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Volatile Composition and Enantiomeric Distribution of Spanish Sage (*Salvia officinalis* Subsp. *lavandulifolia* [Vahl] Gams) From Spain

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Abstract

Background: Spanish sage (*Salvia officinalis* subsp. *lavandulifolia* [Vahl] Gams) is historically an important medicinal plant with a myriad of uses. **Methods:** In this work, 11 *S officinalis* subsp. *lavandulifolia* essential oils (EOs) from Spain were prepared by steam distillation and characterized by gas chromatography-mass spectrometry (GC-MS) and gas chromatography–flame ionization detection (GC-FID). **Results:** The main components of *S officinalis* subsp. *lavandulifolia* EOs were 1,8-cineole (24.34%-33.96%), camphor (23.48%-28.83%) followed by camphene (4.93%-6.43%) and α-pinene (4.29%-6.61%). Enantiomeric analysis of the EOs by chiral gas chromatography–mass spectrometry (CGC-MS) revealed the presence of 17 chiral compounds. Moreover, a hierarchical cluster analysis using the data reported in the literature and those obtained from this study uncovered 3 different chemotypes based on the relative concentrations of the major components. **Conclusion:** The chiral compounds found in the EO can be used in Spanish sage authentication.

Keywords

Spanish sage, *Salvia lavandulifolia*, essential oil, cluster analysis, enantiomeric ratio

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Introduction

The genus *Salvia* L. is one of the famous aromatic genera of the Lamiaceae, represented by more than 900 species distributed worldwide in the temperate, subtropical, and tropical zones.[1,2] *Salvia officinalis* subsp. *lavandulifolia* (Vahl) Gams (synonym: *S. lavandulifolia* Vahl) is a small woody perennial shrub that grows in the Americas, Europe, and Asia.[1] It is also found in the mountains of eastern Spain, southern France, and the northern part of Africa and is cultivated widely in the Mediterranean area.[3] The plant can reach up to 100 cm in height and is characterized by its dense branches, wiry stems, opposite green or gray-white leaves, and vivid blue-purple flowers.[4]

Similar to other *Salvia* species, *S. lavandulifolia* is recognized for its medicinal and economic value. Traditionally, *S. lavandulifolia* was used as gargles for mouth ulcers, common cold, sore throat, premenstrual syndrome (PMS), and menopause.[1] It was also utilized as a food preservative and a flavoring spice. The leaves were used orally as a choleretic and topically as an antiseptic drug.[5] The aqueous extract was hypoglycemic when taken orally.[3] Due to its medicinal value, Spanish sage was subjected to several phytochemical and pharmacological studies. The essential oil (EO) of *S. lavandulifolia* is responsible for its distinctive flavor and aroma and is usually obtained by distillation.[6] Flavonoids, monoterpenoids, and triterpenes were reported from the aerial parts of the plant.[1] *S. lavandulifolia* EO demonstrated moderate anti-inflammatory activity (IC_{50} = 30 μg/mL)[1]; moderate cytotoxicity against human leukemia (HL60), myelogenous leukemia (K562), breast (MCF7), and ovarian (A2780) cancer cell lines[7]; antioxidant[4]; *in vitro* acetylcholinesterase inhibitory activity[8]; antiseptic, analgesic and sedative properties[9]; weak antimalarial activity against *Plasmodium falciparum*[1]; acaricidal toxicity against synanthropic mites[10]; strong antifeedant effects against *Leptinotarsa decemlineata*, *Spodoptera littoralis*, *Myzus persicae*, and *Rhopalosiphum padi*[11]; phytotoxic activity against *Lactuca sativa* and *Lolium perenne*[11]; and antifungal activity against *Fusarium* sp.[11] and *Candida albicans*.[12]

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In a placebo-controlled, double-blind trial, acute administration of a standardized EO extract of *S lavandulifolia* improved the immediate word recall scores in healthy young adult volunteers.[9] Another placebo-controlled, double-blind demonstrated positive acute mood and cognition modulation of a standardized EO extract of *S. lavandulifolia* in 24 healthy young adults.[6] In a recent randomized cross-over, placebo-controlled, and double-blind study, acute administration of *Salvia* mixture (400 mg of *S. officinalis* aqueous extract and 200 mg *S. lavandulaefolia* EO) encapsulated with acacia gum improved cognitive functions (perceived exertion, working memory, and reaction time) in athletes performing a fatiguing cycling task.[13]

There are great variations in the reported major components of *S. lavandulifolia* EO and their percentages in the literature, which can account for the variations in the biological activities of the EO.[1] These variations also suggest the presence of multiple chemotypes. Therefore, the current study aimed to investigate the composition and enantiomeric distribution of *S.lavandulifolia* EOs from Spain over the course of 5 years. A hierarchical cluster analysis based on the EO compositions from this study and from previous reports was also carried out. This study also reports the enantiomeric ratios of the chiral components of Spanish sage EO.

Results and Discussion

S. lavandulifolia *EO Composition*

Eleven hand-harvested *S. lavandulifolia* samples were steam distilled for 3 to 4 h. The average yield was 2.0% to 2.5% (volume per mass fresh plant material). The aroma could be described as camphoraceous, sharp, slightly sweet, 1,8-cineole-like, and slightly herbaceous. The EOs were analyzed by gas chromatography–mass spectrometry (GC-MS) and gas chromatography–flame ionization detection (GC-FID). A total of 89 compounds were identified representing 99.8% to 100.0% of the total EO compositions. Monoterpenoid hydrocarbons, oxygenated monoterpenoids, sesquiterpene hydrocarbons, and oxygenated sesquiterpenoids accounted for 22.3% to 29.1%, 69.1% to 75.8%, 1.5% to 2.6%, and 0.2% to 0.5%, respectively. *S. lavandulifolia* EOs were mainly made of 1,8-cineole (24.3%-34.0%), camphor (23.5%-28.8%) followed by camphene (4.9%-6.4%), and α-pinene (4.3%-6.6%) (major components, Table 1; complete composition, Supplementary Table). The tested samples were in agreement with the ISO 3526 standard for Spanish sage oil.[14]

The EO compositions in this study show similarities and differences from previous reports on Spanish sage EOs. Cutillas et al reported camphor, 1,8-cineole, camphene, and α-pinene as the main components of the EO of *S. lavandulifolia* cultivated in Murcia (Spain).[15] *S. lavandulifolia* EOs from France were mainly made of 1,8-cineole (25.5%), camphor (39.0%) and linalyl acetate (10.2%).[7] Usano-Alemany et al. compared the composition of the steam-distilled *S. lavandulifolia* EOs in different seasons and revealed an important seasonal variation. The major compounds of the EOs were 1,8-cineole (21.4%--33.8%), limonene (5.6%-10.4%), α-pinene (10.5%-17.5%), β-pinene (6.0%-17.3%), myrcene (trace-10.0%), camphor (6.1%-9.4%), and (*E*)-β-caryophyllene (4.0%-8.5%).[16] The main components of *S. lavandulifolia* EO from Castilla–La Mancha, Spain were α-pinene (23.2%), β-pinene (19.2%), limonene (16.6%), (*E*)-β-caryophyllene (8.1%), caryophyllene oxide (5.6%) and viridiflorol (9.7%).[17] On the other hand, β-phellandrene (9.3%), terpineol (12%), and ledol (11%) were the main components of the EO from Solsona, Lerida, Spain.[18] Interestingly, 1,8-cineole (15.5%-55.1%) was the most abundant compound in *S. lavandulifolia* EO cultivated in Cuenca, Spain.[19] The air-dried aerial parts of wild *S lavandulifolia* collected from Morocco had camphor (16%-31%), 1,8–cineole (13%-19%), β-pinene (8%-13%), camphene (5%-11%), and viridiflorol (0%-12%) as the major components.[5] Similarly, wild *S. lavandulifolia* EOs have shown camphor (15.3%), 1,8-cineole (15.0%), α-pinene (11.3%), β-pinene (8.5%), and limonene (7.5%) as the main constituents.[20] The EO obtained from *S. lavandulifolia* from Lublin, Poland contained 1,8-cineole (21.33%), camphor (19.03%), β-pinene (16.15%), thujone (13.08%), and α-pinene (7.22%).[2] (*E*)-β-Caryophyllene (11.87%), spathulenol (8.13%), neomenthol (7.75%), pulegone (6.97%), hexadecanoic acid (6.85%), and germacrene-D (5.70%) were the major constituents of Spanish sage EO from Peru.[1] The EO with acetylcholinesterase inhibitory activity was made of camphor (27%), 1,8-cineole (13%), α- and β-pinene (10%--15%) and bornyl acetate (10%).[8] *S. lavandulifolia* EO from Brazil contained β-thujone (20.0%), camphor (19.0%), α-thujone (19.0%), 1,8-cineole (8.1%), and β-pinene (4.0%).[21] Commercial Spanish sage EO from Germany had camphor (30.5%), 1,8-cineole (24.8%), α-pinene (6.5%), linalool (4.0%), and linalyl acetate (3.5%) as the main components.[22]

Enantiomeric Distribution

The enantiomeric distributions of chiral components of *S. lavandulifolia* EO are shown in Table 2. The results show a total of 17 chiral compounds, namely α-thujene, α-pinene, camphene, sabinene, β-pinene, limonene, *cis*-sabinene hydrate, linalool, camphor, terpinen-4-ol, linalyl acetate, borneol, bornyl acetate, α-terpineol, α-terpinyl acetate, (*E*)-β-caryophyllene, and δ-cadinene. (–)-Linalyl acetate, (–)-bornyl acetate, (+)-α-terpinyl acetate, (–)-(*E*)-β-caryophyllene, and (–)-δ-cadinene were detected as pure enantiomers. Cutillas et al. reported no pure enantiomers and a prevalence of (+)-limonene, (+)-sabinene hydrate, ()-camphor, (+)-terpinen-4-ol, (+)-α-terpineol and (+)-α-terpinyl acetate, (–)-camphene, (–)-β-pinene, (–)-linalool, (–)-bornyl acetate, (–)-borneol, and (–)-β-caryophyllene.[15] These identified chiral compounds and their enantiomeric distributions may serve as a benchmark for *S. lavandulifolia* EO authentication or adulteration detection.

Hierarchical Cluster Analysis

Based on *S. lavandulifolia* EO compositions, a hierarchical cluster analysis of the EOs from literature[4,5,7,16,17,22–27] and from this work was carried out. The dissimilarity index is

Table 1. Major Components in the Essential Oils (EOs) of *Salvia lavandulifolia* (Spanish Sage) Cultivated in Murcia, Spain.

		Percent composition											
RI	Major components[a]	August 2017 S1	November 2017 S2	August 2018 S3	November 2018 S4	August 2019 S5	November 2019 S6	August 2020 S7	November 2020 S8	August 2021 S9	November 2021 S10	November 2021 S11	ISO 3526
931	α-Pinene	5.11	4.81	4.29	5.45	4.40	4.70	6.61	4.39	5.43	5.36	4.61	4.00–11.00
948	Camphene	5.74	6.35	5.72	6.43	5.82	6.06	6.05	6.06	5.00	4.93	6.02	
972	Sabinene	1.22	1.55	1.48	1.44	1.39	1.49	2.11	1.15	0.87	0.89	1.46	0.10–3.50
978	β-Pinene	4.09	4.25	4.09	4.23	3.95	4.15	4.51	3.68	4.42	4.37	4.04	
989	Myrcene	2.59	2.52	2.48	3.50	2.51	2.48	2.35	2.05	2.22	2.34	2.50	
1028	Limonene	4.62	4.65	4.40	4.78	4.45	4.17	4.73	3.72	4.70	4.71	4.25	2.00–6.00
1031	1,8-Cineole	25.94	27.10	26.83	24.34	27.66	26.99	27.34	33.96	28.83	28.60	27.62	10.00–30.00
1099	Linalool	1.93	1.91	2.31	1.75	1.84	1.89	1.63	1.44	1.96	2.17	1.92	0.30–4.00
1138	*trans*-Sabinol	0.81	0.88	0.78	0.86	0.95	0.96	0.91	0.53	0.77	0.76	0.91	
1145	Camphor	26.99	26.06	25.55	23.48	26.54	27.17	27.42	28.83	27.76	27.62	27.01	11.00–36.00
1171	Borneol	2.23	2.47	2.16	2.85	2.55	2.40	2.22	1.33	2.04	2.01	2.46	1.00–7.00
1252	Linalyl acetate	3.86	4.89	6.60	3.67	5.00	4.82	2.66	3.64	3.26	3.35	4.83	0.10–5.00
1282	Bornyl acetate	1.56	1.20	1.24	1.79	1.26	1.21	0.95	0.88	1.37	1.35	1.21	
1285	*trans*-Sabinyl acetate	2.35	2.76	2.94	2.25	2.88	2.90	2.50	2.19	2.08	2.04	2.85	0.50–9.00
1346	α-Terpinyl acetate	3.33	0.92	0.97	4.48	0.96	0.94	0.79	0.68	2.57	2.85	0.94	0.50–9.00
1418	(*E*)-β-Caryophyllene	1.14	1.10	1.16	1.01	1.16	1.13	0.90	0.83	1.11	1.15	1.12	

[a]The major components are the top 16 based on percent composition (average > 0.8%).
Abbreviation: RI, retention index.

very small indicating no significant differences in the EO compositions of the tested samples in this work (Figure 1). The cluster analysis including compositions reported in the literature revealed 3 different clusters (Figure 2): Cluster 1 (camphor/1,8-cineole), Cluster 2 (1,8-cineole/α-pinene/β-pinene), and Cluster 3 (1,8-cineole). All samples reported in this work are in the camphor/1,8-cineole chemotype.

Conclusions

Steam-distilled *S. lavandulifolia* EOs cultivated in southern Spain showed very similar chemical compositions, with 1,8-cineole (24.3%-34.0%), camphor (23.5%-28.8%) followed by camphene (4.9%-6.4%), and α-pinene (4.3%-–6.6%) as the major components. The enantiomeric distributions of chiral components of *S lavandulifolia* EO show a total of 17 chiral compounds that can be used in the Spanish sage EO authentication. There was no significant difference in the EO compositions of the tested samples in this work. The cluster analysis revealed 3 different chemotypes for *S lavandulifolia:* camphor/1,8-cineole chemotype, 1,8-cineole/α-pinene/β-pinene chemotype, and 1,8-cineole chemotype. All samples reported in this work belong to the camphor/1,8-cineole chemotype.

Table 2. Enantiomeric Distributions of Chiral Compounds Present in the Essential Oils (EOs) of *Salvia lavandulifolia* (Spanish Sage) Cultivated in Murcia, Spain.

	Average		
Chiral compounds	(+)	(−)	SD
α-Thujene	69.10	30.90	4.56
α-Pinene	56.38	43.62	1.86
Camphene	44.05	55.95	3.46
Sabinene	60.30	39.70	10.62
β-Pinene	33.99	66.01	3.92
Limonene	78.00	22.00	0.17
cis-Sabinene hydrate	86.04	13.96	1.40
Linalool	15.31	84.69	0.15
Camphor	87.15	12.85	0.65
Terpinen-4-ol	61.31	38.69	2.03
Linalyl acetate	0.00	100.00	0.00
Borneol	34.99	65.01	3.75
Bornyl acetate	0.00	100.00	0.00
α-Terpineol	58.05	41.95	3.34
α-Terpinyl acetate	100.00	0.00	0.00
(*E*)-β-Caryophyllene	0.00	100.00	0.00
δ-Cadinene	0.00	100.00	0.00

Experimental

EO Extraction

Cultivated Spanish sage samples were collected from the hills and mountains around Totana, Murcia, in southern Spain about 600 m above sea level from August to November of 2016 to 2021. The plant samples were handpicked and identified by Mr. Juan Lorente (Figure 3). The fresh aerial parts of Spanish sage samples were steam distilled for 3 to 4 h in industrial scale stills that take up to 1000 kg of plant material. A separate steam generator connected by ducts was used to generate the steam. The steam reached a pressure of 0.4 to 0.7 Mpa and a temperature of 150 to 170 °C. EO samples were numbered from S1 to S11. S1 and S2 are samples collected between August and November 2017, respectively. S3 and S4 are samples collected between August and November 2018, respectively. S5 and S6 are samples collected between August and November 2019, respectively. S7 and S8 are samples collected between August and November 2020, respectively. S9 to S11 are samples collected between August and November 2021, respectively.

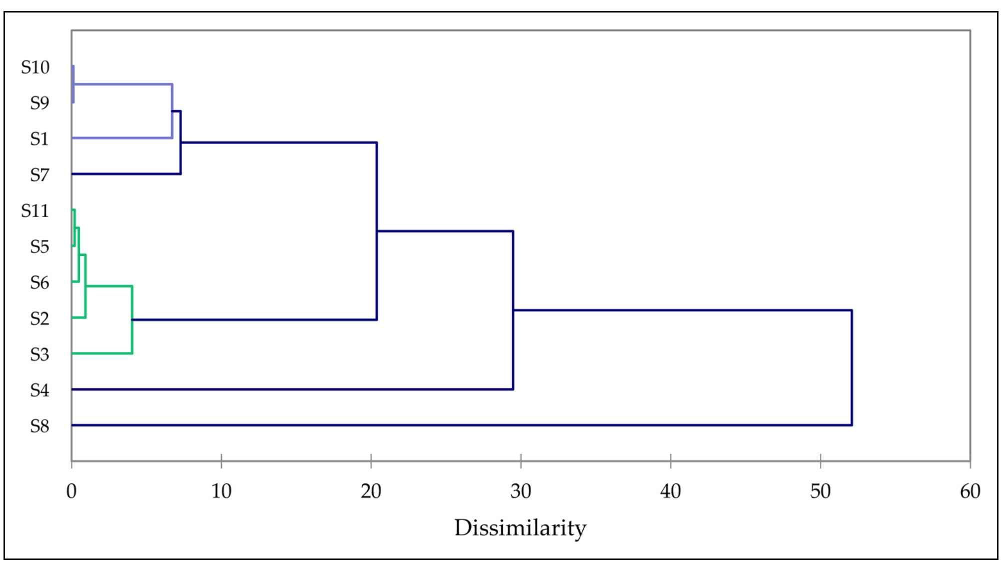

Figure 1. Agglomerative hierarchical cluster (AHC) analysis of *Salvia lavandulifolia* (Spanish sage) essential oils (EOs) cultivated in Murcia, Spain.

GC-MS Analysis

S. lavandulifolia EOs were analyzed by GC-MS as previously described by DeCarlo et al.[30] Shimadzu GC-MS-QP2010 Ultra, electron impact (EI) mode (70 eV, 40-400 *m/z* scan range, 3.0 scan/sec scan rate). ZB-5 ms capillary column (30 m × 0.25 mm, 0.25 μm film thickness); temperature program (50 °C for 2 min, increased 2 °C/min to 260 °C); He carrier gas, column head pressure 552 kPa, flow rate 1.37 mL/min; injector temperature 260 °C, ion source temperature 200 °C. For each sample, 0.3 μL of a 1:10 *v/v* dilution in dichloromethane (DCM) was injected, split ratio of 1:30. The chemical components were identified by comparing mass

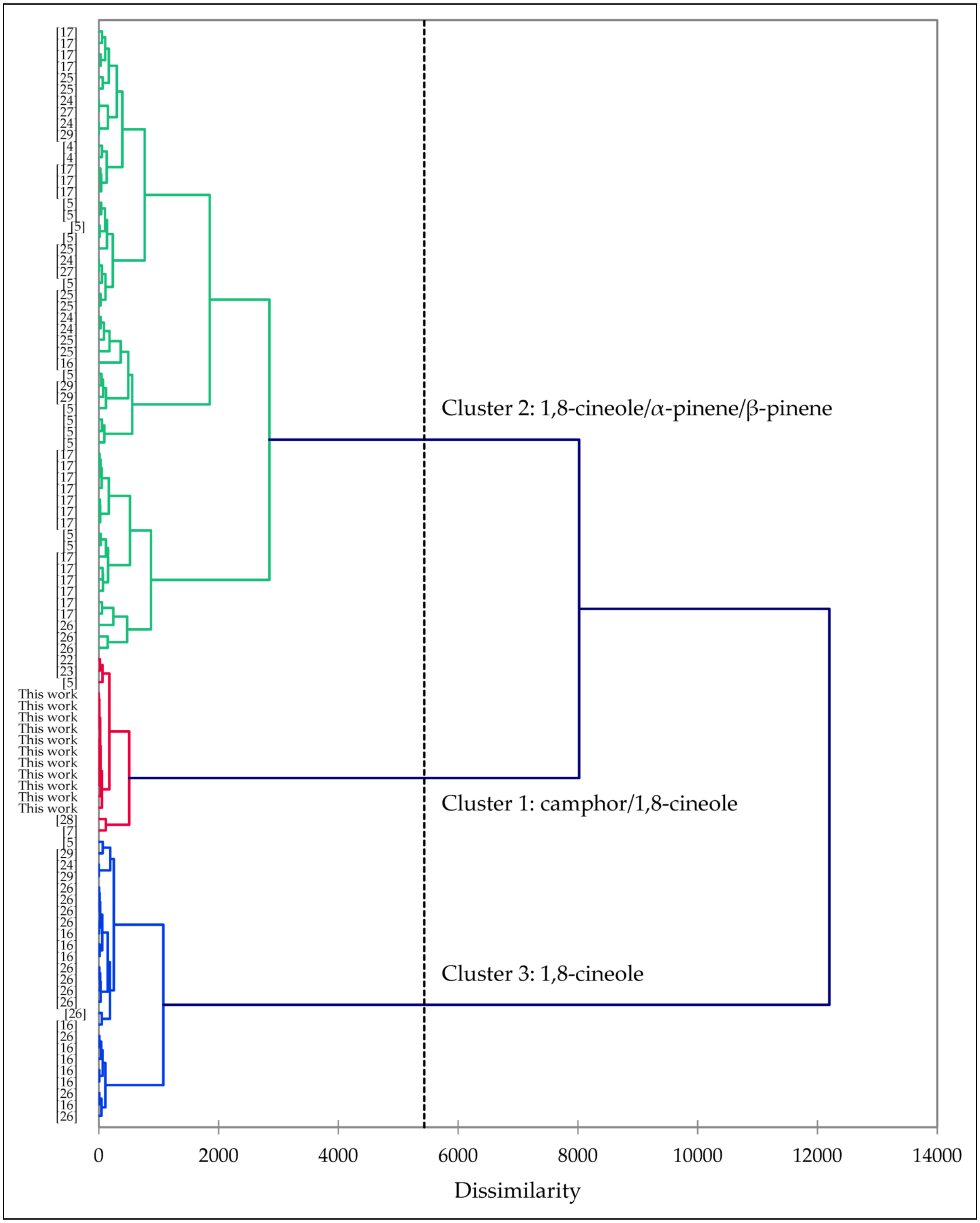

Figure 2. Agglomerative hierarchical cluster (AHC) analysis of *Salvia lavandulifolia* essential oil (EO) compositions from this work and the literature.

Figure 3. Spanish sage (*Salvia lavandulifolia*) plants in the field in Murcia, southern Spain.

spectral fragmentation patterns (> 80% similarity match) and retention indices (RIs) (calculated using a series of homologous C8-C20 *n*-alkanes) with those reported in databases (NIST database, and our in-house library), Lab Solutions GC-MS software (version 4.45).

GC-FID Analysis

Analysis of Spanish sage EOs was carried out as previously described by DeCarlo et al.[30]: Shimadzu GC 2010 FID, ZB-5 capillary column under the same operating conditions as above for GC-MS.

Enantiomeric Analysis by Chiral Gas Chromatography–Mass Spectrometry (CGC-MS)

The enantiomeric analysis of Spanish sage EO was carried out by CGC-MS as previously described by Poudel et al.[31]: Shimadzu GC-MS-QP2010S, EI mode (70 eV), B-Dex 325 chiral capillary GC column, 40 to 400 m/z scan range, 3.0 scan/sec scan rate; column temperature program (50 °C initial temperature, increased 1.5 °C/min to 120 °C, then 2 °C/min to 200 °C, held constant at 200 °C final temperature); He carrier gas, flow rate 1.8 mL/min. For each EO sample, 0.1 μL of 3% *w*/*v* solution was injected, split ratio of 1:45. The enantiomer ratios were determined from peak areas. Enantiomer identification was carried out by comparison of retention times and mass spectral fragmentation patterns with authentic samples obtained from Sigma-Aldrich.

Hierarchical Cluster Analysis

Compositions of *S. lavandulifolia* EOs from the published literature as well as those obtained from this work were used in the cluster analysis. The operational taxonomic units (OTUs) were the EO compositions; the major components (α-pinene, camphene, sabinene, β-pinene, myrcene, *p*-cymene, limonene, 1,8-cineole, γ-terpinene, linalool, *trans*-sabinol, camphor, borneol, terpinen-4-ol, α-terpineol, linalyl acetate, bornyl acetate, *trans*-sabinyl acetate, α-terpinyl acetate, and (*E*)-β-caryophyllene) were used for agglomerative hierarchical cluster (AHC) analysis (XLSTAT Premium, version 2018.5.53172, Addinsoft). Dissimilarity was determined using Euclidean distance, and clustering was defined using Ward's method.

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Author's Contribution

Conceptualization: N.S.D and P.S.; Methodology: A.P.; Software: W.N.S.; Validation: P.S. and A.P.; Formal analysis: A.P. and P.S.; Investigation: N.S.D and P.S.; Data curation: P.S. and W.N. S.; Writing—original draft preparation: N.SD; Writing—review and editing: N.S.D, P.S., and W.N.S.; Supervision: W.N.S. All the authors have read and agreed to the published version of the manuscript.

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Ethical Approval

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Statement of Human and Animal Rights

This article does not contain any studies with human or animal subjects.

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

Data Availability Statement

Data from this investigation are available in the manuscript and Supplemental material.

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Supplemental Material

Supplemental material for this article is available online.

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