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Composition of the essential oil from *Salvia verticillata* L. collected in Eastern Austria

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ABSTRACT

Salvia verticillata (Lamiaceae) is a small herb from the dry grassland in Southern Europe and Western Asia. Inflorescences had about 0.2 % essential oil, leaves 0.1 % and stems less than 0.01%. The essential oils were composed mainly of sesquiterpene hydrocarbons. On average, in all three plant parts the following compounds were identified in decreasing order: germacrene D > (*E*)- β -caryophyllene > bicyclogermacrene > α -humulene > spathulenol > β -bourbonene. In inflorescences, germacrene D, (*E*)- β -caryophyllene and bicyclogermacrene occurred in nearly equal amounts, while in leaves germacrene D accounted often for more than 50% of the essential oil.

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KEYWORDS

Salvia verticillata; whorled clary; Lamiaceae; essential oil; germacrene D; (e)-βcaryophyllene

1. Introduction

Salvia verticillata L., whorled clary (Lamiaceae) is perennial growing on semi-dry meadows and other open landscapes. The leaves are mostly simple and ovatetriangular. The pedicellate lilac-blue flowers are clustered in terminal or axillary racemes formed by superimposed verticillaster. S. verticillata is distributed in Europe and Western Asia from Turkey to Iran and Azerbijan (1).

In Turkey, *S. verticillata* is used in Folk medicine to cure colds and gastrointestinal disorders (2,3). Some biological activities have been reported as antioxidant activity (4,5), weak anti-inflammatory activity (6), and anti-acetylcholinesterase activity (7). Additionally, significant antidepressant effect in mice suggest a therapeutic potential in seizure and depressions (2). The essential oil of *S. verticillata* showed some cytotoxic activity against cancer cell lines (8).

Several reports on essential oils are known from plants of eastern South-Europe (9–12), Turkey (3,13) and Iran (8,14,15) where the whole aerial parts have been analysed. Compounds often recorded were sesquiterpenes as germacrene D, (*E*)- β -caryophyllene, bicyclogermacrene, α -humulene, spathulenol but also monoterpenes as α -pinene, β -pinene and 1,8-cineole.

So, the present study investigates the essential oil composition from inflorescences, stems and leaves of *S. verticillata* collected in Eastern Austria and compares it to published oil composition data from plants of the Mediterranean region and Western Asia.

2. Materials and methods

2.1. Plant material

The plant materials were collected during summer in 2019 and 2021 in Lower Austria on four sites (Siegenfeld SM N 48°02′13′′ E 16° 10′22′′, 350 m; SF N 48°02′49 ′′ E 16° 09′30′′, 416 m, 4 August 2019; Gießhübl G: N 48°06′14′′ E 16°13′05′′, 500 m, 22 August 2021 and Hinterbrühl H: N 48°05′24′′ E 16°15′55′′, 303 m, 25 August 2021) during fully flowering. From the site SM 4 samples were taken at different dates (SM1: 12 June 2019; SM2 and SM3: 30 June 2021 and SM4 12 September 2021).

Three collecting sites were semi-dry meadows and one forest clearing (SF). The plants were separated in stems, leaves and inflorescences and dried in a room in ambient air. The 'Exkursionsflora für Österreich, Liechtenstein und Südtirol' was used to identify the plants (16). Voucher specimens (WU 0157713 for SF, WU0157714 for SM, WU0155898 for G and WU155899 for H) were deposited in the Herbarium of the University of Vienna (WU Generale, https://www.jacq.org/#database).

2.2. Hydrodistillation

For hydrodistillation a Clevenger type apparatus was used. Ten g of leaves or inflorescences or fifteen g of cut stems were distilled with 400 mL water for two hours. The amount of essential oil obtained was read on the capillary of the device. $1000 \,\mu\text{L}$ of hexane were used to wash the

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essential oil fraction out of the apparatus. These fractions were stored at $4-6^{\circ}$ C until analysis within the next days.

2.3. GC-MS and GC-FID

An Agilent Technologies 7890A gas chromatograph equipped with a 5975 C quadrupole mass selective detector (MSD) and a CTC-PAL autosampler (Agilent Technologies, Santa Clara, CA, USA) as described in Chizzola et al., 2018 (17) was used with slight modifications. The separation was done on a $30 \text{ m} \times 0.25 \text{ mm}$ fused silica column coated with 0.25 µm HP5-MS. The temperature program of the oven started isothermal at 50°C for 1 minute, then increased to 220°C at a rate of 5 °C/min and finally further to 280°C to a rate of 15 °C/min. The injector temperature was set at 250°C and the split at 20:1. The injection volume was 1 µL. The total ion current (m/z 40 to 400) from the MSD was used to identify the compounds according to their mass spectra in comparison to the spectral libraries Wiley 275 and NIST20 and their retention indices relative to the n-alkanes (18). Prior analyses hexadecane in hexane as internal standard was added to the samples resulting in a final hexadecane concentration of 0.2 mg/mL. For GC-FID analysis an Agilent Technologies 8690N gas chromatograph was available operated with the same type of column and temperature program as indicated above. The detector was operated at 250°C and supplied with 30 mL/min H₂ and 300 mL/min air. The FID signal without any correction was used to calculate percentage compositions.

2.4. Statistical analysis:

A hierarchical cluster analysis (HCA) and a principal component analysis (PCA) were calculated with SPSS for Windows version 29.0 (SPSS Inc., Chicago, USA). The HCA calculated with 21 samples the squared euklidian distance using linkage between groups based on 17 essential oil compound percentages (bicyclogermacrene, (*E*)- α -bisabolene, β -bourbonene, δ -cadinene, α cadinol, (*E*)- β -caryophyllene, caryophyllene oxide, β copaene, β -elemene, eudesma-4(15),7-dien-1 β -ol, (*E*)- β -farnesene, germacrene D, hexahydrofarnesylacetone, α -humulene, γ -muurolene, α -muurolol, salvial-4(14)-en-1-one, spathulenol). For the PCA the same compounds were taken as variables.

3. Results and discussion

3.1. Essential oil composition and comparison of the plant parts

Salvia verticillata is a Salvia species low on volatile oils. Actually, inflorescences gave about 0.2 % essential oil, leaves 0.1 % while stems had than 0.01% volatiles. Literature reports on essential oils in the aerial parts range from less than 0.05% (10) to 0.3% (13). Essential oil yields decreasing from inflorescences to leaves to stems could also be observed in various other plants as *Achillea collina* (19) or *Melissa officinalis* (17). The wide range of essential oil yields depends on the developmental stages of the plants with different dry matter ratios of stems to leaves to inflorescences. Consequently, plants having a high stem proportion resulted in low essential oil production.

Typical chromatograms of the essential oils from stems, leaves and inflorescences are displayed in figures S1-S3 (supplementary material). Germacrene D was the predominant compound giving median values of 54.0, 38.9 and 21.8 % in the essential oil from leaves, stems and inflorescences, respectively. The highest germacrene D level was found in the leaf oil from 2019 Siegenfeld forest plants (SF 73.7%), the lowest in the inflorescence oils from SM2 plants (11.6%). Literature reports generally a lower germacrene D content in the essential oils from the aerial parts than the present research, ranging from absent (3,20) to 48 % (11).

(*E*)- β -Caryophyllene ranged from 10.5 to 30.2% in the inflorescence, from 3.0 to 10.8 % in the leaf and from 4.6 to 18.1 in the stem essential oils. (*E*)- β - Caryophyllene was the main compound in EOs from Iran reaching percentages of 15–25 % (8,14,15,20) or Serbia with 13.3 % (9).

Actually, bicyclogermacrene was highest in inflorescence oils from 4.5–22.0 % while stems and leaves essential oils contained 0.5 to 8.8 % bicyclogermacrene, the lowest values were in plants from Gießhübl. Literature reports bicyclogermacrene from absent (3,11,20) to 6 % (8,14) to 14.4% (12) to 16.7% (11).

α-Humulene ranged from 5.0 to 15.2 % in inflorescences, from 2.1 to 9.1 % in stems and from 1.0 to 5.0 % in leaves essential oils. α-Humulene contents in *S. verticillata* essential oils from other studies were reported to vary from absent (3,13) to 3–5% (9,12) to 7–10 % (8,11,14,20). Levels up to about 1–2% were found for (*E*)-α-bisabolene, β-bourbonene, δ-cadinene.

Amongst the oxidised sesquiterpenes spathulenol was the most prominent. In inflorescences oil it ranged from 2.8 to 8.9 % and up to 5.5 % in stems or up to 3.9 % in leaves oils. Previous records of spathulenol in *S. verticillata* essential oils mentioned from absent

(10,20) to 6–7% in samples from Iran (8,15) and Serbia (11) to 10% (13) or even to 31% (3) in Turkey. Caryophyllene oxide was in most samples between 1 and 3 %. In stem and leaf essential oils, α -cadinol and eudesma-4(15),7-dien-1 β -ol levels were around 1.5 to 3.2 %, in inflorescences these two compounds had lower levels.

The present *S. verticillata* essential oils were poor in monoterpenes. *p*-Cymene ranged from absent to 2.7 %. Several further monoterpenes as sabinene, β -pinene, myrcene and the ocimenes occurred in the essential oils, their percentage was in each case usually less than 1 %.

One stem oil sample (SM1) showed 38.9% hexadecenoic acid while all other samples were devoid of this compound. In contrast to the present results, the aerial parts from plants collected in Greece very low in essential oil was composed of monoterpenes and lauric acid isopropyl ester and had nearly no sesquiterpenes (10). Further minor compounds listed in Table 1.

On the Siegenfeld meadow site samples were taken in June (SM1, SM2 and SM3) and September (SM4). As some differences in essential oil composition occurred already in two samples (SM2 and SM3) taken on the same date the present data set does not allow to point out a seasonal influence on essential oil production. From July to September the essential oil composition from the aerial parts remained remarkably constant (12). These authors report in leaves 38 % germacrene D but its absence in the inflorescences. Instead, their inflorescences had 10% γ - murolene, which in the present study was usually under 0.5%. Two compounds, 1,8-cineole (10.4%) and δ -elemene (7.4%), present in a flower SPME fraction from Italy (12) could not be

Table 1. Percentual Composition (%) of the essential ons obtaind from innorescences, leaves and sterns of salvia
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	PLovp	DI lit	Inflor. Median	Inflor.	Inflor.	Leaves Median	Leaves	Leaves	Stems Median	Stems Min	Stems Max
- Dia sa s	02C	022		IVIIII			101111		Meulan	101111	IVIAA
a-Pinene Sabinana	930	932	< 0.05	<0.0E	0.2	< 0.05		0.3			
Sabinene 9 Dinono	970	969	0.2	< 0.05	0.7	< 0.05		0.4			
p-rinene Murcono	9/9	974	0.5	< 0.05	1.0	< 0.05		0.2			
nyrcene z Dhallan dran a	995	900	0.1	<0.05	0.0	0.5		0.5			
	1005	1002	0.1	<0.05	0.5	< 0.05	-0.05	0.2	-0.05		1 2
p-Cymene 9 Dhallan dran a	1032	1020	1.2	< 0.05	2.0	1.3	<0.05	Z./	<0.05		1.5
p-Pheliandrene	1030	1025	0.0	< 0.05	0.7	< 0.05	-0.05	1.1	-0.05		0.2
(Z)-p-Ocimene	1042	1032	0.7	< 0.05	1.2	0.6	< 0.05	1.0	< 0.05		0.3
(E)-p-Ocimene	1052	1044	0.6	< 0.05	1.5	0.7	<0.05	1.1	<0.05		0.3
Linalool	1100	1095	0.1	< 0.05	0.5	<0.05		0.1	0.2	.0.05	0.6
Nonanai Biavala alamana	1105	1100	0.1	< 0.05	0.2	0.0	.0.05	0.2	0.3	<0.05	0.6
Bicycloelemene	1342	1336^	0.5	< 0.05	1.0	0.2	< 0.05	0.3	<0.05		0.4
a-Copaene	1380	13/4	0.1	< 0.05	0.5	0.1	< 0.05	0.5		0.05	
β-Bourbonene	1389	1387	0.7	0.1	1.8	1.6	0.7	2./	2.3	< 0.05	5.4
β-Elemene	1396	1389	0.6	0.3	0.9	1.0	0.7	1.3	0.6	< 0.05	1.3
a-Gurjunene	1415	1409	< 0.05		3.5	<0.05		0.2	0.0	< 0.05	0.3
(E)-β-Caryophyllene	1428	1417	19.2	10.5	30.2	9.5	3.0	10.8	12./	4.6	18.1
β-Copaene	1435	1430	0.5	< 0.05	1.7	0.8	0.6	1.7	1.0	< 0.05	2.3
Aromadendrene	1445	1439	0.1	< 0.05	0.5	< 0.05	0.0	0.4	< 0.05	< 0.05	0.4
Isosativene	1457	1441–54*	0.0	< 0.05	0.3	0.3	0.0	0.3	< 0.05	< 0.05	0.7
a-Humulene	1461	1452	10.9	5.0	15.2	4.4	1.0	5.0	6.2	2.1	9.1
<i>(E)</i> -β-Farnesene	1466	1454	0.5	<0.05	1.8	<0.05		0.3	<0.05		0.6
cis-Muurola-4(15),5-diene	1473	1465	0.1	<0.05	0.6	<0.05		0.2			
trans-Cadina-1(6),4-diene	1475	1475	<0.05		0.6	<0.05		0.8			
γ-Muurolene	1483	1478	0.3	<0.05	1.9	0.4		0.5	0.4	<0.05	1.7
Germacrene D	1489	1484	21.8	11.6	39.3	54.0	44.0	73.7	38.9	29.7	55.5
<i>(E,E)-</i> α-Farnesene	1494	1505	<0.05		2.2						
Bicyclogermacrene	1505	1500	19.1	4.5	22.0	5.5	0.5	7.2	6.9	0.5	8.8
MG 202	1514		3.0	<0.05	4.2	2.3	<0.05	4.7	2.0	<0.05	3.7
δ-Cadinene	1529	1522	0.5	0.4	1.9	1.2	1.0	2.7	1.1	<0.05	2.4
<i>(E)</i> -α-Bisabolene	1548	1532–44*	0.4	<0.05	3.6	0.2	<0.05	2.0	0.6	<0.05	2.8
Spatulenol	1583	1577	4.6	0.9	8.9	2.0	<0.05	3.9	4.2	<0.05	5.5
Caryophyllene oxide	1591	1582	1.6	<0.05	2.7	0.8	<0.05	1.1	1.2	<0.05	3.1
Salvial-4(14)-en-1-one	1599	1594	0.3	<0.05	1.5	0.5	<0.05	0.8	0.4	< 0.05	1.3
Isospathulenol	1647	1638–44*	0.3	< 0.05	0.9	0.2	<0.05	0.3	0.0	< 0.05	0.6
a-Muurolol	1650	1644	0.1	<0.05	0.8	0.5	0.3	0.8	0.7	< 0.05	1.3
α-Cadinol	1661	1652	0.6	0.2	1.3	1.9	1.5	2.8	1.7	< 0.05	3.5
Eudesma-4(15),7-dien-1β-ol	1693	1687	0.7	< 0.05	2.3	1.7	0.8	3.2	2.1	< 0.05	5.4
Mintsulfide	1757	1740	< 0.05		0.1	< 0.05		1.0	0.0	< 0.05	1.1
Hexahydrofarnesya-lacetone	1848	1838–47*	1.1	0.3	2.7	0.3	<0.05	0.5	1.1	0.4	5.7
Hexadecanoic acid	1962	1959							<0.05		38.9

RI lit.: Retention indices from literature Adams 2007 or NIST *.

MG 202: M/Z 133 (100), 105 (83), 91 (74), 159 (58), 202 (45), 131 (40), 117 (29), 77 (27), 41 (27), 115 (25), 129 (23), 79 (21).

found in the present study. But when the essential oils of whole aerial parts were analysed these two compounds could not be found (12).

Germacrene D and (E)- β -caryophyllene are widespread sesquiterpenes in essential oils. The essential oils at flowering and fruiting stage from *Ballota nigra* had a similar composition and contained these two compounds additionally to caryophyllene oxide as the main compounds (21). *Salvia nemorosa* is another *Salvia* species low in essential oil. Plants from this species collected in the same region as in the present study had (E)- β -caryophyllene and Germacrene D as main compounds in their leaf oils while the inflorescence oils contained beside these compounds the monoterpene sabinene as main compounds (22).

In contrast an analysis of the head space volatiles from aerial parts of plants collected in Serbia revealed that β -phellandrene accounted for 44–70% of the volatile compounds (23). As in the present research Giuliani et al (2018) found the sesquiterpenes germacrene D, (*E*)- β -caryophyllene and bicyclogermacrene as main compounds in the essential oils from the aerial parts of *S. verticillata* but additionally some monoterpene hydrocarbons as β -phellandrene. In the headspace SPME fraction they found 1,8-cineole (12).

3.2. Multivariate data analyses

To detect similarities between the samples a hierarchical cluster analysis (HCA) has been performed. The

resulting dendrogram is displayed in Figure 1 and shows two main clades. The upper one contains six leaf samples and one stem sample, characterized by their high germacrene D content. The lower clade can be divided in two cluster, one grouping five inflores-cence samples with 16–24% (*E*)- β -caryophyllene and 13–22% bicyclogermacrene and a more heterogenous cluster containing all stem samples, one leaf sample and two inflorescence samples.

Additionally, relations between the individual oil compounds and their repartition on the samples were further evaluated using a principal component analysis (PCA, Figure 2). In this multivariate approach 17 essential oil compounds as variables resulted in 5 components having eigenvalues greater than 1 accounting for 88% of the variance. The first axis accounted for 39.9% and the second for 20.9% of the variance. The first component was positively correlated with the main compounds ahumulene, (E)- β -caryophyllene and bicyclogermacrene. A negative correlation occurred with germacrene D and minor compounds as β -bourbonene, β -copaene, δ cadinene, α -cadinol and eudesma-4(15),7-dien-1 β -ol. The second axis differentiated between high positive loadings of spathulenol and caryophyllene oxide in contrast to the high negative loadings of germacrene D and βelemene.

The PCA scoring plot of the samples in Figure 3 roughly separated the plant organs: Leaves having high levels of germacrene D were placed in the lower left quadrant corresponding to negative



Figure 1. Dendrogram Showing the Similarities Between the Essential Oils from the Plant Parts from the Different Sites. The Sample Code Consisted of the Site Designation and the Plant Part (I: Inflorescence, L: Leaves, S: Stems).



Figure 2. Loading Plot of the Essential Oil Compounds: ACadol: α-Cadinol, Bcaryo: (*E*)-β-Caryophyllene, Bcop: β-Copaene, BElem: β-Elemene, BFarn: (*E*)-β-Farnesene, BiGer: Bicyclogermacrene, Bour: β-Bourbonene, CarOx: Caryophyllene Oxide, Dcadi: α-Muurolol (δ-Cadinol), Eudes: Eudesma-4(15),7-Dien-1β-OI, GerD: Germacrene D, Gmuu: γ-Muurolene, HHFar: Hexaxydrofarnesylacetone, Humul: α-Humulene, MG202: A Sesquiterpene Hydrocarbon, Salv: Salvial-4(14)-En-1-One, Spat: Spathulenol.



Figure 3. Score Plot of the Essential Oil Samples According to the Collecting Sites and Plant Parts Analysed.

component 1 and component 2 loadings. Inflorescences with higher (*E*)- β -caryophyllene, bicyclogermacrene and α -humulene levels were on the right side with positive component 1 scores and stems low in germacrene D but higher in spathulenol, β -bourbonene and eudesma-4(15),7-dien-1 β -ol in the upper part with positive component 2 scorings. Stems which are very low in essential oil and present therefore a higher variability in the relative amounts of the main compounds were placed in all four quadrants.

Altogether, the present EOs with their high germacrene D contents similar to those from Italy (12) and some populations of Serbia (11,18).

4. Conclusion

In conclusion, the essential oil composition of the present samples collected in the restricted area southwest of the city of Vienna appeared to be rather homogenous. A differentiation between the oils from the different plant part was feasible to a large extent. The fingerprint with the four main essential oil compounds germacrene D, (E)- β -caryophyllene, bicyclogermacrene and α humulene might be characteristic for the species in this region.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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