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Original Scientific Paper

A chemometric approach to the headspace sampled volatiles of selected *Salvia* species from Southeastern Serbia

Emilija Kostić[1*], Dušanka Kitić[1], Maja Vujović[1], Marija Marković[2], Aleksandra Pavlović[3] and Gordana Stojanović[3]

1 University of Niš, Faculty of Medicine, Department of Pharmacy, Niš, Serbia
2 University of Niš, Faculty of Sciences and Mathematics, Department of Biology, Niš, Serbia
3 University of Niš, Faculty of Sciences and Mathematics, Department of Chemistry, Niš, Serbia
* Correspondence: emilija293@gmail.com

ABSTRACT:
Headspace sampling is a fast, simple and economical way to prepare plant samples for analysis by gas chromatography. For the first time, the composition of the head space volatiles (HSV) of six *Salvia* species (*S. verticillata, S. glutinosa, S. nemorosa, S. aethiopis, S. amplexicaulis* and *S. officinalis*) in the flowering stage and two (*S. glutinosa* and *S. sclarea*) in the fruiting stage from Southeastern Serbia was analysed using the GC-FID-MS technique after headspace sampling. The chemical composition of the highly volatile compounds of the analysed species varies considerably. Monoterpene hydrocarbons represented the dominant class of volatile compounds in all the *Salvia* species, except for *S. sclarea* and *S. aethiopis*. The content of sesquiterpenes was the highest in *S. aethiopis* (96.9%) and *S. glutinosa* in the flowering phase (29.5%), while in all the other samples that percentage was below 10%. Oxygenated monoterpenes were the most abundant in *S. sclarea*, where the main component was oxygenated monoterpene linalyl acetate (97.7%). The main component of *S. verticillata* was β-phellandrene, and its content varied depending on the plant location and sampling time. The main component of *S. glutinosa* in the flowering phase was limonene (16.6%), and in the fruiting phase sabinene (87.1%). Headspace analysis of the volatile components of *S. aethiopis* was carried out for the first time and the most abundant detected components were sesquiterpenes: (E)-caryophyllene (36.8%), α-copaene (33.4%) and β-elemene (7.3%). The analysis of the principal components was performed to interpret the grouping patterns, as well as to analyse the similarities and differences between the samples in terms of the composition of the volatile components. The samples were grouped into three clusters. The first cluster consisted of samples of *S. verticillata* (S1, S4 and S5) from different locations, the second comprised samples of S. *glutinosa* (S3), *S. aethiopis* (S8), *S. amplexicaulis* (S9) and *S. officinalis* (S10), while samples of *S. nemorosa* (S7) made up the third cluster. The HS-GC-FID-MS technique can be successfully used for the qualitative and quantitative analysis of volatile compounds of different *Salvia* species. The obtained results are important for evaluating the possibility of using different types of sage.

Keywords:
Lamiaceae, HS sampling, PCA, cluster analysis, terpenes

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INTRODUCTION

The genus *Salvia* L. (sage) with approximately 900 species is the largest genus of the Lamiaceae family. Numerous species of this genus are to be found in the tropical and subtropical regions of both hemispheres, and many species inhabit the Mediterranean region. Annual or perennial plants, semi-shrubs and shrubs are often aromatic due to the presence of essential oils. In the flora of Serbia, there are 14 species of this genus (Diklić 1974). The *Salvia* species from the South East region of Serbia are *S. verticillata* L., *S. glutinosa* L., *S. sclarea* L., *S. nemorosa* L., *S. aethiopis* L., *S. amplexicaulis* L. and *S. officinalis* L., which were investigated in this paper. In addition to these species, the following wild-growing species are also present in Serbia: *S. argentea* L., *S. austriaca* Jacq., *S. nutans* L., *S. pratensis* L., *S. virgata* Jacq., *S. viridis* L., and *S. ringens* Sibth. & Sm. Five species are used in traditional Serbian medicine in different forms: *S. glutinosa*, *S. nemorosa*, *S. officinalis*, *S. pratensis* and *S. sclarea* (Sarić 1989). *Salvia officinalis* and *S. sclarea* are referred to as aromatic in a monograph on the aromatic plants of Serbia (Jančić *et al*. 1995). The genus *Salvia* is recognized worldwide for its medicinal and cultural importance, primarily due to its essential oils. The biological and pharmacological roles of the essential oils of the genus *Salvia* are due to the content of secondary metabolites-terpenes (Blumenthal 1998; Božin *et al*. 2007).

According to European Union herbal monographs, the therapeutic applications of the most famous investigated species *S. officinalis* are gastrointestinal disorders, excessive sweating, mouth and throat disorders, skin disorders and minor wounds (European Medicines Agency 2016). The aerial parts of *S. officinalis* are used to treat inflammation, skin and mucus membrane infections, abdominal pain, and excessive sweating in folk medicine in Europe, Asia and Latin America (Ghorbani & Esmaeilizade 2017). Extracts (96% ethanol, 50% ethanol and hot distilled water) of *S. officinalis* collected in Montenegro showed high phenolic content, and high antioxidant, enzyme-inhibiting and cytotoxic activities (Duletić-Laušević *et al*. 2019). Most of the data on the essential oils and antimicrobial activity of *S. officinalis* were obtained in Serbia (Ivanić & Savin 1976; Couladis *et al*. 2002; Veličković *et al*. 2003). The leaves of *S. verticillata* have been traditionally used in cardiovascular disease treatments in the form of decoction in the Kırklareli Province (Turkey) (Kültür 2007), in infusions and decoctions for constipation, colds and nausea in East Anatolia, Turkey (Altunddnag & Ozturk 2011), and infusions for abdominal pain in traditional medicine in Central Anatolia, Turkey (Sezik *et al*. 2001). The leaves of *S. sclarea* are used in infusions to treat the common cold in East Anatolia, Turkey (Altunddnag & Ozturk 2011), as well as anxiety and abdominal pain in the western part of the Central Taurus Mountains (Ozdemir & Alpinar 2015). The anti-inflammatory effect of the *S. sclarea* ethanol extract of aerial parts on lipopolysaccharide-induced periodontitis in rats is also proven in Serbia (Kostić *et al*. 2017). *Salvia nemorosa* herb decoctions and infusions are used to treat the common cold and catarrh in Turkey (Altuddnag & Ozturk 2011). The ethanol extract of the aerial parts of *S. glutinosa* and *S. aethiopis* have strong antimicrobial activity (Veličković *et al*. 2002). The ethanol and water extracts of *S. amplexicaulis* herb showed anti-neurodegenerative effects and tyrosinase inhibition (Alimpić *et al*. 2017).

Headspace (HS) sampling is a fast and cost-effective method for preparing solid, liquid and gas samples for gas chromatography (GC) analysis. It is an effective method for the rapid screening of samples because it provides valuable information about the presence of HSV compounds. It can provide essential data about the value of collected plants and whether they contain HSV or HSV with toxic effects. HS analysis offers a potentially rapid method for the extraction of volatiles and requires small quantities of the plant material. The drawback of HS analysis is complete recovery only for highly volatile materials (Farouk *et al*. 2019).

To the best of our knowledge, there is no published data on the HSV composition of the investigated *Salvia* species from Southeastern Serbia isolated from plant material by HS sampling. This is the first published data about the HSV composition of *S. aethiopis*. This paper aimed to determine the chemical composition of HSV from the aerial parts of *Salvia* spp. from Southeastern Serbia (*S. verticillata, S. glutinosa, S. sclarea, S. nemorosa, S. aethiopis, S. amplexicaulis* and *S. officinalis*) using headspace sampling and GC-FID-MS analysis.

MATERIALS AND METHODS

Plant material. The aerial parts of the plants were collected (about 100 g) from each investigated species from Southeastern Serbia during the summer of 2018. The identification of the plant material followed Diklić (1974). The voucher specimens were deposited in the Herbarium Moesiacum Niš (HMN) (Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš). Detailed information about the collected plant material is given in Table 1 (location, GPS latitude, GPS longitude, voucher number, date of harvesting). The plant material was dried in a dark location at ambient temperature for a week.

HS sample preparation. For the static headspace experiments, 500 mg of milled dry plant material was placed into 20 mL HS vials, and then soaked with 2 mL of distilled water (Ickovski *et al*. 2020; Jovanović *et al*. 2020). The samples were heated at 80°C for 20 min with the following mixing programme: shaking for 5 s, pause for 2 s. The vapour generated from the specimens was

Table 1. Location, date of harvesting and voucher numbers of the analysed *Salvia* spp.

Sample	Species	Location	Latitude	Longitude	Date of harvesting	Voucher number
S1	*Salvia verticillata* (a.p., flowering stage, w.g.)	Bojanine vode, Suva Planina	43.22171	22.11134	09/19/2019	13867
S2	*Salvia glutinosa* (a.p., fruiting stage, w.g.)	Bojanine vode, Suva Panina	43.22171	22.11134	09/19/2019	13868
S3	*Salvia glutinosa* (a.p., flowering stage, w.g.)	Arbinje, Stara Planina	43.30175	22.78151	08/01/2019	13964
S4	*Salvia verticillata* (a.p., flowering stage, w.g.)	Seličevica	43.236073	21.96909	08/01/2019	19693
S5	*Salvia verticillata* (a.p., flowering stage, w.g.)	Temska, Stara Planina	43.26257	22.55272	06/27/2019	13687
S6	*Salvia sclarea* (a.p., fruiting stage, w.g.)	Čiflik, Belava	43.22362	22.40379	07/06/2019	13688
S7	*Salvia nemorosa* (a.p., flowering stage, w.g.)	Vidlič. Basara	43.13724	22.81885	07/06/2019	13691
S8	*Salvia aethiopis* (a.p., flowering stage, w.g.)	Vidlič, Basara	43.13724	22.81885	08/16/2019	13692
S9	*Salvia amplexicaulis* (a.p., flowering stage, w.g.)	Temska, Stara Planina	43.26257	22.55272	06/27/2019	13686
S10	*Salvia officinalis* (a.p., flowering stage, g.s.)	Blato, near Pirot	43.14750	22.49754	07/06/2019	13865

a.p. - aerial parts, w.g. - wild-growing, g.s. - garden specimen

removed from the vials using a gas-tight syringe (90°C) and injected directly into the chromatographic column. All the samples were run in triplicate.

Gas chromatography and gas chromatography-mass spectrometry analysis. GC-MS analyses were performed on an Agilent 7890 gas chromatograph with a 7000B GC-MS-MS triple quadrupole system, operating in MS1 scan mode, and equipped with a fused-silica capillary column Agilent HP-5 MS (30 m × 0.25 mm i.d. × 0.25 m film thickness). The chromatographic analyses were carried out under the following conditions: carrier gas at a flow rate of 1.0 mL/min, the GC oven temperature was maintained at 45°C for 2.25 min and programmed to 290°C at a rate of 4°C/min, the split ratio was adjusted at 40:1, injection volume 1 µL. Post-run: back flash for 1.89 min at 280°C, with helium pressure of 50 psi. The injector temperature was set at 230°C. The ionisation mode was electronic impact at 70 eV. The mass range was set from 40 to 440 Da. The same column and chromatographic conditions were applied for the GC-FID analysis. The FID temperature was 300°C (Jovanović *et al*. 2020).

Identification of components. The components were identified by a comparison of their mass spectra with those of Wiley 6, Adams 2007 and NIST 11, applied on Agilent Mass Hunter Workstation (B.06.00) and AMDIS (2.1, *DTRA*-NIST, 2011) software and confirmed by comparing their calculated retention indexes (relative to C8-C40 n-alkanes) with the Adams 2007 retention indices.

The experimental linear retention indices relative to C8–C40 alkanes on the HP-5MS were computed as:

$I_x = 100n + 100(t_x - t_n) / (t_{n+1} - t_n)$

I_x – the examined compound retention index

n – the number of C-atoms of the alkane eluted immediately before the examined compound

t_x – the retention time of the examined compound

t_n – the retention time of the alkane eluted immediately before the examined compound

t_{n+1} – the retention time of the alkane eluted immediately after the examined compound.

The percentage composition of HSV was computed from the GC-FID peak areas without any corrections. Three samples were taken for each species. The mean values are shown in Table 2.

Statistical data analysis. Principal component analysis (PCA) and agglomerative hierarchical clustering (AHC) of the obtained data were carried out using XLSTAT 2018.5.52459 (Addinsoft 2018). PCA and cluster analysis were used to establish the similarities and the differences between the HSV components of the examined species of the *Salvia* genus. The cluster analysis was performed using Ward's method. The Euclidean distances are presented as the ratio (Dlink/Dmax) ×100, where Dlink is the distance between the variables which are grouped and Dmax is the maximum distance between the variables.

RESULTS AND DISCUSSION

Chemical composition of the HSV of the analysed *Salvia* species. The identified HSV of the investigated *Salvia* species with their retention indices and relative percentages are listed in Table 2.

Table 2 shows that 37 HSV were identified, of which terpenes are the most common. The percentage of identified compounds is high and amounts to over 99% of the HSV compounds in the analysed samples. The main components in all the samples are hydrocarbon monoterpenes, with the exception of *S. aethiopis* (S8) and *S. sclarea* (S6). The sesquiterpene content is highest in the samples of *S. aethiopis* (S8) (96.9%) and *S. glutinosa* (S3)

Table 2. The percentage composition of the HS volatiles of *Salvia* spp. from Southeastern Serbia.

RI[a]	AI[B]	Compound [class of compound]	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
852	847	(*Z*)-Salvene [m]	-[c]	-	-	-	-	-	-	-	-	4.2
862	858	(*E*)-Salvene [m]	-	-	-	-	-	-	-	-	-	0.5
924	921	Tricyclene [m]	-	-	-	-	-	-	-	-	-	1.4
928	924	α-Thujene [m]	2.5	-	-	1.6	1.6	-	17.3		10.2	0.2
936	932	α-Pinene [m]	1.9	0.8	1.4	5.2	21.1	-	0.5	1.3	7.7	**23.0**
975	969	Sabinene [m]	5.5	**87.1**	4.9	2.4	1.7	-	**75.8**	0.8	14.2	-
978	974	β-Pinene [m]	3.0	1.0	2.5	3.6	3.6	-	3.5	0.7	-	8.2
979	974	Camphene [m]	-	-	-	-	-	-	-	-	-	21.6
980	974	1-Octen-3-ol [o]	-	-	-	-	-	-	-	-	18.9	-
985	979	3-Octanone [o]	-	-	-	-	-	-	-	-	2.6	-
992	988	Myrcene [m]	6.0	-	10.5	6.6	6.6	-	0.5	-	4.2	1.3
1006	1002	α-Phellandrene [m]	2.8	-	-	1.4	2.0	-	-	-	-	-
1012	1008	δ-3-Carene [m]	0.7	-	0.9	2.2	-	-	-	-	-	-
1019	1014	α-Terpinene [m]	-	-	-	-	-	-	-	-	-	0.2
1026	1022	*o*-Cymene [m]	0.5	-	-	0.9	0.6	-	-	-	**21.1**	1.1
1031	1025	β-Phellandrene [m]	**43.9**	-	-	**70.4**	**55.5**	-	-	-	-	-
1030	1024	Limonene [m]	-	0.5	**16.6**	-	-	-	-	-	11.2	3.3
1032	1026	1.8-Cineole [ox]	-	-	6.5	-	-	-	-	-	-	6.4
1038	1032	(*Z*)-β-Ocimene [m]	10.3	-	14.9	2.6	1.7	-	-	-	-	0.5
1049	1044	(*E*)-β-Ocimene [m]	12.2	-	1.3	1.4	1.0	-	-	-	-	-
1060	1056	γ-Terpinene [m]	-	-	-	-	-	-	-	-	-	0.3
1090	1086	δ-Terpinene [m]	-	-	-	-	-	-	-	-	-	0.2
1100	1095	Linalool [m]	-	10.6	2.1	-	-	2.0	-	-	-	-
1101	1101	(*Z*)-Thujone [ox]	-	-	-	-	-	-	-	-	-	13.9
1118	1112	(*E*)-Thujone [ox]	-	-	-	-	-	-	-	-	-	3.2
1147	1141	Camphor [ox]	-	-	-	-	-	-	-	-	-	8.1
1169	1165	Borneol [ox]	-	-	-	-	-	-	-	-	-	1.0
1258	1254	Linalyl acetate [ox]	-	-	-	-	-	**97.7**	-	-	-	-
1289	1287	Bornyl acetate [ox]	-	-	-	-	-	-	-	-	-	0.2
1380	1374	α-Copaen [s]	-	-	-	-	-	-	-	33.4	-	-
1387	1388	β-Bourbonene [s]	-	-	1.5	-	-	-	-	0.2	-	-
1395	1389	β-Elemene [s]	-	-	-	-	-	-	-	7.3	-	-
1423	1417	(*E*)-Caryophyllene [s]	0.9	-	14.6	1.2	0.3	-	2.2	36.8	-	0.8
1458	1452	α-Humulene [s]	0.2	-	11.8	-	-	-	-	1.5	-	0.2
1484	1478	γ-Muurolene [s]	7.9	-	1.6	-	-	-	-	10.3	-	-
1503	1500	α- Muurolene [s]	0.3	-	-	-	-	-	-	7.2	-	-
1527	1522	δ-Cadinene [s]	-	-	-	-	-	-	-	0.2	-	-
Monoterpene hydrocarbons [m]			89.3	89.3	61.8	97.7	98.8	0	97.4	2.8	57.0	59.3
Oxygenated monoterpenes and their acetyl derivates [ox]			0	10.5	8.6	0	0	99.7	0	0	0	32.6
Sesquiterpenes hydrocarbons [s]			9.3	0	29.5	1.2	0.3	0	2.20	96.9	0	1.0
Other [o]			0.5	0	0	0.9	0.6	0	0	0	42.6	5.8
Score			99.1	99.8	99.9	99.8	99.7	99.7	99.6	99.7	99.6	99.6

a-RI-experimental linear retention indices relative to C8–C44 alkanes on the HP-5MS;
b-AI –RIs correspond to those listed in Adams 2007 unless otherwise stated;
c- not detected; m-monoterpenes, o-oxygenated monoterpenes, s-sesquiterpenes

(29.5%) in the flowering stage, while in the other species it is below 10%. Oxygenated monoterpenes were present in *S. sclarea* (S6) (99.7%), *S. officinalis* (S10) (32.6%) and in both samples of *S. glutinosa* (S2) (10.5%) and (S3) (8.6%). Non-terpenic aliphatic compounds (3-octanone and 1-octen-3-ol) were present only in the *S. amplexicaulis* samples (S9). The terpenes present in almost all the samples in the flowering stage are α-pinene, myrcene, (*E*)-caryophyllene and sabinene. β-pinene (antifungal, antibacterial properties) was present in all the samples, except in *S. amplexicaulis* (Salehi *et al.* 2019). Myrcene and (*E*)-caryophyllene were absent only in *S. aethiopis*, while sabinene was not present in *S. officinalis*. Sabinene is an unsaturated terpene with a spicy, woody smell. It also possesses antioxidant, anti-inflammatory, antifungal, and antimicrobial properties (Valente *et al.* 2013).

Only samples of *S. glutinosa* and *S. sclarea* were collected in the fruiting stage. The main component in *S. glutinosa* in the fruiting stage was sabinene (87.1%). In *S. sclarea* it was linalyl acetate (97.7%), which is responsible for the anxiolytic effect (Malcolm & Tallian 2017). It has a pleasant fruity odour reminiscent of bergamot mint oil. The high percentage of linalyl acetate indicates that *S. sclarea* from Southeastern Serbia is a high quality raw material for the perfume industry (Elsharif *et al.* 2015). It is mildly toxic to humans, harmful to fish, and highly toxic to daphnia. This is a major component of the essential oil of this plant (Pešić & Banković 2003).

The only species collected and analysed in both the fruiting and flowering stages was *S. glutinosa*. A comparison of the HSV fraction of *S. glutinosa* in its flowering and fruiting stages showed that the main component in the flowering stage was limonene (16.6%) (repellent, sedative effects), while in the fruiting phase it was sabinene (87.1%) (Maia & Moore 2011). Sabinene is also found in the essential oil of *S. glutinosa* in the fruiting phase (Veličković *et al.* 2003). The effect of collection time on chemical constitution and biological activity is highly significant (Tulukcu 2009).

Samples of *S. verticillata* were collected from three different locations in Southeastern Serbia. The main component of the volatile fraction of *S. verticillata* was β-phellandrene (anti-inflammatory, antibacterial and anti-hyperalgesic effects), and the content varied depending on the collection locality (Nasermoadeli *et al.* 2013; Tabanca *et al.* 2017). The content of β-phellandrene in the samples of *S. verticillata* from different locations ranged from 43.9% to 70.4%. The chromatogram of a sample of *S. verticillata* from the Bojanine vode site in the flowering stage is presented in Fig. 1. Other chromatograms are provided as supplementary material.

The HSV fraction of *S. officinalis*, the best-know species of the genus, had the highest content of α-pinene (23%) (antibacterial, antifungal effects) (İşcan *et al.* 2012), camphene (21.6%) (antioxidative effect) (Quintans-Júnior *et al.* 2013), *cis*-thujone (13.9%) (convulsant) (Halicioglu *et al.* 2011) and camphor (8.1%) (decongestant, rubefacient) (Zuccarini 2009). Among the 37 detected compounds, numerous terpenes with important biological activities were present in *S. officinalis* alone: (*E*)- and (*Z*)-salvene, γ- and δ-terpinene, (*E*)- and (*Z*)-thujone, camphor, borneol and bornyl acetate.

The HSV fraction of *S. aethiopis* was analysed for the first time. The most abundant terpenes in *S. aethiopis* are sesquiterpenes (96.9%), and the rest are monoterpenes. Similar results were obtained by Veličković *et al.* (2003), who investigated the essential oil of this plant using a different HSV isolation method. In the HSV fraction of the *S. aethiopis* sample, the main component was *E*-caryophyllene (36.8%), showing anti-inflammatory and antioxidative effects (Scandiffio *et al.* 2020). Among the 37 detected compounds in all the analysed samples, several compounds were present only in *S. aethiopis:* α-copaene (33.4%), β-elemene (7.35%) and δ-cadinene (0.2%). α-Copaene has been proven to increase antioxidant capacity in human lymphocyte cultures (Turkez *et al.* 2014a). Furthermore, it reduces cell proliferation in N2a neuroblastoma cell lines, so further studies of α-copaene as potential anticancer agent were proposed (Turkez *et al.* 2014b). β-elemene is another important sesquiterpene which exhibits anti-proliferative effects on cancer cells, while being less toxic than widely accepted chemotherapeutics (Ziyu *et al.* 2017). Were these activities and the safeness of α-copaene and δ-elemene to be confirmed by other studies, *S. aethiopis* could be used as an important source of these sesquiterpenes.

Rzepa *et al.* (2009) examined the HSV fraction of 20 *Salvia* species growing in Poland by HS–GC–MS. In our study, seven of the same species of *Salvia* (*S. glutinosa, S. officinalis, S. sclarea, S. amplexicaulis, S. verticillata, S. nemorosa*) as in the aforementioned article by Rzepa *et al.* (2009) were examined, and one more species which was analysed for the first time (*S. aethiopis)*, all from Southeastern Serbia. In the samples from Poland, 18 compounds were identified. The main components of the HSV fraction of the *Salvia* species in Poland were identified as α-pinene, β-pinene, camphene, thujol, camphor, β-chamigrene, and cadina-3,9-diene. In the examined *Salvia* samples from the area of Southeast Serbia, 37 compounds were identified, and α-pinene, β-pinene, myrcene and sabinen were present in the largest number of species. The percentage and number of identified compounds in our study is much higher even though a smaller number of species were examined. Thus, although the main HSV are present in the samples from both areas, there are also significant differences.

PCA and cluster analysis of the HS composition of the samples in the same (flowering) phenophase from different species of the *Salvia* genus. PCA analysis of

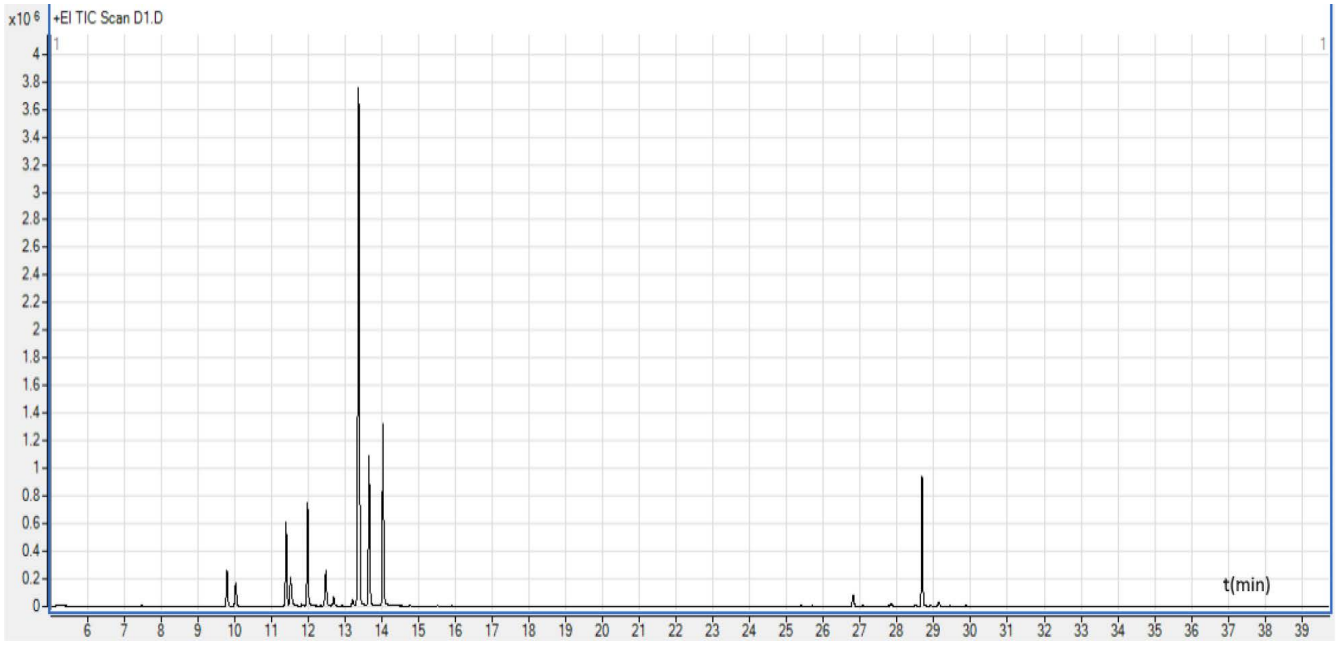

Fig. 1. Gas chromatogram of the volatile fraction determined by means of HS–GC–MS for *Salvia verticillata* (Bojanine vode) in the flowering stage.

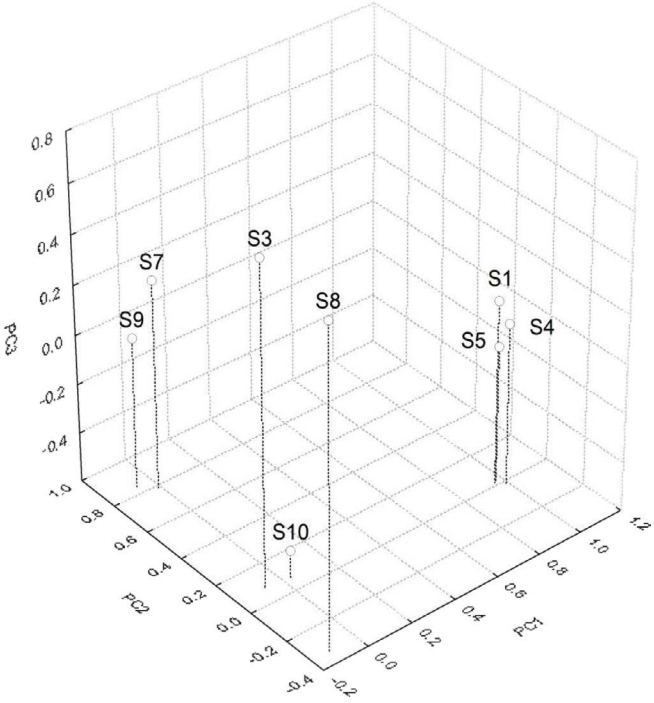

Fig. 2. Three dimensional (3D) PCA map of the samples of *Salvia* species.

the content of the HSV compounds was performed to interpret sample patterns, groupings, similarities, and differences. The principal components with eigenvalues greater than 1 were considered. This led to the formation of three principal components. The first component (eigenvalue 2.861) explains 35.76%, the second (eigenvalue 1.443) 18.04% and the third (eigenvalue 1.283) 16.03% of the total variation of the data. The first three components account for 69.83% of the variance for all of the data (Fig. 2). The PCA biplot indicates the similarities and correlations between the HSV compounds (Fig. 3). From the PCA biplot and the PCA plot for PC1 and PC2 it is obvious that α-phellandrene concentrations are higher for samples S1, S4 and S5 (*S. verticillata*) from various areas of Serbia in the flowering stage. Sabinene concentrations are higher for samples S7 (*S. nemorosa*) and S9 (*S. amplexicaulis*), while the concentrations of the other HSV compounds are higher for samples S3 (*S. glutinosa*), S8 (*S. aethiopis*), S10 (*S. officinalis*) and S9 (*S. amplexicaulis*). The analysed samples of *Salvia* spp. can be classified into three groups based on the HSV compounds. The first group includes three samples of *S. verticillata*, the second comprises samples S7 (*S. nemorosa)* and S9 (*S. amplexicaulis)*, and the third consists of samples S3 (*S. glutinosa),* S8 (*S. aethiopis)* and S10 (*S. officinalis).*

The objective of the cluster analysis was the grouping of the *Salvia* spp. samples based on the content of the analysed HSV compounds. The analysed *Salvia* spp. samples were grouped into three clusters based on the HSV compounds (Dlink/Dmax <50) (Fig. 4). The first cluster is composed of three samples of *S. verticillata*, the second consists of samples S3 (*S. glutinosa*), S8 (*S. aethiopis*), S9 (*S. amplexicaulis)*, and S10 (*S. officinalis)*, while sample S7 (*S. nemorosa)* belongs to the third cluster. The minimum Euclidean distance was recorded between the samples of *S. verticillata* (S1, S4, and S5), so these three samples can be considered to be the most similar in terms of HSV compound content.

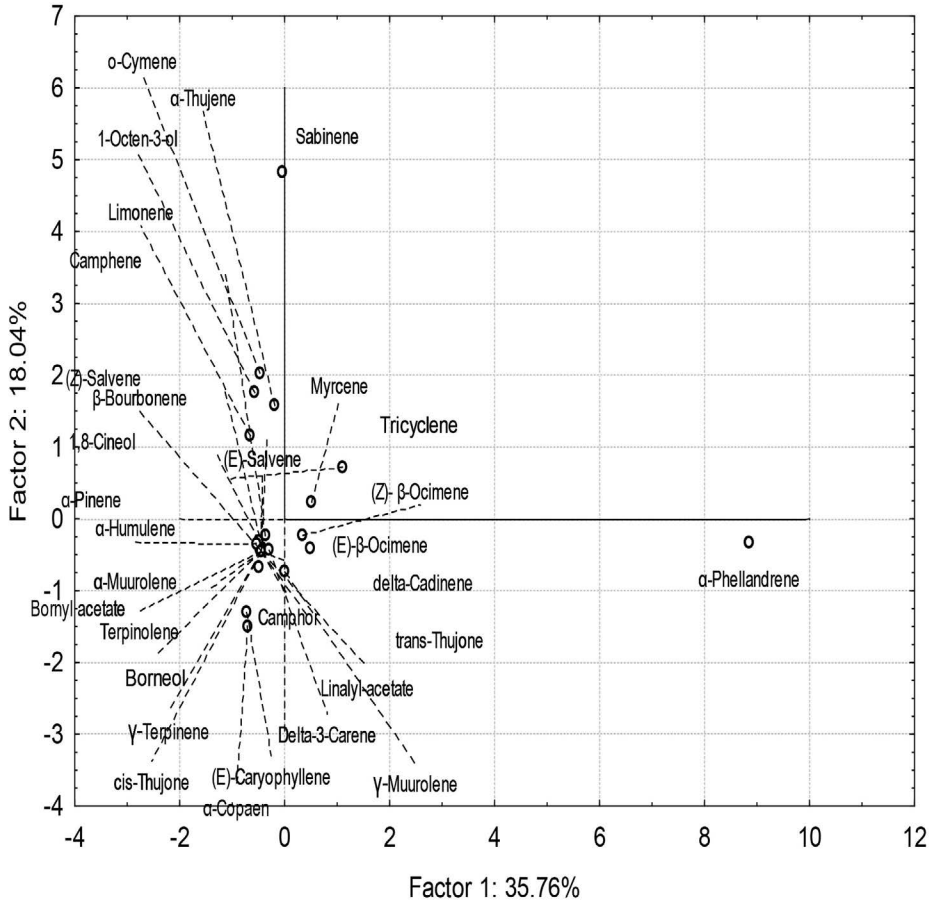

Fig. 3. Principal component analysis (PCA) of the volatile compounds of the samples of *Salvia* species.

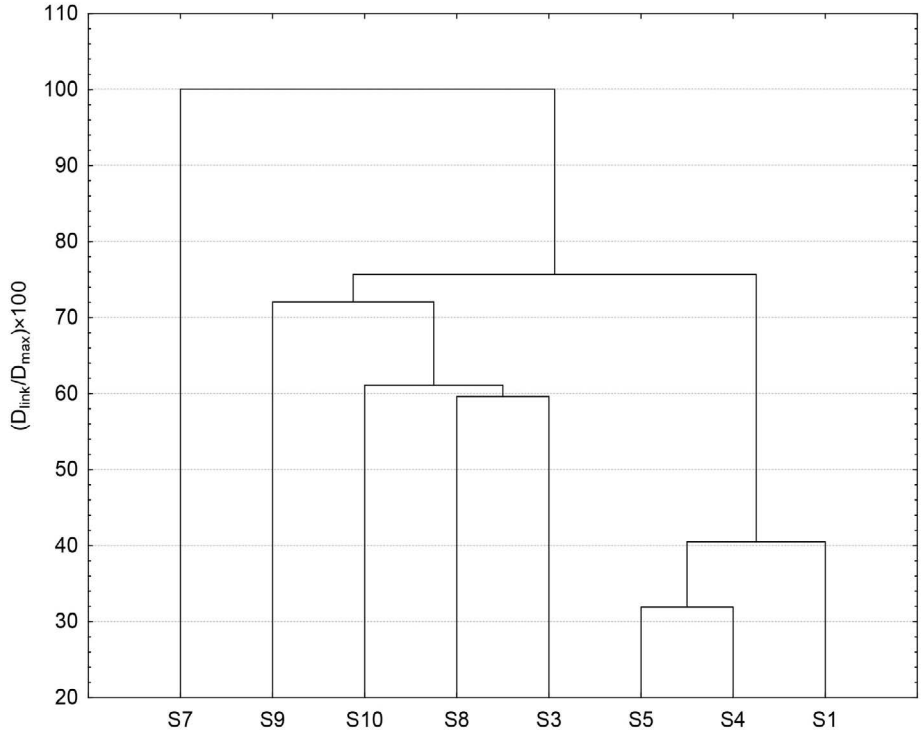

Fig. 4. Dendrogram of HCA (Ward's algorithm) of the samples of *Salvia* species.

The chemical composition of the HSV constituents of *Salvia* species is highly variable, depending on geographic origin, plant part, harvesting, drying, storage, genetic factors and the oil extraction process. Such chemical variability directly affects their biological activity (Nasermoadeli *et al.* 2013; Tabanca *et al.* 2017).

CONCLUSION

This paper provides the results of rapid HS sampling and GC-FID-MS analysis of various HSV components of the *Salvia* species from the area of Southeastern Serbia. *Salvia* species contain numerous compounds with pharmacological activity. The chemical composition of the analysed HSV compounds of the *Salvia* species was very complex, and 37 different HSV compounds were identified. Monoterpene hydrocarbons were the dominant class of HSV compounds in all the investigated *Salvia* species except in *S. sclarea* and *S. aethiopis,* where sesquiterpenes are the most abundant. Oxygenated monoterpenes were present only in four samples: *S. sclarea* (S6), *S. glutinosa* (S2 and S3) and *S. officinalis* (S10). The composition of the volatile components in *S. glutinosa* in the flowering and fruiting phases differ significantly, so we can infer that the effect of collection time is very important. The effect of location was also proved, because the content of β-phellandrene in the samples of *S. verticillata* from different locations ranged from 43.9% to 70.4%. The analytical method was successfully used for the qualitative, quantitative and comparative analysis of the volatile compounds in different *Salvia* spp. More detailed research is necessary to gain a better understanding of the variability in the chemical profile of different *Salvia* species.

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REZIME

Botanica SERBICA

Hemometrijska analiza headspace isparljivih komponenti odabranih vrsta roda *Salvia* iz jugoistočne Srbije

Emilija Kostić, Dušanka Kitić, Maja Vujović, Marija Marković, Aleksandra Pavlović i Gordana Stojanović

Headspace uzorkovanje je brz, jednostavan i ekonomičan način za pripremu biljnih uzoraka za analizu gasnom hromatografijom. Prvi put je izvršena analiza sastava lako isparljivih komponenti šest vrsta roda *Salvia* (*S. verticillata, S. glutinosa, S. nemorosa, S. aethiopis, S. amplexicaulis* i *S. officinalis*) u fazi cvetanja i dve vrste (*S. glutinosa* i *S. sclarea)* u fazi plodonošenja iz jugoistočne Srbije, tehnikom HS-GC-FID-MS. Hemijski sastav lako isparljivih jedinjenja ispitivanih vrsta je bio veoma različit. Ugljovodonični monoterpeni su bili dominantna klasa isparljivih jedinjenja kod svih vrsta *Salvia*, osim u uzorcima *S. sclarea* i *S. aethiopis*. Sadržaj seskviterpena bio je najveći kod *S. aethiopis* (96,9%) i *S. glutinosa* u fazi cvetanja (29,5%), dok je u svim ostalim uzorcima taj procenat bio manji od 10%. Oksigenovani monoterpeni su bili najzastupljeniji kod *S. sclarea*, čija je glavna komponenta bio oksigenovani monoterpen linalilacetat (97,7%). Glavna komponenta vrste *S. verticillata* bio je β-felandren, ali je njegov sadržaj varirao u zavisnosti od lokacije i vremena branja biljke. Glavna komponenta u fazi cvetanja *S. glutinosa* bio je limonen (16,6%), dok je u fazi fruktifikacije sabinen (87,1%). Prvi put je izvršena HS-GC-FID-MS analiza vrste *S. aethiopis*, a najzastupljenije komponente su bile seskviterpeni: (E)-kariofilen (36,8%), α-kopaen (33,4%) i β-elemen (7,3%). Analiza glavnih komponenti izvršena je u cilju interpretacije obrazaca grupisanja, kao i analize sličnosti i razlika uzoraka u pogledu sastava isparljivih komponenata. Uzorci su grupisani u tri klastera. Prvi klaster su činili uzorci vrste *S. verticillata* sa različitih lokacija (S1, S4, S5), drugi su činili uzorci *S. glutinosa* (S3), *S. aethiopis* (S8), *S. amplexicaulis* (S9) i *S. officinalis* (S10), dok je uzorak *S. nemorosa* (S7) bio u trećem klasteru. HS-GC-FID-MS tehnika se može uspešno koristiti za kvalitativnu i kvantitativnu analizu analize isparljivih jedinjenja različitih vrsta *Salvia*. Dobijeni rezultati su važni za procenu mogućnosti upotrebe različitih vrsta žalfija.

Ključne reči: Lamiaceae, HS uzorkovanje, PCA, klaster analiza, terpeni