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Research Article

Study on the histochemical localization of essential oil and evaluation of the chemical profiles of wild populations of *Salvia nemorosa* L.

Stanislava Ivanova[1,2,3], Ralitsa Parova[1], Zoya Dzhakova[1,3], Diana Karcheva-Bahchevanska[1,2,3], Kalin Ivanov[1,2,3], Rumyana Etova[4], Raina Ardasheva[3,5]

1 *Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Plovdiv, 4002 Plovdiv, Bulgaria*
2 *Research Institute, Medical University of Plovdiv, 4002 Plovdiv, Bulgaria*
3 *PERIMED-2, BG16RFPR002-1.014-0007, Central District, Vasil Aprilov Blvd. 15A, 4002 Plovdiv, Bulgaria*
4 *Department of Epidemiology and Disaster Medicine, Faculty of Public Health, Medical University of Plovdiv, 4002 Plovdiv, Bulgaria*
5 *Department of Medical physics and Biophysics, Faculty of Pharmacy, Medical University of Plovdiv, 4002 Plovdiv, Bulgaria*

Corresponding author: Zoya Dzhakova (zoya.dzhakova@mu-plovdiv.bg)

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Abstract

Salvia nemorosa L. is a widely distributed but comparatively understudied *Salvia* species. This work investigated the tissue localization of lipophilic constituents and profiled essential oil (EO) composition of wild Bulgarian populations. Stems were hand-sectioned and stained with Sudan III to visualize lipid deposits by light microscopy. EOs isolated from the collected flowering aerial parts from four sites were obtained by 4 h hydrodistillation and analyzed by gas chromatography–mass spectrometry (GC–MS); constituents were identified by RI and library match. Results: Sudan III staining revealed abundant red–orange lipid droplets distributed throughout the internal and epidermal tissues, including within the glandular trichomes. Across sites, 31–45 volatile compounds were identified, representing 91.96–97.71% of the total oils. Profiles were dominated by sesquiterpenes: germacrene D (17.64–41.34%) and β-caryophyllene (9.50–22.38%) were major constituents, with site-specific contributions of sabinene (up to 21.89%) and bicyclogermacrene (up to 7.67%). Sesquiterpene hydrocarbons (SH) comprised 50.96–70.81% of each EO, followed by oxygenated sesquiterpenes (8.19–25.58%). Monoterpenes were present in low concentrations in all samples, except for the Ivan Vazovo EO sample. Conclusion: *Salvia nemorosa* L. from Bulgaria exhibits SH-dominant chemotypes with marked geographic variation. The high levels of germacrene D and β-caryophyllene support the species as a potential source of bioactive sesquiterpenes and merit further biological evaluation.

Keywords

Salvia nemorosa L., *Salvia*, natural products, essential oil, histochemical analysis, gas chromatography–mass spectrometry (GC–MS)

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PENSOFT

Introduction

Salvia is regarded as the largest genus of the Lamiaceae family, comprising nearly 1,000 different species (Yang et al. 2024). The genus is of great importance for pharmaceutical research, the food industry, and cosmetics. Some of the most important species include *S. officinalis* L., *S. stepposa* Des.-Shost., *S. verticillata* L., *Salvia nemorosa* L., and others. Many plant species are rich sources of essential oil (EO) and phytochemical compounds characterized by a wide range of biological activity (Dimitrova and Slavova 2005; Tomova et al. 2021; Petkov et al. 2022; Kokova and Apostolova 2023; Gvozdeva et al. 2025), while the Lamiaceae remains the dominant family as a source of EO.

Salvia nemorosa L. (Lamiaceae) is a perennial herbaceous plant reaching a height of 30–50 cm, native to expansive regions of Central Europe and Western Asia. The species is widespread, from sea level to about 2,000 m altitude. The plant is commonly known as "woodland sage" and is a good example of a less studied member of the *Salvia* genus. The species exhibits dark purple stems that arise in long, upright inflorescences bearing flowers in shades ranging from occasional white to pale pink, mauve, blue, and purple. It blooms from June through October. Adaptable to various growing conditions, *Salvia nemorosa* L. (*S. nemorosa* L.) thrives in moderately moist, well-drained soils and performs well under both full sun and partial shade. It shows a distinct preference for sandy or gravelly substrates yet can tolerate drier conditions when necessary. Moreover, it integrates effectively with other perennial species in mixed plantings along the margins of forest gardens or woodland areas, thereby enhancing pollination of nearby crops (Yang et al. 2024).



Figure 1. Wild growing *Salvia nemorosa* L.

The plant exhibits astringent, antiseptic, carminative, and disinfecting properties. It is classified as an aromatic herb due to its potent, nearly camphoraceous scent. The flavor is slightly bitter, and the herb is noted for its stimulating, warming effects. It is rich in phenolic compounds, flavonoids, terpenoids, and EO, with its phytochemical profile predominantly comprising caffeic acid, luteolin, apigenin, ferulic acid, quercetin, kaempferol, *p*-hydroxybenzoic acid, rosmarinic acid, germacrene D, β-caryophyllene, spathulenol, and sabinene. Extracts of *S. nemorosa* L. have been investigated for their antioxidant, antimicrobial, antifungal, antidiabetic, and analgesic activities. It exhibits acetylcholinesterase inhibitory activity and demonstrates potential antidiabetic effects (Selvaraj et al. 2023).

There are 19 naturally occurring species from the genus *Salvia* in Bulgaria, 3 of which are protected by the Biodiversity Act.

The aim of this study was to perform a histochemical analysis of the localization of essential oil in *S. nemorosa* L. and to evaluate the chemical profiles of *S. nemorosa* EO. Although the number of studies on *S. nemorosa* L. is limited, some researchers consider that the plant species could be a novel and important source of bioactive agents with significant therapeutic potential (Bahadori et al. 2017).

This is the first study evaluating the EO of *S. nemorosa* L. from Bulgaria. The study supports current research on *S. nemorosa* L.

Materials and methods

Plant material

The aerial parts of wild *S. nemorosa* L. were collected in June 2025 during the flowering period from four different locations in Bulgaria, as shown in Table 1. The plants were authenticated according to Stoyanov K. et al. (2021), and voucher specimens were deposited in the Herbarium of the University of Agriculture, Plovdiv, Bulgaria.

Microscopic histochemical analysis

In order to soften the tissues, the plant material was fixed in a solution of 50% ethanol and glycerol in a 7:3 ratio for 24 h. Hand-cut transverse stem sections were prepared.

Table 1. Sample origin, coordinates, collection period, and voucher specimens of *Salvia nemorosa* L.

Sample origin	Coordinates	Soil type	Collection period	Voucher №	Abbreviation
Perushtica	42°04'53"N, 24°33'14"E	Cinnamon forest soils	June	063615	SN1
Asenovgrad	41°59'59.784"N, 24°54'48.78"E	Clay-rich and naturally enriched with Pb and other heavy metals	June	063602	SN2
Ivan Vazovo	42°27'18"N, 24°47'25"E	Cinnamon forest soils	June	063614	SN3
Slavovica	42°19'03.5"N, 24°04'36.9"E	Cinnamon forest soils	June	063603	SN4

Histochemistry for lipid accumulation was performed using Sudan III (Merck KGaA, Darmstadt, Germany). Sections were treated with Sudan III in 70% ethanol for 30 min, rinsed briefly in 80% ethanol, and mounted in 50% glycerol (Pellicciari and Biggiogera 2017). Observation of the sections was performed with a light microscope (Leica DM 2000 LED, Leica Microsystems, Wetzlar, Germany) equipped with a digital camera (Leica DMC 2900) and image processing software (Leica Application Suite, LAS).

Isolation of the essential oil

The flowering air-dried aerial parts of *S. nemorosa* L. were hydrodistilled using a Clevenger-type apparatus for 4 h to obtain the EOs. The collected EOs were dried over anhydrous sodium sulfate and stored in dark glass vials at 4 °C until GC–MS analysis.

Chemicals and reagents used for the chemical analyses

The retention indices (RIs) were determined using the following hydrocarbons: nonane (≥99%), decane (≥99%), undecane (≥99%), dodecane (99%), tridecane (≥99%), tetradecane (≥99%), and hexadecane (≥99%), purchased from Merck KGaA (Darmstadt, Germany). For the dilution of the essential oil, hexane (Thermo Fisher Scientific GmbH, Bremen, Germany) was used.

Chromatographic conditions

The analysis of the EOs was carried out using gas chromatography–mass spectrometry (GC–MS). The volatile compounds were separated using a Bruker Scion 436-GC SQ MS (Bremen, Germany) equipped with a Zebron ZB-5MSplus capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). Helium was used as the carrier gas with a constant flow rate of 1 mL/min. The injection volume was 1 µL. The injector temperature was set to 250 °C, and the split ratio was 1:30. The oven temperature was set at 60 °C for 2 min, increased to 110 °C at a rate of 10 °C/min, and then increased to 180 °C at a rate of 5 °C/min. Finally, the oven temperature was raised to 280 °C at a rate of 15 °C/min and held for 2 min. The detector temperature was set to 300 °C. The collected mass spectra were recorded in full-scan mode with a mass range of 50–350 m/z. The constituents of the EOs were identified by comparison of their mass spectra and RI with the Wiley NIST11 Mass Spectral Library (NIST11/2011/EPA/NIH) and literature data. The RI values were calculated using the retention times of the C8–C30 *n*-alkane series injected under the same described conditions. The percentage of the relative peak area represents the average value of three measurements. The standard error of the mean was not included, as it did not exceed 2%.

Results

Histochemical localization of essential oil

Hand-cut transverse sections of the stem of *S. nemorosa* L. were stained with Sudan III to detect lipids. Essential oils are produced and deposited in multiple secretory structures, including secretory glandular trichomes, as well as in internal individual parenchymal EO cells, secretory cavities, and ducts. Treatment of tissues with Sudan III solution positively correlated with the presence of lipid structures, indicated by the appearance of orange-stained droplets. Due to the lipophilic nature of EO compounds, their presence was confirmed in the histochemical analysis. The epidermal cells of the stem are polygonal in outline, with thick walls, numerous coverings, and occasional glandular trichomes (Fig. 2A, B, D–F; Pellicciari and Biggiogera 2017).

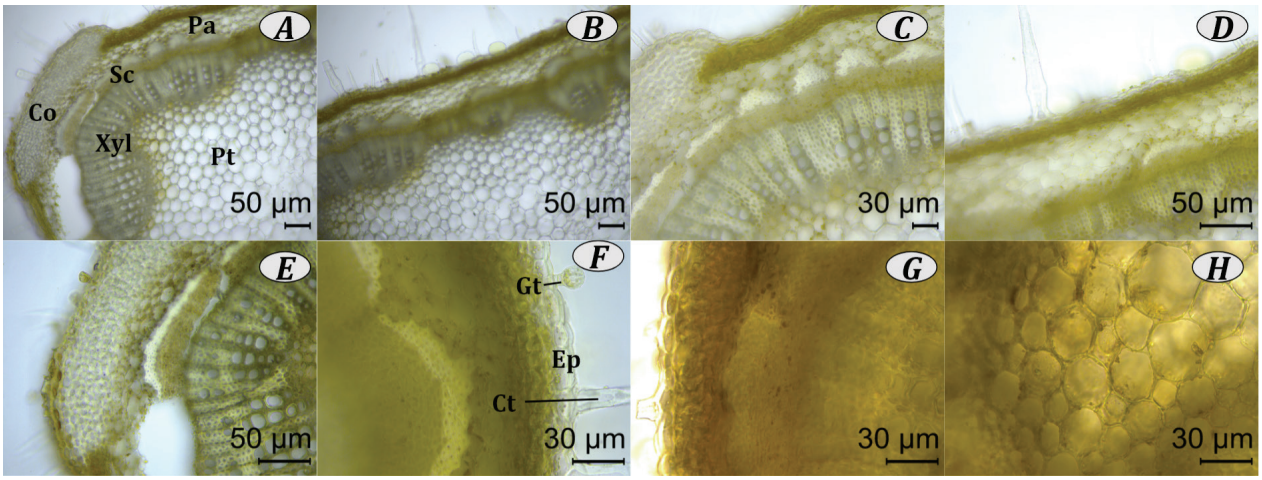


Figure 2. Histochemical observations of lipids stained with Sudan III in the stem of *S. nemorosa* L. (**A**) General aspect of transverse stem section, showing the presence of collenchyma (Co), sclerenchyma (Sc), parenchyma (Pa), xylem (Xyl), and pith region (Pt). (**B, C**) Details of stem. (**E–H**) Positive red–orange staining droplets demonstrate the presence of lipid components. (**F**) Epidermis (Ep) with covering trichome (Ct) and glandular trichome (Gt). Scale bars: 50 µm (**A, B, D, E**); 30 µm (**C, F, G, H**).

Chemical profiles

The EOs were obtained from the aerial parts of four wild populations of *S. nemorosa* L., and their phytochemical composition was determined by gas chromatography–mass spectrometry (GC–MS). A total of 33, 31, 45, and 32 volatile compounds were found in *S. nemorosa* EOs, representing 95.99%, 95.34%, 97.71%, and 91.96% of the total oil content for SN1, SN2, SN3, and SN4, respectively. The chemical composition of *S. nemorosa* EOs is presented with retention indices, formulas, classes of compounds, and percentages of total EO in Table 2.

Table 2. Volatile constituents of *S. nemorosa* EOs from different locations in Bulgaria, as a percentage of total EO.

№	Compound	RI	Formula	Class of compound	SN1 (%)	SN2 (%)	SN3 (%)	SN4 (%)
1	α-Thujene	945	$C_{10}H_{16}$	MH	0.99	-	4.49	-
2	α-Pinene	951	$C_{10}H_{16}$	MH	-	0.13	0.15	-
3	Sabinene	983	$C_{10}H_{16}$	MH	6.88	0.11	21.89	0.16
4	1-Octen-3-ol	986	$C_8H_{16}O$	O	-	0.15	0.12	-
5	β-Pinene	988	$C_{10}H_{16}$	MH	0.35	0.17	0.34	0.19
6	β-Myrcene	993	$C_{10}H_{16}$	MH	-	-	1.11	-
7	α-Phellandrene	1014	$C_{10}H_{16}$	MH	-	-	0.05	-
8	α-Terpinene	1027	$C_{10}H_{16}$	MH	0.87	-	1.98	-
9	*p*-Cymene	1035	$C_{10}H_{14}$	MH	-	-	0.12	-
10	Limonene	1042	$C_{10}H_{16}$	MH	0.29	-	0.25	-
11	β-Phellandrene	1044	$C_{10}H_{16}$	MH	-	-	0.20	-
12	Eucalyptol	1046	$C_{10}H_{18}O$	MO	0.40	-	0.17	0.06
13	γ-Terpinene	1065	$C_{10}H_{16}$	MH	2.02	-	3.55	-
14	Terpinolene	1082	$C_{10}H_{16}$	MH	0.36	-	0.67	-
15	Terpinen-4-ol	1165	$C_{10}H_{18}O$	MO	2.03	-	2.83	-
16	α-Terpineol	1179	$C_{10}H_{18}O$	MO	-	-	0.08	-
17	Bornyl acetate	1299	$C_{12}H_{20}O_2$	MO	0.69	-	-	-
18	α-Cubebene	1340	$C_{15}H_{24}$	SH	0.40	0.29	0.24	0.23
19	α-Ylangene	1365	$C_{15}H_{24}$	SH	-	-	0.08	-
20	α-Copaene	1373	$C_{15}H_{24}$	SH	1.59	1.39	0.77	1.18
21	β-Bourbonene	1383	$C_{15}H_{24}$	SH	4.79	1.09	0.91	1.04
22	β-Elemene	1387	$C_{15}H_{24}$	SH	-	1.21	0.35	1.08
23	α-Gurjunene	1409	$C_{15}H_{24}$	SH	-	-	-	0.17
24	β-Caryophyllene	1424	$C_{15}H_{24}$	SH	22.38	13.91	9.50	11.65
25	β-Copaene	1434	$C_{15}H_{24}$	SH	1.18	0.79	0.32	0.67
26	Aromadendrene	1444	$C_{15}H_{24}$	SH	-	-	0.43	-
27	(*E*)-β-Farnesene	1454	$C_{15}H_{24}$	SH	-	1.25	6.90	2.04
28	Humulene	1464	$C_{15}H_{24}$	SH	1.23	1.86	0.84	1.76
29	Alloaromadendrene	1478	$C_{15}H_{24}$	SH	0.30	-	0.55	2.55
30	γ-Muurolene	1484	$C_{15}H_{24}$	SH	2.66	2.08	1.20	1.68
31	Germacrene D	1493	$C_{15}H_{24}$	SH	25.74	37.08	17.64	41.34
32	β-Selinene	1501	$C_{15}H_{24}$	SH	-	0.40	-	0.53
33	γ-Amorphene	1503	$C_{15}H_{24}$	SH	1.18	0.55	0.74	0.49
34	Bicyclogermacrene	1508	$C_{15}H_{24}$	SH	1.45	1.10	7.67	1.05
35	δ-Amorphene	1515	$C_{15}H_{24}$	SH	0.38	0.14	0.20	0.26
36	γ-Cadinene	1526	$C_{15}H_{24}$	SH	1.11	0.92	0.64	0.74
37	δ-Cadinene	1531	$C_{15}H_{24}$	SH	3.22	2.70	1.98	2.35
38	Nerolidol	1573	$C_{15}H_{26}O$	SO	-	0.99	-	0.68
39	Spathulenol	1598	$C_{15}H_{24}O$	SO	0.85	0.95	3.74	1.06
40	Caryophyllene oxide	1605	$C_{15}H_{24}O$	SO	5.77	7.35	1.05	4.66
41	Globulol	1608	$C_{15}H_{26}O$	SO	-	-	0.27	-
42	Salvial-4(14)-en-1-one	1616	$C_{15}H_{24}O$	SO	0.44	1.73	0.14	1.13
43	Ledene oxide II	1640	$C_{15}H_{24}O$	SO	0.64	1.71	0.22	1.14
44	τ-Muurolol	1670	$C_{15}H_{26}O$	SO	0.69	0.73	0.39	0.58
45	Cubenol	1673	$C_{15}H_{26}O$	SO	0.39	1.08	0.23	0.76
46	α-Cadinol	1683	$C_{15}H_{26}O$	SO	1.61	2.61	0.69	2.02
47	14-Hydroxy-9-epi-(*E*)-caryophyllene	1687	$C_{15}H_{24}O$	SO	2.70	7.98	0.99	2.37
48	Hexahydrofarnesyl acetone	1835	$C_{18}H_{36}O$	SO	-	0.45	0.47	1.13
49	Phytol	1960	$C_{20}H_{40}O$	DH	0.41	2.44	0.56	5.21
	Terpene classes							
	Monoterpene hydrocarbons (MH)				11.76	0.41	34.80	0.35
	Oxygenated monoterpenes (MO)				3.12	-	3.08	0.06
	Sesquiterpene hydrocarbons (SH)				67.61	66.76	50.96	70.81
	Oxygenated sesquiterpenes (SO)				13.09	25.58	8.19	15.53
	Diterpene hydrocarbons (DH)				0.41	2.44	0.56	5.21
	Others				-	0.15	0.12	-
	Total identified (%)				95.99	95.34	97.71	91.96

The chromatograms from the GC–MS analysis of the EOs are presented in Figs 4–7.

The dominant terpene classes in *S. nemorosa* EOs are SH. The percentage of SH of the total oil content in all four samples – SN1, SN2, SN3, and SN4 – ranges from 50.96 to 70.81%, as shown in Fig. 3.

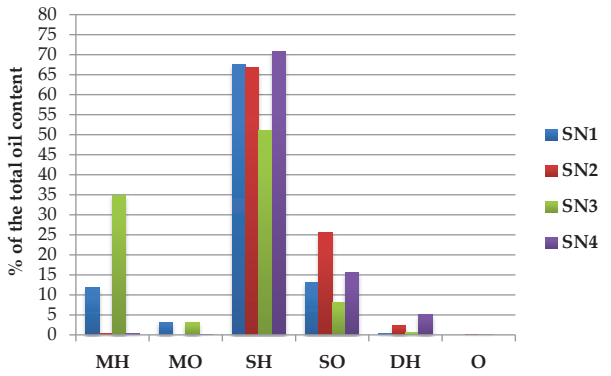


Figure 3. Main classes of volatile compounds in the analyzed SN1, SN2, SN3, and SN4 samples, where MH – monoterpene hydrocarbons; MO – oxygenated monoterpenes; SH – sesquiterpene hydrocarbons; SO – oxygenated sesquiterpenes; DH – diterpene hydrocarbons; O – others.

The concentration of MH is nearly negligible for samples SN2 and SN4 (0.41 and 0.35%, respectively). In contrast, sample SN1 has a MH content of 11.76%,

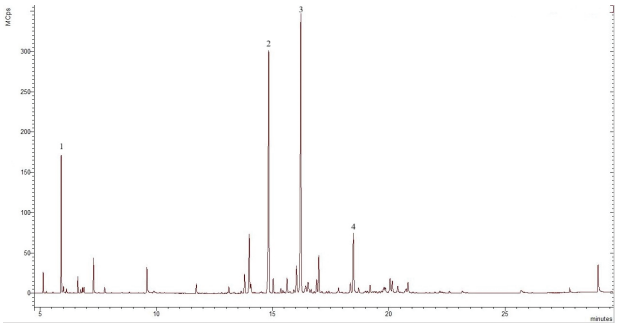


Figure 4. GC–MS chromatogram of analyzed *Salvia nemorosa* EO from the Perushtica region (SN1), where MCps is mega counts per second, and the numbers refer to the following compounds: 1-sabinene, 2-β-caryophyllene, 3-germacrene D, 4-caryophyllene oxide.

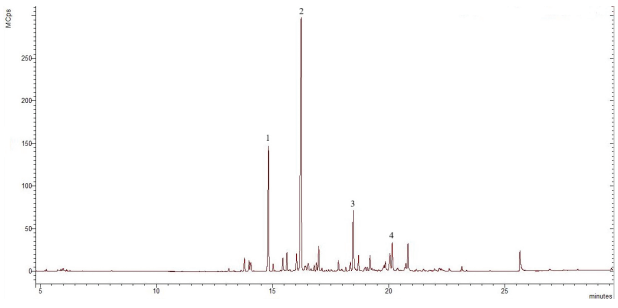


Figure 5. GC–MS chromatogram of analyzed *Salvia nemorosa* EO from the Asenovgrad region (SN2), where MCps is mega counts per second, and the numbers refer to the following compounds: 1-β-caryophyllene, 2-germacrene D, 3-caryophyllene oxide, 4-14-hydroxy-9-epi-(*E*)-caryophyllene.

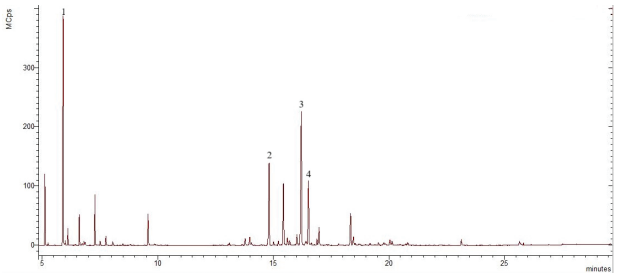


Figure 6. GC–MS chromatogram of analyzed *Salvia nemorosa* EO from the Ivan Vazovo region (SN3), where MCps is mega counts per second, and the numbers refer to the following compounds: 1-sabinene, 2-β-caryophyllene, 3-germacrene D, 4-bicyclogermacrene.

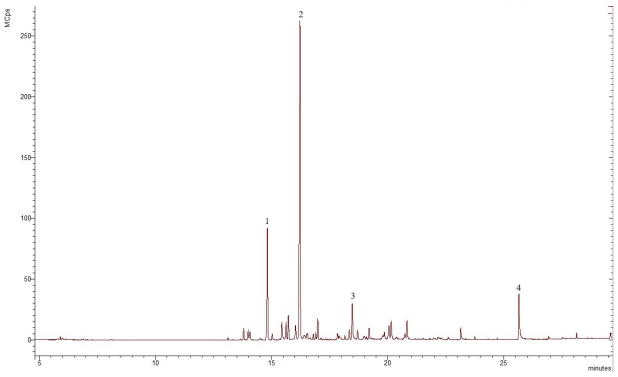


Figure 7. GC–MS chromatogram of analyzed *Salvia nemorosa* EO from the Slavovica region (SN4), where MCps is mega counts per second, and the numbers refer to the following compounds: 1-β-caryophyllene, 2-germacrene D, 3-caryophyllene oxide, 4-phytol.

while in sample SN3, MH are among the predominant compounds, comprising 34.80% of the total oil content. Significant variability is also observed in the total content of SO (8.19–25.58%). The amount of DH ranges from 0.41 to 5.21%. Oxygenated monoterpenes are either completely absent or found in very low concentrations in all samples, ranging from 0.06% to 3.12%, followed by the other compounds (0.12–0.15%).

Among the representatives of SH, germacrene D and β-caryophyllene are found in the highest percentages in all EOs. The concentration of germacrene D in SN2 and SN4 samples is 37.08% and 41.34%, respectively, which is almost double that in SN1 (25.74%) and SN3 (17.64%). The bicyclic sesquiterpene β-caryophyllene is found in samples SN2, SN3, and SN4 in comparable amounts (9.5–13.91%), whereas in sample SN1, the concentration is 22.38%. Bicyclogermacrene is present above 5% of the total oil content only in SN3 (7.67%). Other SH that are approximately or slightly above 5% include β-bourbonene in the SN1 sample (4.79%) and (*E*)-β-farnesene in the SN3 sample (6.9%).

Caryophyllene oxide, an SO, is a major compound in SN1 (5.77%), SN2 (7.35%), and SN4 (4.66%) samples. Another prominent SO found in the SN2 sample is 14-hydroxy-9-epi-(*E*)-caryophyllene (7.98%). Other SO compounds in the EOs composition are spathulenol, salvial-4(14)-en-1-one, leden oxide II, cubenol, and α-cadinol. Among the representatives of MH, sabinene is one of the main components in the SN1 (6.88%) and

SN3 (21.89%) EO samples. The majority of MH found in amounts exceeding 1% were present in the SN3 sample, including α-thujene (4.49%), β-myrcene (1.11%), α-terpinene (1.98%), and γ-terpinene, which appeared in both SN1 and SN3 samples. Only terpinen-4-ol from MO was present in slightly higher amounts in SN1 (2.03%) and SN3 (2.83%) samples. Phytol is the only representative of DH found in all four samples, with the highest percentage of total oil content observed in SN4 (5.21%).

Discussion

The present study provides the first combined histochemical and chemical analysis of wild *S. nemorosa* L. populations from Bulgaria. Sudan III staining confirmed the accumulation of lipophilic secondary metabolites within both epidermal and internal tissues, consistent with the distribution of secretory structures reported for other *Salvia* species. The presence of glandular trichomes suggests that EOs are produced and stored in specialized organs adapted for defense and ecological interactions.

Research on the chemical composition of *S. nemorosa* EO has been conducted in various countries, including Iran, Serbia, Romania, and Austria. Two independent studies from Iran found that β-caryophyllene (18.78–60.6%) and caryophyllene oxide (6.8–27.0%) were the main components of *S. nemorosa* EO, while the other major components differed (Mirza and Sefidkon 1999; Rajabi et al. 2014). According to Rajabi et al. (2014), the components above 5% of the total oil content were spathulenol, bicyclogermacrene, and germacrene D, while Mirza et al. (1999) identified germacrene B, *cis*-β-farnesene, germacrene D, and spathulenol. Another study from Iran examined separately the EOs extracted from the flowers and leaves, respectively, showing that in both, the main components were caryophyllene oxide (28.2–45.0%) and spathulenol (23.0–57.8%) (Bahadori et al. 2017). Caryophyllene oxide was also the predominant compound in *S. nemorosa* EOs from two Serbian populations, with concentrations ranging from 13.4% to 23.4%. In the first population, β-caryophyllene (10.0%) was also a primary component, while spathulenol (11.2%) was the main compound in the second population (Chalchat et al. 2004, Malenčić et al. 2004). Chizola et al. (2012) reported that the EOs extracted separately from the stems, leaves, and flowers of *S. nemorosa* L. were primarily composed of β-caryophyllene (stems: 8.9–25.6%; leaves: 14.4–41.0%; flowers: 6.9–11.8%) and germacrene D (stems: 6.6–8.0%; leaves: 14.0–38.3%; flowers: 8.7–13.4%). Additionally, other detected constituents included salvial-4(14)-en-1-one, hexadecanoic acid, caryophyllene oxide, sabinene, β-bourbonene, terpinene, and α-thujene.

In the present study, GC–MS analysis demonstrated that all four populations share a sesquiterpene hydrocarbon–dominant profile (50.96–70.81% of the EOs), with germacrene D (17.64–41.34%) and β-caryophyllene (9.50–22.38%) as major constituents. These compounds are well known for antimicrobial, anti-inflammatory, and antioxidant properties, which may explain the ethnobotanical use of *Salvia* taxa in traditional medicine. The predominance of sesquiterpenes distinguishes *S. nemorosa* L. from more widely studied species such as *S. officinalis* L., where monoterpenes like α-thujone and 1,8-cineole are abundant (Craft et al. 2017; Khedher et al. 2017; El Euch et al. 2019). This chemotaxonomic difference underlines the metabolic diversity within the genus.

Despite this general pattern, notable interpopulation variability was observed. The Ivan Vazovo sample (SN3) contained high concentrations of sabinene (21.89%) and bicyclogermacrene (7.67%), indicating a site-specific chemotype. Environmental factors (soil, altitude, microclimate) and genetic diversity may underlie such variations. Similar geographic chemotypes have been described in *S. sclarea* L. and *S. nemorosa* L. populations from other regions, supporting the view that local adaptation shapes EO profiles.

From a practical perspective, the high content of β-caryophyllene, a selective CB2 receptor agonist with potential nutraceutical applications, enhances the value of *S. nemorosa* L. as a renewable source of bioactive sesquiterpenes. Germacrene D, also abundant, has been implicated in plant defense against herbivores and in allelopathic interactions, suggesting ecological as well as pharmacological significance. The variability among Bulgarian populations indicates that targeted selection of sites or chemotypes could optimize yields of desired constituents for industrial or therapeutic uses.

Conclusion

Salvia nemorosa L. populations from Bulgaria exhibit consistent sesquiterpene-rich EO profiles with clear population-level chemotypic variation. The predominance of sesquiterpenes distinguishes *S. nemorosa* L. from the more widely studied *S. officinalis* L., where monoterpenes such as α-thujone and 1,8-cineole are typically abundant. These results expand the phytochemical knowledge of *S. nemorosa* L. and underline its potential as a source of bioactive sesquiterpenes. Future work should include broader geographic sampling, seasonal variation studies, and bioactivity assays to fully evaluate the pharmacological potential of this underutilized sage.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

The authors declared that no experiments on animals were performed for the present study.

The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

Use of AI

No use of AI was reported.

Author contributions

Conceptualization, S.I. and D.K.-B.; methodology, S.I., D.K.-B., and Z.D.; software, S.I. and Z.D.; formal analysis, S.I., D.K.-B., K.I., Z.D., R.P., R.E., and R.A.; investigation, S.I., D.K.-B., K.I., Z.D., R.P., R.E., and R.A.; resources, R.P.; data curation, S.I., D.K.-B., K.I., Z.D., R.P., R.E., and R.A.; writing – original draft preparation, S.I., D.K.-B., Z.D., and R.P.; writing – review and editing, S.I., D.K.-B., and Z.D.; visualization, S.I., and D.K.-B.; supervision, S.I., and K.I. All authors have read and agreed to the published version of the manuscript.

Author ORCIDs

Stanislava Ivanova https://orcid.org/0000-0003-1282-7868
Ralitsa Parova https://orcid.org/0009-0001-1816-7728
Zoya Dzhakova https://orcid.org/0000-0002-6405-8549
Diana Karcheva-Bahchevanska https://orcid.org/0000-0001-8721-4463
Kalin Ivanov https://orcid.org/0000-0002-5689-2920
Rumyana Etova https://orcid.org/0000-0002-3804-8777
Raina Ardasheva https://orcid.org/0000-0002-6854-8916

Data availability

All of the data that support the findings of this study are available in the main text.

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