agronomy

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Characterization of Essential Oils from Seven *Salvia* Taxa from Greece with Chemometric Analysis

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Abstract: Over the years, several studies have investigated the essential oils (EOs) of *Salvia* taxa, revealing significant chemical variability in their composition. The present study focused on the characterization of the EOs of seven *Salvia* taxa growing wild in Greece, namely *S. aethiopis* L., *S. argentea* L., and *S. sclarea* L. (*Aethiopis* section); *S. officinalis* L. subsp. *officinalis* and *S. tomentosa* Mill. (*Eusphace* section); *S. verticillata* L. subsp. *verticillata* (*Hemisphace* section); and *S. amplexicaulis* Lam. (*Plethiosphace* section). Chemometric analysis, including PCA, HCA, and a clustered heat map, were applied to identify possible relationships among the samples based on their constituents, chemical groups, and thujone contents. The analysis classified the samples into two distinct groups based on their chemical classes; Group I (Svert, Sarg, Sampl, and Sath) was characterized by the highest amounts of sesquiterpene hydrocarbons (42.7–88.0%), followed by oxygenated sesquiterpenes (6.7–41.6%) and monoterpenes (0–17.2%), while Group II (Soff, Stom, and SScl) showed the highest amounts of oxygenated monoterpenes (47–66.4%), followed by monoterpene hydrocarbons (4.9–22.7%), sesquiterpenes (3.2–15.3%), and oxygenated diterpenes (3.5–9.0%). Regarding thujone content, two major groups were detected. The first group comprised Sscl, Svert, Sarg, Sampl, and Sath while the second group comprised Soff and Stom (Subgenus *Salvia*/Section *Eusphace*), which exhibited the highest percentages of thujones. These findings provide a basis for further investigation into taxonomic studies of the *Salvia* genus.

Keywords: sage; volatile compounds; chemical composition; GC-MS; sesquiterpenes; monoterpenes; thujones

Academic Editors: Kunmei Chen and Ping Chen

Received: 3 December 2024
Revised: 6 January 2025
Accepted: 14 January 2025
Published: 17 January 2025

Citation: Tziakas, S.; Tomou, E.-M.; Fraskou, P.; Goula, K.; Dimakopoulou, K.; Skaltsa, H. Characterization of Essential Oils from Seven *Salvia* Taxa from Greece with Chemometric Analysis. *Agronomy* **2025**, *15*, 227. https://doi.org/10.3390/agronomy15010227

1. Introduction

Salvia L. (sage) is the largest genus within the Lamiaceae family and one of the largest plant genera in the world [1]. This mega-genus comprises approximately 1000 species with a nearly cosmopolitan distribution [2,3]. Like many other large genera, it is non-monophyletic [2,4], with a circumscription that remains unresolved. According to Will and Claßen-Bockhoff [4], the genus should break into six genera. On the contrary, Drew et al. [3] suggested a broader, united *Salvia* and proposed a treatment with six subgenera. This concept was followed also by other researchers [5,6], who raised the number of subgenera to eleven. Three species radiations have occurred in the genus that correspond to the three major

species diversity centers: Central and South America (ca. 500 species), East Asia (ca. 90 species), and Southwest Asia and the Mediterranean region (ca. 250 species) [2,6]. In the latter area and, more specifically, in Greece, 21 species and 24 taxa occur [7]. Among them, there are taxa with a broad distribution in all the floristic regions of the country, such as *S. fruticosa* Mill., *S. verbenaca* L., or *S. viridis* L.; taxa with a restricted distribution in Greece, found in only one or two floristic regions, such as *S. napifolia* Jacq. and *S. pratensis* subsp. *haematodes* (L.) Arcang.; and two Greek endemic taxa: *S. eichleriana* Halácsy and *S. teddii* Turrill [7].

The seven *Salvia* species included in this study (Figure 1) belong to the *Salvia* s.s. [4] and four different sections: sect. *Aethiopis* Benth., sect. *Eusphace* Benth., sect. *Hemisphace* Benth., and sect. *Plethiosphace* Benth. (Table 1). The four sections differ in several morphological features. Regarding the morphology of the corolla, the upper lip can be straight (sections *Eusphace*, *Hemisphace,* and *Plethiosphace*) or falcate (sections *Aethiopis* and *Plethiosphace*), and the tube may have (sections *Eusphace* and *Hemisphace*) or not have (sections *Aethiopis* and *Plethiosphace*) a ring of hairs. The staminal connective is shorter or equal to the filament (sect. *Eusphace*), longer than the filament (sections *Aethiopis* and *Plethiosphace*), or not articulating with the filament (sect. *Hemisphace*), and its arms can be unequal, with the shorter arm dolabriform (sections *Aethiopis* and *Plethiosphace*); unequal, with the shorter arm subulate (sect. *Hemisphace*); or subequal (sect. *Eusphace*) [8].

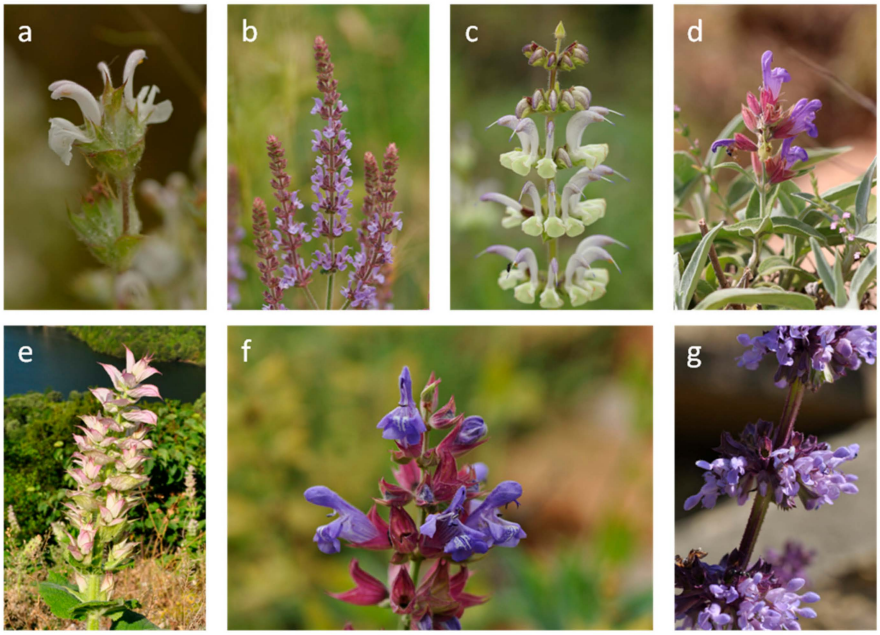

Figure 1. Pictures of the seven examined *Salvia* taxa: (**a**) *S. aethiopis*, (**b**) *S. amplexicaulis*, (**c**) *S. argentea*, (**d**) *S. officinalis* subsp. *officinalis*, (**e**) *S. sclarea*, (**f**) *S. tomentosa*, and (**g**) *S. verticillata* subsp. *verticillata*. Photos were provided by K. Goula.

Salvia EOs have garnered significant interest in their potential health benefits, exhibiting several pharmacological activities, including notable antimicrobial and anti-inflammatory effects [9,10]. For instance, sage EO have shown inhibitory activity against Gram-positive (such as *Bacillus subtilis*) and Gram-negative (like *Escherichia coli* and *Salmonella* species) bacteria, as well as against fungus species (e.g., *Candida albicans*) [10]. Moreover, *Salvia* EOs have been reported to possess antioxidant, anticholinesterase, antimutagenic, and cytotoxic activities [9]. *Salvia* leaves and EOs have been used extensively since ancient times in culinary applications (e.g., as spices and flavoring agents), as well as in perfumery, cosmetics, and the food industry [9]. Over the years, numerous *Salvia* taxa have been investigated for the chemical diversity of their EOs [10,11]. It is important to highlight the considerable infrageneric variability in the volatile constituents and the

chemical classes (e.g., monoterpenoids, sesquiterpenoids, and diterpenoids) of *Salvia* EOs, which is commonly attributed to factors such as genetic differences, geographical origins, environmental conditions, harvesting times, and the types of plant material used [12–14].

Thujone naturally occurs as a variable mixture of α-thujone (*cis*-thujone) and β-thujone (*trans*-thujone) [15]. It is present in various medicinal plants and their EOs, including cedar leaf, sage, tansy, wormwood, thyme, and rosemary, in highly variable concentrations [15]. Additionally, it has been reported that the EO of *S. officinalis* contains constituents like thujone, which can have toxic effects at high doses [10]. Although differing perspectives have emerged recently, thujone is still regarded as a potent neurotoxicant [15]. To effectively regulate the thujone content and optimize the safe use of thujone in plant materials, further studies are needed, encompassing phytochemistry, toxicology, and pharmacokinetics.

The present study aimed to investigate the EOs of seven *Salvia* taxa growing wild in Greece, with a focus on examining the chemical variability in their compositions. Using chemometric analysis, the study also explored potential relationships among the samples based on their constituents, chemical classes, and thujone contents.

Table 1. Botanical names, sections, collection sites in Greece, and voucher specimen numbers of the seven investigated *Salvia* taxa. Specific names follow the Vascular Plants of Greece checklist [7] and section names follow Bentham [16], as updated in recent literature [3–5].

Taxon	Section	Collection Site	Voucher Specimens No
S. aethiopis L.	*Aethiopis* Benth.	Krinida-Serres	TzSa_001
S. amplexicaulis Lam	*Plethiosphace* Benth.	Krinida-Serres	TzSa_002
S. argentea L.	*Aethiopis* Benth.	Kosani	TzSa_003
S. officinalis L. subsp. *officinalis*	*Eusphace* Benth.	Delvinaki-Pogoniani	TzSa_004
S. sclarea L.	*Aethiopis* Benth.	Krinida-Serres	TzSa_005
S. tomentosa Mill.	*Eusphace* Benth.	Kosani	TzSa_006
S. verticillata L. subsp. *verticillata*	*Hemisphace* Benth.	Negades-Ioannina	TzSa_007

2. Materials and Methods

2.1. Plant Materials

Flowering aerial parts of seven *Salvia* taxa were collected from June to July of 2021. A detailed list of samples, collection sites, and voucher specimen numbers is provided in Table 1. Voucher specimens were identified by Dr. K. Goula and have been deposited in the Herbarium of the Section of Pharmacognosy and Chemistry of Natural Products, Department of Pharmacy, NKUA.

2.2. EOs Isolation

All collected plant materials were air-dried at room temperature for 10 days and then were comminuted. About 20 g from each plant was used and the EOs were obtained by hydro-distillation in a modified Clevenger apparatus for 3 h according to the Hellenic Pharmacopoeia [17]. Gas Chromatography (GC)-grade n-pentane was used for the collection of the EOs, with the addition of anhydrous sodium sulfate to reduce any moisture. The EOs were subsequently analyzed by Gas Chromatography–Mass Spectrometry (GC-MS) and finally stored at −20 °C.

2.3. GC-MS Analysis

GC–MS analysis was carried out using a Hewlett-Packard 7820A-5977B MSD system (Agilent Technologies, Santa Clara, CA, USA) operating in EI mode (70 eV), equipped with a HP-5MS fused silica capillary column (30 m × 0.25 mm; film thickness 0.25 μm), and a split–splitless injector. The temperature program was from 60 °C at the time of the injection,

raised to 300 °C at a rate of 3 °C/min, and subsequently held at 300 °C for 10 min. Helium was used as a carrier gas at a flow rate of 2.0 mL/min. The injected volume of the samples was 1 μL.

Retention index (RI) values were calculated using a linear equation by Van den Dool and Kratz [18] based on a homologous series of n-alkanes from C9 to C24. The identification of the chemical components was based on comparison of RI values and mass spectra fragmentation patterns with those reported in the NIST/NBS and Wiley libraries, as well as with those described by Adams [19] and other literature data.

2.4. Statistical Analysis

Hierarchical cluster analysis (HCA) was applied to form major groups for the 138 studied chemical compounds, the 9 chemical groups, and the thujones. Following this, Principal Component Analysis (PCA) was performed to reduce the dimension data by linear combinations between variables. Finally, heatmaps were plotted by combining HCA with chemical compounds, chemical groups, and thujones. Correlation coefficient was calculated to estimate the direction and strength of each relationship. All analysis was conducted in IBM SPSS Statistics for Windows, version 28 (IBM Corp., Armonk, NY, USA).

3. Results and Discussion

3.1. Chemical Analysis of EOs

The yields (*v*/*w*) of the EOs from the seven taxa under study were 0.05% (*S. amplexicaulis*), 0.1% (*S. verticillata* subsp. *verticillata*, *S. argentea*, and *S. sclarea*), 0.2% (*S. aethiopis*), and 0.3% (*S. officinalis* subsp. *officinalis* and *S. tomentosa*). In total, 138 compounds were identified in the present analysis (Tables 2–5).

3.1.1. Subgenus Sclarea

Section Aethiopis

Overall, 49 compounds were identified in the *S. aethiopis* EO (Saeth), representing 98.2% of the total component (Table 2). The main chemical constituents (>5.0%) were (*E*)-caryophyllene (34.6%), germacrene D (17.3%), α-copaene (14.6%), and α-humulene (8.6%). The EO exhibited high levels of sesquiterpene hydrocarbons (88.0%), followed by oxygenated sesquiterpenes (6.7%) (Table 2), while monoterpenes were present in low amounts. These findings align with previous studies identifying (*E*)-caryophyllene as the major component, but with some differences in the percentages of the rest of the main compounds [11,20–22]. Bicyclogermacrene and bornyl acetate were detected in low amounts (0.2 and 0.4%, respectively) whereas linalool was not found. Previous work had also reported the sesquiterpenoids as the main chemical classes followed by monoterpenoids in *S. aethiopis* EO collected from Greece [11].

The chemical constituents of the *S. argentea* EO (Sarg) are presented in Table 2. A total percentage of 86.6%, represented by 55 compounds, was identified. Sesquiterpene hydrocarbons constituted the major chemical class with a percentage of 42.7%, followed by oxygenated sesquiterpenes (16.9%), monoterpene hydrocarbons (14.0%) and oxygenated monoterpenes (10.6%) (Table 2). Oxygenated diterpenes were also found in low amounts (2.2%). The most abundant constituents (>5.0%) were germacrene B (8.9%), caryophyllene oxide (8.6%), α-copaene (8.3%), germacrene D (8.0%), δ-cadinene (6.0%), α-pinene (5.7%), and viridiflorol (5.7%). Significant variations could be observed in the chemical constituents and classes across previous studies that had investigated the EO of *S. argentea* [11,23–29]. The EOs of two *S. argentea* population samples from Greece, one cultivated and one wild-growing, had been previously investigated [11]. Although sesquiterpene hydrocarbons were also the predominant class, quantitative variations had been observed in the rest of

the main categories. Furthermore, differences had been noticed in the principal constituents (>5.0%) among the two samples. Consequently, variations in the EO constituents had been noted compared to our results. These differences could likely be attributed to the different geographical regions where the samples were collected.

Overall, 47 compounds were found, representing 96.9% of the *S. sclarea* EO (Sscl) (Table 2). The major constituents were linalool acetate (30.3%), linalool (19.9%), germacrene D (8.8%), α-terpineol (7.8%), and sclareol (7.1%). Oxygenated monoterpenes constituted the main group (66.4%), followed by sesquiterpene hydrocarbons (13.4%) and oxygenated diterpenes (9.0%). Monoterpene hydrocarbons and oxygenated sesquiterpenes were detected in low amounts (4.9 and 3.2%, respectively). Linalool and linalyl acetate have been reported as the most characteristic and dominant volatiles of *S. sclarea* EO (ranges: 6.5–24.0% and 56.0–78.0%, respectively) while the concentration of sclareol varies from 0.4 to 2.6% [30]. In this study, sclareol was detected in significant amounts (7.1%). Our findings align broadly with previous reports on *S. sclarea* EOs from Greece [11,30–33]. However, noticeable variations were observed in the relative percentages of individual components. The sample could be classified as the linalyl acetate/linalool chemotype based on Sharopov and Setzer [34].

Table 2. Chemical composition of *Salvia aethiopis*, *S. argentea*, and *S. sclarea* EOs.

No	RI_L	RI_C	Compounds	Saeth	Sarg	Sscl
1	924	923	α-thujene	tr	tr	-
2	932	930	α-pinene	0.2	5.7	0.1
3	946	945	camphene	0.1	1.7	tr
4	969	968	sabinene	tr	0.8	tr
5	974	972	β-pinene	0.2	5.0	0.1
6	988	987	myrcene	0.1	0.3	1.5
7	1014	1013	α-terpinene	tr	-	tr
8	1020	1023	p-cymene	0.7	0.1	-
9	1024	1025	limonene	-	-	0.4
10	1026	1028	1,8-cineole	0.8	2.7	-
11	1032	1033	*cis*-ocimene	-	-	0.8
12	1044	1043	*trans*-ocimene	-	0.1	1.5
13	1054	1053	γ-terpinene	tr	0.2	tr
14	1086	1087	terpinolene	-	0.1	0.5
15	1095	1096	linalool	-	0.1	19.9
16	1100	1101	nonanal	-	-	tr
17	1101	1103	*cis*-thujone	0.1	2.2	-
18	1102	1105	isopentyl isovalerate	0.4	-	-
19	1112	1111	*trans*-thujone	0.1	0.2	-
20	1122	1120	α-campholenal	tr	0.1	-
21	1135	1131	*trans*-pinocarveol	tr	0.1	-
22	1140	1137	*trans*-verbenol	-	tr	-
23	1141	1140	camphor	0.2	0.1	-
24	1160	1159	pinocarvone	tr	0.1	-
25	1165	1163	borneol	tr	0.7	tr

Table 2. *Cont.*

No	RI_L	RI_C	Compounds	Saeth	Sarg	Sscl
26	1172	1170	*cis*-pinocamphone	-	-	0.1
27	1174	1172	terpinen-4-ol	tr	tr	0.1
28	1186	1183	α-terpineol	tr	0.1	7.8
29	1194	1191	myrtenol	-	tr	-
30	1195	1194	myrtenal	tr	0.1	-
31	1227	1225	nerol	-	-	1.3
32	1228	1230	bornyl formate	-	0.4	-
33	1254	1253	linalool acetate	tr	-	30.3
34	1283	1279	iso-bornyl acetate	-	3.7	-
35	1284	1285	bornyl acetate	0.4	-	-
36	1335	1332	δ-elemene	-	0.1	-
37	1345	1350	α-cubebene	0.5	-	-
38	1359	1360	neryl acetate	-	-	2.4
39	1374	1375	α-copaene	14.6	8.3	0.9
40	1379	1378	geranyl acetate	-	-	4.5
41	1387	1386	β-bourbonene	0.6	0.5	-
42	1387	1388	β-cubebene	5.0	0.4	0.2
43	1389	1390	β-elemene	1.2	0.7	0.3
44	1417	1415	(*E*)-caryophyllene	34.6	4.7	1.9
45	1430	1428	β-copaene	0.2	-	tr
46	1434	1430	γ-elemene	-	1.2	-
47	1442	1440	6,9-guaiadiene	-	1.0	-
48	1452	1449	α-humulene	8.6	0.9	0.1
49	1453	1453	geranylacetone	-	0.2	-
50	1454	1455	*trans*-β-farnesene	-	0.1	-
51	1480	1481	germacrene D	17.3	8.0	8.8
52	1489	1486	β-selinene	-	-	tr
53	1496	1497	viridiflorene	-	0.2	-
54	1500	1499	bicyclogermacrene	0.2	-	0.7
55	1505	1503	(*E*,*E*)-α-farnesene	-	0.6	0.2
56	1508	1506	germacrene A	0.5	0.4	tr
57	1513	1510	γ-cadinene	0.7	-	-
58	1522	1523	δ-cadinene	4.0	6.0	0.3
59	1544	1540	α-calacorene	tr	0.5	-
60	1548	1546	elemol	-	-	tr
61	1557	1550	1,5-epoxysalvial-4(14)-ene	-	-	0.1
62	1559	1556	germacrene B	-	8.9	-
63	1564	1566	β-calacorene	-	0.2	-
64	1574	1566	germacrene D-4-ol	0.3	-	-
65	1577	1575	spathulenol	-	0.2	0.6
66	1582	1580	caryophyllene oxide	4.3	8.6	0.6
67	1590	1587	β-copaen-4-α-ol	-	0.3	-
68	1592	1590	viridiflorol	-	5.7	-

Table 2. *Cont.*

No	RI_L	RI_C	Compounds	Saeth	Sarg	Sscl
69	1594	1593	salvial-4(14)-en-1-one	0.5	-	0.3
70	1608	1605	humulene epoxide II	0.6	1.3	-
71	1627	1625	1-*epi*-cubenol	0.2	-	-
72	1639	1636	caryophylla-4(12), 8(13)-dien-5α-ol or caryophylla-4(12), 8(13)-dien-5β-ol	0.1	0.2	-
73	1644	1645	α-muurolol	0.2	-	-
74	1649	1647	β-eudesmol	-	-	0.9
75	1652	1650	α-cadinol	0.2	-	0.2
76	1652	1651	α-eudesmol	-	-	0.5
77	1660	1655	*cis*-calamenen-10-ol	-	0.3	-
78	1668	1666	*trans*-calamenen-10-ol	-	0.3	-
79	1691	1690	vulgarol B	0.1	-	-
80	1711	1715	valerenol	0.2	-	-
81	1826	1820	8,13-epoxy-15,16-dinorlab-12-ene	0.1	-	1.0
82	1886	1882	(5*E*,9*Z*)-farnesylacetone	0.1	-	-
83	1987	1985	manool oxide	-	-	0.2
84	2009	2006	13-*epi*-manool oxide	-	0.1	tr
85	2056	2052	manool	-	2.1	0.7
86	2149	2150	abienol	-	-	tr
87	2222	2218	sclareol	-	-	7.1
			Total	98.2	86.6	96.9
			Grouped components	Saeth	Sarg	Sscl
			Monoterpene Hydrocarbons	1.3	14.0	4.9
			Oxygenated Monoterpenes	2.0	10.6	66.4
			Sesquiterpene Hydrocarbons	88.0	42.7	13.4
			Oxygenated Sesquiterpenes	6.7	16.9	3.2
			Oxygenated Diterpenes	0.1	2.2	9.0
			Hydrocarbons–Ketones	0.1	0.2	-
			Hydrocarbons–Aldehydes	-	-	tr
			Total	98.2	86.6	96.9

RIc = calculated retention indices using an n-alkane standard solution C9–C24 in HP-5 MS column; RI_L = literature retention indices; tr = traces (% < 0.05).

Section Plethiosphace

In total, 16 components were identified in the *S. amplexicaulis* EO (Sampl), representing 97.3% of the oil (Table 3). Germacrene D was the major compound (28.6%), followed by caryophyllene oxide (15.0%), spathulenol (14.6%), salvial-4(14)-en-1-one (12.0%), and (*E*)-caryophyllene (7.8%). The EO was rich in sesquiterpenes, with sesquiterpene hydrocarbons being in higher amounts (55.7%), whereas oxygenated sesquiterpenes were in the percentage of 41.6%. Our results are in accordance with a previous study on *S. amplexicaulis* EOs from wild-growing samples of Greece [11] where the primary constituents identified were germacrene D (4.0–40.2%), caryophyllene oxide (6.8–35.1%), and (*E*)-caryophyllene (5.7–14.8%). Additionally, spathulenol was present in all samples, being the major component in one sample. Salvial-4(14)-en-1-one was present in four samples, with its amounts

ranging from 3.4 to 7.0%. Moreover, one sample exhibited a high concentration of viridiflorol (12.1%) while two EOs contained it at lower levels, ranging from 0.4% to 0.6%. However, viridiflorol was not detected in our study. Such variations in the chemical composition of the EOs could be attributed to several factors such as the origin of the plant material and the microclimate. Sesquiterpenes were the dominant group in the EOs from the wild-growing samples, with oxygenated sesquiterpenes being more abundant in three samples (samp1: 64.5%, samp2: 61.8%, and samp4: 45.4%), whereas sesquiterpene hydrocarbons predominated in two samples (samp3: 58.4% and samp5: 72.2%) [11]. Further studies are needed to gain a deeper understanding of the variability in the chemical profile in *S. amplexicaulis* EOs.

Table 3. Chemical composition of *Salvia amplexicaulis* EO.

No	RI_L	RI_C	Compounds	Sampl
1	1345	1350	α-cubebene	tr
2	1374	1375	α-copaene	1.9
3	1387	1386	β-bourbonene	3.7
4	1389	1390	β-elemene	tr
5	1417	1415	(*E*)-caryophyllene	7.8
6	1452	1449	α-humulene	tr
7	1458	1459	*allo*-aromadendrene	3.2
8	1478	1475	γ-muurolene	4.2
9	1480	1481	germacrene D	28.6
10	1500	1501	α-muurolene	3.0
11	1513	1510	γ-cadinene	tr
12	1522	1523	δ-cadinene	3.3
13	1577	1575	spathulenol	14.6
14	1582	1580	caryophyllene oxide	15.0
15	1594	1593	salvial-4(14)-en-1-one	12.0
16	1711	1715	valerenol	tr
Total				97.3
Grouped components				Svert
Sesquiterpene Hydrocarbons				55.7
Oxygenated Sesquiterpenes				41.6
Total				97.3

RIc = calculated retention indices using an n-alkane standard solution C9–C24 in HP-5 MS column; RI_L = literature retention indices; tr = traces (% < 0.05).

3.1.2. Subgenus Salvia

Section Eusphace

Overall, 66 compounds were identified in the *S. officinalis* subsp. *officinalis* EO (Soff), representing 96.8% of the total component (Table 4). The main chemical constituents (>5.0%) were 1,8-cineole (14.7%), *cis*-thujone (11.1%), borneol (9.4%), (*E*)-caryophyllene (9.1%), manool (8.5%), camphor (7.8%), viridiflorol (7.2%), α-pinene (7.0%), and α-humulene (5.8%). Oxygenated monoterpenes constituted the dominant chemical group (47.0%), followed by monoterpene hydrocarbons (17.5%), sesquiterpene hydrocarbons (15.3%), oxygenated sesquiterpenes (8.5%), and oxygenated diterpenes (8.5%). These findings align with previous studies in *S. officinalis* EO, but with some differences in the percentages of

the compounds [12,35–38]. Schmiderer et al. [36] reported high variability in the major monoterpenes (i.e., 1,8-cineole, α-thujone, β-thujone, and camphor) and sesquiterpenes (β-caryophyllene, α-humulene, and viridiflorol) in EOs of *S. officinalis* populations from Albania. They also observed that the composition, particularly of certain major monoterpenes, was influenced by the geographical origin. In addition, oxygenated monoterpenes were the major chemical class of *S. officinalis* EO in a previous study [37].

The total percentage of 97.6%, represented by 72 compounds, was identified in the EO of *S. tomentosa* (Stom) (Table 4). Specifically, 1,8-cineole (18.2%), *cis*-thujone (17.9%), α-pinene (9.2%), viridiflorol (8.5%), borneol (6.7%), and (*E*)-caryophyllene (5.3%) were the main constituents. The EO was rich in oxygenated monoterpenes (54.1%), monoterpene hydrocarbons (22.7%), oxygenated sesquiterpenes (9.8%), and sesquiterpene hydrocarbons (7.5%). Hanlidou et al. [39] investigated 19 samples of *S. tomentosa* collected from different geographical locations in Greece (Thrace and Thassos Island), highlighting significant variation in the EO composition among the populations. Two oil types were found, mainly varying in the contents of 1,8-cineole, *cis*-thujone, and α-/β- pinenes. Viridiflorol and (*E*)-caryophyllene were detected in lower amounts (ranges: 0–1.2% and 0–4.0%, respectively) while they were main compounds in our sample. Regarding the content of *cis*-/*trans*-thujones, they found high amounts of *cis*-thujone (range: 6.1–24.3%) and lower percentages of *trans*-thujone (range: 1.0–3.7%) in the samples that originated from Thassos Island.

In previous studies, *cis*-/*trans*-thujones were reported in the EOs of *S. officinalis* and *S. tomentosa* [12,35–39]. Our findings revealed that *S. officinalis* subsp. *officinalis* and *S. tomentosa* EOs exhibited higher concentrations of *cis*-thujone (Soff: 11.1% and Stom: 17.9%) than *trans*-thujone (Soff: 1.4% and Stom: 3.7%). Schmiderer et al. [36] reported that α- and β-thujone are biosynthetically closely related, with α-thujone being mostly the dominant compound of the two.

Table 4. Chemical composition of *Salvia officinalis* subsp. *officinalis* (Soff), and *S. tomentosa* (Stom) EOs.

No	RI_L	RI_C	Compounds	Soff	Stom
1	921	920	tricyclene	0.2	0.2
2	924	923	α-thujene	0.1	0.1
3	932	930	α-pinene	7.0	9.2
4	946	945	camphene	3.4	3.2
5	969	968	sabinene	tr	-
6	974	972	β-pinene	4.7	4.9
7	979	980	3-octanone	tr	tr
8	988	987	myrcene	0.7	1.0
9	1002	1003	α-phellandrene	0.1	0.2
10	1008	1007	δ-3-carene	-	tr
11	1014	1013	α-terpinene	0.3	0.5
12	1020	1023	p-cymene	tr	1.1
13	1026	1028	1,8-cineole	14.7	18.2
14	1032	1033	*cis*-ocimene	0.1	1.1
15	1044	1043	*trans*-ocimene	tr	0.2
16	1054	1053	γ-terpinene	0.5	0.7
17	1065	1062	*cis*-sabinene hydrate	0.1	tr
18	1067	1068	*cis*-linalool oxide	tr	tr
19	1086	1087	terpinolene	0.4	0.3

Table 4. *Cont.*

No	RI_L	RI_C	Compounds	Soff	Stom
20	1098	1099	*trans*-sabinene hydrate	tr	tr
21	1101	1103	*cis*-thujone	11.1	17.9
22	1112	1111	*trans*-thujone	1.4	3.7
23	1122	1120	α-campholenal	tr	tr
24	1128	1126	*allo*-ocimene	-	tr
25	1135	1131	*trans*-pinocarveol	-	0.1
26	1141	1140	camphor	7.8	3.7
27	1145	1143	camphene hydrate	tr	0.1
28	1149	1147	*neo*-3-thujanol	-	tr
29	1155	1153	isoborneol	tr	tr
30	1158	1156	*trans*-pinocamphone	0.1	0.2
31	1165	1163	borneol	9.4	6.7
32	1172	1170	*cis*-pinocamphone	tr	0.1
33	1174	1172	terpinen-4-ol	0.2	0.4
34	1179	1178	p-cymen-8-ol	tr	tr
35	1186	1183	α-terpineol	0.2	0.4
36	1194	1191	myrtenol	tr	0.1
37	1195	1194	myrtenal	-	tr
38	1207	1212	*trans*-piperitol	tr	tr
39	1215	1216	*trans*-carveol	tr	tr
40	1226	1223	*cis*-carveol	tr	-
41	1284	1285	bornyl acetate	2.0	2.2
42	1289	1290	thymol	tr	tr
43	1289	1293	*trans*-sabinyl acetate	-	0.1
44	1295	1294	3-thujanol acetate	tr	-
45	1298	1296	carvacrol	tr	0.1
46	1324	1320	myrtenyl acetate	tr	0.1
47	1339	1335	*trans*-carvyl acetate	-	tr
48	1345	1350	α-cubebene	-	tr
49	1356	1353	eugenol	tr	-
50	1373	1370	α-ylangene	-	0.1
51	1374	1373	isoledene	tr	-
52	1374	1375	α-copaene	tr	0.1
53	1387	1386	β-bourbonene	-	tr
54	1408	1406	(*Z*)-caryophyllene	tr	tr
55	1409	1410	α-gurjunene	tr	-
56	1417	1415	(*E*)-caryophyllene	9.1	5.3
57	1419	1417	β-ylangene	tr	-
58	1431	1430	β-gurjunene	tr	-
59	1439	1438	aromadendrene	0.2	-
60	1452	1449	α-humulene	5.8	1.0
61	1458	1459	*allo*-aromadendrene	0.1	0.1
62	1478	1475	γ-muurolene	-	0.2

Table 4. *Cont.*

No	RI_L	RI_C	Compounds	Soff	Stom
63	1480	1481	germacrene D	tr	-
64	1483	1482	α-amorphene	-	tr
65	1489	1486	β-selinene	tr	tr
66	1496	1497	viridiflorene	0.1	0.1
67	1500	1501	α-muurolene	tr	0.1
68	1513	1510	γ-cadinene	tr	0.1
69	1521	1520	*trans*-calamenene	-	0.1
70	1522	1523	δ-cadinene	tr	0.3
71	1537	1535	α-cadinene	-	tr
72	1544	1540	α-calacorene	-	tr
73	1577	1575	spathulenol	tr	tr
74	1582	1580	caryophyllene oxide	0.6	0.6
75	1592	1590	viridiflorol	7.2	8.5
76	1602	1595	ledol	0.1	0.1
77	1608	1605	humulene epoxide II	0.5	0.2
78	1639	1636	caryophylla-4(12),8(13)-dien-5α-ol or caryophylla-4(12),8(13)-dien-5β-ol	0.1	0.1
79	1640	1641	*epi*-α-muurolol	-	0.1
80	1649	1647	β-eudesmol	tr	-
81	1652	1650	α-cadinol	-	tr
82	1652	1651	α-eudesmol	tr	-
83	1666	1662	14-hydroxy-(*Z*)-caryophyllene	-	0.2
84	2056	2052	manool	8.5	3.5
			Total	96.8	97.6
			Grouped components	Soff	Stom
			Monoterpene Hydrocarbons	17.5	22.7
			Oxygenated Monoterpenes	47.0	54.1
			Sesquiterpene Hydrocarbons	15.3	7.5
			Oxygenated Sesquiterpenes	8.5	9.8
			Oxygenated Diterpenes	8.5	3.5
			Hydrocarbons–Ketones	tr	tr
			Phenylpropanoids	tr	-
			Total	96.8	97.6

RIc = calculated retention indices using an n-alkane standard solution C9–C24 in HP-5 MS column; RI_L = literature retention indices; tr = traces (% < 0.05).

3.1.3. Subgenus Leonia

Section Hemisphace

In total, 64 compounds were identified in the EO of *S. verticillata* subsp. *verticillata* (Svert), which presented about 90.9% of the total composition of the oil (Table 5). The major constituents were (*E*)-caryophyllene (27.1%), α-humulene (14.6%), β-phellandrene (10.3%), and germacrene D (9.3%). Sesquiterpene hydrocarbons (62.0%) were the dominant chemical class, followed by monoterpene hydrocarbons (17.2%) and oxygenated sesquiterpenes (10.5%). Our findings present differences with previous studies on *S. verticillata* EO [11].

However, the composition of *S. verticillata* EOs is generally characterized by a high degree of complexity [11]. It is noteworthy to mention that a significant chemical polymorphism has been reported, which can be attributed to factors such as the geographical origins of the samples, the specific plant parts analyzed, and the techniques employed [40,41]. Further studies are needed to gain a deeper understanding of the variability in the chemical profile of *S. verticillata*. Previous studies also reported qualitative and quantitative differences in the EO compositions of *S. verticillata* populations collected from Greece [11].

Table 5. Chemical composition of *Salvia verticillata* subsp. *verticillata* EO.

No	RI_L	RI_C	Compounds	Svert
1	924	923	α-thujene	0.7
2	932	930	α-pinene	1.1
3	946	945	camphene	tr
4	969	968	sabinene	0.5
5	974	972	β-pinene	0.3
6	979	980	3-octanone	tr
7	988	987	myrcene	1.3
8	1002	1003	α-phellandrene	0.7
9	1008	1007	δ-3-carene	0.1
10	1014	1013	α-terpinene	0.1
11	1020	1023	p-cymene	0.4
12	1025	1027	β-phellandrene	10.3
13	1032	1033	*cis*-ocimene	0.8
14	1044	1043	*trans*-ocimene	0.6
15	1054	1053	γ-terpinene	0.2
16	1086	1087	terpinolene	0.1
17	1095	1096	linalool	tr
18	1100	1101	n-nonanal	0.3
19	1165	1166	n-nonanol	tr
20	1174	1172	terpinen-4-ol	0.1
21	1186	1183	α-terpineol	tr
22	1201	1203	n-decanal	0.1
23	1266	1268	n-decanol	tr
24	1289	1290	thymol	tr
25	1345	1350	α-cubebene	0.6
26	1356	1353	eugenol	tr
27	1373	1370	α-ylangene	0.2
28	1374	1375	α-copaene	0.5
29	1387	1386	β-bourbonene	0.6
30	1387	1388	β-cubebene	0.4
31	1389	1390	β-elemene	0.1
32	1408	1406	(*Z*)-caryophyllene	0.1
33	1417	1415	(*E*)-caryophyllene	27.1
34	1430	1428	β-copaene	1.0
35	1431	1430	β-gurjunene	tr

Table 5. *Cont.*

No	RI_L	RI_C	Compounds	Svert
36	1439	1438	aromadendrene	0.2
37	1452	1449	α-humulene	14.6
38	1454	1455	*trans*-β-farnesene	0.7
39	1465	1460	*cis*-muurola-4(14),5-diene	0.4
40	1480	1481	germacrene D	9.3
41	1487	1484	*trans*-β-ionone	0.4
42	1493	1490	*trans*-muurola-4(14),5-diene	0.2
43	1500	1499	bicyclogermacrene	1.4
44	1500	1501	α-muurolene	0.7
45	1513	1510	γ-cadinene	1.2
46	1521	1520	*trans*-calamenene	0.2
47	1522	1523	*δ*-cadinene	2.1
48	1533	1530	10-*epi*-cubebol	tr
49	1533	1532	*trans*-cadina-1,4-diene	0.1
50	1537	1535	α-cadinene	0.1
51	1544	1540	α-calacorene	0.1
52	1561	1558	(*E*)-nerolidol	0.2
53	1564	1566	β-calacorene	0.1
54	1577	1575	spathulenol	2.2
55	1582	1580	caryophyllene oxide	3.9
56	1594	1593	salvial-4(14)-en-1-one	0.6
57	1608	1605	humulene epoxide II	1.7
58	1627	1625	1-*epi*-cubenol	0.1
59	1639	1636	caryophylla-4(12), 8(13)-dien-5α-ol or caryophylla-4(12), 8(13)-dien-5β-ol	0.4
60	1640	1641	*epi*-α-muurolol	0.6
61	1652	1650	α-cadinol	0.4
62	1666	1662	14-hydroxy-(*Z*)-caryophyllene	0.4
63	1913	1911	(5*E*,9*E*)-farnesylacetone	0.1
64	1942	1948	phytol	0.2
			Total	90.9
			Grouped components	Svert
			Monoterpene Hydrocarbons	17.2
			Oxygenated Monoterpenes	0.1
			Sesquiterpene Hydrocarbons	62.0
			Oxygenated Sesquiterpenes	10.5
			Oxygenated Diterpenes	0.2
			Hydrocarbons–Alcohols	tr
			Hydrocarbons–Aldehydes	0.4
			Hydrocarbons–Ketones	0.5
			Phenylpropanoids	tr
			Total	90.9

RIc = calculated retention indices using an n-alkane standard solution C9–C24 in HP-5 MS column; RI_L = literature retention indices; tr = traces (% < 0.05).

3.2. Chemometric Analysis

3.2.1. Chemical Compounds

The hierarchical cluster analysis (HCA) (Figure 2) showed the formation of two major groups for the 138 chemical compounds studied. The first group (Group I) comprised *S. officinalis* subsp. *officinalis* (Soff), *S. tomentosa* (Stom), *S. verticillata* subsp. *verticillata* (Svert), *S. argentea* (Sarg), *S. amplexicaulis* (Sampl), and *S. aethiopis* (Sath) while the second group (Group II) comprised only *S. sclarea* (SScl).

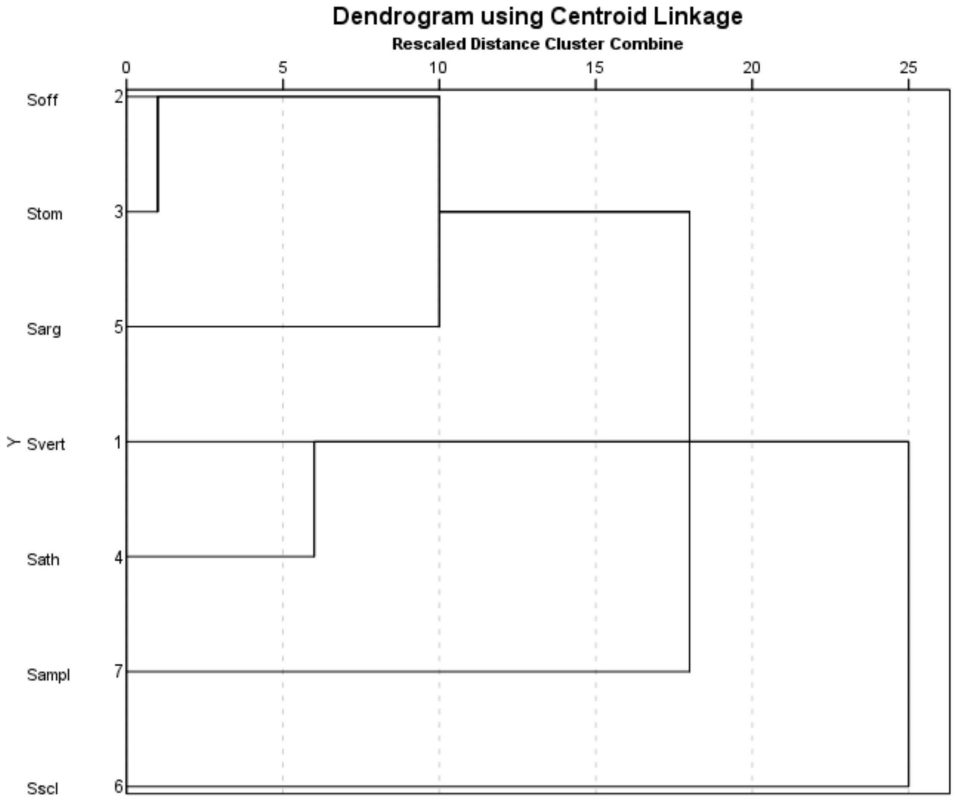

Figure 2. Hierarchical cluster analysis of 138 chemical compounds.

The PCA elucidated 71% of data variability (Figure 3). The main contributions to each of the three principal components (PCs) and the direction of loadings are shown in the Supplementary Materials (Table S1). For example, a contribution over 0.9 to the first PC was observed for ledol, *trans*-piperitol, p-cymen-8-ol, *trans*-carveol, *cis*-linalool oxide, isoborneol, tricyclene, *trans*-sabinene hydrate, 1,8-cineole, *cis*-thujone, bornyl acetate, borneol, myrtenyl acetate, carvacrol, camphene hydrate, *trans*-pinocamphone, α-terpinene, γ-terpinene, camphene, and *trans*-thujone; to the second PC, it was observed for α-phellandrene, α-thujene, *epi*-α-muurolol, (*Z*)-caryophyllene, α-cadinene, 14-hydroxy-(*Z*)-caryophyllene, δ-3-carene, *trans*-cadina-1,4-diene, *trans*-calamenene, α-ylangene, n-decanal, (*E*)-nerolidol, β-phellandrene, *trans*-β-ionone, *trans*-muurola-4(14),5-diene, 10-*epi*-cubebol, (5*E*,9*E*)-farnesylacetone, *cis*-muurola-4(14),5-diene, n-decanol, nonanol, phytol, and β-copaene; a contribution over −0.8 to the third PC was observed for α-cadinol, β-eudesmol, α-terpineol, linalool acetate, geranyl acetate, sclareol, 1,5-epoxysalvial-4(14)-ene, limonene, elemol, abienol, neryl acetate, manool oxide, nerol, 8,13-epoxy-15,16-dinorlab-12-ene, and linalool. The clustered heatmap of chemical compounds is shown in the Supplementary Materials (Table S2).

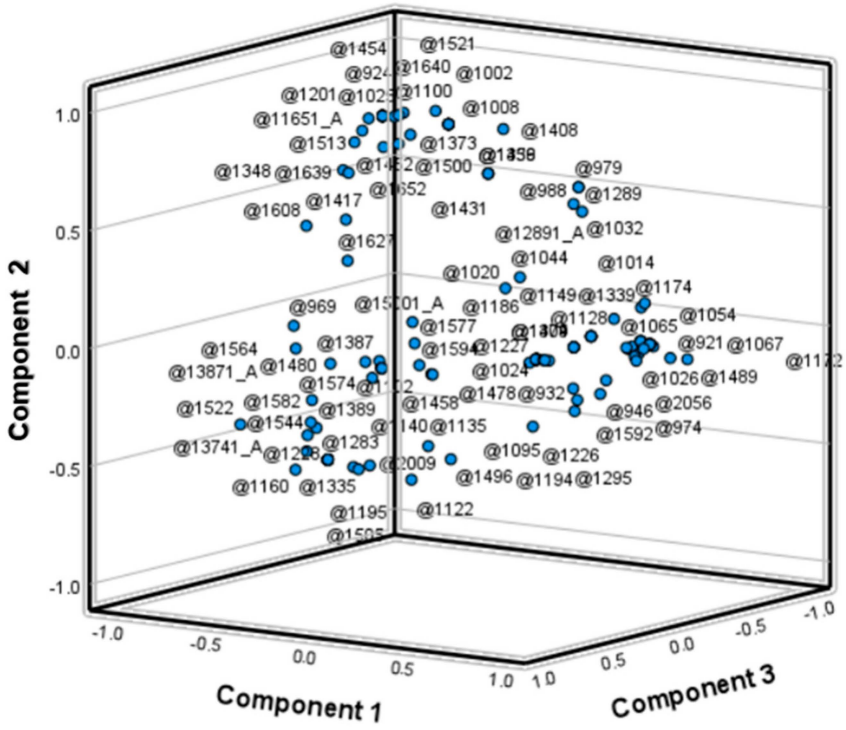

Figure 3. Contributions of chemical compounds to principal components. The values correspond to the RI_L of the chemical compounds (see Supplementary Materials, Table S1).

3.2.2. Chemical Groups

The hierarchical cluster analysis (HCA) (Figure 4) showed the formation of two major groups for the nine studied chemical groups (Hydrocarbons–Ketones/HK, Hydrocarbons–Alcohols/Halc, Hydrocarbons–Aldehydes/HAld, Monoterpene Hydrocarbons/MH, Sesquiterpene Hydrocarbons/SH, Oxygenated Sesquiterpenes/OS, Phenylpropanoids/Ph, Oxygenated Monoterpenes/OM, and Oxygenated Diterpenes/OD). The first group (Group I) comprised *S. verticillata* subsp. *verticillata* (Svert), *S. argentea* (Sarg), *S. amplexicaulis* (Sampl), and *S. aethiopis* (Sath) while the second group (Group II) comprised *S. officinalis* subsp. *officinalis* (Soff), *S. tomentosa* (Stom), and *S. sclarea* (SScl).

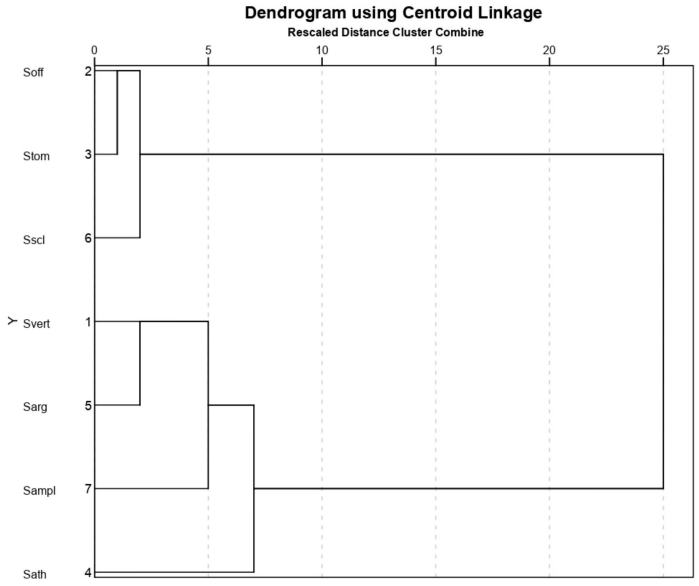

Figure 4. Hierarchical cluster analysis of nine chemical groups.

The PCA elucidated 88% of data variability. The main contribution to the first principal component (PC) was observed for Hydrocarbons–Ketones (HK), Hydrocarbons–Alcohols (HAlc), and Hydrocarbons–Aldehydes (HAld). Positive loadings were observed for Monoterpene Hydrocarbons (MH), Sesquiterpene Hydrocarbons (SH), Oxygenated Sesquiterpenes (OS), HAlc, HAld, HK, and Phenylpropanoids (Ph). Negative loadings were observed for Oxygenated Monoterpenes (OM) and Oxygenated Diterpenes (OD). The main contribution to the second PC was observed for MH and Ph. Positive loadings in Group II were found for MH, OM, OD, HAlc, HAld, HK, and Ph while negative loadings were observed for SH and OS (Figure 5).

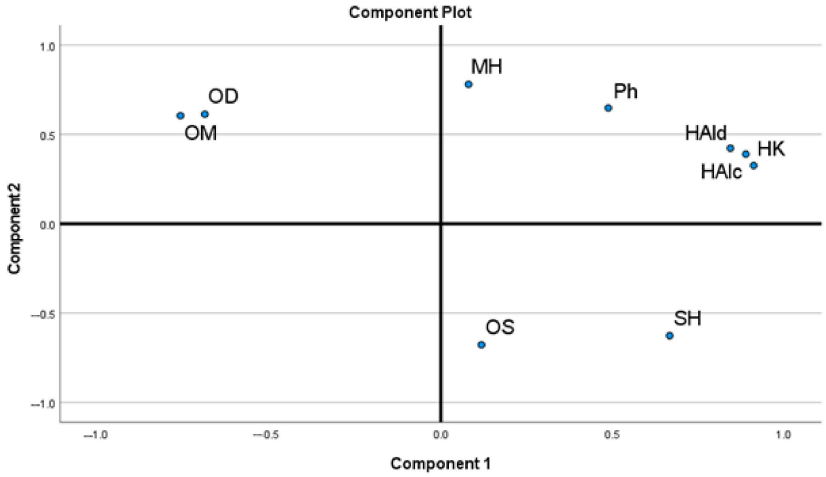

Figure 5. Contributions of chemical groups to principal components.

Group I (Svert, Sarg, Sampl, and Sath) was characterized by the highest amounts of SH (42.7–88%), OS (6.7–41.6%), MH (0–17.2%), and OM (0–10.6%). Group II (Soff, Stom, and SScl) was characterized by the highest amounts of OM (47–66.4%), MH (4.9–22.7%), SH (7.5–15.3%), OS (3.2–9.8%), and OD (3.5–9%). The analysis of the mean contents and standard deviations of the chemical groups showed that Group I was statistically different (*t*-test, $p < 0.05$) from Group II by the contents of OM (I = 3.2 ± 5%; II = 55.8 ± 9.8%), SH (I = 62.1 ± 19.04%; II = 12.1 ± 4.1%), and OD (I = 0.6 ± 1.05%; II = 7 ± 3.04%) (Figure 6).

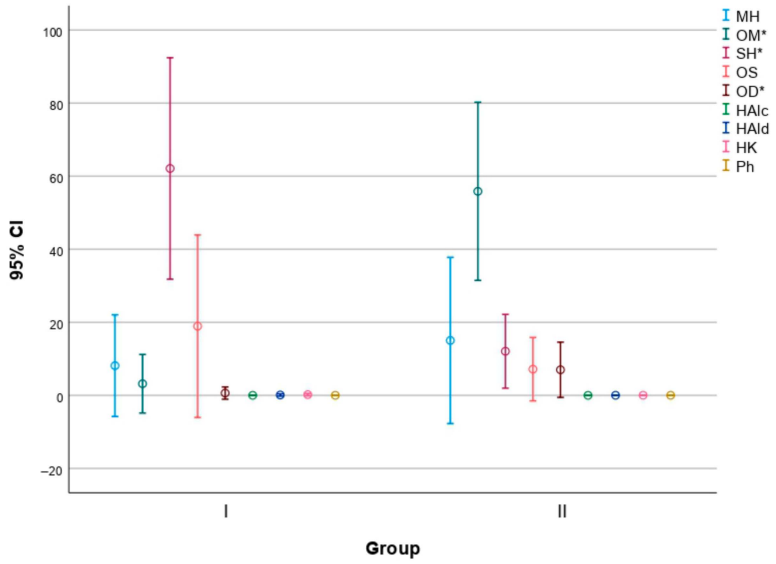

Figure 6. Chemical classes of two groups concerning the chemical groups. Means and 95% confidence intervals are given. Chemical groups marked with asterisks (*) differed statistically significantly in the *t*-test ($p < 0.05$).

Utilizing additional multivariate analyses in the heatmap analysis combined alongside HCA with the chemical groups revealed varying color intensities across different samples, gradually increasing from the lowest to the highest grade. Blue indicated low correlations while red represented high correlations. The clustered heatmap (Figure 7) confirmed the previously mentioned clustering outcomes observed in both the HCA and PCA.

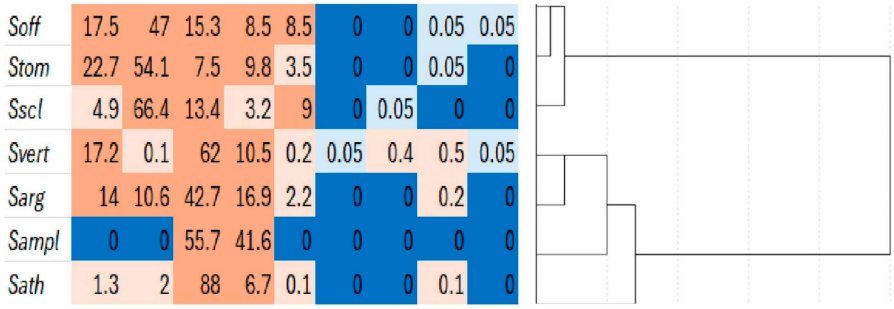

Figure 7. Clustered heat map of chemical groups.

3.2.3. Thujone Content

The hierarchical cluster analysis (HCA) (Figure 8) showed the formation of two major groups for the thujones. The first group (Group I) comprised *S. sclarea* (SScl), *S. verticillata* subsp. *verticillata* (Svert), *S. argentea* (Sarg), *S. amplexicaulis* (Sampl), and *S. aethiopis* (Sath) while the second group (Group II) comprised *S. officinalis* subsp. *officinalis* (Soff) and *S. tomentosa* (Stom), which belong to the subgenus *Salvia* in the section *Eusphace*.

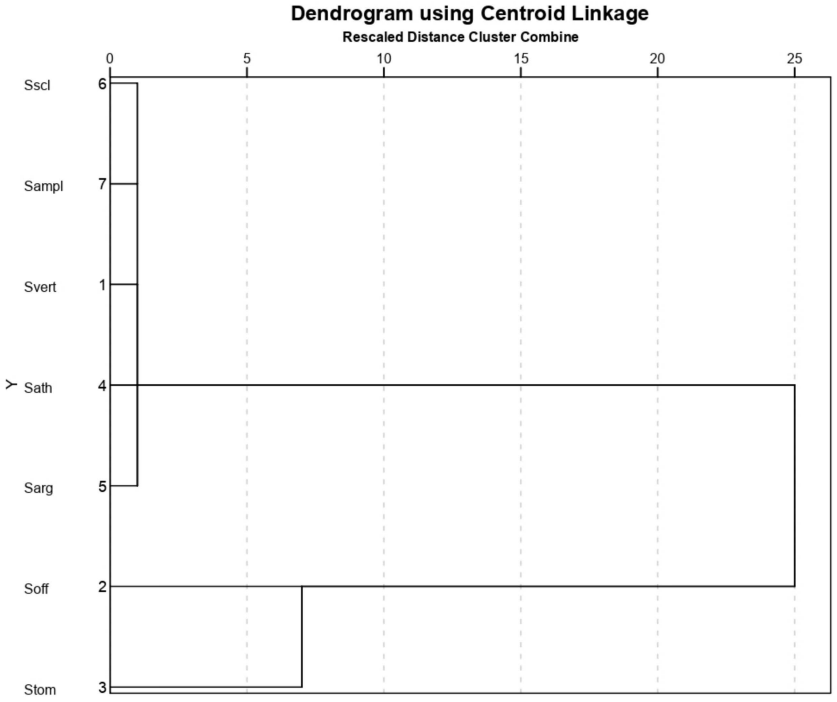

Figure 8. Hierarchical cluster analysis of thujones.

Only one component was extracted by the PCA that elucidated 99.3% of data variability. Group I (Sscl, Svert, Sarg, Sampl, and Sath) was characterized by the lowest amounts of *cis*-thujone (0–2.2%) and *trans*-thujone (0–0.2%). Group II (Soff and Stom) was characterized by the highest amounts of *cis*-thujone (11.1–17.9%) and *trans*-thujone (1.4–3.7%). The analysis of the mean contents and standard deviations of the thujones showed that Group I was statistically different (*t*-test, $p < 0.05$) from Group II by the contents of *cis*-thujone (I = 0.5 ± 0.97%; II = 14.5 ± 4.8%) and *trans*-thujone (I = 0.6 ± 0.09%; II = 2.6 ± 1.6%) (Figure 9).

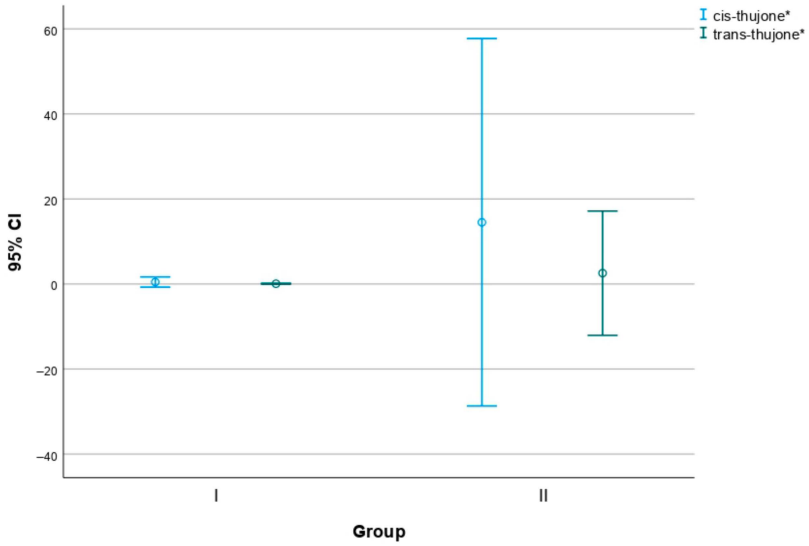

Figure 9. Chemical classes of two groups concerning the thujones. Means and 95% confidence intervals are given. Thujones marked with asterisks (*) differed statistically significantly in the *t*-test ($p < 0.05$).

Applying additional multivariate analyses in the heatmap analysis combined with HCA with the thujones, the color pattern corresponding to different samples varied with color intensity and increased gradually from the lowest to highest grade (blue indicated low correlations while red indicated high correlations). The clustered heatmap (Figure 10) confirmed the abovementioned clustering results for HCA and PCA.

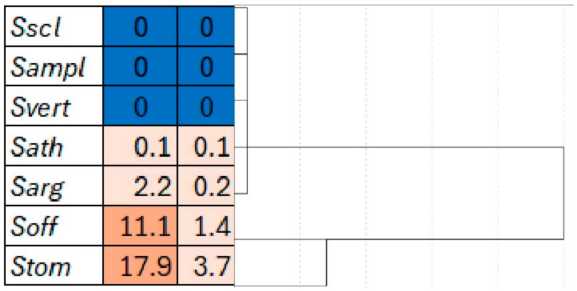

Figure 10. Clustered heat map of thujones.

4. Conclusions

This study investigated the EO compositions of seven wild *Salvia* taxa belonging to three subgenera (i.e., *Sclarea, Salvia,* and *Leonia*) and four different sections (i.e., *Aethiopis*, *Eusphace*, *Hemisphace*, and *Plethiosphace*). In total, 138 compounds were identified in the seven *Salvia* EOs. However, our results revealed substantial variability within the genus, with notable qualitative and quantitative differences observed in the volatile compounds and their relative chemical groups. Moreover, the levels of thujones, *cis*- and *trans*-thujones, were also determined in all samples, being in high amounts in *S. officinalis* subsp. *officinalis* and *S. tomentosa*.

Applying chemometric analysis (including PCA, HCA, and clustered heat map analysis), the samples were categorized into different groups based on their constituents, chemical groups, and thujone contents. Two distinct groups were formed based on their chemical classes, from which Group I was characterized by the highest amounts of sesquiterpene hydrocarbons, followed by oxygenated sesquiterpenes and monoterpenes, while Group II showed the highest levels of oxygenated monoterpenes, followed by monoterpene hydrocarbons, sesquiterpenes, and oxygenated diterpenes. In addition, two major groups were

also detected for the thujone content in which *S. sclarea*, *S. verticillata* subsp. *verticillata*, *S. argentea*, *S. amplexicaulis*, and *S. aethiopis* were categorized into the first group (Group I), with the lowest amounts of *cis*- and *trans*-thujones, while the second group (Group II) comprised *S. officinalis* subsp. *officinalis* and *S. tomentosa* with high amounts of these thujones.

These findings underscore the significant infrageneric chemical variability in *Salvia* EOs, emphasizing the importance of prior chemical profiling before recommending their application in phytomedicine or the food industry. The present paper provides new insights into the chemical diversity of *Salvia* EOs, contributing to a deeper understanding of their compositions and potential applications. Further studies are necessary to better understand the variability in the chemical profile of the EOs of *Salvia* taxa.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy15010227/s1, Table S1: Chemical compound contribution to each PCA component. Table S2: Clustered heat map of chemical compounds.

Author Contributions: Conceptualization, E.-M.T. and H.S.; methodology, E.-M.T. and H.S.; validation, E.-M.T. and K.G.; formal analysis, K.D.; investigation, S.T., E.-M.T. and P.F.; writing—original draft preparation, E.-M.T., K.G. and K.D.; writing—review and editing, H.S.; visualization, E.-M.T. and K.D.; supervision, E.-M.T. and H.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Acknowledgments: The authors wish to thank Ioannis Zalidas, Elli Lampretsa, and Panagiotis Dimos for the collection of plant materials.

Conflicts of Interest: The authors declare no conflicts of interest.

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