AkiNik
American Journal of Essential Oils and Natural Products
Available online at www.essencejournal.com
A J E O N P
American Journal of Essential Oils and Natural Products

ISSN: 2321-9114
AJEONP 2025; 13(1): 15-21

Received: 08-10-2024
Accepted: 11-11-2024

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Essential oil composition of *Salvia nemorosa* cv. "May Night" cultivated in Kuna, Idaho

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DOI: https://doi.org/10.22271/23219114.2025.v13.i1a.266

Abstract

Salvia nemorosa is a popular landscape plant that is attractive to pollinators. In this work, *Salvia nemorosa* cv. "May Night" was collected from two different years, 2023 and 2024. The essential oil was obtained by hydrodistillation and analyzed by gas chromatographic methods. The major components from the 2023 sample were (*E*)-β-caryophyllene (22.4%), sabinene (18.4%), germacrene D (17.1%), and caryophyllene oxide (5.7%). In contrast, the major components in the 2024 sample were sabinene (29.9%) and germacrene D (18.1%), along with large concentrations of two diterpenoids, levopimaradiene and levopimarinal (9.0% and 8.1%, respectively), but low concentrations of (*E*)-β-caryophyllene (0.3%) and caryophyllene oxide (0.1%). Multivariate analyses of *S. nemorosa* essential oils from this work and from the literature reveal vast differences in compositions and at least three different chemical groupings (a sabinene-rich group, a (*E*)-β-caryophyllene-rich group, and a spathulenol/caryophyllene oxide group).

Keywords: Gas chromatography, enantiomer, chiral, seasonal variation, violet sage, woodland sage

1. Introduction

Salvia L. is the largest genus of the Lamiaceae with around 800-900 species [1]. *Salvia nemorosa* L. (woodland sage, violet sage) is native to Europe and west-central Asia, but has been introduced to North America as an ornamental [2]. It is a fibrous-rooted, multi-branched perennial that grows 0.5-1.0 m tall and 0.6 m wide. The leaves are ovate-lanceolate, 5-10 cm long. The flowers, 1.3 cm long, are lavender to violet blue; blooming from May into October (Figure 1) [3]. Several cultivars are popular landscape plants. The flowers are attractive to pollinators and the leaves are aromatic when bruised. The purpose of this work is to determine the essential oil composition of *S. nemorosa* cv. "May Night" cultivated in Kuna, Idaho. In Idaho, this fragrant plant is attractive to bees, butterflies, and hummingbirds.

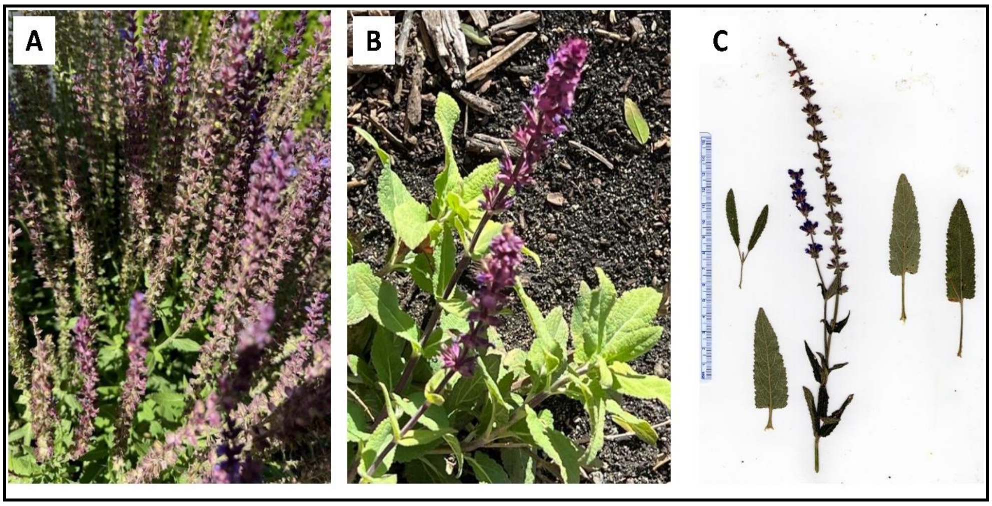

Fig 1: *Salvia nemorosa* cv. "May Night". **A:** Plant at the time of collection (photograph by K. Swor). **B:** Closeup of the leaves and flowers (photograph by K. Swor). **C:** Scan of the pressed plant.

2. Materials and Methods

2.1 Plant Material and Essential Oil

The plant was started from a seedling obtained from Jordan's Garden Center, Boise, Idaho. Aerial parts of the plant were sampled on 30 October 2023 and 19 June 2024. The fresh plant material was immediately frozen (–20 °C) and stored frozen until distilled. The fresh/frozen aerial parts (287.3 g and 207.2 g, respectively) were chopped and hydrodistilled using a Likens-Nickerson apparatus with continuous extraction of the distillate with dichloromethane [4] to give 1.435 g and 1.468 g colorless essential oils, respectively.

2.2 Gas Chromatographic Analysis

The essential oils were subjected to gas chromatography with flame ionization detection (GC-FID) and with mass spectral detection (GC-MS), as well as enantioselective GC-MS, as previously reported [5].

2.3 Multivariate Analyses

Agglomerative hierarchical cluster (AHC) analysis and principal component analysis (PCA) were carried out using XLSTAT v. 2018.1.1.62926 (Addinsoft, Paris, France). The AHC analysis was carried out using the percentages of the five most abundant components [(*E*)-β-caryophyllene, caryophyllene oxide, spathulenol, germacrene D, and sabinene] from this study in addition to compositions previously reported [6–15]. Dissimilarity was used to determine clusters considering Euclidean distance and Ward's method was used to define agglomeration. The PCA, type correlation, was carried out to verify the chemical associations (clusters) from the AHC analysis.

3. Results and Discussion

3.1 Essential Oil Composition

Hydrodistillation of the aerial parts of *S. nemorosa* gave colorless essential oils in yields of 0.499 and 0.708%. Gas chromatographic analysis led to identification of 75 compounds accounting for 99.3% and 99.1% of the total composition for the two samples (Table 1).

There have been several previous investigations of *S. nemorosa* from various geographical locations [6–15]. A perusal of these data reveals that the volatile phytochemical makeup of this species shows much variation. In order to place the essential oil of *S. nemorosa*, "May Night" from Idaho into perspective, an agglomerative hierarchical cluster (AHC) analysis was carried out based on the top five major components in the compositions [(*E*)-β-caryophyllene, caryophyllene oxide, spathulenol, germacrene D, and sabinene] (Figure 2). Based on the AHC analysis, the compositions can be grouped into three general clusters: (1) a cluster dominated by (*E*)-β-caryophyllene, (2) a sabinene cluster, and (3) a cluster dominated by the oxygenated sesquiterpenoids spathulenol and caryophyllene oxide.

A principal component analysis (PCA) confirms the three clusters and their component correlations (Figure 3). Interestingly, the two samples from this work (2023 and 2024), although having qualitative similarities, are remarkably different quantitatively and occupy different clusters. Rajabi and co-workers [14] examined four different accessions from different geographical locations in Iran and found notable differences in their concentrations. Likewise, Mahdieh and co-workers also noted composition differences in *S. nemorosa* essential oils from different geographical areas of Iran [11]. While the differences in the samples from Iran can likely be explained by the very different geographical locations and conditions, the two samples from Idaho were collected from the same individual plant. The compositional differences are likely due to seasonal effects; the 2023 sample was collected in October, near the end of the flowering season, while the 2024 sample was collected in June, near the beginning of the flowering season.

3.2 Enantiomeric Distributions

Enantioselective GC-MS was carried out on both the 2023 and 2024 essential oil samples (Table 2). The (–)-enantiomer was the major for α-pinene, β-pinene, limonene, and germacrene D, while the (+)-enantiomer dominated sabinene. Only one enantiomer observed for α-thujene, but (–)-α-thujene seems to be the most common enantiomer [21]. Only one enantiomer was observed for 1-octen-3-ol, but (–)-1-octen-3-ol is common in the Lamiaceae [21]. Likewise, only one enantiomer was detected for (*E*)-β-caryophyllene, but (–)-(*E*)-β-caryophyllene is the common enantiomer in higher plants, while (+)-(*E*)-β-caryophyllene has been found in liverworts [21]. Although the concentrations of the major components showed wide variation, the enantiomeric distributions of the chiral components remained consistent. As far as we are aware, there have been no previous reports on the enantiomeric distribution in *S. nemorosa*. There have been several investigations on enantiomeric distribution in other *Salvia* species, however (Table 3). The enantiomeric distributions for α-pinene, sabinene, and limonene are inconsistent throughout the genus. The (–)-enantiomers are consistently the major for β-pinene and for germacrene D, however.

Table 1: Chemical composition (%) of *Salvia nemorosa* cv. "May Night" aerial parts essential oils.

RI$_{\text{calc}}$	RI$_{\text{db}}$	Compounds	2023	2024
758	759	1-Pentanol	0.4	tr
800	800	Octane	0.4	0.1
805	802	Hexanal	0.4	0.1
852	849	(2*E*)-Hexenal	3.7	0.3
925	925	α-Thujene	2.5	2.8
932	932	α-Pinene	1.2	0.6
947	950	Camphene	-	tr
971	971	Sabinene	18.4	29.9
976	978	β-Pinene	2.2	2.2
979	978	1-Octen-3-ol	1.4	0.6
988	989	Myrcene	0.6	1.0
1005	1006	α-Phellandrene	-	tr
1017	1017	α-Terpinene	0.2	0.3
1025	1025	*p*-Cymene	0.1	0.1
1029	1030	Limonene	1.8	2.1
1030	1031	β-Phellandrene	0.2	0.1
1031	1032	1,8-Cineole	0.2	0.2
1035	1034	(*Z*)-β-Ocimene	0.1	tr
1045	1045	(*E*)-β-Ocimene	0.2	0.1
1048	1045	Phenylacetaldehyde	0.3	0.1
1057	1057	γ-Terpinene	0.5	1.0
1070	1069	*cis*-Sabinene hydrate	0.3	0.5
1084	1086	Terpinolene	0.2	0.3
1098	1099	Linalool	-	0.1
1101	1099	*trans*-Sabinene hydrate	0.2	0.5
1106	1105	Nonanal	2.3	0.8
1124	1124	*cis*-*p*-Menth-2-en-1-ol	-	0.1
1142	1142	*trans*-*p*-Menth-2-en-1-ol	-	0.1
1147	1145	Camphor	-	0.1
1181	1180	Terpinen-4-ol	0.3	1.4
1195	1195	α-Terpineol	-	0.2
1283	1282	Bornyl acetate	0.2	tr
1331	1335	δ-Elemene	-	0.1
1332	1331	4-Terpinenyl acetate	0.1	-
1343	1344	α-Cubebene	0.1	0.1
1345	1346	α-Terpinyl acetate	0.1	0.3
1375	1375	α-Copaene	0.4	0.3
1378	1379	(*E*)-β-Damascenone	-	0.1
1383	1382	β-Bourbonene	1.3	1.4
1386	1385	α-Bourbonene	0.1	0.1
1387	1387	β-Cubebene	0.2	0.1
1389	1390	β-Elemene	0.4	0.3
1415	1419	β-Ylangene	-	0.4
1419	1417	(*E*)-β-Caryophyllene	22.4	0.3
1429	1430	β-Copaene	0.4	0.4
1443	1439	(*Z*)-β-Farnesene	0.1	-
1443	1447	*iso*-Germacrene D	-	0.1
1452	1452	(*E*)-β-Farnesene	1.7	4.3
1455	1454	α-Humulene	1.3	0.1
1458	1461	4,6,8,10-Tetramethyltridecane	-	0.2
1474	1475	γ-Muurolene	0.3	0.3
1480	1480	Germacrene D	17.1	18.1
1491	1490	γ-Amorphene	0.3	0.1
1495	1497	Bicyclogermacrene	0.2	0.1
1498	1497	α-Muurolene	0.2	0.1
1512	1512	γ-Cadinene	0.2	0.2
1515	1515	Cubebol	0.2	tr
1517	1518	δ-Cadinene	0.8	0.5
1577	1576	Germacrene D-4-ol	0.2	0.2
1584	1587	Caryophyllene oxide	5.7	0.1
1595	1593	Salvial-4(14)-en-1-one	0.3	0.2
1611	1611	Humulene epoxide II	0.2	-
1628	1629	*iso*-Spathulenol	-	0.2
1644	1643	τ-Cadinol	1.4	tr
1645	1645	τ-Muurolol	0.4	0.1
1648	1651	α-Muurolol (= δ-Cadinol)	0.4	-

1657	1655	α-Cadinol	0.9	0.3
1909	1907	Isopimara-9(11),15-diene	-	0.3
1991	1994	Manoyl oxide	0.4	1.6
2051	2049	Abietatriene	-	2.1
2063	2040	Levopimaradiene	0.5	9.0
2108	2109	Phytol	-	0.9
2144	2143	Serratol	0.3	-
2262	2266	Dehydroabietal	0.7	2.6
2283	2265	Levopimarinal	1.7	8.1
		Monoterpene hydrocarbons	28.2	40.6
		Oxygenated monoterpenoids	1.3	3.3
		Sesquiterpene hydrocarbons	47.6	27.4
		Oxygenated sesquiterpenoids	9.7	1.0
		Diterpenoids	3.7	24.5
		Benzenoid aromatics	0.3	0.1
		Others	8.5	2.1
		Total identified	99.3	99.1

RI$_{calc}$ = Retention index calculated with respect to a homologous series of *n*-alkanes on a ZB-5ms column [16]. RI$_{db}$ = Reference retention index from the databases [17–20]. 2023 = sample collected on 30 October 2023. 2024 = sample collected on 19 June 2024. tr = trace (< 0.05%). - = not detected.

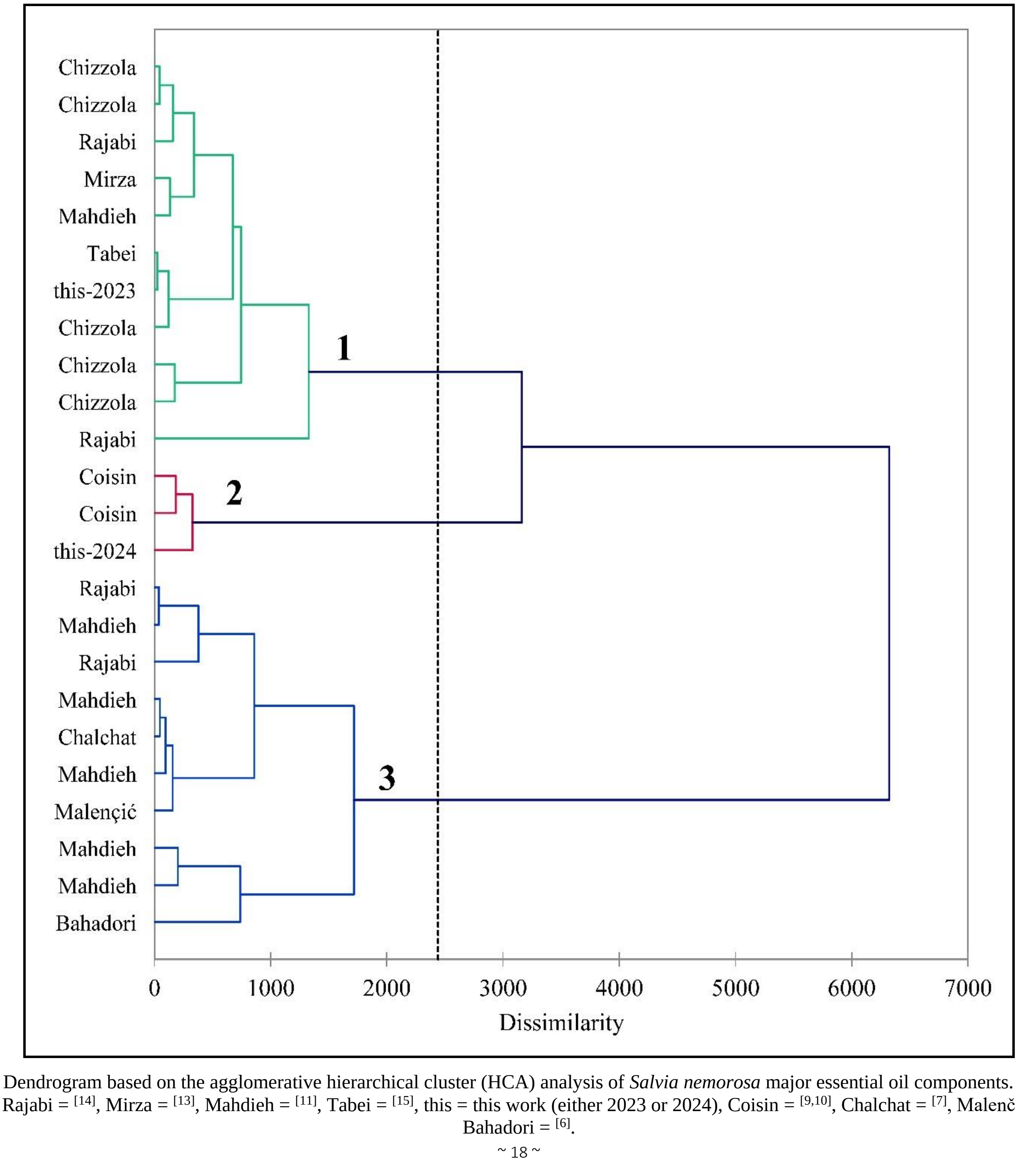

Fig 2: Dendrogram based on the agglomerative hierarchical cluster (HCA) analysis of *Salvia nemorosa* major essential oil components. Chizzola = [8], Rajabi = [14], Mirza = [13], Mahdieh = [11], Tabei = [15], this = this work (either 2023 or 2024), Coisin = [9,10], Chalchat = [7], Malenčić = [12], Bahadori = [6].

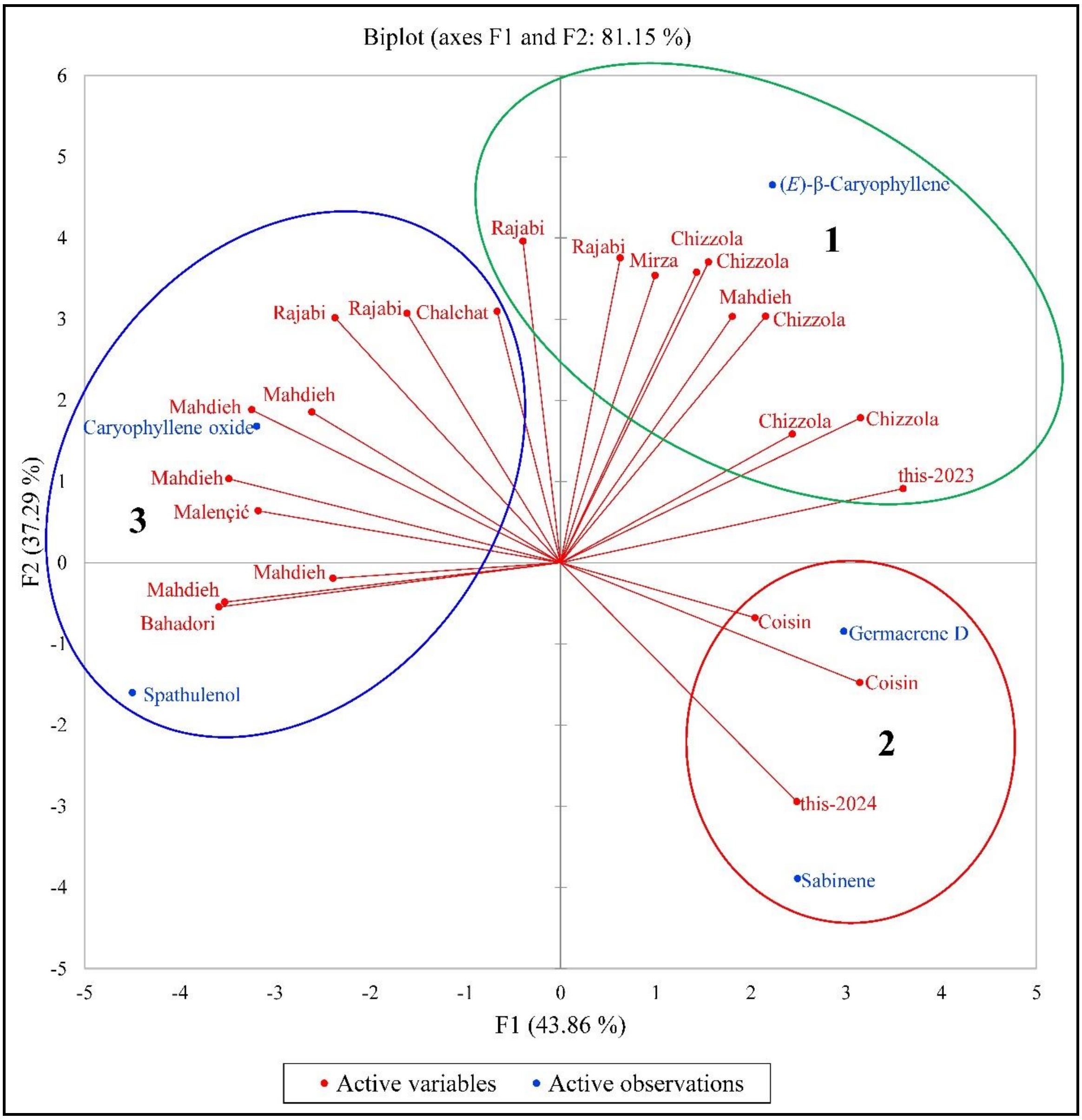

Fig 3: Biplot based on the principal component analysis of *Salvia nemorosa* major essential oil components. Chizzola = [8], Rajabi = [14], Mirza = [13], Mahdieh = [11], Tabei = [15], this = this work (either 2023 or 2024), Coisin = [9, 10], Chalchat = [7], Malenčić = [12], Bahadori = [6].

Table 2: Enantiomeric distribution (ED, %) of chiral components in *Salvia nemorosa* cv. "May Night" aerial parts essential oils.

Compounds	RI$_{calc}$	RI$_{db}$	ED (%)	
			2023	**2024**
(+)-α-Thujene	nd	950	0.0	0.0
(–)-α-Thujene	950	951	100.0	100.0
(–)-α-Pinene	977	976	67.0	68.4
(+)-α-Pinene	982	982	33.0	31.6
(+)-Sabinene	1019	1021	94.1	96.7
(–)-Sabinene	1030	1030	5.9	3.3
(+)-β-Pinene	1026	1027	17.5	13.0
(–)-β-Pinene	1032	1031	82.5	87.0
(–)-Limonene	1074	1073	62.1	64.5
(+)-Limonene	1081	1081	37.9	35.5
(–)-1-Octen-3-ol	1218	1218	100.0	100.0
(–)-(E)-β-Caryophyllene	1461	1461	100.0	100.0
(+)-(E)-β-Caryophyllene	nd	na	0.0	0.0
(+)-Germacrene D	1518	1519	9.1	14.1
(–)-Germacrene D	1522	1522	90.9	85.9

RI$_{calc}$ = Retention index determined with respect to a homologous series of *n*-alkanes on a Restek B-Dex 325 column. RI$_{db}$ = Retention index from our own in-house database. nd = not detected. na = not available.

Table 3: Enantiomeric distribution of chiral terpenoid components in *Salvia* species.

***Salvia* species**	**Component enantiomeric distribution, (+) : (–)**						**Ref.**
	α-Thujene	**α-Pinene**	**Sabinene**	**β-Pinene**	**Limonene**	**Germacrene D**	
Salvia nemorosa [a]	0.0 : 100.0	32.3 : 67.7	95.4 : 4.6	15.2 : 84.8	36.7 : 63.3	11.6 : 88.4	This work
Salvia albimaculata	-	-	-	-	68.5 : 31.5	-	[22]
Salvia bracteata	-	93.2 : 6.8	-	9.5 : 90.5	-	-	[22]
Salvia lavandulifolia	69.1 : 30.9	56.4 : 43.6	60.3 : 39.7	34.0 : 66.0	78.0 : 22.0	-	[23]
Salvia leucantha	-	4.3 : 95.7	30.0 : 70.0	-	-	1.6 : 98.4	[24]
Salvia potentillifolia	-	5.6 : 94.4	-	3.9 : 96.1	-	-	[22]
Salvia sclarea	-	10.2 : 89.8	51.3 : 48.7	4.5 : 95.5	68.3 : 31.7	0.2 : 99.8	[25]
Salvia tomentosa	-	37.7 : 62.3	-	8.0 : 92.0	-	-	[22]
Salvia wiedemannii	-	97.5 : 2.5	-	13.5 : 86.5	-	-	[22]

4. Conclusions

This is the first report on the essential oil composition of *Salvia nemorosa* cv. "May Night". The *S. nemorosa* species shows wide variation in composition, which can be attributed to geographical, genetic, and seasonal differences. Furthermore, there is wide variation in enantiomeric distribution of chiral terpenoid components with the genus *Salvia*. Clearly, more work is needed to further delineate the volatile phytochemical characteristics in both the genus *Salvia* and *S. nemorosa*.

5. Acknowledgments

This work was carried out as part of the activities of the Aromatic Plant Research Center (APRC, https://aromaticplant.org/).

6. Funding

This research received no external funding.

7. Conflicts of Interest

The authors declare no conflicts of interest.

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