both hybrids using the Past3 software package. The principal component analysis (PCA) was performed selecting the two highest principal components (PCs) obtained by the linear regressions. The hierarchical cluster analysis (HCA) was performed by the Ward's method. Statistically significant differences among the hybrids related to the aroma profiles and the EO compositions were assessed through the one-way PERMANOVA with Bray-Curtis-based similarity. The contribution of each compound in percentage to the observed dissimilarity was evaluated by means of the Similarity Percentage Analysis (SIMPER, Euclidean distance). The value for the statistical significance was $p < 0.05$. These analyses were performed with Past software (version 3) (Hammer et al., 2001).

2.3. Micromorphological analysis

At least five leaves per hybrid were observed to assess the level of variability of the glandular trichome morphotypes, their distribution pattern and the chemical nature of the secretory products. The observations were carried out by means of scanning electron microscopy (SEM) and light microscopy (LM) through histochemical procedures.

SEM - Small pieces of leaves were fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer at pH 7.2 for 7 days at room temperature. Therefore, they were dehydrated in an ascending ethanol series up to absolute and dried using a critical point dryer apparatus. The samples, mounted on aluminium stubs and coated with gold, were observed with a Scanning Electron Microscope Gaia 3 (Tescan s.r.o, Brno, Czech Republic) FIB-SEM (Focused Ion Beam-Scanning Electron Microscope) operating at the voltage of 10 kV and in high-vacuum mode with secondary electron (SE) detector.

LM - Fresh material was frozen, cryostat-cut in semi-thin sections (20-25 µm thick) and stained with the following dyes: Fluoral Yellow-088 for total lipids (Brundrett et al., 1991), Nile Red for neutral lipids (Greenspan et al., 1985), Nadi reagent for terpenes (David and Carde, 1964), Ruthenium Red for acid polysaccharides (Jensen, 1962), Alcian Blue for muco-polysaccharides (Beccari and Mazzi, 1966), Ferric Trichloride for polyphenols (Gahan, 1984) and aluminium trichloride for flavonoids (Guerin et al. 1971). Control procedures were contemporarily carried out for all the employed histochemical assays.

Primary fluorescence of the secretory products was also evaluated under UV and Blue lights. Observations were made with a Leitz DM-RB Fluo optic microscope.

3. Results

3.1 Phytochemical analysis

3.1.1 Salvia x mendizabalii

The VOC profile and the EO compositions of *S×m* are reported in Table 1. A total of 30 different compounds were isolated in the VOC profile accounting for 99.9% of the identified fraction. The spontaneous emission was dominated by monoterpenes, both hydrocarbons (65.8%) and oxygenated compounds (33.3%), reaching almost the totality of the identified fraction (99.1%). High percentages of α-pinene (*3*, 28.2%), camphor (*22*, 19.8%) and camphene (*4*, 11.5%) were detected, followed by 1,8-cineole (*14*, 8.4%), limonene (6.1%), sabinene (*9*) and δ-3-carene (*10*) in equal amounts (5.2%).

The EO analyses evidenced that the dried samples had a more complex profile in comparison to the fresh ones, accounting for a greater number of compounds [38 constituents (99.9%) *vs* 33 (99.4%), respectively], and for a higher EO yield (1.0% w/w *vs* 0.4% w/w, respectively). Monoterpenes dominated in both EOs (96.0%) and the major compounds resulted camphor (*22*, 25.9 *vs* 29.7), α-pinene (*3*, 18.2% *vs* 21.0%), 1,8-cineole (*14*, 11.1 *vs* 13.6%) and camphene (*4*, 8.1% *vs* 7.0%). Camphor (*22*) and α-pinene (*3*) showed a decrease of about 13.0% from fresh to dried samples, while an increase of 22.5% and 15.7% were observed in the other two main compounds, respectively.

In the fresh samples, it was noteworthy the occurrence of four exclusive constituents with sabinene (*9*, 2.5%) and limonene (*13*, 3.2%) present in higher percentages, while β-pinene (*5*, 2.6%) was characteristic of the dried samples, out of nine exclusive compounds in lower amounts.

Table 1. *Salvia x mendizabalii*: chemical composition of the leaf spontaneous volatile emission (VOC) and of the essential oil (EO) profiles obtained from fresh and dried leaves as identified by GC-MS analysis (Average of triplicate analyses ± standard deviation)

	Compound[$]	**Class**	**LRI**[a]	**VOC**	**EO**	
					Fresh	**Dried**
1	tricyclene	mh	927	0.5±0.20	0.2±0.15	0.3±0.07
2	*α*-thujene	mh	930	0.6±0.24	0.1±0.12	0.2±0.02
3	*α*-pinene	mh	939	28.2±1.83	21.0±1.37	18.2±1.81
4	camphene	mh	954	11.5±1.53	7.0±0.59	8.1±0.29
5	*β*-pinene	mh	979	-	-	2.6±0.14
6	3-octanone	nt	984	0.1±0.09	-	0.1±0.06
7	myrcene	mh	991	1.8±0.39	1.2±0.14	1.1±0.06
8	*α*-phellandrene	mh	1003	0.6±0.10	0.3±0.07	0.4±0.02
9	sabinene	mh	1007	5.2±0.57	2.5±0.26	-
10	*δ*-3-carene	mh	1011	5.2±0.37	2.8±0.22	2.7±0.08
11	*α*-terpinene	mh	1017	0.3±0.08	0.5±0.08	0.6±0.02

12	*p*-cymene	mh	1025	3.3±0.51	1.3±0.26	1.9±0.18
13	limonene	mh	1029	6.1±0.67	3.2±0.28	-
14	1,8-cineol	om	1030	8.4±2.76	11.1±0.76	13.6±0.69
15	*γ*-terpinene	mh	1060	0.8±0.08	0.6±0.03	0.7±0.01
16	*cis*-sabinene hydrate	om	1070	0.5±0.10	0.2±0.13	0.3±0.03
17	terpinolene	mh	1089	1.7±0.25	1.4±0.25	1.3±0.04
18	linalool	om	1097	0.3±0.12	0.5±0.46	0.9±0.06
19	*trans*-sabinene hydrate	om	1098	0.2±0.04	-	0.2±0.07
20	chrysanthenone	om	1128	0.4±0.05	0.3±0.01	0.7±0.08
21	*trans*-pinocarveol	om	1139	0.1±0.07	0.1±0.10	-
22	camphor	om	1146	19.8±0.01	29.7±0.78	25.9±0.14
23	pinocarvone	om	1165	-	-	0.1±0.02
24	borneol	om	1169	1.2±0.34	5.0±0.64	5.5±0.51
25	terpinen-4-ol	om	1177	0.3±0.07	1.0±0.17	1.5±0.20
26	*p*-cymen-8-ol	om	1183	-	-	-
27	*α*-terpineol	om	1189	0.1±0.12	0.8±0.18	1.7±0.07
28	dihydrocarveol	om	1194	-	0.1±0.23	-
29	myrtenol	om	1196	-	-	0.3±0.05
30	verbenone	om	1205	0.9±0.41	4.2±0.88	5.9±0.62
31	*iso*bornyl acetate	om	1286	1.1±0.23	0.8±0.08	1.3±0.12
32	*α*-coapene	sh	1377	0.1±0.07	0.1±0.08	0.1±0.02
33	*β*-caryophyllene	sh	1419	0.4±0.07	1.0±0.06	0.7±0.06
34	*α*-humulene	sh	1455	0.1±0.03	0.5±0.11	0.4±0.04
35	*γ*-muurolene	sh	1480	-	0.1±0.11	0.2±0.03
36	valencene	sh	1496	-	-	-
37	*trans*-*γ*-cadinene	sh	1516	-	0.1±0.10	0.2±0.03
38	*δ*-cadinene	sh	1523	0.1±0.07	0.2±0.27	0.4±0.01
39	caryophyllene oxide	os	1583	-	1.0±0.20	1.0±0.09
40	viridiflorol	os	1593	-	-	0.2±0.27
41	humulene *epoxide* II	os	1608	-	0.5±0.12	0.3±0.04
42	cubenol	os	1647	-	-	0.1±0.08
43	intermedeol	os	1667	-	-	0.2±0.02
44	14-hydroxy-9-*epi*-caryophyllene	os	1670	-	-	0.1±0.06
45	Unknown			-	0.6±0.60	-
	Yield				**0.4±0.08**	**1.0±0.02**
	Number of identified compounds			**30**	**33**	**38**
	Monoterpene Hydrocarbons (mh)			65.8±1.35	42.1±1.50	38.2±2.63
	Oxygenated Monoterpenes (om)			33.3±1.55	53.8±1.63	57.9±1.97
	Sesquiterpene Hydrocarbons (sh)			0.7±0.28	2.0±0.34	1.9±0.31
	Oxygenated Sesquiterpene (os)			-	1.5±0.31	1.8±0.45
	Non-terpene Derivatives			0.1±0.09	-	0.1±0.06
	Total Identified (%)			**99.9±0.10**	**99.4±0.60**	**99.9±0.10**

[$]: Only compounds with relative percentages > 0.1% were included in the table.
[a]: LRI: linear retention indices relative to *n*-alkane on the DB5 column

3.1.2 Salvia x lavandulacea

The VOC profile and the EO compositions of *S*×*l* are reported in Table 2. A total of 29 different compounds accounting for 100.0% of the volatiles were identified in the SPME profile. Monoterpene hydrocarbons (77.9%) were the most represented class followed by the oxygenated monoterpenes (21.2%). Among the hydrocarbons, α-pinene (*4*, 24.5%), myrcene (*9*, 13.5%), camphene (*5*, 9.4%), β-pinene (*8*, 8.4%) and limonene (*13*, 5.2%) showed the highest amounts, whereas camphor (*24*, 11.4%) dominated among the oxygenated derivatives, followed by 1,8-cineole (*14*, 5.6%).

The EOs from the fresh and dried samples displayed a comparable number of compounds (38 *vs* 39, respectively) and a similar amount of the identified fraction (99.5% *vs* 99.9%, respectively). Despite this behaviour, the EO yields showed an increase of about 50.0% with drying (0.6 *vs* 0.9% (w/w)). Monoterpenes were the major class with a slight difference in the relative percentages of each hydrocarbons and oxygenated compounds (55.2% and 41.0%, respectively, in the fresh samples, while 53.7% and 43.3%, respectively, in the dried samples). Camphor (*24*) was the main compound in both EOs, with an increase in the relative amounts of about 15.0% with drying (24.0% *vs* 27.5%). The drying process caused also an increase of β-pinene (*8*, 3.8% *vs* 6.1%) and camphene (*5*, 8.8% *vs* 9.8%) and a decline of other compounds, *e.g.* *γ*-terpinene (*17*, 3.7% *vs* 2.0%), terpinen-4-ol (*29*, 2.6% *vs* 1.9%), limonene (*13*, 3.0% *vs* 2.6%) and *α*-pinene (*4*, 13.1% *vs* 12.2%). It is noteworthy the invariable amount of myrcene (*9*) and 1,8-cineole (*14*) in both fresh and dried samples.

In the PCA analysis, performed on the EO compositions of all the analysed samples, the first two axes explained for more than 93.0% of the total variability, indicating significant statistical differences between the EO compositions of the two hybrids (Fig. 1). The score plot showed a distribution of the samples based on their different compositions revealing that the fresh samples of *S*×*m* were characterized by camphor and α-pinene, whereas 1,8-cineole and verbenone characterized the dried ones. All the dried samples of *S*×*l*, as well as one of the fresh samples (*S*×*l*_Fr3), occurred in the upper right side of the score plot; all of them displayed myrcene as the main characteristic compound. The remaining samples (*S*×*l*_Fr2 and *S*×*l*_Fr1) were located on the right lower quadrant, showing β-pinene and γ-terpinene as distinctive components. HCA analysis (Fig. 2) confirmed the PCA results and evidenced two distinct groups, A and B. Group A was formed by *S*×*l*, whereas group B was represented by *S*×*m*. Each group was divided into two homogeneous subgroups, each constituted by samples with the same state of conservation (fresh *vs* dried).

The one-way PERMANOVA test evidenced no significant differences between the aroma profiles of the two hybrids, as well as between the EO compositions of the fresh and the dried samples within each hybrid. Conversely, high significant differences resulted between the EOs of the examined hybrids (*p*-value: 0.0019<<<0.05 criterion of significance). The SIMPER analysis pointed out eighteen compounds responsible for more than 90% of the dissimilarity between the EOs (Table 3), with myrcene and α-pinene being the major contributors (22.6% and 11.5%, respectively). All the compounds differed significantly among the samples, except for camphor and sabinene.

Table 2. *Salvia x lavandulacea*: chemical composition of the leaf spontaneous volatile emission (VOC) and of the essential oil (EO) obtained from fresh and dried leaves as identified by GC-MS analysis (Average of triplicate analyses ± standard deviation).

	Compounds[$]	Class	LRI[a]	VOC	EO	
					fresh	dry
1	Santolina triene	mh	909			0.1±0.02
2	tricyclene	mh	927	0.4±0.06	0.3±0.04	0.4±0.03
3	α-thujene	mh	930	8.9±0.31	1.9±0.22	1.4±0.06
4	α-pinene	mh	939	24.5±2.36	13.1±1.86	12.2±0.78
5	camphene	mh	954	9.4±0.06	8.8±0.54	9.8±0.56
6	Thuja-2,4(10)-diene	mh	960			0.2±0.02
7	sabinene	mh	975	0.2±0.04	0.4±0.04	0.4±0.08
8	β-pinene	mh	979	8.4±1.12	3.8±0.23	6.1±0.25
9	myrcene	mh	991	13.5±1.2	14.9±0.93	14.8±0.04
10	α-phellandrene	mh	1003	0.2±0.03	0.3±0.09	0.2±0.03
11	α-terpinene	mh	1017	1.2±0.01	1.8±0.18	1.0±0.03

12	p-cymene	mh	1025	0.4±0.18	1.7±0.50	1.9±0.06
13	limonene	mh	1029	5.2±0.12	3.0±0.17	2.6±0.20
14	1,8-cineole	om	1031	5.6±0.28	7.5±0.28	7.2±0.20
15	(*Z*)-β-ocimene	mh	1037	0.6±0.30	0.4±0.13	0.1±0.01
16	(*E*)-β-ocimene	mh	1050	0.1±0.08		
17	γ-terpinene	mh	1060	3.2±0.17	3.7±0.37	2.0±0.03
18	*cis*-sabinene hydrate	om	1070	1.2±0.12	0.7±0.05	0.4±0.04
19	terpinolele	mh	1089	1.4±0.08	1.1±0.23	0.5±0.03
20	linalool	om	1097	0.7±0.12	0.8±0.71	0.6±0.17
21	*trans*-sabinene hydrate	om	1098	0.3±0.14	0.3±0.18	0.3±0.20
22	chrysanthenone	om	1128	0.3±0.01	0.3±0.11	0.3±0.08
23	Trans-pinocarveol	om	1139			0.1±0.01
24	camphor	om	1146	11.4±1.10	24.0±2.06	27.5±0.41
25	*trans*-pinocamphone	om	1163			0.1±0.01
26	pinocarvone	om	1165		0.1±0.08	0.1±0.01
27	borneol	om	1169	0.3±0.05	2.1±0.41	2.2±0.12
28	*cis*-pinocamphone	om	1175		0.7±0.19	0.6±0.03
29	terpinen-4-ol	om	1177	0.5±0.06	2.6±0.68	1.9±0.26
30	α-terpineol	om	1189	0.2±0.31	0.7±0.30	0.6±0.11
31	myrtenol	om	1196			0.4±0.01
32	verbenone	om	1205	0.1±0.08	0.6±0.06	0.5±0.05
33	isobornyl acetate	om	1286	0.6±0.04	0.6±0.01	0.5±0.03
34	α-copaene	sh	1377		0.1±0.06	
35	β-caryophyllene	sh	1419	0.7±0.01	1.3±0.05	1.2±0.08
36	α-humulene	sh	1455	0.1±0.01	0.5±0.01	0.2±0.03
37	γ-muurolene	sh	1480		0.1±0.08	0.1±0.01
38	*trans*-γ-cadinene	sh	1514			0.1±0.04
39	β-curcumene	sh	1516		0.1±0.10	
40	δ-cadinene	sh	1523	0.1±0.01	0.1±0.14	0.2±0.04
41	caryophyllene oxide	os	1583		0.6±0.22	0.9±0.15
42	humulene epoxide II	os	1608		0.2±0.04	0.1±0.04
43	caryophylla-4(14),8(15)-dien-5-ol	os	1641		0.1±0.09	
44	*epi*-α-bisabolool	os	1685		0.2±0.38	
45	Unknown				0.8±0.75	
	Yield				**0.6±0.01**	**0.9±0.02**
	Number of identified compounds			**29**	**38**	**39**
	Monoterpene Hydrocarbons (mh)			77.9±0.76	55.2±2.01	53.7±1.66
	Oxygenated Monoterpenes (om)			21.2±0.75	41.0±0.44	43.3±1.09
	Sesquiterpene Hydrocarbons (sh)			0.9±0.01	2.2±0.25	1.9±0.25
	Oxygenated Sesquiterpene (om)				1.1±0.72	1.0±0.25
	Total Identified (%)			**100±0.01**	**99.5±0.02**	**99.9±0.08**

[$] :Only compounds with relative percentages > 0.1% were included in the table.
[a]: LRI: linear retention indices relative to *n*-alkane on the DB5 column

Table 3. List of the compounds responsible for the dissimilarity in the EO compositions of *S. x lavandulacea* and *S. x mendizabalii* according to SIMPER analysis.

	Compounds	Contrib. %	Cumulative %	***S* × *l***	***S* × *m***	Stat. Sign.
1	myrcene	22.6	22.6	14.8	1.2	*
2	α-pinene	11.5	34.1	12.7	19.6	*
3	1,8-cineol	8.3	42.4	7.3	12.3	*
4	verbenone	7.4	49.8	0.6	5.0	*
5	β-pinene	6.0	55.8	4.9	1.3	*
6	borneol	5.1	60.9	2.2	5.3	*
7	camphor	4.7	65.6	25.7	27.8	ns
8	δ-3-carene	4.5	70.1	0.0	2.7	*
9	γ-terpinene	3.6	73.7	2.8	0.7	*
10	camphene	3.0	76.7	9.3	7.6	ns
11	limonene	2.7	79.4	2.8	1.6	*
12	α-thujene	2.5	81.9	1.7	0.1	*
13	sabinene	2.1	84.0	0.4	0.0	ns
14	4-terpineol	1.6	85.6	2.2	1.3	*
15	α-terpinene	1.4	87.0	1.4	0.6	*
16	isobornyl acetate	1.3	88.3	0.3	1.1	*
17	α-terpineol	1.1	89.4	0.6	1.3	*
18	*cis*-pinocamphone	1.0	90.4	0.6	0.0	*

Stat. Sign.: Statistical significance: *: p<0.05 criterion of significance; ns: not significant

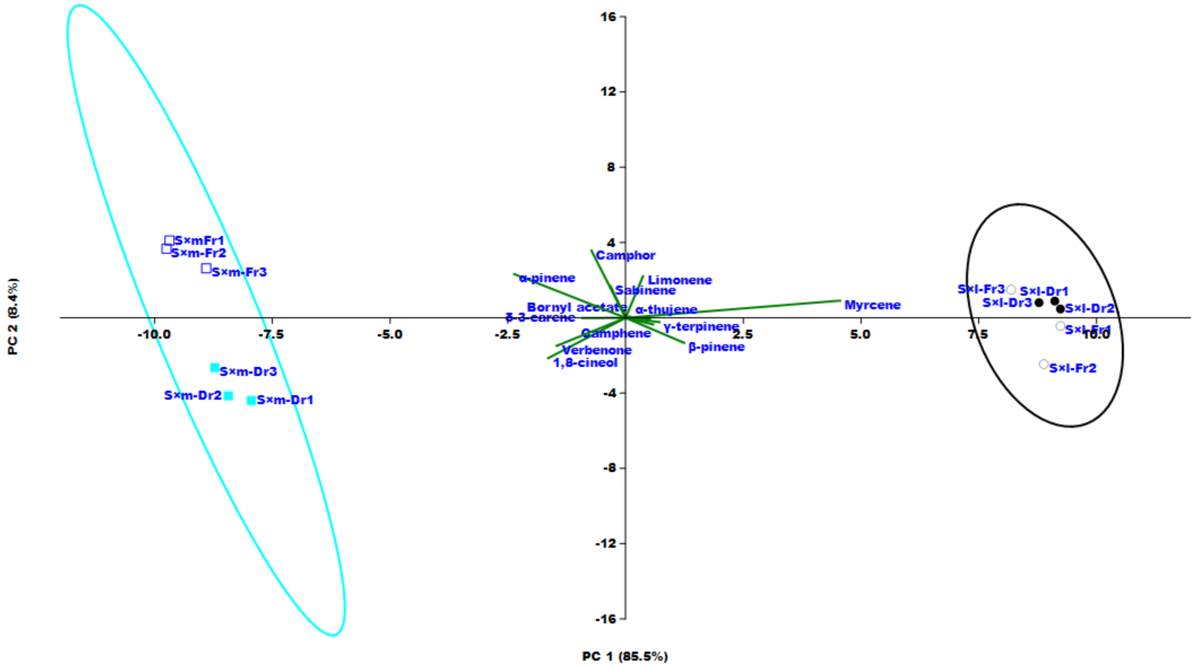

Figure 1. Score plot of the principal compound analysis (PCA) of the EOs obtained from the fresh and dried samples of *S. x lavandulacea* and *S. x mendizabalii*. Dr1 Dr2 Dr3, Fr1 Fr2 Fr3 represent the three replicates of the dried (Dr) and fresh (Fr) samples of the two hybrids.

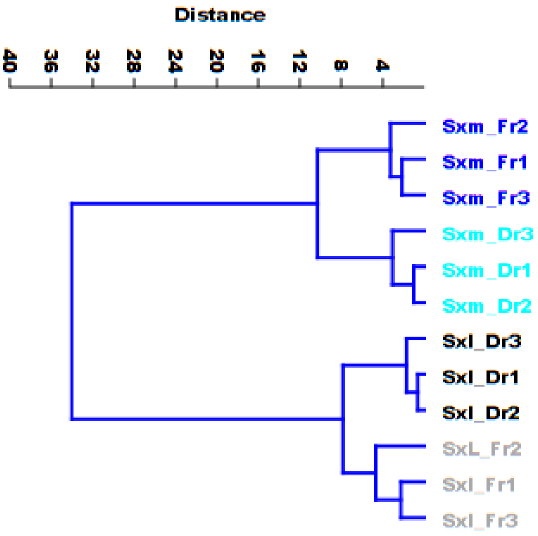

Figure 2. HCA Dendrogram of the essential oils (EOs) from both *S. x lavandulacea* and *S. x mendizabalii*. Dr1 Dr2 Dr3, Fr1 Fr2 Fr3 represent the three replicates of the dried (Dr) and fresh (Fr) samples of the two hybrids.

3.2 Micro-morphological analysis

The leaf *indumentum* of the investigated hybrids consisted of both non-glandular and glandular trichomes (Fig. 3, A-I). Abundant non-glandular dendritic trichomes with smooth cuticle were observed in both hybrids, especially on the abaxial lamina (Fig. 3 A-B, D); they were sporadic on the adaxial side and in this case they were located at the edges (Fig. 3 A, E). These projections generally presented pointed apices at all the branches and the main axes were perpendicular to the epidermis or point apically toward the top of the organ forming acute angles to the surface (Fig. 3, C-D, F).

The glandular trichomes included the peltate type and two types of capitates.

The peltate hair, present on both hybrids (Fig. 3 A-B, D-E, G-H) was constituted by a basal epidermal cell, a neck-cell and by a multicellular glandular head made up of 8 (*S*×*m*) until 16 secreting cells (*S*×*l*, Fig. 3 H). The head was surrounded by a wide subcuticular chamber in which the secretory products were accumulated. The responses to all the lipophilic stainings were positive as well as to $AlCl_3$, indicating the presence of terpenes and of major flavonoidic derivatives (Fig. 3 J-K, Table 4).

The short capitate was widespread in both hybrids (Fig. 3 A-B, D-E, G-H). It was constituted by an elongated basal cell, a neck-stalk cell and by a glandular head of 1-2 cells surrounded by a thin subcuticular space. The secreted material proved positive only to the lipophilic dyes, indicating the exclusive production of terpenes (Fig. 3 L, Table 4).

The medium-long capitate, occurring only on the leaf adaxial side of *S*×*m* (Fig. 3 D, I),was made up of a basal cell, a stalk cell, a neck cell and by a globose head of 1-2 secretory cells surrounded by a wide storing chamber. The secretion proved positive to all the lipophilic stainings, particularly to the Nadi reagent, indicating that they were typical terpene producers (Fig. 3 M, Table 4).

Table 4. Results of the histochemical tests on the leaf glandular trichomes in *S. x lavandulacea* and *S. x mendizabalii*.

Stainings	Target-compounds	***S. x lavandulacea***		***S. x mendizabalii***		
		peltate	**short capitate**	**peltate**	**short capitate**	**medium capitate**
Fluoral yellow-088	Total lipids	++	++	++	++	++
Nile Red	Neutral lipids	+	+	+	+	+
Nadi reagent	Terpenoids	++	++	++	++	++
Ruthenium Red	Acid polysaccharides	–	–	–	–	–
Alcian Blue	Muco-polysaccharides	–	–	–	–	–
Ferric Tricholoride	Polyphenols	+	–	+	–	–
Aluminium Trichloride	Flavonoids	+	–	+	–	–

(–) negative; (+) positive; (++) strongly positive

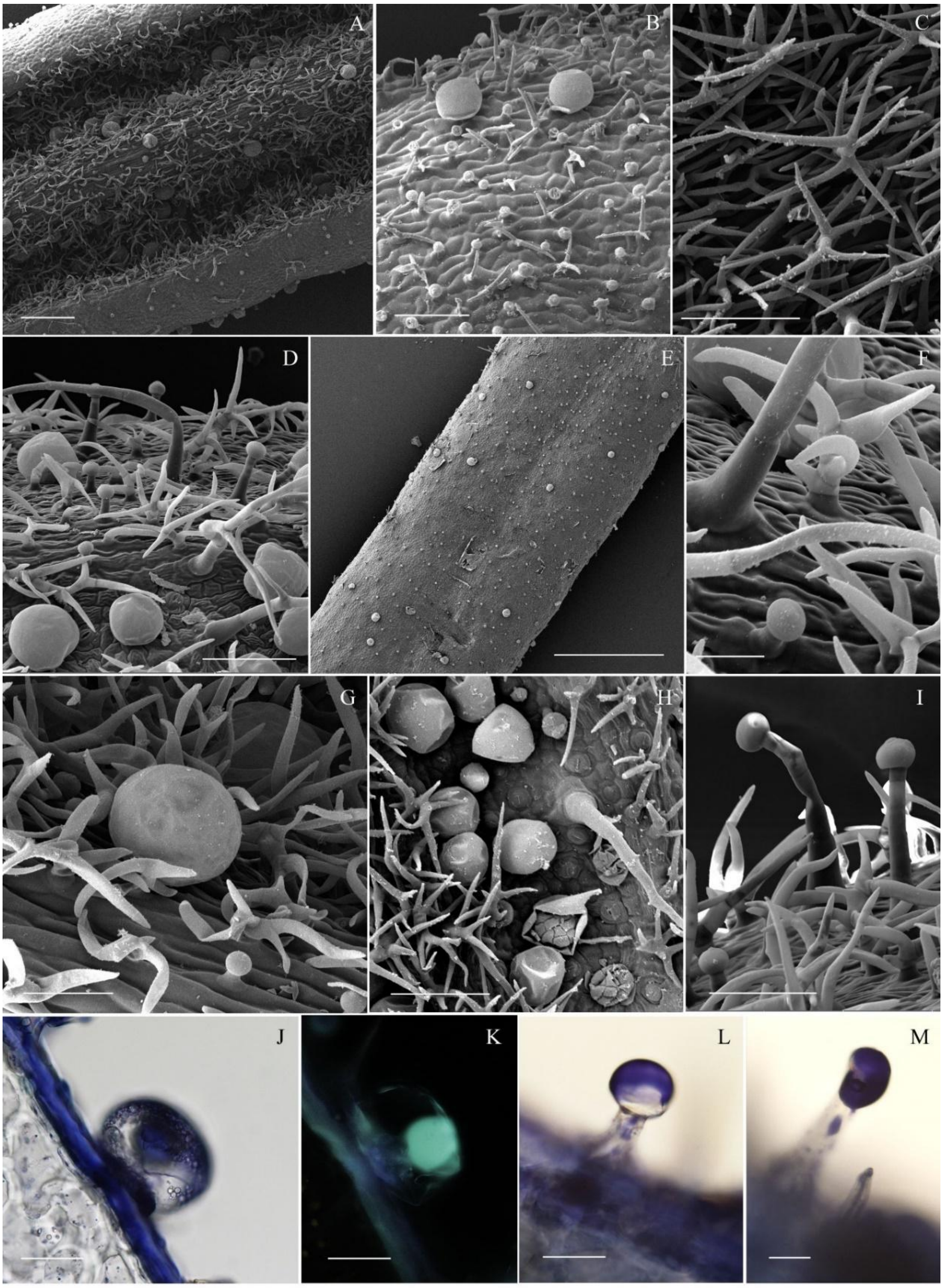

Figure 3. **A-C.** Leaf of *S. x lavandulacea*: abaxial surface (A), adaxial surface at the edges (B), non-glandular dendritic trichomes (C). **D-F.** Leaf of *S. x mendizabalii*: abaxial surface (D), adaxial surface (E), non-glandular dendritic trichomes (F). **G-I.** Glandular trichomes: peltate and short capitate in *S. x lavandulacea* (G), peltates and short capitates in *S. x mendizabalii* (H), short capitates and medium capitates in *S. x mendizabalii* (I). **J-M**. Histochemistry of the glandular trichomes: peltate, Nadi reagent (J) and Aluminium Trichlorides (K); short capitate, Nadi reagent (L); medium capitate, Nadi reagent (M). A-I SEM. J-M. LM.

Scale bars: A, 200 μm; B-D, G-I, 100 μm; E, 1mm; F, 20 μm; J-K, 50 μm; L-M, 25 μm.

4. Discussion

4.1 Phytochemical analysis

The total number of the isolated compounds, the dominant terpenoidic classes, and the major components of the aroma profiles, although present in relatively variable percentages, resulted consistent between the two hybrids. The common compounds included the dominant constituents, while the exclusive compounds were 5 in *S*×*m* and 4 in *S*×*l*, with a prevailing presence of δ-3-carene and β-pinene in the two hybrids, respectively. The other exclusive compounds occurred in relative percentages lower than 1.0%.

The literature on the phytochemistry of rosemary is rich, but only few contributions focused on the aroma profile (D'auria and Racioppi, 2015; Bendif *et al.*, 2017; Carruba *et al.*, 2020). The first authors reported that the fresh leaves of *S. rosmarinus* (common parent of both hybrids) from Italy were characterized by α-pinene (23.9%), limonene (8.3%) and β-pinene (8.0%) as the main constituents. The two hybrids showed a similar α-pinene amount, while limonene was present in lower relative percentages in our samples. Noteworthy is the lack of β-pinene in *S*×*m*.

Bendif *et al.* (2017) studied the leaf aroma profile of *S. jordanii* (one of the parental species of *S*×*l*) from Algeria. They pointed out, in agreement with our results on *S*×*l*, that the main classes were monoterpene hydrocarbons (67.0%) and oxygenated monoterpenes (31.9%), with camphene (37.4%) as the main compound instead of α-pinene evidenced herein.

The experimental investigation was also associated to a literature research on the ecological role of the main compounds. Among the major common ones, α-pinene, camphene, 1,8-cineole and limonene are documented as attractants towards member of the genus *Bombus* and different bees (Williams et al., 1983; Borg-Karlson et al., 1996; Granero et al. 2005). Camphene plays an attractive role towards pollinators in several species of Sileneae (Prieto-Benítez et al. 2015).

Volatiles biosynthesis is also a defensive response: the production of 1,8-cineole, as an example, seems to be induced by herbivory (Bedoya-Pérez et al., 2014), whereas the synthesis of camphor displays a repellent function and toxicity against red fire ants (Zhang et al., 2014).

Among the main exclusive compounds, β-pinene which characterizes the aroma profile of *S*×*l* was demonstrated to be involved in the flower attraction by bumblebees and honeybees (Aronne *et al.*, 2014; Hetherington-Rauth and Ramirez, 2016). Information on the ecological properties of δ-3-carene which characterizes *S*×*m* are lacking.

Concerning the EOs, the statistical data processing indicated significant differences among the hybrids, also allowing to evidence the main compounds that characterized the various samples: camphor and α-pinene for the fresh samples of *S*×*m*; 1,8-cineole and verbenone for the dried ones; myrcene for the dried samples of *S*×*l*, as well as one of the fresh samples; β-pinene and γ-terpinene for the remaining fresh samples of *S*×*l*.

The essential oil composition of the species belonging to the Rosemary complex was the subject of considerable research in recent years. Cano and Sánchez (1993) investigated the oil compositions of Spanish samples of both hybrids, together with two of the parental species. These authors evidenced that *S*×*l* EO were dominated by 1,8-cineole (18.8%), camphor (17.0%), α-pinene (15.1%) and camphene (9.6%). *S. jordanii* EO was slightly different, with 1,8-cineole detected in fourth position after the other three compounds in the same order. All these constituents were also identified in our study, even though with different relative percentages; noteworthy the difference in the amount of myrcene, which was present in a very low percentage in comparison to the Spanish samples (0,85% *vs* 14.9%). The same authors investigated also *S*×*m* and one of its parents, *S. granatensis*, showing consistent compositions with camphor, α-pinene, camphene and 1,8-cineole as dominant constituents. These data were in agreement with our results.

Recent studies focused on the EOs of *S. jordanii* from Algeria (Benbelaïd *et al.*, 2016; Bendif *et al.*, 2017). The former authors studied the EO obtained from the whole aerial parts, revealing the highest amount of camphor (37.8%) followed by 1,8-cineole (17.4%), camphene (13.3) and α-pinene (10.9%). Bendif *et al.* (2017) extracted EOs from different plant parts, detecting camphor (41.2% in stems; 36.9% in leaves and 29.7% in flowers) followed by camphene (10.0%, 15.6%, and 13.1%, respectively) and α-pinene (7.8%, 17.8%, and 15.1%, respectively), as dominant constituents.

S. rosmarinus is a common parental species for the studied hybrids. Raeisi *et al.* (2016) reported α-pinene (22.8%) and 1,8-cineole (24.3%) as the major compounds followed by camphor (12.1%). A more recent study by Feriotto *et al.* (2017) highlighted that half of the identified fraction was dominated by 1,8-cineole (50.6%), camphene (13.3%) and α-pinene (10.1%).

In general, the literature survey indicated a high variability in the EOs composition within the Rosemary complex; however, some considerations emerge: *(i)* the main terpenoidic class was invariably represented by monoterpenes, both hydrocarbons and oxygenated derivatives; *(ii)* the principal volatile compounds were, although represented in comparatively different amounts, camphor, 1,8-cineole, α-pinene and camphene.

Concerning the biological activity of the main compounds, camphor is known for its broad range of insecticidal and antibacterial activities and is used as acaricidal agent against house dust mites (Jeon et al., 2014). 1,8-cineole and α-pinene showed antimicrobial, insecticidal and repellent, cytotoxic and anti-inflammatory activities (Barbosa Damasceno et al., 2019). Camphene, a bycyclic monoterpene, evidenced significant antidiabetic, antidyslipemic and antioxidant properties (Mishra et al., 2018) together with a larvicidal and ovicidal activities against *Helicoverpa armigera* (Benelli et al., 2018).

In both hybrids, the drying process implied the increase or the decline of several volatiles, including the major ones. Therefore, the sensory profile of the oils was affected, as evidenced in a previous paper on the variation of the sensory quality of the EOs of *R. officinalis* following different drying techniques (Szumny et al., 2010).

In *S*×*m*, camphor and α-pinene showed a decrease from fresh to dried samples, while increases were observed for 1,8-cineole and camphene. Therefore, the drying of fresh samples implied decreases of some sensory attributes: *camphor odour* (camphor), *woody* (α-pinene), but increases of some others: *fruity*, *sweet* (1,8-cineole) and *vanilla* (camphene).

In *S*×*l*, the relative amounts of camphor (descriptor: *camphor odour*), β-pinene (*woody*) and camphene (*vanilla*) increased with drying, whereas a decrease was detected for limonene (*citrus*) and α-pinene (*woody*). Noteworthy is the invariable amount of 1,8-cineole (*fruity*, *sweet*).

4.2 Micro-morphological analysis

The observed glandular trichomes showed overall morphological features comparable to those already known in the literature for the Rosemary complex (Giuliani and Maleci Bini, 2008; Hallahan, 2000; Werker, 2000).

The micro-morphological observations on leaves proved the occurrence of abundant non-glandular dendritic hairs in both hybrids, in accordance with literature data on rosemary (Maleci Bini and Giuliani, 2006; Boix et al., 2011). A previous taxonomic paper (Rosuà, 1981) defined the leaf adaxial surface as cano-tomentose in *S*×*m* and almost hairless in *S*×*l*. On the contrary, our observations proved that the features of the non-glandular *indumentum* are not discriminant for the recognition of the examined hybrids.

Concerning the glandular hairs, the peltates and the short capitates, common to both hybrids, were widespread in the Lamiaceae family. The peltates however differed in the examined hybrids since the number of the head secreting cells was eight in *S*×*m* and sixteen in *S*×*l*. Their productivity in secondary metabolites was instead homogeneous, since major terpenoidic and flavonoidic fractions were detected in agreement with previous results on *R. officinalis* (Marin et al., 2006; Boix et al., 2011). The short capitates synthetized exclusively terpenes, whereas in most of the investigated Lamiaceae species they were typical polysaccharides producers (Giuliani and Maleci Bini, 2008). The medium-long capitates occurred exclusively on the leaf adaxial side of *S*×*m*, thus constituting a diagnostic micro-character.

5. Conclusions

The present contribution on *S. x mendizabalii* and *S. x lavandulacea* combined for the first time a phytochemical and morphological investigation on the leaves, with the aim to sketch a link between the productivity in volatile compounds and the glandular *indumentum.*

From the phytochemical perspective, the aroma profiles of the examined hybrids resulted consistent with regards to the total number of the isolated compounds, the dominant terpenoidic classes and the high number of common components, including the major ones. Variability was related to the occurrence of exclusive compounds, the main one being δ-3-carene and β-pinene in *S. x mendizabalii* and *S. x lavandulacea*, respectively.

The EO compositions were characterized by significant statistical differences, however a general phytochemical affinity emerged due to the occurrence of major common compounds, *i.e.* camphor, 1,8-cineole, α-pinene and camphene, also in accordance with the literature data on the EOs of the species belonging to *S.* subgenus *rosmarinus*.

The drying process, implying the increase or the decline of several volatiles in the oils of both hybrids, affected their sensory attributes, and potentially also their biological activity; in particular, in *S. x mendizabalii* the camphor odour became more intense, whereas in *S. x lavandulacea* declined, while the vanilla flavour increased in both hybrids.

From the micromorphological perspective, three morphotypes of glandular trichomes were described and all resulted responsible for the productivity of the volatile components characterized herein.

Disclosure statement

No potential conflict of interest was reported by the authors.

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***Declaration of Interest Statement**

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

For all the authors,

Prof. Gelsomina Fico

*Credit Author Statement

Author contribution statement

B.N., L.P. and C.G. conceived and designed the experiments and analysis. B.N. performed the phytochemical survey. M.C.S. and C.G. carried out the micromorphological survey. B.N. and CG wrote the manuscript. All authors discussed the results and commented the manuscript.