



original scientific paper

Ratar. Povrt. 2024, 61(3): 61-73

doi: 10.5937/ratpov61-50284

Manuscript submitted: 6 April 2024

Manuscript accepted: 16 August 2024

Copyright © 2024 at the authors

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>)



Influence of environmental factors on the accumulation volatile compounds in *Salvia officinalis* essential oil and hydrolate

Milica Acimović^{ID 1*} · Jovana Stanković Jeremić^{ID 2} · Mirjana Cvetković^{ID 2} · Marina Todosijević^{ID 3} · Ljubodrag Vujisić^{ID 3} · Vladimir Sikora^{ID 1} · Biljana Lončar^{ID 4}

¹Institute of Field and Vegetable Crops, Novi Sad, Serbia

²University of Belgrade, Institute of Chemistry, Technology and Metallurgy, Belgrade, Serbia

³University of Belgrade, Faculty of Chemistry, Belgrade, Serbia

⁴University of Novi Sad, Faculty of Technology, Novi Sad, Serbia

* Corresponding author: milica.acimovic@ifvcns.ns.ac.rs

Summary: The study investigated the cultivation, chemical composition, and potential applications of *Salvia officinalis* L. cultivar “Primorska” over three years. The research revealed that the essential oil of *S. officinalis* contains significant amounts of oxygenated monoterpenes, including *α*-thujone (19.9-29.0%), camphor (15.8-19.6%), *β*-thujone (12.3-13.3%), and 1,8-cineole (8.8-11.3%). The content of these compounds varied across years and correlated with temperature fluctuations. The hydrolate derived from *S. officinalis* exhibited a similar chemical profile to the essential oil, with camphor emerging as the most abundant compound (42.4-49.6%). Statistical analysis revealed correlations between temperature, precipitation, and the content of various compounds in both the essential oil and hydrolate. Temperatures are in strong negative correlation with *α*-thujone ($K_T = -36.500$), and in positive correlation with camphor ($K_T = 9.5$), *β*-thujone ($K_T = 4.500$) and 1,8-cineole ($K_T = 5.000$) in essential oil. In hydrolates is also noted similar trend of correlations: negative correlations between temperature and *α*-thujone ($K_T = -32.000$), and positive correlations temperature and camphor ($K_T = 34.000$), *β*-thujone ($K_T = 7.000$), and 1,8-cineole ($K_T = 7.000$). *S. officinalis* essential oil is highly valued in the food and pharmaceutical industry, as well as in cosmetics, perfumery and aromatherapy. Numerous studies have indicated its antioxidant, antimicrobial, anti-inflammatory and anticancer potential. The study also highlighted potential applications of *S. officinalis* hydrolate in cosmetics, food sanitation, and pharmaceuticals due to its antioxidant properties and moderate antimicrobial effects. Overall, the research contributes to a deeper understanding of *S. officinalis* products' chemical composition and underscores the potential of its hydrolate as a valuable resource in multiple industries. Further exploration into its applications and benefits will drive innovation in product development and utilization.

Keywords: climate change, correlations, cultivars, essential oils, hydrolates, hydrosol, sage, *Salvia officinalis*, volatile oil

Introduction

Salvia officinalis L. (Lamiaceae), commonly known as sage (*eng.*), is a perennial low shrub native to the Mediterranean basin, but it is also domesticated in temperate regions, where it is successfully adopted and cultivated as an ornamental, culinary, medicinal, and aromatic plant (Vosoughi et al. 2018, Giannoulis et al. 2021, Tibaldi et al. 2022). Since prehistoric times until today, this plant represents one of the most important medicinal plant species (Grdiša et al. 2015). Ancient Greeks and Romans used *S. officinalis* as a condiment (Andrews, 1956), in medieval times, it was a must-have plant in “Hortulus” according to Charlemagne (Hansox and Hocking, 1957), and contemporary it is a very popular home remedy, mainly consumed as herbal tea (infusion), against influenza, for oral hygiene, for improving cognitive function, liver status and treatment of diabetes (Lima et al. 2005, Eidi et al. 2005, Beheshti-Rouy et al. 2015, Dinel et al. 2020, Abou Baker et al. 2021).

S. officinalis is rich in polyphenols (Duletić-Laušević et al. 2019, Boufadi et al. 2021) and essential oil (Awen et al. 2011, Baydar et al. 2013). It possesses a strong, spicy, woody, refreshing scent, and aroma profile was determined by *α*-thujone (or *cis*-thujone), *β*-thujone (or *trans*-thujone), and camphor (Pachura et al. 2022). Thujone-containing plants (sages, tansy, wormwood, cedar) are generally considered as anthelmintic, psychedelic, uterine and antidote to opium and other CNS depressant poisons (Albert-Puleo 1978). However, today, they are renowned as the highly toxic and possibly psychoactive constituents (Radulović et al. 2017, Rasmussen and Rosenfeld, 2020). Therefore, their content in *S. officinalis* is limited according to ISO standard (9909:1997) to 18.0-43.0% for *α*-thujone and 3.0-8.5% for *β*-thujone.

Global climate change shortens the growing season, declines productivity, and the ranges of some plant species are shifting, which is especially noticeable in the case of Mediterranean plant species (Matula et al. 2023). This shift in the area of growth and commercial cultivation leads to the requirement to monitoring the quality of the obtained raw materials. Additionally, zero-waste management technologies have led to an increase in the popularity of hydrolates as a value-added product (Aćimović et al. 2020b). Many aromatic plants with a limited cultivation area outside Serbia, among term roses (Božanić Tanjga et al. 2022, Aćimović et al. 2023), lavender (Aćimović et al. 2022b, Kiproviski et al. 2023), immortelle (Aćimović et al. 2021, Aćimović et al. 2022c) and sage (Aćimović et al. 2022a), have great potential for cultivation as essential oil bearing plants in the agro-ecological conditions of Serbia, if is selected appropriate chemotype/variety adapted to the microclimatic and soil conditions of a particular locality. All this points to the necessity of developing a national policy and strategy for the management and mapping of medicinal plants in Serbia, but also to education farmers species ecological requirements, cultivation and postharvest processing of promising plant species that are in demand on the international market.

The aim of this study was to evaluate the influence of environmental factors on the chemical composition of the essential oil of *S. officinalis* grown under continental conditions. For this purpose, an experiment with *S. officinalis* cultivar “Primorska” was established at the Institute of Field and Vegetable Crops, Novi Sad; northern Serbia. Plant material was collected at the end of August during all three successive years, analyzed by GC-MS and correlated with main climatic parameters (temperature and precipitation) for that growing season.

Material and method

Plant material and weather conditions

Salvia officinalis L. cultivar “Primorska” were grown for three successive years (2018/19, 2019/20 and 2020/2021) at the Institute of Field and Vegetable Crops Novi Sad (Department for Alternative crops located in Bački Petrovac, 45.3367898, 19.6682531). Voucher specimens (2-1548)

were confirmed and deposited at the BUNS Herbarium (Herbarium of the Department of Biology and Ecology, Faculty of Science, University of Novi Sad). The plantation was established in the spring of 2017, on chernozem calcareous galeyc soil type (detailed information about soil conditions is given by Aćimović et al. 2022a). Seedlings produced in a greenhouse were planted at a distance of 70×30 cm. Irrigation and manual weed control (weeding and hoeing) was performed during all growing years. Temperature and precipitation for experimental years are shown in climate graphs (figures 1-3). The harvest was carried out manually, with a sickle, at the end of August after the flowering and fruiting of the plants.

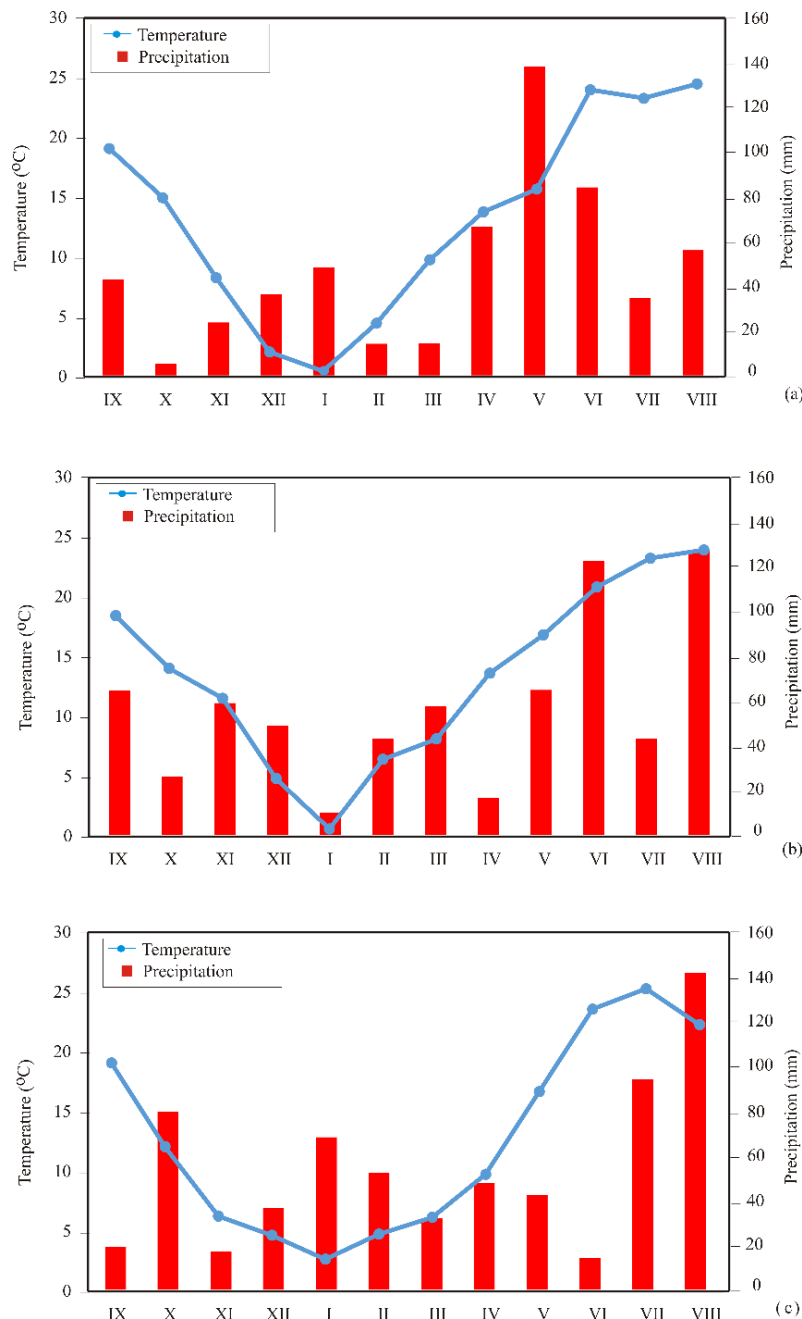


Figure 1. Climate graphs during: (a) 1st, (b) 2nd, and (c) 3rd growing season (data obtained from the meteorological station within the experimental field)

Steam distillation

After harvesting, the above-ground part of the *S. officinalis* was placed in a solar dryer, and after reaching a constant weight, it was transferred to further processing. Each year, one distillation was done, for which 100 kg of dry plant material was used. The plant material was placed in a cylindrical vessel with a lid and supplied with steam. The steam, together with the volatile compounds of *S. officinalis* after passing through the cooler and condenser turns into a liquid and collects in a Florentine flask. Essential oil floats on the surface of the water phase as it is lighter than water, and is easily decanted and further dewatered in a separatory funnel with the addition of anhydrous sodium sulphate. The condensed water that remains in the Florentine flask, commonly known as hydrolate, contains a small amount of volatile compounds that are hydro soluble and give it a flavor and fragrance that is very similar to the corresponding essential oil. Due to the very small amount of volatile compounds (less than 0.1%), the hydrolate represents wastewater. The amount of hydrolate obtained in one process in the small-scale distillation unit at the Institute of Field and Vegetable Crops Novi Sad, is limited by the volume of the Florentine flask (20 liters). After the distillation process was completed, the hydrolate was filled into the sterile plastic bottles through filter paper.

Chemical characterization of volatile compounds

For chemical characterization of volatile compounds from essential oils and recovery essential oil from hydrolates was used multiple analytical devices which combines Agilent 7890A gas chromatograph, flame ionization detector with nonpolar HP-5MS fused-silica capillary column and Agilent 5973 mass spectrometry detector. Terms and conditions for GC-FID-MS analysis are given in Aćimović et al. 2020a.

Statistical Analysis

A linear regression model was constructed to forecast the content of active compounds in the essential oil and hydrolate composition of *S. officinalis* over three years based on temperature and precipitation data. The corresponding regression coefficients were derived using the linear equation: $x = a + b_T T + b_P P$, where x represents either the content of essential oil active compounds or hydrolate, 'a' denotes the intercept, 'b_T' and 'b_P' denote coefficients associated with temperature and precipitation, while 'T' and 'P' represent the respective amounts of temperature and precipitation. Statistical processing of the gathered data was carried out using the STATISTICA 10.0 software package.

Results and discussion

S. officinalis essential oil

In the *S. officinalis* essential oil dominate oxygenated monoterpenes (65.5-71.4%), among them, α -thujone (19.9-29.0%), camphor (15.8-19.6%), β -thujone (12.3-13.3%) and 1,8-cineole (8.8-11.3%) were leading (Table 1). The investigation of *S. officinalis* essential oils from various European countries showed that the principal compounds were 1,8-cineole, camphor, α -thujone and β -thujone (Raaijmakers et al. 2007). Similar composition referred other authors too (Mot et al. 2022, Mehmet et al. 2024). However, stress conditions, such as temperature, water-deficit stress and salinity stress, can influence the differences in *S. officinalis* secondary metabolites profile (Karalić et al. 2022, Mohammadi-Cheraghabadi et al. 2021, Sheikhalipour et al. 2024).

According to ISO 9909 standard, the α -thujone content is between 18.0-43.0%. In this study, the content of α -thujone, as the most abundant compound in all three years, was in the range

of recommended values, and it could be concluded that temperatures are in strong negative correlation with this compound ($K_T = -36.500$). The highest content of α -thujone is noted in the first investigated year (29.0%), in the year when the lowest average temperature was recorded (12.9 °C). However, this year the lowest temperature was recorded in the winter months (December, January and February), so this probably negatively influences essential oil quality, i.e. higher accumulation of α -thujone in essential oil. Bearing in mind that *S. officinalis* is a plant of the Mediterranean climate, this contributes to understanding the continental climate (with lower temperatures during the winter months) is not suitable for growing this plant.

Moreover, camphor content is limited to 4.5-24.5% according to ISO 9909 standard, and the content of this component in all three examined years was within the limit values. Results showed that camphor is in positive correlation to the temperature ($K_T = 9.5$).

ISO 9909 standard limits β -thujone content between 3.0 and 8.5%, while in our sample its content was significantly higher, between 12.3 and 13.3%. From this it can be concluded that our samples do not meet the standard, because the content of β -thujone is significantly higher, and it is positively correlated with temperatures ($K_T = 4.500$); the higher the mean annual temperature, the higher the content of this component in the essential oil.

The content of 1,8-cineole is limited to 5.5-13.0% according to ISO 9909 standard, and in this study content was ranged between 8.8 and 11.3%, which is within the recommended limits. However, it is noted positive correlation with temperature ($K_T = 5.000$).

Too high or too low a concentration of certain compounds in an essential oil can affect the loss of medicinal properties or even poisonousness (Lakušić et al. 2013). Therefore, the ISO Standard recommends limit values within which the essential oil exhibits biological activity, and is acceptable for application in various industries. In the food industry, *S. officinalis* essential oil is used as natural preservative and flavoring agents (Porte et al. 2013), while in pharmaceutical industry it has a wide application, as antioxidant (Rguez et al. 2019, Hay et al. 2018), antimicrobial (Hay et al. 2018, Aćimović 2022a), anti-inflammatory (de Melo et al. 2012, Boufadi et al. 2021) and anticancer (Loizzo et al. 2007, Garcia et al. 2016) agents. In cosmetics, perfumery and aromatherapy, *S. officinalis* essential oil is highly appreciated because of its characteristic aromatic profile (Jonas and Schieberle 2021, Mot et al. 2022).

S. officinalis hydrolate

In the *S. officinalis* hydrolate also dominated oxygenated monoterpenes (93.7-97.0%), among them same dominant compounds as in essential oil, but in different proportions; camphor (42.4-49.6%), α -thujone (13.4-19.8%), 1,8-cineole (12.3-20.5%) and β -thujone (5.7-7.1%) (Table 2). In previous studies, camphor is also noted as the most abundant compound in *S. officinalis* hydrolate (Smail et al. 2011, Baydar et al. 2013). On the other side, there are reported that 1,8-cineole was the main compound of *S. officinalis* hydrolate as well as in essential oil (Ovidi et al. 2021).

Camphor is almost insoluble in water (Chen et al. 2019), but hydrolates are acidic liquids that change the solubility of some compounds (Aćimović et al. 2020). However, there are previous reports that camphor could be found in other hydrolates as a dominant compound, such as *Picea glauca* (65.0%) (Garneau et al. 2012) and *Rosmarinus officinalis* (29.7-51.9%) (Hay et al. 2018, Tomi et al. 2016), while is also noted in other hydrolates but in lower concentrations, for example lavender (13.4%-19.6%) (Baydar and Kineci 2009, Garzoli et al. 2020) and spearmint (13.5%) (Zekri et al. 2014).

The analysis of the influence of temperature and precipitation on the accumulation of camphor in the hydrolate ($K_T = 34.000$) shows the same trend as with the essential oil. In addition, temperature is negatively correlated with the content of α -thujone in the hydrolate ($K_T = -32.000$),

the same as in the case of the essential oil. The same can be said for 1,8-cineole ($K_T=7.000$) and β -thujone ($K_T=7.000$).

Table 1. *Salvia officinalis* essential oil composition

Compound	RI	1 st	2 nd	3 rd	K_T	K_P
Normonoterpene hydrocarbons						
<i>cis</i> -Salvene	846	0.5	0.1	0.3	-2.000	-0.003
<i>trans</i> -Salvene	856	0.1	nd	nd	-0.500	-0.001
Total		0.6	0.1	0.3	-2.500	-0.004
Monoterpene hydrocarbons						
Tricyclene	920	0.2	0.1	0.2	-0.500	-0.001
α -Thujene	924	0.2	0.1	0.2	-0.500	-0.001
α -Pinene	931	4.3	2.8	4.3	-7.500	-0.011
Camphene	946	6.2	4.7	6.3	-7.500	-0.010
Sabinene	970	0.1	0.1	0.1	0.000	0.000
β -Pinene	974	2.2	2.4	3.9	1.000	0.005
Myrcene	988	1.2	0.8	1.0	-2.000	-0.003
α -Phellandrene	1004	0.1	tr	0.1	0.000	0.000
α -Terpinene	1014	0.2	0.2	0.2	0.000	0.000
<i>p</i> -Cymene	1022	0.7	0.2	0.4	-2.500	-0.004
Limonene	1025	3.1	2.7	3.1	-2.000	-0.003
<i>cis</i> - β -Ocimene	1034	nd	0.1	nd	0.500	0.001
γ -Terpinene	1055	0.4	0.4	0.4	0.000	0.000
Terpinolene	1086	0.2	0.3	0.3	0.500	0.001
Total		19.1	14.9	20.5	-21.000	-0.027
Oxygenated monoterpenes						
1,8-Cineole	1028	8.8	9.8	11.3	5.000	0.012
Linalool	1099	0.3	0.4	0.5	0.500	0.001
α -Thujone	1106	29.0	21.7	19.9	-36.500	-0.069
β -Thujone	1115	12.3	13.2	13.3	4.500	0.008
<i>iso</i> -3-Thujanol	1132	0.1	0.2	nd	0.500	0.001
<i>trans</i> -Sabinol (OH vs. IPP)	1138	0.1	nd	nd	-0.500	-0.001
Camphor	1143	17.7	19.6	15.8	9.500	0.010
<i>trans</i> -Pinocamphone	1158	tr	0.1	nd	1.000	0.003
Borneol	1163	1.6	3.5	3.6	9.500	0.017
<i>cis</i> -Pinocamphone	1168	nd	0.2	nd	1.000	0.001
Terpinen-4-ol	1174	0.3	nd	nd	-1.500	-0.003
α -Terpineol	1188	0.1	0.1	nd	0.000	0.000
Myrtenol	1194	0.1	0.1	nd	0.000	0.000
Neral	1239	0.1	nd	nd	-0.500	-0.001
Geranial	1269	0.1	nd	nd	-0.500	-0.001
Bornyl acetate	1284	0.6	1.6	1.1	5.000	0.008

<i>trans</i> -Sabinyl acetate (AC vs. IPP)	1291	0.2	nd	nd	-1.000	-0.002
Total		71.4	70.5	65.5	-4.500	-0.018
Sesquiterpene hydrocarbons						
<i>α</i> -Copaene	1369	nd	nd	0.1	0.000	0.000
<i>trans</i> -Caryophyllene	1417	2.4	5.5	4.2	15.500	0.025
<i>α</i> -Humulene	1452	3.2	4.8	4.0	8.000	0.013
<i>allo</i> -Aromadene	1459	0.1	0.1	0.1	0.000	0.000
<i>γ</i> -Muurolene	1472	nd	0.1	nd	0.500	0.001
Viridiflorene	1494	0.1	0.1	nd	0.000	0.000
<i>δ</i> -Cadinene	1518	nd	0.1	0.1	0.500	0.001
Total		5.8	10.7	8.5	24.500	0.040
Oxygenated sesquiterpenes						
Caryophyllene oxide	1581	0.2	0.3	nd	0.500	0.000
Viridiflorol	1589	1.6	nd	nd	-8.000	-0.014
Humulene epoxide II	1606	0.4	nd	0.4	-2.000	-0.003
13- <i>epi</i> -Manool	2054	nd	nd	0.3	0.000	0.001
Total		2.2	0.3	0.7	-9.500	-0.016

RI—Retention index; nd – not detected; tr – trace (less than 0.05%)

Considering the similar chemical composition of the essential oil and the hydrolate, the hydrolate of *S. officinalis* has great potential for use as a product with added value, through re-distillation and obtaining a secondary essential oil by cohabitation (similar to rose hydrolate), or in other industries processes. Research has shown that *S. officinalis* hydrolate has good antioxidant potential (Ovidi et al. 2021) and low to moderate antimicrobial properties (Ovidi et al. 2021, Aćimović et al. 2022), but can be used as a food sanitizer (fresh cut carrots and apples), by washing for 60 minutes, which achieves a significant reduction of *Salmonella* Typhimurium inoculum (Tornuk et al. 2011). However, *S. officinalis* hydrosol appears as a high potency cytotoxic agent on the hypothalamic cell line (Hypo-E22) (Politi et al. 2022).

Table 2. *Salvia officinalis* hydrolate composition

Compound	RI	1 st	2 nd	3 rd	K _T	K _P
Other						
3-Hexanol	795	nd	nd	0.1	0.000	0.000
Isovaleric acid	828	0.1	0.2	nd	0.500	0.001
Furfural	830	0.1	nd	nd	-0.500	-0.001
<i>cis</i> -3-Hexenol	847	nd	0.3	0.3	1.500	0.003
<i>trans</i> -3-Hexenol	850	0.1	nd	nd	-0.500	-0.001
<i>cis</i> -2-Hexenol	861	nd	nd	0.1	0.000	0.000
1-Octen-3-ol	975	0.3	0.2	0.3	-0.500	-0.001
6-methyl-5-Hepten-2-one	985	0.4	nd	nd	-2.000	-0.004
Benzyl alcohol	1036	0.1	nd	nd	-0.500	-0.001
Benzene acetaldehyde	1042	0.2	0.1	0.1	-0.500	-0.001
<i>p</i> -vinyl-Guaiacol	1314	0.1	nd	nd	-0.500	-0.001

Total		1.4	0.8	0.9	-3.000	-0.005
Monoterpene hydrocarbons						
<i>p</i> -Cymene	1023	0.1	nd	nd	-0.500	-0.001
Total		0.1	nd	nd	-0.500	-0.001
Oxygenated monoterpenes						
1,8-Cineole	1031	13.7	12.3	20.5	7.000	0.003
<i>cis</i> -Linalool oxide (furanoid)	1071	0.3	0.4	0.4	0.500	0.001
<i>trans</i> -Linalool oxide (furanoid)	1089	0.3	0.4	0.3	0.500	0.001
Linalool	1103	0.5	0.4	0.8	-0.500	0.000
α -Thujone	1108	19.8	13.4	13.9	-32.000	-0.057
β -Thujone	1117	7.1	5.7	6.8	7.000	0.010
<i>iso</i> -3-Thujanol	1140	0.3	0.4	0.4	0.500	0.001
Camphor	1150	42.8	49.6	42.4	34.000	0.047
Isoborneol	1159	0.1	0.1	nd	0.000	0.000
<i>trans</i> -Pinocamphone	1160	0.1	0.2	0.1	0.500	0.001
Borneol	1167	4.7	10.5	9.1	29.000	0.049
<i>cis</i> -Pinocamphone	1169	nd	0.3	0.1	1.500	0.002
<i>trans</i> -Linalool oxide (pyranoid)	1174	0.1	nd	nd	-0.500	-0.001
Terpinen-4-ol	1177	1.2	0.9	1.3	-1.500	-0.002
<i>p</i> -Cymen-8-ol	1185	0.4	nd	nd	-2.000	-0.004
α -Terpineol	1191	0.4	0.5	0.4	0.500	0.001
<i>p</i> -Mentha-1,5-dien-8-ol	1192	0.2	nd	nd	-1.000	-0.002
Myrtenol	1197	0.2	0.3	0.2	0.500	0.001
<i>exo</i> -2-Hydroxycineole	1210	0.1	nd	nd	-0.500	-0.001
<i>trans</i> -Carveol	1219	0.2	0.2	0.2	0.000	0.000
<i>cis-p</i> -Mentha-1(7),8-dien-2-ol	1228	0.1	nd	nd	-0.500	-0.001
<i>cis</i> -Carveol	1230	0.1	nd	nd	-0.500	-0.001
Neral	1241	0.1	nd	nd	-0.500	-0.001
Carvone	1244	0.1	nd	nd	-0.500	-0.001
Geraniol	1254	0.1	nd	nd	-0.500	-0.001
Geranial	1271	0.3	nd	nd	-1.500	-0.003
Bornyl acetate	1286	0.1	0.2	0.1	0.500	0.001
Thymol	1293	0.1	nd	nd	-0.500	-0.001
Carvacrol	1302	0.1	0.3	nd	1.000	0.001
Piperitenone	1342	0.1	nd	nd	-0.500	-0.001
Total		93.7	96.1	97.0	12.000	0.023
Phenylpropanoids						
Eugenol	1358	0.1	0.1	0.1	0.000	0.000
Total		0.1	0.1	0.1	0.000	0.000
Oxygenated sesquiterpens						
Humulene epoxide II	1609	0.1	0.1	nd	0.000	0.000

Total		0.1	0.1	nd	0.000	0.000
--------------	--	------------	------------	-----------	--------------	--------------

RI—Retention index; nd – not detected

Conclusions

Salvia officinalis a typical Mediterranean plant, is well adapted to a dry and hot summer and a cool and rainy winter. In its essential oil dominate oxygenated monoterpenes such as α -thujone, camphor, β -thujone and 1,8-cineole. Results showed a strong negative correlation between α -thujone and temperatures, while camphor, β -thujone and 1,8-cineole were in positive correlation with this parameter. By comparison with the ISO standard, it was determined that the content of α -thujone, camphor and 1,8 cineole is within the recommended values, while the content of β -thujone is significantly higher. Practically, it can be said that the production of *S. officinalis* in continental climate is limited by the high content of β -thujone, and such an essential oil does not meet the criteria to be used in the food and pharmaceutical industry, neither in cosmetics, perfumery, or aromatherapy. On the other side, *S. officinalis* hydrolate also contains oxygenated monoterpenes as the dominant class, with camphor, α -thujone, 1,8-cineole and β -thujone. It could be said that essential oil and corresponding hydrolate contain the same dominant compounds, but in slightly different proportions. However, there is no quality assessment standard for hydrolates, while interest in this by-product has been increasing in recent years, both in terms of chemical characterization and finding potential applications.

Author contributions: Milica Aćimović: Conceptualization, Project administration, Writing – original draft, Funding acquisition; Jovana Stanković Jeremić: Formal analysis, Validation, Funding acquisition; Mirjana Cvetković: Investigation, Validation, Funding acquisition; Marina Todosijević: Methodology, Writing – review & editing, Funding acquisition; Ljubodrag Vujisić: Supervision, Funding acquisition; Vladimir Sikora: Resources, Visualization, Funding acquisition; Biljana Lončar: Data curation, Software, Funding acquisition.

Competing interests' statement: No competing interests were disclosed.

Funding statement: This research has been financially supported by the Ministry of Science, Technological Development and Innovation of Republic of Serbia (Contract No: 451-03-66/2024-03/200032 (Institute of Field and Vegetable Crops Novi Sad), 451-03-66/2024-03/200026 (University of Belgrade, Institute of Chemistry, Technology and Metallurgy), 451-03-66/2024-03/200168 (University of Belgrade, Faculty of Chemistry), and 451-03-66/2024-03/200134 (University of Novi Sad, Faculty of Technology).

References

- Abou Baker, D., Amarowicz, R., Kandeil, A., Ali, M., & Ibrahim, E. (2021). Antiviral activity of *Lavandula angustifolia* L. and *Salvia officinalis* L. essential oils against avian influenza H5N1 virus. *Journal of Agriculture and Food Research*, 4, 100135. <https://doi.org/10.1016/j.jafr.2021.100135>
- Aćimović, M., Ljujić, J., Vulić, J., Zheljazkov, V.D., Pezo, L., Varga, A., & Tumbas Šaponjac, V. (2021). *Helichrysum italicum* (Roth) G. Don essential oil from Serbia: Chemical composition, classification and biological activity – May it be a suitable new crop for Serbia? *Agronomy*, 11, 1282. <https://doi.org/10.3390/agronomy11071282>
- Aćimović, M., Lončar, B., Stanković Jeremić, J., Cvetković, M., Pezo, L., Pezo, M., Todosijević, M., & Tešević, V. (2022b). Weather conditions influence on lavandin essential oil and hydrolate quality. *Horticulturae*, 8, 281. <https://doi.org/10.3390/horticulturae8040281>
- Aćimović, M., Pezo, L., Čabarkapa, I., Trudić, A., Stanković Jeremić, J., Varga, A., Lončar, B., Šovljanski, O., & Tešević, V. (2022a). Variation of *Salvia officinalis* L. essential oil and hydrolate composition and their antimicrobial activity. *Processes*, 10, 1608. <https://doi.org/10.3390/pr10081608>
- Aćimović, M., Pezo, L., Tešević, V., Čabarkapa, I., & Todosijević, M. (2020a). QSRR model for predicting retention indices of *Satureja kitaibelii* Wierzb. ex Heuff.

- essential oil composition. *Industrial Crops and Products*, 154, 112752.
<https://doi.org/10.1016/j.indcrop.2020.112752>
- Aćimović, M., Raičević, V., Božanić Tanjga, B., Lončar, B., Šuput, D., Šovljanski, O., Tomić, A., Cvanić, T., Radovanović, K., & Kiprovski, B. (2023). Profiling of *Rosa hybrida* cv. Mileva essential oil, evaluation of its bioactivity *in vitro*, chemometric analysis and comparison to other non-commercial roses. *Journal of Essential Oil Bearing Plants*, 26, 848-861.
<https://doi.org/10.1080/0972060X.2023.2258910>
- Aćimović, M., Tešević V., Smiljanić, K., Cvetković, M., Stanković, J., Kiprovski, B., & Sikora V. (2020b). Hydrolates – By-products of essential oil distillation: Chemical composition, biological activity and potential uses. *Advanced technologies*, 9, 54–70.
<https://doi.org/10.5937/savteh2002054A>
- Aćimović, M., Zeremski, T., Šovljanski, O., Lončar, B., Pezo, L., Zheljaskov, V.D., Pezo, M., Šuput, D., & Kurunci, Z. (2022). Seasonal variations in essential oil composition of immortelle cultivated in Serbia. *Horticulturae*, 8, 1183.
<https://doi.org/10.3390/horticulturae8121183>
- Albert-Puleo M. (1978). Mythobotany, pharmacology, and chemistry of thujone-containing plants and derivatives. *Economic Botany*, 32, 65–74.
<http://doi.org/10.1007/BF02906731>
- Andrews, A.C. (1956). Sage as a condiment in the graeco-roman era. *Economic Botany*, 10, 263–266.
<https://doi.org/10.1007/BF02899005>
- Awen, B.Z., Unnithan, C.R., Ravi, S., Kermagy, A., Prabhu, V., & Hemlal H. (2011). Chemical composition of *Salvia officinalis* essential oil of Libya. *Journal of Essential Oil Bearing Plants*, 14, 89–94.
<https://doi.org/10.1080/0972060X.2011.10643905>
- Baydar, H., & Kineci, S. (2009) Scent composition of essential oil, concrete, absolute and hydrosol from lavandin (*Lavandula × intermedia* Emeric ax Loisel.). *Journal of Essential Oil Bearing Plants*, 12, 131–136.
<https://doi.org/10.1080/0972060X.2009.10643702>
- Baydar, H., Sangun, M.K., Erbas, S., & Kara, N. (2013). Comparison of aroma compounds in distilled and extracted products of sage (*Salvia officinalis* L.). *Journal of Essential Oil Bearing Plants*, 16, 39–44.
<https://doi.org/10.1080/0972060X.2013.764175>
- Beheshti-Rouy, M., Azarsina, M., Rezaie-Soufi, L., Alikhani, M.Y., Roshanaie, G., & Komaki, S. (2015). The antibacterial effect of sage extract (*Salvia officinalis*) mouthwash against *Streptococcus mutans* in dental plaque: a randomized clinical trial. *Iranian Journal of Microbiology*, 7, 173–177.
- Boufadi, M.Y., Keddari, S., Moulai-Hacene, F., & Chaa, S. (2021). Chemical composition, antioxidant and anti-inflammatory properties of *Salvia officinalis* extract from Algeria. *Pharmacognosy Journal*, 13, 506–615.
- Božanić Tanjga, B., Lončar, B., Aćimović, M., Kiprovski, B., Šovljanski, O., Tomić, A., Travičić, V., Cvetković, M., Raičević, V., & Zeremski, T. (2022). Volatile profile of garden rose (*Rosa hybrida*) hydrosol and evaluation of its biological activity *in vitro*. *Horticulturae*, 8, 895.
<https://doi.org/10.3390/horticulturae8100895>
- Chen, J., He, J., Li, N., Zheng, H., & Zhao, S. (2019). Determination and correlation of solubility of borneol, camphor, and isoborneol in different solvents. *Journal of Chemical and Engineering Data*, 64, 1826–1833.
<https://doi.org/10.1021/acs.jced.9b00045>
- de Melo, G.A.N., Fonseca, J.P., Farinha, T.O., do Pinho, R.J., Damião, M.J., Grespan, R., da Silva, E.L., Bersani-Amado, C.A., & Cuman R.K.N. (2012). Anti-inflammatory activity of *Salvia officinalis* L. *Journal of Medicinal Plants Research*, 6, 4934–4939.
<https://doi.org/10.5897/JMPR12.251>
- Dinel, A.L., Lucas, C., Guillemet, D., Layé, S., Pallet, V., & Joffre, C. (2020). Chronic supplementation with a mix of *Salvia officinalis* and *Salvia lavandulaefolia* improves morris water maze learning in normal adult C57Bl/6J mice. *Nutrients*, 12, 1777.
<https://doi.org/10.3390/nu12061777>
- Duletić-Laušević, S., Alimpić Aradski, A., Živković, J., Gligorijević, N., Šavikin, K., Radulović, S., Čočić, D., & Marin, P. (2019). Evaluation of bioactivities and phenolic composition of extracts of *Salvia officinalis* L. (Lamiaceae) collected in Montenegro. *Botanica Serbica*, 43, 47–58.
<https://doi.org/10.2298/BOTSERB1901047D>
- Eidi, M., Eidi, A., & Zamanizadeh, H. (2005). Effect of *Salvia officinalis* L. leaves on serum glucose and insulin in healthy and streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology*, 100, 310–313.
<https://doi.org/10.1016/j.jep.2005.03.008>
- Garcia, C.S.C., Menti, C., Lambert, A.P.F., Barcellos, T., Moura, S., Calloni, C., Branco, C.S., Salvador, M., Roesch-Ely, M., & Henriques, J.A.P. (2016). Pharmacological perspectives from Brazilian *Salvia officinalis* (Lamiaceae): antioxidant, and antitumor in mammalian cells. *Annals of the Brazilian Academy of Sciences*, 88, 281–292.
<http://dx.doi.org/10.1590/0001-3765201520150344>
- Garneau, F.X., Collin, G., Gagnon, H., & Pichette, A. (2012). Chemical composition of the hydrosol and the essential oil of three different species of the Pinaceae family: *Picea glauca* (Moench) Voss., *Picea mariana* (Mill.) B.S.P., and *Abies balsamea* (L.) Mill. *Journal of Essential Oil Bearing Plants*, 15, 227–236.
<https://doi.org/10.1080/0972060X.2012.10644040>
- Giannoulis, K., Skoufogianni, E., Bartzialis, D., Solomou, A., & Danalatos, N. (2021). Growth and productivity of *Salvia officinalis* L. under Mediterranean climatic conditions depends on biofertilizer, nitrogen fertilization, and sowing density. *Industrial Crops and Products*, 160, 113136.
<https://doi.org/10.1016/j.indcrop.2020.113136>
- Grdiša, M., Jug-Dujaković, M., Lončarić, M., Carović-Stanko, K., Ninčević, T., Liber, Z., Radosavljević, I., &

- Šatović, Z. (2015). Dalmatian sage (*Salvia officinalis* L.): A review of biochemical contents, medical properties and genetic diversity. *Agriculturae Conspectus Scientificus*, 80, 69–78.
- Hansox, W.I., & Hocking, G.M. (1957). Garden sage. *Economic Botany*, 11, 64–74.
<https://doi.org/10.1007/BF02985331>
- Hay, Y.O., Abril-Sierra, M.A., Sequeda-Castaneds, L.G., Bonnafoos, C., & Raynaud, C. (2018). Evaluation of combinations of essential oils and essential oils with hydrosols on antimicrobial and antioxidant activities. *Journal of Pharmacy and Pharmacognosy Research*, 6, 216–230.
- International Organization for Standardisation (1997). ISO 9909:1997. Oil of Dalmatian Sage (*Salvia officinalis* L.). International Organization for Standardisation (ISO): London, UK.
- Jonas, M., & Schieberle, P. (2021). Characterization of the key aroma compounds in fresh leaves of garden sage (*Salvia officinalis* L.) by means of the sensomics approach: Influence of drying and storage and comparison with commercial dried sage. *Journal of Agricultural and Food Chemistry*, 69, 5113–5124.
<http://dx.doi.org/10.1021/acs.jafc.1c01275>
- Karalija, E., Dahija, S., Tarkowski, P., & Čavar Zeljković, S. (2022). Influence of climate-related environmental stresses on economically important essential oils of Mediterranean *Salvia* sp. *Frontiers in Plant Science*, 13, 864807. <https://doi.org/10.3389/fpls.2022.864807>
- Kiprovski, B., Zeremski, T., Varga, A., Čabarkapa, I., Filipović, J., Lončar, B., & Aćimović, M. (2023). Essential oil quality of lavender grown outside its native distribution range: a study from Serbia. *Horticulturae*, 9, 816.
<https://doi.org/10.3390/horticulturae9070816>
- Lakušić, B., Ristić, M., Slavkovska, V., Stojanović, D., & Lakušić D. (2013) Variations in essential oil yields and compositions of *Salvia officinalis* (Lamiaceae) at different developmental stages. *Botanica Serbica*, 37, 127–139.
- Lima, C., Andrade, P., Seabra, R., Fernandes-Ferreira, M., & Pereira-Wilson, C. (2005). The drinking of a *Salvia officinalis* infusion improves liver antioxidant status in mice and rats. *Journal of Ethnopharmacology*, 97, 383–389.
<https://doi.org/10.1016/j.jep.2004.11.029>
- Loizzo, M.R., Tundis, R., Menichini, F., Saab, A.M., Statti, G.A., & Menichini, F. (2007). Cytotoxic activity of essential oils from Labiatae and Lauraceae families against in vitro human tumor models. *Anticancer Research*, 27, 3293–3300.
- Matula, R., Knířová, S., Vítámvás, J., Šrámek, M., Kníř, T., Ulbrichová, I., Svoboda, M., & Plichta, R. (2023). Shifts in intra-annual growth dynamics drive a decline in productivity of temperate trees in Central European Forest under warmer climate. *Science of the Total Environment*, 905, 166906.
<https://doi.org/10.1016/j.scitotenv.2023.166906>
- Mehmet, N.İ., Şengül, K., & Özlem, T. (2024). Interactive effects of number of plants and nitrogen fertilization on quality parameters of *Salvia officinalis* L. cultivated in semi-arid environments. *Journal of Essential Oil Bearing Plants*,
<https://doi.org/10.1080/0972060X.2024.2307906>
- Mohammadi-Cheraghabadi, M., Modarres-Sanavy, S.A.M., Sefidkon, F., Mokhtassi-Bidgoli, A., & Hazrati, S. (2021). Phytochemical and physiological changes in *Salvia officinalis* L. under different irrigation regimes by exogenous applications of putrescine. *Saudi Journal of Biological Sciences*, 28, 7227–7240.
<https://doi.org/10.1016/j.sjbs.2021.08.024>
- Mot, M.D., Gavrilas, S., Lupitu, A.I., Moisa, C., Chambre, D., Tit, D.M., Bogdan, M.A., Bodescu, A.M., Copolovici, L., Copolovici, D.M., & Bungau, S.G. (2022). *Salvia officinalis* L. essential oil: characterization, antioxidant properties, and the effects of aromatherapy in adult patients. *Antioxidants*, 11, 808.
<https://doi.org/10.3390/antiox11050808>
- Ovidi, E., Laghezza Masci, V., Zambelli, M., Tiezzi, A., Vitalini, S., & 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.
<https://doi.org/10.3390/plants10040707>
- Pachura N., Zimmer A., Grzywna K., Figiel A., Szumny A., & Łyczko J. (2022). Chemical investigation on *Salvia officinalis* L. Affected by multiple drying techniques – The comprehensive analytical approach (HS-SPME, GC–MS, LC-MS/MS, GC-O and NMR). *Food Chemistry*, 397, 133802.
<https://doi.org/10.1016/j.foodchem.2022.133802>
- Politi, M., Ferrante, C., Menghini, L., Angelini, P., Flores, G.A., Muscatello, B., Braca, A., & De Leo, M. (2022). Hydrosols from *Rosmarinus officinalis*, *Salvia officinalis*, and *Cupressus sempervirens*: Phytochemical analysis and bioactivity evaluation. *Plants*, 11, 349.
<https://doi.org/10.3390/plants11030349>
- Porte, A., Godoy, R.L.O., & Maia-Porte, L.H. (2013). Chemical composition of sage (*Salvia officinalis* L.) essential oil from the Rio de Janeiro State (Brazil). *Revista Brasileira de Plantas Medicinais, Campinas*, 15, 438–441.
- Raal, A., Orav, A., & Arak, E. (2007). Composition of the essential oil of *Salvia officinalis* L. from various European countries. *Natural Product Research*, 21, 406–411. <https://doi.org/10.1080/14786410500528478>
- Radulović, N., Genčić, M., Stojanović, N., Randjelović, P., Stojanović-Radić, Z., & Stojiljković, N. (2017). Toxic essential oils. Part V: Behaviour modulating and toxic properties of thujones and thujone-containing essential oils of *Salvia officinalis* L., *Artemisia absinthium* L., *Thuja occidentalis* L. and *Tanacetum vulgare* L. *Food and Chemical Toxicology*, 105, 355–369.
<http://dx.doi.org/10.1016/j.fct.2017.04.044>

- Rasmussen, L.H., & Rosenfeld, M. (2020). A rapid GC-FID method for determination of sabinene, β -pinene, α -thujone and β -thujone in the essential oil of Kitchen Sage (*Salvia officinalis* L.). *Journal of Chromatography B*, 1149, 122159. <https://doi.org/10.1016/j.jchromb.2020.122159>
- Rguez, S., Msaada, K., Daami-Remadi, M., Chayeb, I., Bettaieb Rebey, I., Hammami, M., Laarif, A., & Hamrouni-Sellami, I. (2019). Chemical composition and biological activities of essential oils of *Salvia officinalis* aerial parts as affected by diurnal variations. *Plant Biosystems*, 153, 264–272. <https://doi.org/10.1080/11263504.2018.1473305>
- Sheikhalipour, M., Kulak, M., Mohammadi, S.A., Esmailpour, B., Nouraein, M., Kocak, M.Z., Farajzadeh, S.M., Gohari, G., Fotopoulos, V., & Vita, F. (2024). Foliar application of either melatonin or sodium nitroprusside regulates the antioxidant status, and the morpho-physiological attributes and essential oil production in sage (*Salvia officinalis* L.) under salinity stress. *Scientia Horticulturae*, 323, 112526. <https://doi.org/10.1016/j.scienta.2023.112526>
- Smail, A., Badiacirc, L., & Maria, G.M. (2011). Antioxidant activity of some Moroccan hydrosols. *Journal of Medicinal Plant Research*, 5, 6688–6696. <https://doi.org/10.5897/JMPR11.1176>
- Tibaldi, G., Hazrati, S., Hosseini, S.J., Ertani, A., Bulgari, R., & Nicola S. (2022). Cultivation techniques and drying process can affect the inflorescence essential oil composition of three selections of *Salvia officinalis*. *Industrial Crops and Products*, 183, 114923. <https://doi.org/10.1016/j.indcrop.2022.114923>
- Tomi, K., Kitao, M., Konishi, N., Murakami, H., Matsumura, Y., & Hayashi, T. (2016). Enantioselective GC-MS analysis of volatile components from rosemary (*Rosmarinus officinalis* L.) essential oils and hydrosols. *Bioscience, Biotechnology and Biochemistry*, 80, 840–847. <https://doi.org/10.1080/09168451.2016.1146066>
- Tornuk, F., Cankurt, H., Ozturk, I., Sagdic, O., Bayram, O., & Yetim, H. (2011). Efficacy of various plant hydrosols as natural food sanitizers in reducing *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on fresh cut carrots and apples. *International Journal of Food Microbiology*, 148, 30–35. <https://doi.org/10.1016/j.ijfoodmicro.2011.04.022>
- Vosoughi, N., Gomarian, M., Pirbalouti, A.G., Khaghani, S., & Malekpoor, F. (2018). Essential oil composition and total phenolic, flavonoid contents, and antioxidant activity of sage (*Salvia officinalis* L.) extract under chitosan application and irrigation frequencies. *Industrial Crops and Products*, 117, 336–374. <https://doi.org/10.1016/j.indcrop.2018.03.021>
- Zekri, N., Amalich, S., Elbelghiti, M.A., & Zair, T. (2014). Phytochemical screening and chemical composition of essential oils and hydrosols of *Mentha* species from Morocco. *Advances in Environmental Biology*, 8, 10–18.

Uticaj klimatskih faktora na akumulaciju isparljivih komponenti u etarskom ulju i hidrolatu *Salvia officinalis*

Milica Aćimović · Jovana Stanković Jeremić · Mirjana Cvetković · Marina Todosijević · Ljubodrag Vujisić · Vladimir Sikora · Biljana Lončar

Sažetak: Ova studija se bavila ispitivanjem hemijskog sastava i etarskog ulja i hidrolata *Salvia officinalis* L. sorta “Primorska” gajene u Srbiji tokom tri godine. Takođe, bitna celina u ovom radu je i predočavanje potencijalne primene dobijenih proizvoda. Istraživanje je pokazalo da etarsko ulje *S. officinalis* sadrži značajne količine oksidovanih monoterpena, kao što su α -tujon (19.9-29.0%), kamfor (15.8-19.6%), β -tujon (12.3-13.3%), i 1,8-cineol (8.8-11.3%). Sadržaj ovih komponenti je varirao tokom godina i bio je u korelaciji sa klimatskim promenama, posebno variranja temperature. Hidrolat, dobijen kao nuzproizvod prilikom destilacije etarskog ulja *S. officinalis* pokazao je sličan hemijski profil kao i etarsko ulje, pri čemu je kamfor bio najzastupljeniji (42.4-49.6%). Statističkom analizom utvrđena je korelacija između temperature, padavina i različitih jedinjenja, kako u etarskom ulju, tako i u hidrolatu. U etarskom ulju temperatura je bila u jakoj negativnoj korelaciji sa α -tujonom ($K_T=-36.500$), i u pozitivnoj korelaciji sa kamforom ($K_T=9.5$), β -tujonom ($K_T=4.500$) i 1,8-cineolom ($K_T=5.000$). U hidrolatu je takođe zabeležen sličan trend korelacija: negativna između temperature i α -tujona ($K_T=-32.000$), i pozitivne korelacije temperature i kamfora ($K_T=34.000$), β -tujona ($K_T=7.000$), i 1,8-cineola ($K_T=7.000$). Etarsko ulje *S. officinalis* je veoma cenjeno u prehrambenoj i farmaceutskoj industriji kao i u kozmetici, parfimeriji i aromaterapiji. Brojne studije su pokazale njegov antioksidativni, antimikrobni, antiinflamatorni i antikancerogeni

potencijal. Ova studija je takođe istakla potencijalnu primenu hidrolata *S. officinalis* u kozmetici, sanitaciji hrane i farmaceutskim proizvodima zbog njegovih antioksidativnih svojstava i umerenog antimikrobnog potencijala. Sveukupno, ovo istraživanje doprinosi boljem razumevanju hemijskog sastava etarskog ulja i hidrolata *S. officinalis* i naglašava potencijal primene hidrolata kao vrednog resursa u različitim granama industrije. Dalja istraživanja u ovoj oblasti podstakće inovacije u razvoju i korišćenju proizvoda na bazi etarskog ulja i hidrolata *S. officinalis*.

Ključne reči: esencijalna ulja, hidrolati, hidrosol, isparljivo ulje, klimatske promene, korelacije, *Salvia officinalis*, sorte, žalfija