Milica G. Acimovic, Biljana Lj. Loncar, Valtcho D. Jeliazkov, Lato L. Pezo, Jovana P. Ljujic, Ana R. Miljkovic & Ljubodrag V. Vujisic (2022) Comparison of Volatile Compounds from Clary Sage (Salvia sclarea L.) Verticillasters Essential Oil and Hydrolate, Journal of Essential Oil Bearing Plants, 25:3, 555-570, DOI: 10.1080/0972060X.2022.2105662.

Comparison of Volatile Compounds from Clary Sage (*Salvia sclarea* L.) Verticillasters Essential Oil and Hydrolate

Milica G. Aćimović[1]*, Biljana Lj. Lončar[2], Valtcho D. Jeliazkov[3], Lato L. Pezo[4], Jovana P. Ljujić[5], Ana R. Miljković[6], Ljubodrag V. Vujisić[5]

[1]Institute of Field and Vegetable Crops Novi Sad, Novi Sad, Serbia; ORCID ID: 0000-0002-5346-1412

[2]Faculty of Technology, University of Novi Sad, Serbia; ORCID ID: 0000-0003-2994-6871

[3]Oregon State University; ORCID ID: 0000-0002-3479-9653

[4]Institute of General and Physical Chemistry, University of Belgrade, Belgrade, Serbia; ORCID ID: 0000-0002-0704-3084

[5]Department of Organic Chemistry, Faculty of Chemistry, University of Belgrade, Serbia; ORCID ID (Jovana): 0000-0002-0215-0155; ORCID ID (Ljubodrag): 0000-0001-7625-7484;

[6]Faculty of Medicine, University of Novi Sad, Serbia; ORCID ID: 0000-0001-6540-5349

Abstract

The volatile compounds of essential oil (EO) and corresponding hydrolate (HY) extracted by steam distillation from clary sage (*Salvia sclarea* L.) cv "Domaća mirisna" grown in Serbia were identified using gas chromatography/mass spectrometry (GC/MS). The most abundant compounds of EO were linalyl acetate (43.5%) and linalool (25.9%), followed by α-terpineol, germacrene D, and geranyl acetate. In the corresponding HY (recovered water-soluble fraction of EO) the dominant were linalool (63.3%) and *α*-terpineol (26.8%), followed by geraniol. These differences in composition between clary sage EO and HY could be explained by linalyl acetate's low water solubility. Clustering of 55 clary sage EO accessions (from literature) shows the presence of several chemotypes: linalyl acetate+linalool, linalyl acetate+sclareol, linalool+geranyl acetate, germacrene D+*β*-caryophyllene, caryophyllene oxide, and three unspecified chemotypes (geranyl acetate, methyl chavicol, and *α*-terpineol). According to this classification, clary sage cv "Domaća mirisna" belongs to a moderate linalyl acetate chemotype (between 19.8 and 45.7%). Further investigations need to focus on clary sage HY and their potential applications because HYs could increase economic gain as a by-product. However, their utilization for other purposes (cosmetic, postharvest fruit processing, organic agriculture, *etc.*) could be a safe solution for wastewater produced during EO distillation.

Keywords: volatile compounds, hydrolate, steam distillation, chemotype, linalyl acetate, linalool, *α*-terpineol

Introduction

Clary sage (*Salvia sclarea* L.) is a biannual or perennial herbaceous plant from the Lamiaceae family, cultivated as an ornamental plant and an industrial crop in temperate and sub-temperate climatic regions[1]. Its shoot is covered with glandular trichomes, leaves form leaf rosette in the

first year, while from the second year onwards, they are arranged along with the stem in pairs. Leaves are simple and big, wide ovate, acute on the top. The leaf surface is curly and covered with hairs, due to this, clary sage is also called Bear's ear[2]. It is lilac or white inflorescences arranged in verticillasters are attractive to bees and used as herbal tea against periodontal diseases (gingivitis, stomatitis, and aphthae), colds, and stomach aches[3].

Clary sage is an economically significant essential oil (EO) bearing plant. According to literature, essential oil content in fresh clary sage verticillasters varied between 0.1-1.0% v/w, depending on variety[4,5], agrotechnical measures (mineral nutrition, application of herbicides, plant density, shading, propagation)[5-9], environmental conditions[10-12], harvesting stage[13,14], *etc*. Because of its refreshing and long-lasting scent, clary sage EO is widely used in aromatherapy, perfumery, food, alcoholic beverage, and tobacco industries[3]. Furthermore, clary sage is also a significant source of sclareol, which is the major compound in concrete[15]. Sclareol is the starting material for synthesizing ambergris-like odorants Ambrox®, a valuable compound used in perfumery, and in cleaning products and household care (air fresheners, disinfectants, laundry detergents)[16]. Utilization of residual biomass after distillation and EO production of clary sage for further conversion into value-added products such as sclareol, and other diterpenes (sclerenbol, ambecler, hetabrol, and tabeceron) is a known and applied technique[17-19].

Hydrolate (HY) obtained as a by-product during distillation, without further processing, could be used as valuable material. However, linalool which was previously reported as the main compound in clary sage HY has been used extensively in pharmaceutical and cosmetic preparations (in perfumes, hygiene products, and cleaning agents)[20]. Furthermore, considering that clary sage EO is added to fortified wines and liqueurs because of its musky note, it is assumed that its corresponding HY could be used for flavoring soft drinks because of its similar aroma[20,21]. In addition, a large number of biological activities of clary sage EO[3], indicate the

high biological perspective of its HY. All these potential applications of clary sage HY need to be further studied in detail.

The principle of by-products synergy, which turns HYs into valuable raw material for application in other branches of industry, is of great importance considering economic gain, environmental sustainability, and social benefits[19]. Studies showed that HYs could be used as an alternative for antimicrobial agents against common food-borne and spoilage pathogens[22], and as natural disinfectants for areas in contact with food[23-25]. For example, plant HYs could be used for decontamination and preservation of processed carrots, lettuce, parsley, apple, and strawberry fruit against pathogens during storage[26-30]. Further, they can be incorporated into functional beverages for the treatment or prevention of hyperlipidemia, cardiovascular and neurological disorders, mental health, as well as for women's hormonal and reproductive health conditions[31-33]. Lemongrass HY could be used as natural flavoring matter for herbal ice cream[34], while cinnamon HY could be applied for coating eggs with pectin to increase shelf life during storage[35]. Moreover, HYs have the potential for application in organic agriculture as natural pest control agents[36], or control phytopathogens[37]. In addition, HYs can be used in aromatherapy[38], as well as in the cosmetic industry as a replacement for the water phase in body gels[39].

Considering this, and especially the rising popularity of HYs, this study aimed to determine the chemical composition of clary sage EO and its corresponding HY as a by-product of the same process. Furthermore, a comparison with data from literature was made to find a correlation between clary sage EO and HY, as well as to classify clary sage chemotypes according to references collected from scientific databases (Scopus, Web of Science, PubMed, ScienceDirect, Directory of Open Access Journals, JSTOR).

Material and method

Plant material

Clary sage cv "Domaća mirisna" was cultivated at experimental plots at the Institute of Field and Vegetable Crops Novi Sad (Department of Vegetable and Alternative Crops Bački Petrovac) during 2020 (three years old crop). In the complete flowering stage, the upper parts with verticillaster were harvested (from experimental plot 15×10 m) early in the morning, and immediately transported to a distillation unit.

Steam distillation

A total of 100 kg of fresh plant material was distilled during 3 h as previously described[21]. Briefly, plant material was placed in a stainless still distillation vessel, hermetically sealed, and supplied with steam. The steam passes through plant material, and further water vapour and entrained volatiles go through the condenser and cooler, and finally collected in the Florentine glass flask. After this process was completed, EO together with HY was decanted from the Florentine flask, and placed in a separation funnel overnight. The average yield of EO was 0.39%, according to three replications. Hydrolate dripped through filter paper into a sterile plastic bottle. Anhydrous sodium sulphate was added to the funnel to remove water traces from the EO. The Likens-Nickerson extraction procedure was applied to recover EO remaining in HY as we previously described[40]. Briefly, total of 400 ml of HY were used for simultaneous distillation and extraction with dichloromethane by the Likens-Nickerson apparatus for 2 h.

Analysis of volatile compounds

Both EO (diluted with dichloromethane), as well as recovery EO obtained from HY were analyzed using Agilent 7890A gas chromatograph (GC) equipped with a flame ionization detector (FID), a split/splitless injector, and a nonpolar HP-5MS fused-silica capillary column (30 m × 0.25 mm × 0.25 μm). Helium was used as the carrier gas with an inlet pressure of 19.6

psi (constant pressure mode, 1 mL/min flow rate at 210 °C). Splitless injections with an injection volume of 1 μL were used for all analyses. The oven temperature was linearly programmed from 60 to 300 °C at a rate of 3 °C/min. All GC parameters set according to Adams Retention Times Locked (RTL) GC/MS library conditions in order to obtained comparison of retention times with library data. Gas chromatographic-mass spectrometric (GC-MS) analysis was performed using an Agilent 7890A GC coupled to an Agilent 5973 MSD spectrometer. The column and analysis conditions were the same as in GC-FID. The components were identified based on three-way comparison: comparison of obtained retention times with R_t of authentic standards from Adams Retention Times Locked GC/MS library data; comparison of retention indices with literature RI's (Adams ver. 4 and NIST RI ver. 17 databases) and with comparison of obtained EI mass spectra with reference spectra (Adams ver. 4, Wiley ver. 7, and NIST ver. 17 databases). The relative percentage of the identified compounds of the EO was computed from the peak area from GC-FID chromatogram.

Statistics

The colour plot diagram as well as the phylogenetic cluster tree were calculated and plotted using R software 4.0.3. Principal component analysis (PCA) was done with the StatSoft Statistica, ver. 10.0, Palo Alto, CA, USA.

Results and discussion

According to Table 1, in the clary sage EO from this study there are detected 30 compounds (comprising 97.7%), and the most abundant were linalyl acetate (43.5%) and linalool (25.9%). A significantly lower number of compounds (15, comprising 99.8%) were detected in HY, and the dominant were linalool (63.3%) and α-terpineol (26.8%). However, according to the two previous conducted research (comparing EO and HY composition of clary sage), as well as

from this study, in the EO the dominant compound was linalyl acetate, which content varied between 43.0 and 60.6%, while linalool content varied between 11.1 and 27.1% (Table 1). Linalool content in all three HY samples varied from 62.5 to 89.5%, while *α*-terpineol varied between 10.5 and 26.8%. Oxygenated monoterpenes were dominant in clary sage sample EO and HY from Serbia, with 82.2% and 93.5%, respectively, as well as from India (80.4% and 90.3%, respectively)[41] and Italy (76.6% and 100.0%, respectively)[42].

Table 1. Chemical composition of clary sage essential oil (EO) and corresponding hydrolate (HY) from this study and from literature.

No	Compound	RI_{exp}	RI_{lit}	This study		Verma[41]		Ovidi et al.[42]	
				EO1	HY1	EO2	HY2	EO3	HY3
1	3-*Z*-Hexenol^{O}	848	850	nd	0.7	nd	nd	nd	nd
2	2-*Z*-Hexenol^{O}	858	854	nd	0.6	nd	nd	nd	nd
3	*n*-Hexanol^{O}	859	863	nd	0.3	nd	nd	nd	nd
4	1-Octen-3-ol^{O}	974	974	nd	0.1	nd	nd	nd	nd
5	Myrcene^{MT}	988	988	1.0	nd	7.3	2.0	nd	nd
6	Limonene^{MT}	1025	1024	0.4	tr	3.1	2.2	0.6	nd
7	*Z*-*β*-Ocimene^{MT}	1034	1032	0.2	nd	2.0	0.5	1.5	nd
8	*E*-*β*-Ocimene^{MT}	1044	1044	0.6	nd	4.8	1.2	0.8	nd
9	*Z*-Linalool oxide (furanoid)^{OMT}	1068	1067	tr	0.5	0.8	tr	nd	nd
10	Terpinolene^{MT}	1086	1086	0.1	nd	nd	nd	nd	nd
11	*E*-Linalool oxide (furanoid)^{OMT}	1087	1088	nd	0.5	nd	nd	nd	nd
12	Linalool^{OMT}	1099	1095	25.9	63.3	27.1	62.5	11.1	89.5
13	Menthol^{OMT}	1172	1167	nd	0.1	nd	nd	nd	nd
14	Terpinen-4-ol^{OMT}	1175	1174	tr	0.3	nd	nd	nd	nd
15	*α*-Terpineol^{OMT}	1188	1186	5.0	26.8	2.1	20.6	1.5	10.5
16	Linalool formate^{OMT}	1214	1214	0.1	nd	nd	nd	nd	nd
17	Nerol^{OMT}	1225	1227	0.9	1.9	0.2	1.8	nd	nd
18	Linalyl acetate^{OMT}	1256	1254	43.5	nd	43.0	nd	60.6	nd
19	Geraniol^{MT}	1257	1249	nd	4.6	0.7	4.8	nd	nd
20	Thymol^{OMT}	1292	1289	nd	0.1	nd	nd	nd	nd
21	*δ*-ElemeneST	1335	1335	0.3	nd	nd	nd	nd	nd
22	Neryl acetate^{OMT}	1362	1359	2.1	nd	1.3	0.2	nd	nd
23	*α*-CopaeneST	1373	1374	1.0	nd	nd	nd	1.8	nd
24	Geranyl acetate^{OMT}	1381	1379	4.4	tr	3.1	0.4	1.4	nd
25	*β*-CubebeneST	1388	1387	0.4	nd	nd	nd	2.7	nd
26	*β*-ElemeneST	1389	1389	0.3	nd	nd	nd	nd	nd
27	*E*-CaryophylleneST	1417	1417	2.4	nd	1.4	tr	3.4	nd
28	*β*-CopaeneST	1427	1430	0.2	nd	nd	nd	6.7	nd
29	*α*-HumuleneST	1452	1452	0.2	nd	nd	nd	nd	nd
30	Germacrene DST	1480	1484	5.0	nd	0.1	nd	nd	nd
31	*β*-SelineneST	1487	1489	0.2	nd	nd	nd	nd	nd
32	BicyclogermacreneST	1496	1500	0.8	nd	nd	nd	nd	nd
33	*E*,*E*-α-FarneseneST	1508	1505	0.1	nd	nd	nd	nd	nd
34	*δ*-CadineneST	1522	1522	0.3	nd	nd	nd	1.0	nd
35	Spathulenol^{OST}	1576	1577	0.3	nd	nd	nd	nd	nd
36	Caryophyllene oxide^{OST}	1580	1582	0.2	nd	nd	0.1	nd	nd

37	β-Eudesmol[OST]	1648	1649	0.2	nd	nd	nd	nd	nd
38	Sclareol[OD]	2223	2222	1.6	nd	nd	tr	nd	nd
	Monoterpene hydrocarbons	%		2.3	4.6	17.4	6.1	6.0	-
		No compounds		5	2	5	5	3	
	Oxygenated monoterpenes	%		82.2	93.5	80.4	90.3	76.6	100.0
		No compounds		10	9	7	6	4	2
	Sesquiterpene hydrocarbons	%		10.9	-	1.5	-	17.4	-
		No compounds		12	-	2	-	5	-
	Oxygenated sesquiterpenes	%		0.7	-	-	0.1	-	-
		No compounds		3	-	-	1	-	-
	Oxigenated diterpenes	%		1.6	-	-	-	-	-
		No compounds		1	-	-	-	-	-
	Other*	%		-	1.7	-	-	-	-
		No compounds		-	4	-	-	-	-
	Total identified (SUM %)			**97.7**	**99.8**	**99.2**	**96.5**	**100.0**	**100.0**

RI_{exp} – Experimentally obtained Retention Index, RI_{lit} – Retention Index from the literature, nd – not detected, * – aliphatic hydrocarbons, aldehydes, alcohols, acids, as well as their esters, and alkyl-aromatic alcohols.

The difference between the chemical composition of clary sage EO and HY composition could be explained by data that linalool is well soluble in water (1.336 mg/ml), while linalyl acetate is practically water-insoluble (0.054 mg/ml)[43]. Taking into account that clary sage HY contains a large quantity of linalool, it is occurring as a valuable by-product, especially because of the large-scale production of EO by steam distillation in a significantly larger proportion than EO. Linalool possesses a typical flowery fresh odour and it is widely used in the flavor and fragrance industry, but it also expresses various biological activities. Further, linalool is a key compound for the industrial production of other fragrance compounds such as geraniol, nerol, and citral[44]. Potential applications of clary sage HY could be for the production of soaps, detergents and shampoos as a replacement for the water phase, as well as for further industrial processes such as re-extraction of pure linalool.

Fig. 1 shows dendrograms of cluster analysis for the tested clary sage samples. The complete linkage algorithm and City block (Manhattan) distances were used to measure proximity among the samples. City block distances (shown on the ordinate axis) are measured as the average difference across dimensions of the tested samples.

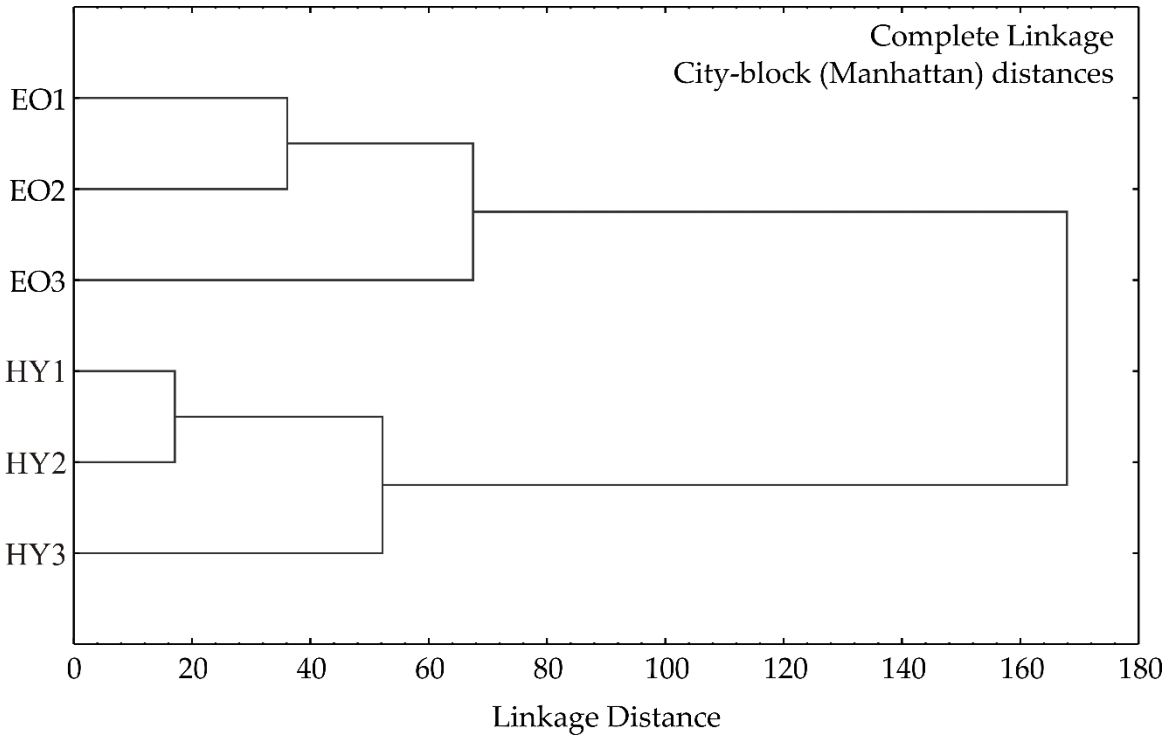


Figure 1. Cluster analysis of the clary sage samples

The dendrogram presented in Figure 1 is based on the chemical composition of clary sage EO and HY samples. The resulting dendrogram showed two main clusters, the first cluster contained EOs samples (EO1-EO3). The second cluster comprised HY samples (HY1-HY3). The linkage distance (shown on the ordinate axis) between the two main clusters was evident (nearly 170).

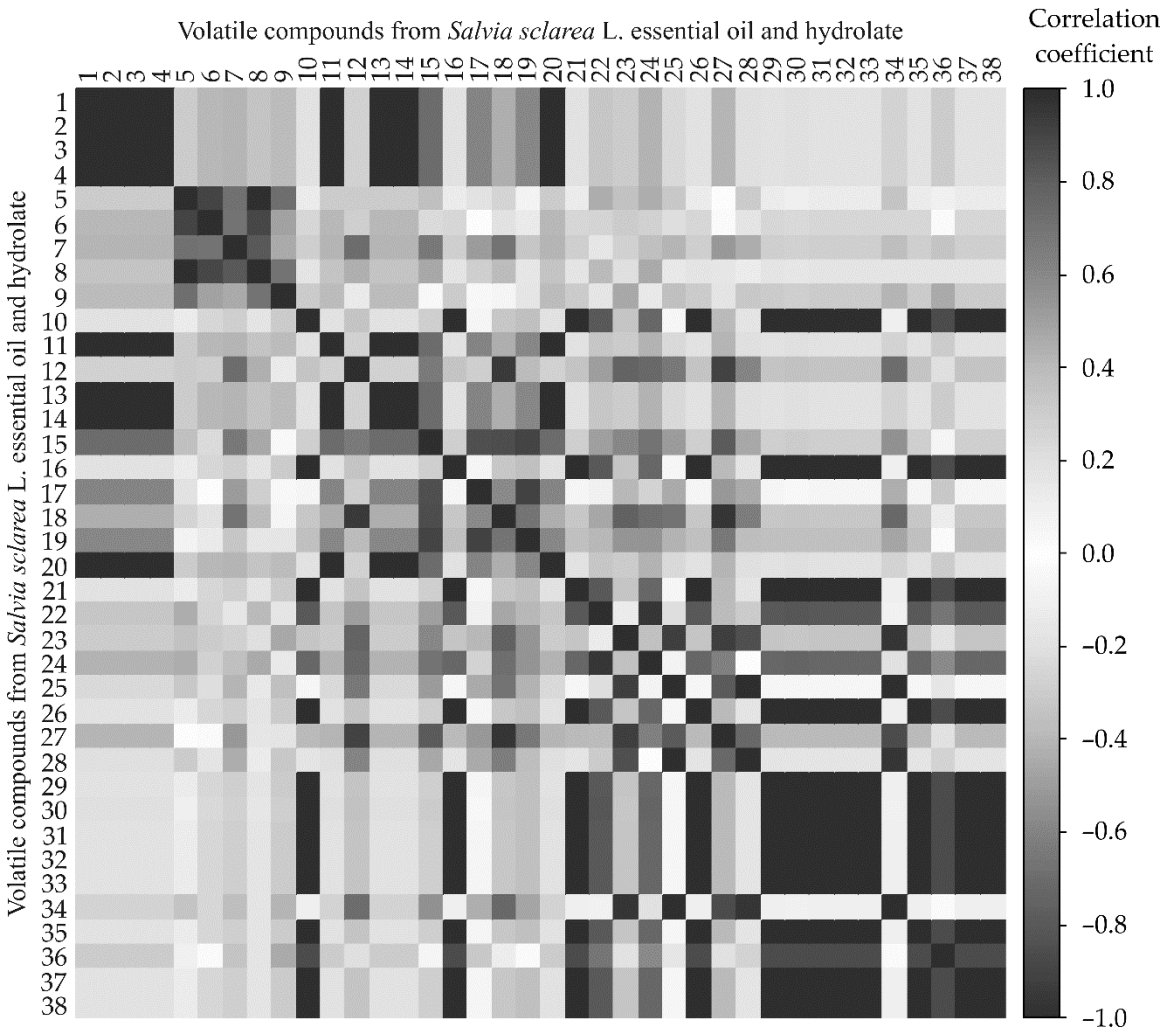
According to the chemical composition (GC-MS chromatography data), which were presented in Table 2, Pearson's coefficients of correlation between clary sage essential oil and corresponding hydrolate samples showed that a strong correlation existed between EO samples (statistically significant at $p \leq 0.001$ level) and hydrolate samples (also at $p \leq 0.001$ level). 0.901 - 0.977 for clary sage EO and 0.957-0.995 for hydrolate samples. High correlations were observed between EO1-3 and HY1-3 samples, statistically significant at $p \leq 0.01$ level.

Table 2. Correlation matrix between clary sage essential oil and hydrolate samples

	EO2	EO3	HY1	HY2	HY3
EO1	0.977^{+}	0.917^{+}	0.463*	0.471*	0.487*
EO2		0.901^{+}	0.459*	0.480*	0.500*
EO3			0.123	0.127	0.143
HY1				0.995^{+}	0.957^{+}
HY2					0.976^{+}

170 $^{+}$correlation statistically significant at $p<0.001$ level, *correlation statistically significant at
171 $p<0.01$ level
172

173 The correlation analysis was also conducted to analyze the similarities in active compounds
174 content of the clary sage EO and HY, and the results were illustrated in Figure 2. The darker
175 blue tone of the squares, which shows a relation between two samples, presents a stronger
176 correlation between these samples. The lighter color suggests a specific distinction between
177 samples, a lower correlation between two samples. On the other hand, the red color describes
178 a negative correlation between the compounds discovered by GC-MS. The result presented in
179 Figure 2 was based on all the data in Table 1 (EO 1-3; HY 1-3).



180

181 **Figure 2. Correlation between volatile compounds of clary sage essential oil and**

182 **hydrolate samples**

183

184 The PCA of the essential oil compounds in samples explained that the first two principal

185 components summarized 71.64% of the total variance in the 38 parameters (active compounds).

186 The EO and HY samples were different mainly in the content of: *δ*-cadinene, *E*-caryophyllene,

187 *α*-copaene, geranyl acetate, *β*-cubebene, nerol, linalyl acetate, geraniol, thymol, *α*-copaene, *E*-

188 linalool oxide (furanoid), linalool, menthol, terpinene-4-ol, *α*-terpineol, 3-*Z*-hexenol, 2-*Z*-

189 hexenol, *n*-hexanol, and 1-octen-3-ol.

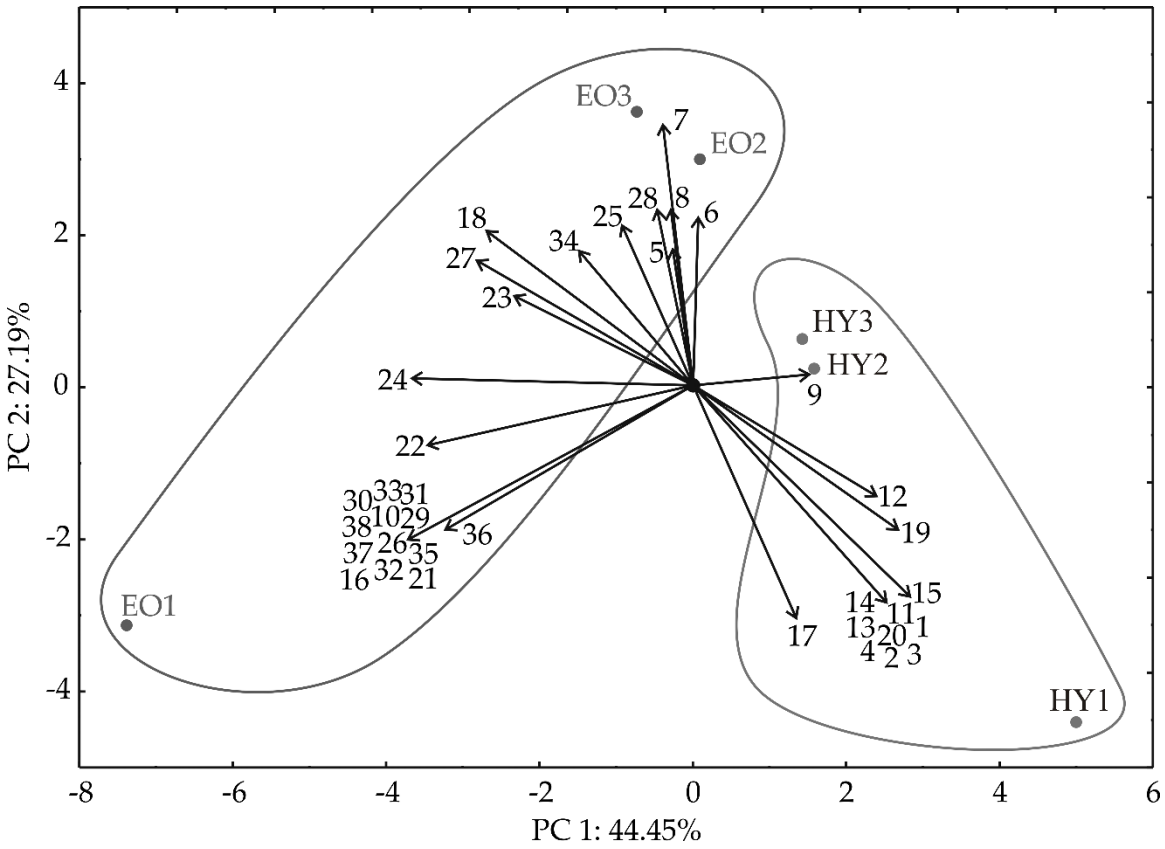


Figure 3. The PCA biplot diagram describing the relations between essential oil compounds of clary sage essential oil and hydrolate

Same as in the case of EO content, the chemical composition of EO primarily depends on many factors, such as genotype (origin/population/hybrid)[45-51] as well as on environmental conditions (growing season, elevation, soil conditions *i.e.*, non-polluted soils or polluted with heavy metal, or salinity)[51-56] and growing technology (propagation, plant density, fertilization, plants health condition, *etc.*)[6,8,9,57,58,59]. Further, harvest (plant part, *i.e.* inflorescence or leaf, position of inflorescence on stem *i.e.* primary or secondary, phenological stage *i.e.* full blooming or seed ripening)[4,10,13,14,47,51,60,61,62] and postharvest processing (storage period, extraction method, distillation time)[13,21,41,63,64] play a significant role in EO quality. In addition, differences in the chemical composition of EO could occur as a consequence of the applied analysis technique[65,66].

A review of the 39 scientific papers dealing with clary sage EO composition and one from this study was used to construct an unrooted tree (Table 3; Figure 4). Clustering of 55 accessions shows the presence of several chemotypes of clary sage EO according to dominant compounds.

Table 3. Chemical composition of clary sage essential oil (10 compounds presented with more than 1.5% in average) according to reference review (references are sorted from oldest to newest)

Nº	REFERENCE	linalyl acetate	linalool	α-terpineol	geranyl acetate	germacrene D	sclareol	β-caryophyllen	geraniol	caryophyllene oxide	neryl acetate	SUM of 10 compounds
1	Elnir et al.[46]	0.3	1.7	0.3	38.3	0.8	0.0	0.0	24.3	0.0	3.0	68.7
2	Elnir et al.[46]	35.0	31.0	10.3	5.4	2.7	0.0	0.0	5.7	0.0	2.6	92.7
3	Elnir et al.[46]	12.6	26.6	3.7	20.2	2.6	0.0	0.0	12.8	0.0	2.6	81.1
4	Elnir et al.[46]	4.2	22.0	2.5	22.5	4.8	0.0	0.0	22.0	0.0	1.9	79.9
5	Dzumayev et al.[50]	25.0	34.0	11.0	5.4	0.0	0.0	0.8	0.0	0.0	3.2	79.4
6	Souleles and Argyriadou[66]	14.3	17.2	15.1	7.5	0.0	5.2	1.2	6.5	1.5	5.2	73.7
7	Moretti et al.[67]	19.2	9.9	7.5	0.2	0.2	0.0	0.2	0.1	0.0	0.4	37.7
8	Peana et al.[68]	12.7	2.6	47.4	1.3	1.6	0.0	2.9	0.6	0.0	2.1	71.2
9	Pitarokili et al.[44]	19.8	30.4	5.1	12.1	2.6	3.5	2.0	4.2	0.7	7.8	88.2
10	Pitarokili et al.[44]	31.1	18.5	7.6	4.5	0.0	5.6	2.3	0.0	2.3	2.0	73.9
11	Lorenzo et al.[51]*	45.1	17.0	1.7	1.6	13.2	1.2	3.9	0.0	0.0	1.1	84.8
12	Fraternale et al.[69]	20.9	24.5	9.8	6.3	0.9	1.8	3.0	1.2	5.3	3.6	77.3
13	Farkaš et al.[60]	13.7	18.9	6.5	4.3	5.0	15.7	2.1	0.0	0.8	2.2	69.2
14	Tognolini et al.[70]	67.5	8.8	0.8	2.4	0.0	0.0	0.0	0.0	0.3	1.3	81.1
15	Cai et al.[47]	49.8	28.1	5.1	2.8	0.3	0.0	0.8	2.2	0.8	1.6	91.5
16	Cai et al.[47]	29.5	17.0	3.2	1.7	0.5	0.0	0.6	1.4	0.5	1.0	55.4
17	Cai et al.[47]	51.6	28.8	4.4	2.3	0.6	0.0	1.0	2.1	0.7	1.3	92.8
18	Cai et al.[47]	48.2	28.5	5.0	2.8	1.3	0.0	1.3	2.5	0.5	1.5	91.6
19	Ogutcu et al.[71]	5.5	1.2	1.6	2.2	24.7	0.0	16.2	0.0	1.9	1.1	54.4
20	Schmiderer et al.[15]	57.7	13.5	3.0	0.4	2.9	7.5	1.7	0.0	0.0	2.0	88.7
21	Džamić et al.[72]	52.8	18.2	5.0	0.0	0.8	0.1	1.8	0.0	0.3	0.5	79.5
22	Saharkhiz et al.[61]	23.1	30.0	11.1	8.4	0.6	0.0	0.0	0.0	0.0	4.7	77.9
23	Kuzma et al.[9]	2.6	38.6	14.3	5.8	0.6	0.6	1.1	7.7	2.2	3.0	76.5
24	Verma[41]	43.0	27.1	2.1	3.1	0.1	0.0	1.4	0.7	0.0	1.3	78.8
25	Yadav et al.[48]	51.2	23.6	3.8	3.3	1.3	1.3	3.2	0.0	1.2	1.4	90.3
26	Yadav et al.[48]	60.8	14.5	1.8	2.2	2.6	1.3	1.9	0.0	0.0	0.9	86.0
27	Yadav et al.[48]	45.7	29.8	5.3	3.0	0.2	2.3	0.3	0.0	0.0	1.5	88.1
28	Verma et al.[13]	28.0	34.3	5.0	3.5	0.4	1.5	0.0	6.7	0.0	1.7	81.1
29	Sharopov and Setzer[12]	39.2	12.5	5.5	3.5	11.4	1.2	2.4	0.0	0.2	1.9	77.8
30	Sharma and Kumar[57]	28.8	4.0	1.0	6.0	0.5	7.0	2.6	0.0	3.7	2.7	56.3
31	Kumar et al.[8]	21.6	31.9	13.3	7.0	0.2	6.4	2.4	0.0	0.4	3.5	86.7
32	Hristova et al.[64]	56.9	20.8	2.6	1.2	5.1	0.2	3.4	0.0	0.2	0.7	91.1
33	Yuce et al.[73]	0.0	0.0	0.0	0.0	1.3	11.5	5.1	0.0	24.1	0.0	42.0
34	Sharopov et al.[74]	36.3	23.5	8.1	2.3	0.5	14.6	0.6	0.0	1.1	1.1	88.1
35	Dogan et al.[11]	11.3	8.5	4.5	0.0	0.7	0.0	1.8	0.0	15.5	0.0	42.3
36	Andrade et al.[75]	60.1	28.8	5.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	94.0
37	Zutic et al.[49]	56.0	17.4	4.2	3.9	0.0	3.5	0.7	0.0	1.8	2.0	89.5
38	Zutic et al.[49]	41.5	17.9	5.7	5.9	3.6	7.5	2.6	0.0	0.6	2.8	88.1
39	Safaei-Ghomi et al.[59]	0.0	0.0	0.0	0.0	20.9	0.0	9.7	0.0	0.0	0.0	30.6
40	Koutsaviti et al.[63]	21.9	19.7	6.8	4.4	4.4	13.2	0.7	2.3	0.1	2.3	75.8
41	Kumar et al.[52]	26.8	31.2	0.0	5.1	3.1	0.0	1.0	1.7	0.0	2.5	71.4

42	Raafat and Habib[45]	1.0	38.1	13.4	4.9	2.0	0.1	1.0	5.7	1.3	2.5	70.0
43	Raafat and Habib[45]	35.3	10.8	6.5	3.5	10.6	1.2	2.3	4.4	0.5	1.8	76.9
44	Tuttolomondo et al.[4]**	38.8	22.4	5.8	2.6	4.4	5.5	2.7	0.0	0.0	1.5	83.7
45	Tuttolomondo et al.[4]**	39.0	25.6	7.2	3.0	4.5	2.9	2.0	0.0	0.0	1.7	85.9
46	Tuttolomondo et al.[4]**	41.6	24.0	6.5	2.7	3.6	3.2	2.2	0.0	0.0	1.5	85.3
47	Karayel[6]	6.0	10.1	2.7	1.7	1.9	0.0	3.4	1.6	19.4	0.9	47.7
48	Kostova et al.[76]	40.3	22.7	7.7	4.6	0.0	0.0	1.5	0.0	0.1	2.8	79.7
49	El-Gohary et al.[10]	10.6	6.5	2.1	0.9	0.6	33.9	1.3	0.0	2.7	0.5	59.1
50	Grigoriadou et al.[56]	21.4	3.4	0.0	0.0	5.9	5.3	11.3	0.0	11.4	1.1	59.8
51	Ovidi et al.[42]	62.6	11.1	1.5	1.4	0.0	0.0	3.4	0.0	0.0	0.0	80.0
52	Tasheva et al.[77]	34.6	17.7	4.8	0.0	0.0	0.0	5.6	0.8	0.0	2.3	65.8
53	Raveau et al.[53]	53.0	10.0	1.9	3.8	14.8	0.3	3.2	0.0	0.3	0.0	87.3
54	Aćimović et al.[21]	40.3	28.6	8.4	4.0	0.0	2.6	0.0	0.0	0.8	2.1	86.8
55	This study	43.5	25.9	5.0	4.4	5.0	1.6	2.4	0.0	0.2	2.1	90.1
	AVERAGE	**31.6**	**19.4**	**6.0**	**4.5**	**3.2**	**3.1**	**2.3**	**2.1**	**1.9**	**1.9**	

*average value of the essential oil percentage composition from the plants harvested at full flowering stage during two years (1999 and 2000)

**average value of the essential oil percentage composition obtained from inflorescences forming on the main and secondary stem

In 36 accessions, linalyl acetate and linalool were the most abundant compounds, and this chemotype (hereinafter linalyl acetate + linalool) is the most valuable for industrial application. According to relative ratio of this two compounds, this chemotype could be divided into two subgroup: moderate (19.8-45.7%)[4,8,12,13,21,41,45-51,53,62,67,68,69,70] and high (45.1-67.5%)[15,42,48,49,50,52,54,65,71,72,73] content of linalyl acetate, while linalool is ranged between 8.8 and 34.3%. The market value of linalyl acetate is considerably higher than that of linalool, so the chemotypes with a higher portion of this component are more appreciated[74].

Other chemotypes are: linalyl acetate + sclareol (10.6-28.8% and 5.2-33.9%, respectively)[10,57,58,61,64,75], linalool + geranyl acetate (22.0-38.6% + 4.9-22.5%, respectively)[9,46,47], germacrene D + *β*-caryophyllene (20.9-24.7% and 9.7-16.2%, respectively)[60,76], caryophyllene oxide chemotype (15.5-24.1%)[6,11,77] and three unspecified accessions with dominant geranyl acetate[47], methyl chavicol[78], and α-terpineol[79].

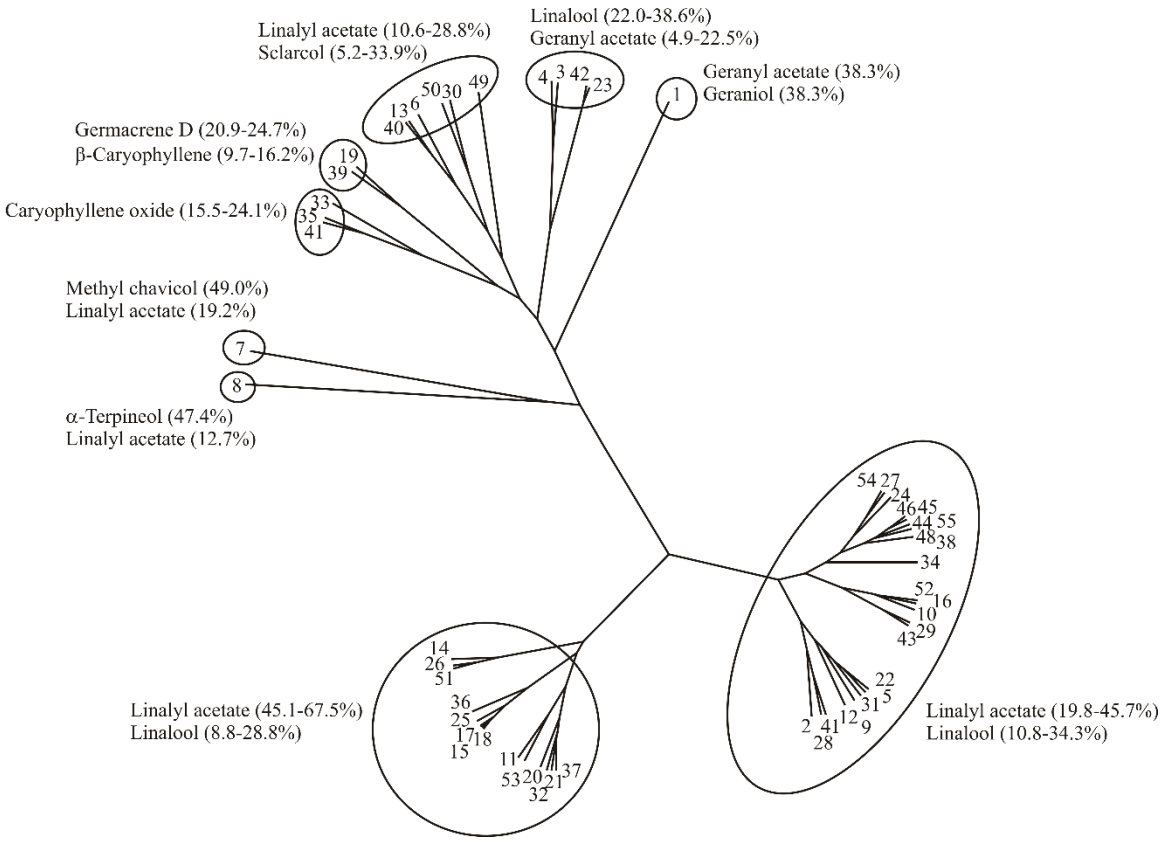


Figure 4. Unrooted cluster tree for the chemical composition of clary sage essential oil compounds in accordance with the literature and presented study (samples are coded compatible to Table 3)

Since clary sage EO and HY have a similar chemical composition with linalyl acetate and linalool as dominant compounds, HY occurs as a potentially valuable by-product, especially in light of the green economy and waste management. Clary sage EO has many proven biological activities and wide applications in different industries; however, further investigations need to focus on the biological activities of HY as well as their incorporation in different types of products and practical implementation.

Conclusion

The relatively easy cultivation of clary sage with low labor cost, the stable international market of EO and sclareol, as well as tolerance of this crop to air and soil heavy metal pollution,

encouraged producers to expand the cultivation area of this plant[7,80-83]. The additional economic gain obtained from HY could be a further financial achievement and an environmentally safe solution for wastewater generated during EO extraction.

Acknowledgments: This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Contract numbers: 451-03-68/2022-14/200032 and 451-03-68/2022-14/200168.

References

1 **Yaseen, M., Kumar, B., Ram, D., Singh, M., Anand, S., Yadav, H.K. and Samad, A. (2015).** Agro morphological, chemical and genetic variability studies for yield assessment in clary sage (*Salvia sclarea* L.). Ind Crops Prod. 77: 640–647. DOI: 10.1016/j.indcrop.2015.09.047

2 **Koplan, F. and Cakir, E.A. (2019).** Morphological characteristics of some *Salvia* L. taxa in Sakarya Province (Turkey). Eurasian J Forest Sci. 7(2): 133–144. DOI: 10.31195/ejejfs.559428

3 **Aćimović, M., Kiprovski, B., Rat, M., Sikora, V., Popović, V., Koren, A. and Brdar-Jokanović, M. (2018).** *Salvia sclarea*: Chemical composition and biological activity. J Agron Technol Eng Manag. 1(1): 18–28.

4 **Tuttolomondo, T., Iapichino, G., Licata, M., Virga, G., Leto, C. and La Bella, S. (2020).** Agronomic evaluation and chemical characterization of Sicilian *Salvia sclarea* L. accessions. Agronomy. 10(8): 1114. DOI: 10.3390/agronomy10081114

5 **Yaseen, M., Singh, M., Ram, D. and Singh, K. (2014).** Production potential, nitrogen use efficiency and economics of clary sage (*Salvia sclarea* L.) varieties as influenced by

nitrogen levels under different locations. Ind Crops Prod. 54: 86–91. DOI: 10.1016/j.indcrop.2014.01.002

6 **Karayel, H.B. (2020).** Effect of natural boron mineral use on the essential oil ratio and component of Musk sage (*Salvia sclarea* L.). Open Chem. 18(1): 732–739. DOI: 10.1515/chem-2020-0134

7 **Zhalnov, I. and Zheljazkov, V.D. (2016).** Potential herbicides for weed control in clary sage (*Salvia sclarea*). Ed: Zheljazkov VD, Cantrell CL. In book: Medicinal and Aromatic Crops: Production, Phytochemistry, and Utilization. Washington, DOI: 10.1021/bk-2016-1218.ch007

8 **Kumar, R., Sharma, S. and Pathania, V. (2013).** Effect of shading and plant density on growth, yield and oil composition of clary sage (*Salvia sclarea* L.) in north western Himalaya. J Essent Oil Res. 25(1): 23–32. DOI: 10.1080/10412905.2012.742467

9 **Kuzma, L., Kalemba, D., Rozalski, M., Rozalska, B., Wieckowska-Szakiel, M., Krajewska, U. and Wysokinska, H. (2009).** Chemical composition and biological activities of essential oil from *Salvia sclarea* plants regenerated *in vitro*. Molecules. 14(4): 1438–1447. DOI: 10.3390/molecules14041438

10 **El-Gohary, A.E., Amer, H.M., Salama, A.B., Wahba, H.E. and Khalid, K.A. (2020).** Characterization of the essential oil components of adapted *Salvia sclarea* L. (clary sage) plants under Egyptian environmental conditions. J Essent Oil-Bear Plants. 23(4): 788–794. DOI: 10.1080/0972060X.2020.1818635

11 **Dogan, G., Hayta, S., Yuce, E. and Bagci, E. (2015).** Composition of the essential oil of two Salvia taxa (*Salvia sclarea* and *Salvia verticillata* subsp. *verticillata*) from Turkey. Natural Science and Discovery. 1(3): 62–67. DOI: 10.20863/nsd.23928

12 **Sharopov, F.S. and Setzer, W.N. (2012).** The essential oil of *Salvia sclarea* L. from Tajikistan. Rec Nat Prod. 6(1): 75–79.

13 **Verma, R.S., Chauhan, A., Rahman, L., Verma, R.K. and Singh, A. (2011).** Aroma profile of clary sage (*Salvia sclarea* L.): influence of harvesting stage and postharvest storage in Uttarakhand Hills. Med Aromat Plant Sci Biotechnol. 5(2): 139–142.

14 **Pešić, P. and Banković, V. (2003).** Investigation on the essential oil of cultivated *Salvia sclarea* L. Flavour Fragrance J. 18(3): 228–230. DOI: 10.1002/ffj.1202

15 **Schmiderer, C., Grassi, P., Novak, J., Weber, M. and Franz, C. (2008).** Diversity of essential oil glands of clary sage (*Salvia sclarea* L., Lamiaceae). Plant Biol. 10(4): 433–440. DOI: 10.1111/j.1438-8677.2008.00053.x

16 **Ncube, E.N., Steenkamp, L. and Dubery, I.A. (2020).** Ambrafuran (AmbroxTM) synthesis from natural plant product precursors. Molecules. 25: 3851. DOI: 10.3390/molecules25173851.

17 **Ilieva, S. (1979).** New *Salvia sclarea* L. cultivars developed by hybridization. Acta Hortic. 96: 197–204. DOI: 10.17660/ActaHortic.1980.96.18

18 **Laville, R., Castel, C., Fattarsi, K., Roy, C., Legendre, L., Delbecque, C., Garry, P.P., Audran, A. and Fernandez, X. (2013).** Low sclareol by-product of clary sage concrete: chemical analysis of a waste product of the perfume industry. Flavour Fragrance J. 28(2): 93–101. DOI: 10.1002/ffj.3133

19 **Saha, A. and Basak, B.B. (2020).** Scope of value addition and utilization of residual biomass from medicinal and aromatic plants. Ind Crops Prod. 145: 111979. DOI: 10.1016/j.indcrop.2019.111979

20 **Aćimović, M., Tešević, V., Smiljanić, K., Cvetković, M., Stanković, J., Kiprovski, B. and Sikora, V. (2020).** Hydrolates – by-products of essential oil distillation: chemical composition, biological activity and potential uses. Advanced Technologies. 9(2): 54–70; DOI: 10.5937/savteh2002054A

317 21 **Aćimović, M., Cvetković, M., Stanković Jeremić, J., Pezo, L., Varga, A., Čabarkapa,**
318 **I. and Kiprovski, B. (2022).** Biological activity and profiling of *Salvia sclarea* essential
319 oil obtained by steam and hydrodistillation extraction methods via chemometric tools.
320 Flavour Fragrance J. 37(1): 20–32. doi:10.1002/ffj.3684
321 22 **Yavuzer, E. and Kuley Boga, E. (2020).** Testing the antimicrobial effects of some
322 hydrosols on food borne-pathogens and spoilage bacteria. LimnoFish. 6(1): 47–51. DOI:
323 10.17216/limnofish.618101
324 23 **Chorianopoulos, N.G., Giaouris, E.D., Skandamis, P.N., Haroutounian, S.A. and**
325 **Nychas, G.J.E. (2008).** Disinfectant test against monoculture and mixed-culture biofilms
326 composed of technological, spoilage and pathogenic bacteria: bactericidal effect of
327 essential oil and hydrosol of *Satureja thymbra* and comparison with standard acid-base
328 sanitizers. J Appl Microbiol. 104(6): 1586–1596. DOI: 10.1111/j.1365-
329 2672.2007.03694.x.
330 24 **Karampoula, F., Giaoris, E., Deschamps, J., Doulgeraki, A.I., Nychas, G.J.E. and**
331 **Dubois-Brissonnet, F. (2016).** Hydrosol of *Thymbra capitata* is a highly efficient
332 biocide against *Salmonella enterica* serovar Typhimurium biofilms. Appl Environ
333 Microbiol. 82(17): 5309–5319. DOI: 10.1128/AEM.01351-16
334 25 **Garcia-Romo, J.S., Yepiz-Gomez, M.S., Plascencia-Jatomea, M., Santacruz-**
335 **Ortega, H.D.C., Burgos-Hernandez, A., Leon, J.R.G., Cinco-Moroyoqui, F.J. and**
336 **Borboa-Flores, J. (2018).** Compounds with in vitro antibacterial activity from hydrosol
337 of *Lippia palmeri* and morphometric changes on *Listeria monocytogenes*. Biotecnia.
338 20(3): 35–42. DOI: 10.18633/biotecnia.v20i3.713
339 26 **Tornuk, F., Cankurt, H., Ozturk, I., Sagdic, O., Bayram, O. and Yetim, H. (2011).**
340 Efficacy of various plant hydrosols as natural food sanitizers in reducing *Escherichia coli*

O157:H7 and *Salmonella* Typhimurium on fresh cut carrots and apples. Int J Food Microbiol. 148(1): 30–35. DOI: 10.1016/j.ijfoodmicro.2011.04.022

27 **Xylia, P., Clark, A., Chrysargyris, A., Romanazzi, G. and Tzortzakis, N. (2019).** Quality and safety attributes on shaded carrots by using *Origanum majorana* and ascorbic acid. Postharvest Biol Technol. 155: 120–129. DOI: 10.1016/j.postharvbio.2019.05.015

28 **Ozturk, I., Tornuk, F., Caliskan-Aydogan, O., Durak, M.Z. and Sagdic, O. (2016).** Decontamination of iceberg lettuce by some plant hydrosols. LWT - Food Sci Technol. 74, 48–54. DOI: 10.1016/j.lwt.2016.06.067

29 **Tornuk, F. and Dertli, E. (2015).** Decontamination of *Escherichia coli* O157:H7 and *Staphylococcus aureus* from fresh-cut parsley with natural plant hydrosols. J Food Process Preserv. 39: 1587–1594. DOI:10.1111/jfpp.12387

30 **Zatla, A.T., Dib, M.E.A., Djabou, N., Ilias, F., Costa, F. and Muselli, A. (2017).** Antifungal activities of essential oils and hydrosol extracts of *Daucus carota* subsp. *sativus* for the control of fungal pathogens, in particular gray rot of strawberry during storage. J Essent Oil Res. 29(5): 391–399. DOI: 10.1080/10412905.2017.1322008

31 **Hamedi, A., Afifi, M. and Etemadfard, H. (2017).** Investigating chemical composition and indications of hydrosol soft drinks (aromatic waters) used in Persian folk medicine for women's hormonal and reproductive health conditions. J Evidence-Based Complementary Altern Med. 22(4): 824–839. DOI: 10.1177/2156587217717413

32 **Hamedi, A., Moheimani, S.M., Sakhteman, A., Etemadfard, H. and Moein, M. (2017).** An overview on indications and chemical composition of aromatic waters (hydrosols) as functional beverages in Persian nutrition culture and folk medicine for hyperlipidemia and cardiovascular conditions. J Evidence-Based Complementary Altern Med. 22(4): 544–561. DOI: 10.1177/2156587216686460

33 **Hamedi, A., Pasdaran, A., Zabarjad, Z. and Moein, M. (2017).** A survey on chemical constituents and indications of aromatic waters soft drinks (hydrosols) used in Persian nutrition culture and folk medicine for neurological disorders and mental health. J Evidence-Based Complementary Altern Med. 22(4): 744–752. DOI: 10.1177/2156587217714145

34 **Kumar, R., Atanu, J., Ankit, D. and Satish, P. (2018).** Suitability of type of herb and its form as flavoring in herbal ice cream. Int J Chem Stud. 6(5): 1562–1567.

35 **Didar, Z. (2019).** Effects of coating with pectin and *Cinnamomum verum* hydrosol included pectin on physical characteristics and shelf life of chicken eggs stored at 30°C. Nutr Food Sci Res. 6: 39–45.

36 **Petrakis, E.A., Kimbaris, A.C., Lykouressis, D.P., Polissiou, M.G. and Perdikis, D.Ch. (2015).** Hydrosols evaluation in pest control: insecticidal and settiling inhibition potential against *Mysus persicae* (Sulzer). J Appl Entomol. 139(4): 260–267. DOI: 10.1111/jen.12176

37 **Tabti, L., Dib, M.E.A., Djabou, N., Benyelles, N.G., Paoilini, J., Costa, J. and Muselli, A. (2014).** Control of fungal pathogens of *Citrus sinensis* L. by essential oil and hydrosol of *Thymus capitatus* L. J Appl Bot Food Qual. 87: 279–285. DOI: 10.5073/JABFQ.2014.087.039

38 **Dyer, J., Ashley, S. and Shaw, C. (2008).** A study to look at the effects of a hydrolate spray on hot flushes in women being treated for breast cancer. Complement Ther Clin Pract. 14(4): 273–279. DOI: 10.1016/j.ctcp.2008.02.003

39 **Kunicka-Styczynska, A., Smigielski, K., Prusinowska, R., Rajkowska, K., Kusmider, B. and Sikora, M. (2014).** Preservative activity of lavender hydrosols in moisturizing body gels. Lett Appl Microbiol. 60(1): 27–32. DOI: 10.1111/lam.12346

40 **Aćimović, M., Stanković Jeremić, J., Todosijević, M., Kiprovski, B., Vidović, S., Vladić, J. and Pezo, L. (2022).** Comparative study of the essential oil and hydrosol composition of sweet wormwood (*Artemisia annua* L.) from Serbia. Chem Biodivers. 19: e202100954. DOI: 10.1002/cbdv.202100954.

41 **Verma, R.S. (2010).** Chemical investigation of decanted and hydrophilic fractions of *Salvia sclarea* essential oil. Asian J Tradit Med. 5(3): 102–107.

42 **Ovidi, E., Masci, V.L., Zambelli, M., Tiezzi, A., Vitalini, S. and Garzoli, S. (2021).** *Laurus nobilis*, *Salvia sclarea* and *Salvia officinalis* essential oils and hydrolates: evaluation of liquid and vapor phase chemical composition and biological activities. Plants. 10: 707. DOI: 10.3390/plants10040707

43 **Krzysztof, C. (2006).** Aqueous solubility of liquid monoterpenes at 293 K and relationship with calculated log P value. J Pharm Soc Jpn J Pharm Soc Jpn. 126(4):307-309.

44 **Kamatou, G.P.P., Viljoen, A.M. (2008).** Linalool – A Review of a Biologically Active Compound of Commercial Importance. Nat Prod Commun. 3(7): 1183-1192.

45 **Pitarokili, D., Couladis, M., Petsikos-Panayotarou, N. and Tzakou, O. (2002).** Composition and antifungal activity on soil-borne pathogens of the essential oil of *Salvia sclarea* from Greece. J Agric Food Chem. 50(23): 6688–6691. DOI: 10.1021/jf020422n

46 **Raafat, K. and Habib, J. (2018).** Phytochemical compositions and antidiabetic potentials of *Salvia sclarea* L. essential oil. J. Oleo Sci. 67(8): 1015-1025. DOI: 10.5650/jos.ess17187

47 **Elnir, O., Ravid, U., Putievsky, E. and Dudai, N. (1991).** The chemical composition of two clary sage chemotypes and their hybrids. Flavour Fragrance J. 6(2): 153–155. DOI: 10.1002/ffj.2730060212

48 **Cai, J., Lin, P., Zhu, X. and Su, Q. (2006).** Comparative analysis of clary sage (*S. sclarea* L.) oil volatiles by GC-FTIR and GC-MS. Food Chem. 99(2): 401–407. DOI: 10.1016/j.foodchem.2005.07.041

49 **Yadav, A., Chanotiya, C.S. and Singh, A.K. (2010).** Terpenoid compositions and enantio-differentiation of linalool and sclareol in *Salvia sclarea* L. from three different climatic regions in India. J Essent Oil Res. 22(6): 589–592. DOI: 10.1080/10412905.2010.9700406

50 **Zutic, I., Nitzan, N., Chaimovitsh, D., Schechter, A. and Dudai, N. (2016).** Geographical location is a key component to effective breeding of clary sage (*Salvia sclarea*) for essential oil composition. Isr J Plant Sci. 63(2): 134–141. DOI: 10.1080/07929978.2016.1141602

51 Dzumayev, K.K., Tsibulskaya, I.A., Zenkevich, I.G., Tkachenko, K.G. and Satzyperova, I.F. (1995). Essential oils of *Salvia sclarea* L. produced from plants grown in Southern Uzbekistan. J Essent Oil Res. 7(6): 597–604. DOI: 10.1080/10412905.1995.9700513

52 **Lorenzo, D., Paz, D., Davies, P., Villamil, J., Vila, R., Canigueral, S. and Dellacassa, E. (2004).** Characterization and enantiometric distribution of some terpenes in the essential oil of a Uruguayan biotype of *Salvia sclarea* L. Flavour Fragrance J. 19(4): 303–307. DOI: 10.1002/ffj.1282

53 **Kumar, R., Kaundal, M., Sharma, S., Thakur, M., Kumar, N., Kaur, T., Vyas, D. and Kumar, S. (2017).** Effect of elevated [CO_2] and temperature on growth, physiology and essential oil composition of *Salvia sclarea* L. in the western Himalayas. J Appl Res Med Aromat Plants. 6: 22–30. DOI: 10.1016/j.jarmap.2017.01.001

54 **Raveau, R., Fontaine, J., Verdin, A., Mistrulli, L., Laruelle, F., Fourmentin, S. and Sahraoui, A.L.H. (2021).** Chemical composition, antioxidant and anti-inflammatory activities of clary sage and coriander essential oils produced on polluted and amended soils-phytomanagement approach. Molecules. 26: 5321. DOI: 10.3390/molecules26175321

55 **Angelova, V., Ivanova, R., Todorov, G. and Ivanov, K. (2016).** Potential of *Salvia sclarea* L. for phytoremediation of soils contaminated with heavy metals. International Scholarly and Scientific Research and Innovation. 10: 780–790.

56 **Taarit, M.B., Msaada, K., Hosni, K. and Marzouk, B. (2011).** Physiological changes and essential oil composition of clary sage (*Salvia sclarea* L.) rosette leaves as affected by salinity. Acta Physiol Plant. 33(1): 153–162. DOI: 10.1007/s11738-010-0532-8

57 **Grigoriadou, K., Trikka, F.A., Tsoktouridis, G., Krigas, N., Sarropoulou, V., Papanastasi, K., Maloupa, E. and Makris, A.M. (2020).** Micropropagation and

449 cultivation of *Salvia sclarea* for essential oil and sclareol production in northern Greece.
450 In vitro Cell Dev Biol Plant. 56(1): 51–59. DOI: 10.1007/s11627-019-10040-4
451 58 **Sharma, S. and Kumar, R. (2012).** Effect of nitrogen on growth, biomass and oil
452 composition of clary sage (*Salvia sclarea* Linn.) under mid hills of north western
453 Himalayas. Indian J Nat Prod Resour. 3(1): 79–83.
454 59 **Hudaib, M., Bellardi, M.G., Rubies-Autonell, C., Fiori, J. and Cavrini, V. (2001).**
455 Chromatographic (GC-MS, HPLC) and virological evaluation of *Salvia sclarea* infected
456 by BBWV-I. Farmaco. 56(3): 219–227. DOI: 10.1016/s0014-827x(01)01038-2
457 60 **Safaei-Ghomi, J., Masoomi, R., Kashi, F.J. and Batooli, H. (2016).** Bioactivity of the
458 essential oil and methanol extracts of flowers and leaves of *Salvia sclarea* L. from central
459 Iran. J Essent Oil-Bear Plants. 19(4): 885–896. DOI: 10.1080/0972060X.2016.1195292
460 61 **Farkaš, P., Holla, M., Tekel, J., Mellen, S. and Vaverkova, Š. (2005).** Composition of
461 the essential oils from the flowers and leaves of *Salvia sclarea* L. (Lamiaceae) cultivated
462 in Slovak Republic. J Essent Oil Res. 17(2): 141–144. DOI:
463 10.1080/10412905.2005.9698858
464 62 **Saharkhiz, M.J., Ghani, A. and Hassanzadeh-Khayyat, M. (2009).** Changes in
465 essential oil content and composition of clary sage (*Salvia sclarea*) aerial parts during
466 different phenological stages. Med Aromat Plant Sci Biotechnol. 3(1): 90–93.
467 63 **Ronyai, E., Simandi, B., Lemberkovics, E., Veress, T. and Patiaka, D. (1999).**
468 Comparison of the volatile composition of clary sage oil obtained by hydrodistillation
469 and supercritical fluid extraction. J Essent Oil Res. 11(1): 69–71. DOI:
470 10.1080/10412905.1999.9701074
471 64 **Koutsaviti, A., Tzini, D.I. and Tzakou, O. (2016).** Greek *Salvia sclarea* L. essential
472 oils: effect of hydrodistillation time, comparison of the aroma chemicals using
473 hydrodistillation and SH-SPME techniques. Rec Nat Prod. 10(6): 800–805.

65 **Hristova, Y., Gochev, V., Wanner, J., Jirovetz, L., Schmidt, E., Girova, T. and Kuzmanov, A. (2013).** Chemical composition and antifungal activity of essential oil of *Salvia sclarea* L. from Bulgaria against clinical isolates of *Candida* species. J BioSci Biotech. 2(1): 39–44.

66 **Esteban, J.L., Martinez-Castro, I., Morales, R., Fabrellas, B. and Sanz, J. (1996).** Rapid identification of volatile compounds in aromatic plants by automatic thermal desorption – GC-MS. Chromatographia. 43(1/2): 63–72.

67 **Fraternale, D., Giamperi, L., Bucchini, A., Ricci, D., Epifano, F., Genovese, S. and Curini, M. (2005).** Composition and antifungal activity of essential oil of *Salvia sclarea* from Italy. Chem Nat Compd. 41(5): 604–606. DOI: 10.1007/s10600-005-0221-9

68 **Sharopov, F.S., Satyal, P., Setzer, W.N. and Wink, M. (2015).** Chemical compositions of the essential oils of three *Salvia* species cultivated in Germany. Am J Essent Oil Nat Prod. 3(2): 26–29.

69 **Kostova, I., Lasheva, V., Fidan, H., Georgieva, D., Damyanova, S. and Stoyanova, A. (2020).** Effect of clary sage (*Salvia sclarea* L.) essential oil on paper packing materials. Ukr Food J. 9(2): 287–297. DOI: 10.24263/2304-974X-2020-9-2-3

70 **Tasheva, S., Gandova, V., Prodanova-Stefanova, V., Marinova, K., Dimov, M., Dobreva, K. and Stoyanova, A. (2021).** Investigation of the thermodynamic and thermal properties of clary sage (*Salvia sclarea* L.) essential oil and its main components. E3S Web of Conferences. 286: 02003. DOI: 10.1051/e3sconf/202128602003

71 **Tognolini, M., Barcelli, E., Ballabeni, V., Bruni, R., Bianchi, A., Chiavarini, M. and Impicciatore, M. (2006).** Comparative screening of plant essential oils: phenylpropanoid moiety basic core for antiplatent activity. Life Sci. 78(13): 1419–1432. DOI: 10.1016/j.lfs.2005.07.020.

72 **Džamić, A., Soković, M., Ristić, M., Grujić-Jovanović, S., Vukojević, J. and Marin, P. (2008).** Chemical composition and antifungal activity of *Salvia sclarea* (Lamiaceae) essential oil. Arch Biol Sci. 60(2): 233–237. DOI: 10.2298/ABS0802233D

73 **Andrade, M.A., Azevedo, C.S., Motta, F.N., Santos, M.L., Silva, C.L., Santana, J.M. and Bastos, I. (2016).** Essential oils: *in vitro* activity against *Leishmania amazonensis*, cytotoxicity and chemical composition. BMC Complementary Altern Med. 16: 444. DOI: 10.1186/s12906-016-1401-9

74 **Martin, A., Silva, V., Pérez, L., García-Serna, J. and Cocero, M. J. (2007).** Direct synthesis of linalyl acetate from linalool in supercritical carbon dioxide: a thermodynamic study. Chem Eng Technol. 30(6): 726–731. DOI: 10.1002/ceat.200600407

75 **Souleles, C. and Argyriadou, N. (1997).** Constituents of the essential oil of *Salvia sclarea* growing wild in Greece. Int J Pharmacogn (Lisse, Neth). 35(3), 218–220. DOI: 10.1076/phbi.35.3.218.13295

76 **Ogutcu, H., Sokmen, A., Sokmen, M., Polissiou, M., Serkedjieva, J., Daferera, D., Sahin, F., Baris, O. and Gulluce, M. (2008).** Bioactivities of the various extracts and essential oils of *Salvia limbata* C.A.Mey. and *Salvia sclarea* L. Turk J Biol. 32(3): 181–192.

77 **Yuce, E., Yildirim, N., Yildirim, N.C., Paksoy, M.Y. and Bagci, E. (2014).** Essential oil composition, antioxidant and antifungal activities of *Salvia sclarea* L. from Munzur Valley in Tunceli, Turkey. Cell Mol Biol. 60(2): 1–5.

78 **Moretti, M., Peana, A. and Satta, M. (1997).** A study on anti-inflammatory and peripheral analgesic action of *Salvia sclarea* oil and its main components. J Essent Oil Res. 9(2): 199–204. DOI: 10.1080/10412905.1997.9699459

79 **Peana, A., Moretti, M. and Juliano, C. (1999).** Chemical composition and antimicrobial action of the essential oils of *Salvia desoleana* and *S. sclarea*. Planta Med. 65(8): 752–754. DOI: 10.1055/s-2006-960857

80 **Caissard, J.C., Olivier, T., Delbecque, C., Palle, S., Garry, P.P., Audran, A., Valot, N., Moja, S., Nicole, F., Magnard, J.L., Legrand, S., Baudino, S. and Jullien, F. (2012).** Extracellular localization of the diterpene sclareol in clary sage (*Salvia sclarea* L., Lamiaceae). PLoS One. 7(10): e48253. DOI: 10.1371/journal.pone.0048253

81 **Carrubba, A., La Tore, R., Piccaglia, R. and Marotti, M. (2006).** Modification over years of volatile compounds and agronomic features in a Sicilian clary sage biotype. Acta Hort. 723, 203–208. DOI: 10.17660/ActaHortic.2006.723.24

82 **Zheljazkov, V.D. and Nielsen, N.E. (1996).** Growing clary sage (*Salvia sclarea* L.) in heavy metal-polluted areas. Acta Hort. 426: 309-328. DOI: 10.17660/ActaHortic.1996.426.36

83 **Sperdouli, I., Adamakis, I.D.S., Dobrikova, A., Apostolova, E., Hanc, A. and Moustakas, M. (2022).** Excess zinc supply reduces cadmium uptake and mitigates cadmium toxicity effects on chloroplast structure, oxidative stress, and photosystem II photochemical efficiency in *Salvia sclarea* plants. Toxics. 10(1): 36. DOI: 10.3390/toxics10010036.