Botanica SERBICA

(2024) 48 (2): 173–184

DOI: https://doi.org/10.2298/BOTSERB2402173S
journal homepage: botanicaserbica.bio.bg.ac.rs

Original Scientific Paper

Clinopodium menthifolium subsp. *menthifolium* in the Central Balkan Peninsula - essential oil composition in relation to climatic conditions

Violeta Slavkovska*, Miloš Zbiljić and Danilo Stojanović

Department of Botany, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia
* Correspondence: violetas@pharmacy.bg.ac.rs

ABSTRACT:
The compositional dependency of the essential oil of *Clinopodium menthifolium* subsp. *menthifolium* (Lamiaceae) on ecological conditions has not yet been investigated. In pursuit of this objective, we assessed the quantity and quality of the essential oil in plants from 11 natural populations from the Central Balkans and one cultivated plant. In order to determine the correlations between essential oil variations and environmental conditions, each habitat was characterised by 36 climatic and 19 bioclimatic parameters. Despite inhabiting diverse climatic zones, altitudes, and biogeographical regions, no significant differences were observed in the yield and qualitative and quantitative composition of the essential oils among the analysed plants. All the samples exhibited essential oil yield ≥ 0.5%, V/w, with piperitone epoxide as the major compound. Among the identified compounds, only limonene and (*E*)-caryophyllene demonstrated dependence on bioclimatic parameters. The bioclimatic parameters which influence the presence of a larger number of compounds are annual temperature range and the precipitation of the wettest quarter. The consistent quantity and quality of the essential oils of *C. menthifolium* subsp. *menthifolium* indicate their probable adaptive significance and could serve as chemotaxonomic features of this taxon.

Keywords:
Calamintha sylvatica, volatile compounds, bioclimatic parameters, Central Balkans, piperitone epoxide, limonene.

UDC: 582.929.4:581.135.5

Received: 31 January 2024
Revision accepted: 24 June 2024

INTRODUCTION

Due to the significant presence of essential oils, certain species within the *Clinopodium* L. genus hold considerable importance as aromatic plants. Notably, in the central region of the Balkan Peninsula, *C. menthifolium* (Host) Stace, *C. nepeta* (L.) Kuntze, *C. vardarense* (Šilić) Govaerts and *C. grandiflorum* (L.) Kuntze are present. In earlier classification systems, all these species were classified under the genus *Calamintha* Miller (Bentham 1848; Ball & Getliffe 1972; Šilić 1979; Doroszenko 1986).

Clinopodium menthifolium exhibits a broad ecological amplitude. It grows on limestone, shale and sandy substrates, within open oak forests or communities dominated by common and hop-hornbeam, in thickets, but never in open habitats, occurring individually or in smaller groups. These habitats are mainly found at altitudes of up to 1300 m (Šilić 1979).

There are three subspecies, *C. menthifolium* subsp. *menthifolium, C. menthifolium* subsp. *ascendens* (Jord.) Govaerts and *C. menthifolium* subsp. *hirtum* (Briq.) Govaerts. The typical subspecies is distributed throughout Europe, ranging north from southern England and extending eastward to Turkey. *C. menthifolium* subsp. *ascendens* is distributed in Western and Southern Europe, extending eastward to Turkey, Iran, and North Africa. The third subspecies is an endemic of Greece (Ball & Getliffe 1972; Doroszenko 1986; Euro+Med 2006+).

In the Central part of the Balkan Peninsula, only *C. menthifolium* subsp. *menthifolium* is present. According to the results of previous research on the essential oils of this taxon, the oil content was higher than 0.5%, with piperitone epoxide as the dominant component with the absence or a very low percentage (below 1%) of pulegone (Hanlidou *et al.* 1991; Kitić *et al.* 2001; Mimica-Dukić *et al.* 2004; Karousou *et al.* 2012; Milenković *et al.* 2018). On the other hand, other aromatic species within the *Clinopodium* (formerly *Calamintha*) genus are characterised by pulegone as the dominant component of the essential oil (Ševarda *et al.* 1987; Akgül *et al.* 1991; Ristorcelli *et al.* 1996, Kitić *et al* 2002a, b, 2005; Karousou *et al.* 2012; Milenković *et al.* 2018). Piperitone epoxide was found only as an accompanying component in the oils of *C. nepeta* (Ristorcelli *et al.* 1996; Karousou *et al.* 2012).

Although the presence and the quality of the essential oils are predominantly constant characteristics of aromatic taxa, the oil content and the quantity of some components may depend on environmental conditions and/or the phenological stage (Li & Zidorn 2022). Research carried out by Lakušić *et al.* (2012) demonstrated significant variations in the quantity of major components of the essential oil of *Rosmarinus officinalis* L. depending on the climate characteristics of habitats in the Balkan Peninsula. Additionally, significant differences in the chemical composition of the essential oils of *Satureja horvatii* Šilić (Lakušić *et al.* 2011), *Salvia officinalis* L. (Lakušić *et al.* 2013) and *Lavandula angustifolia* Mill. (Lakušić *et al.* 2014) were found at different developmental stages of the plants. Considering that these taxa are woody perennials, the question regarding the influence of climatic factors on perennial herbaceous species arises. This research question has not yet been investigated for aromatic species within the genus *Clinopodium*.

The aim of this study was to determine the quantity, composition and variability of the essential oil of *C. menthifolium* subsp. *menthifolium* within the central Balkan Peninsula, as well as to elucidate the extent to which the observed variability in the oil is influenced by diverse habitat conditions.

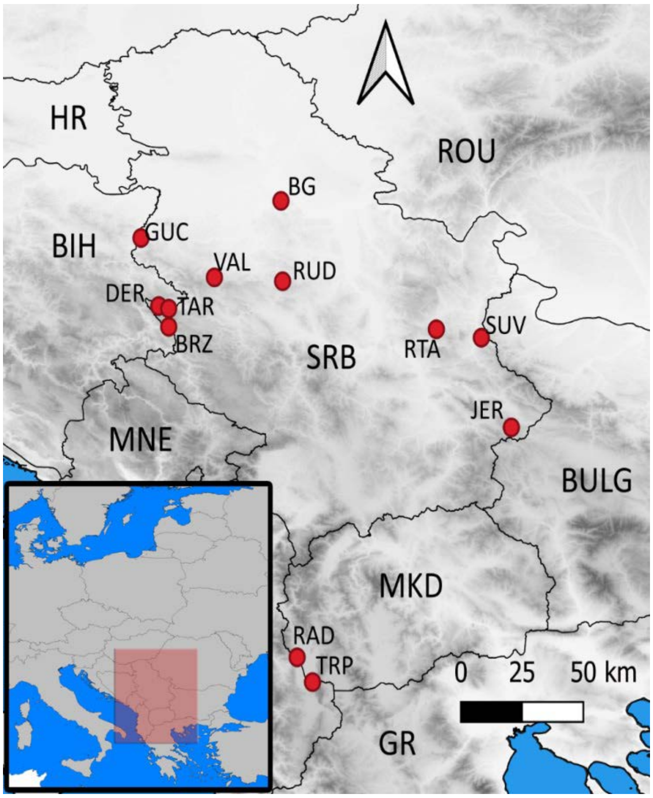

Fig. 1. Geographical distribution of the populations of *Clinopodium menthifolium* subsp. *menthifolium* used in this study. All the acronyms of the sampled populations are given in Table 1. HR – Croatia; BIH – Bosnia and Hercegovina; MNE – Montenegro; MKD – North Macedonia; GR – Greece; BULG – Bulgaria; ROU – Romania.

MATERIALS AND METHODS

Plant material. Samples of *Clinopodium menthifolium* subsp. *menthifolium* were collected from 11 natural populations and one cultivated plant in a private garden in Belgrade (Fig. 1). The cultivated plant originates from the Rapajin gorge in Lika (Croatia), from where it was transferred in October 2012. Pooled samples were collected from each locality. The content and quality of the essential oils of the cultivated plant were monitored in the second, third and ninth year of cultivation. The plants from all populations were collected during the flowering stage, from 1997 to 2022. The voucher specimens are deposited in the Herbarium of the Department of Botany, University of Belgrade - Faculty of Pharmacy (HFF) (Table 1).

Isolation of essential oils. The essential oils were isolated from air-dried above ground plant material by hydro-distillation in a Clevenger-type apparatus according to the standard procedure reported in the European Pharmacopoeia 10th edition (European Pharmacopoeia 2020). The amount of distilled material varied and depended on the size of the population. It ranged from 20 g to 100 g of dry plant material. The distillation time was 2 h. The essential oils were dissolved with n-hexane and dried over anhydrous sodium sulphate and stored at -40°C prior to analysis. The oil yield was expressed in ml/100 g of the dry weight of the plant material.

GC-FID/MS analysis. Gas chromatographic analysis (GC-FID/MS) was performed on an Agilent 6890N gas chromatograph equipped with a flame-ionisation detector (FID) and Agilent 5975C MS detector, using a capillary column HP-5MS (30 m × 0.25 mm i.d., film thickness 0.25 μm). The essential oil samples were dissolved in hexane (1%) and 1 μl was injected in split mode (10:1). The carrier gas was He with a constant flow rate of 1.0 mL/min, the injector temperature 200°C and the oven was linearly heated from 60°C to 280°C at a rate of

Table 1. Characteristics of the collection sites of the studied *Clinopodium menthifolium* subsp. *menthifolium* populations; date of collection; voucher numbers

No.	Locality	Acronym	Latitude	Longitude	Altitude (m a.s.l.)	Climate type	Altitude zone	Choria	Date of collection	Voucher number
1	Gučevo	GUC	44.50	19.19	175	humid continental	submontanus	Illyrian	22.09.2022.	HFF 4298
2	Valjevo	VAL	44.18	19.87	486	humid continental	submontanus	Illyrian	22.08.2008.	HFF 4295
3	Derventa Canyon	DER	43.95	19.35	780	humid continental	montanus	Illyrian	28.09.2004.	HFF 1251
4	Mt. Tara	TAR	43.93	19.45	872	humid continental	montanus	Illyrian	05.10.1997.	HFF 1241
5	Beli Rzav Canyon	BRZ	43.78	19.45	732	humid continental	montanus	Illyrian	15.09.2020.	HFF 4224
6	Mt. Rudnik	RUD	44.15	20.50	727	humid continental	montanus	Illyrian	15.09.2010.	HFF 3251
7	Mt. Rtanj	RTA	43.76	21.93	700	arid continental	montanus	Balkan	18.08.2008.	HFF 4296
8	Suvodol	SUV	43.69	22.34	463	arid continental	submontanus	Balkan	20.07.2009.	HFF 4297
9	Jerma Gorge	JER	42.97	22.62	672	arid continental	montanus	Balkan	15.07.1997.	HFF 1253
10	Radožda	RAD	41.12	20.64	724	humid sub-Mediterranean	montanus	Albanian	20.08.1997.	HFF 1259
11	Trpejca	TRP	40.92	20.78	773	humid sub-Mediterranean	montanus	Albanian	23.08.2002.	HFF 1252
12	Belgrade	BG1[a]/ BG2[b] /BG3[c]	44.80	20.49	111	arid continental	submontanus	Illyrian	27.08.2014./ 21.07.2015./ 01.09.2021.	HFF 3683

[a]Plant material collected in 2014.; [b] Plant material collected in 2015.; [c] Plant material collected in 2021.

3°C/min. The transfer line temperatures were set at 250°C for MS and 300°C for FID. EI mass spectra (70 eV) were acquired over the *m/z* range of 35–550.

The essential oil's constituents were identified by comparing their retention indices (RI) and mass spectra with those from NIST/NBS, Wiley libraries and the literature. The linear RIs were determined in relation to a homologous series of *n*-alkanes (C_8–C_{40}) run under the same operating conditions, according to the formula provided by Van den Dool and Kratz as given in Adams (2017). The quantitative analysis was based on the calculation of the peak areas obtained from the FID data.

Ecological analysis. To describe the climate characteristics and establish the relationships between the variations in the essential oils and the climatic conditions in which the analysed plants thrive, the habitats of each sample were characterised according to altitudinal zones and climatic types and biogeographical choria (Table 1).

Two altitude zones are defined according to the altitude of the sample localities: *submontanus* 100–500 m and *montanus* 500–1000 m. For each sample locality, 36 climatic and 19 bioclimatic parameters (Supplementary Table 1) were extracted from the WorldClim set of global climate layers using DIVA-GIS 7.5 software (Hijmans *et al.* 2012). The climate types were defined based on modified Walter climate diagrams generated from 36 climate parameters (Fig. S1) and the key for identifying climate types and variants given in Zbiljić *et al.* (2023). All the samples were classified into three biogeographic choria (*Illyrian*, *Balkan* and *Albanian*) according to the Comparative Chorology of Central European Flora (Meusel *et al.* 1965).

Statistical analysis. Multivariate analyses were performed to identify the structure of variability and to measure the distances among the samples. In both multivariate analyses, the Belgrade samples (BG1, BG2 and BG3) were pooled and analysed as one sample (BG), with the average values calculated from the three samples from different years for all the identified compounds. Principal Component Analysis (PCA) was performed to determine the overall variability, while Cluster Analysis, utilising the unweighted pair-group method (UPGMA) with the Euclidean similarity index was used to determine the sample distances. All the identified compounds were included in the multivariate analysis without prior logarithmisation, executed using Past 4.17 (Hammer 2001). Analysis of Variance (ANOVA) was carried out to ascertain the compound contributions to group separation. Box-and-whiskers plots, with Kruskal-Wallis H-statistics values, were generated to delineate the variation and separation contribution of the selected compounds among *a priori* defined groups. The most significant compounds according to their frequency and quantity in the samples were presented in the box-and-whiskers plots. Regression analyses (simple linear regression) were performed to determine the degree of dependence of the variation in the chemical composition of the essential oils on the 19 bioclimatic parameters. Only nine compounds (limonene, γ-terpinene, *cis*-sabinene hydrate, menthone, terpinen-4-ol, piperitone epoxide, piperitenone, piperitenone oxide, and (*E*)-caryophyllene) registered with at least 5% in a sample were correlated with the bioclimatic parameters using Pearson's correlation matrices. Statistica v.8.0 was utilised for the creation of the box-and-whiskers plots and regressions. (StatSoft 2007).

RESULTS AND DISCUSSION

Climatic analysis. Ten of the twelve analysed samples grow in the temperate zone, while the remaining two thrive in the sub-Mediterranean zone. The samples growing in the temperate zone are divided into two climatic variants, humid continental and arid continental. The sub-Mediterranean samples thrive under the influence of a humid sub-Mediterranean climate (Table 1). The humid continental climate, under which the samples from western and central Serbia thrive, is characterised by cold winters and warm summers, with constant rainfall throughout the year, increasing slightly in late summer and early autumn, with no dry or semi-dry periods. The arid continental climate which can be observed in Belgrade and the eastern parts of Serbia is characterised by cold winters and warm summers, with significantly less precipitation compared to western and central Serbia. In Suvodol, Jerma and Belgrade, there is a semi-arid period in summer. In the localities of Radožda and Trpejca, the climatic conditions are those of a humid, sub-Mediterranean type with mild winters, warm summers and a considerable amount of precipitation throughout the year. However, the distribution of precipitation at these localities differs considerably from others. In particular, there is a semi-dry period in summer, while perhumid months prevail in late autumn and early winter (> 100 mm of rainfall per month) (Fig. S1).

The cluster analysis of the localities based on 36 climatic parameters revealed that the analysed samples are divided into two clusters, with the larger cluster further subdivided into two subclusters. The most distant cluster is formed by the localities of Radožda (RAD) and Trpejca (TRP), which are located in the zone of a humid sub-Mediterranean climate variant, while the second cluster includes samples which thrive in a temperate zone. The second cluster is clearly divided into two subclusters, one includes localities with an arid continental climate and the other localities with a humid continental climate variant (Fig. 2A).

Essential oil yield and chemical composition. The yield and the essential oil composition of the studied populations are given in Table 2.

The quantity of essential oil ranged from 0.5%, V/w (the populations from Gučevo Mountain and Derventa Canyon) to 1.8%, V/w (the population from Beli Rzav Canyon). The yield of the essential oil showed almost the same range of variation among the plants collected from humid continental (0.5‑1.8%, V/w, mean 0.9 ± SD 0.5) and arid continental (0.7–1.3%, V/w, mean 0.9 ± SD 0.3) climatic conditions, and it was relatively uniform among the plants from humid sub-Mediterranean areas (1.3–1.5%, V/w, mean 1.4 ± SD 0.1). Given that all the studied populations across diverse climatic conditions exhibit a substantial and approximately uniform quantity of essential oil during the flowering stage, it could be inferred that the oil content found in *C. menthifolium* subsp. *menthifolium* represents a stable adaptive trait.

Fifty constituents were identified in all the analysed samples (Table 2). The qualitative composition of the essential oils among the *C. menthifolium* subsp. *menthifolium* populations exhibited notable similarity. In all the populations, monoterpene compounds were dominant, particularly oxygenated monoterpenes of menthane type (47.9‑80.7%). The quantity of sesquiterpenes was relatively low across all the populations, ranging from 0.7% to 16.1%.

The composition of the essential oil was consistent across all the studied populations. The notably dominant component in all the populations was piperitone epoxide, with quantities ranging from 41.8% (the population from Suvodol - SUV) to 71.9% (the population from Derventa Canyon -DER). The Rtanj (RTA), Valjevo (VAL) and Belgrade (BG1/BG2/BG3) populations exhibited a high content (more than 10%) of limonene, the Suvodol (SUV) population of limonene and piperitenone oxide, the Jerma Gorge (JER) population of *cis*-sabinene hydrate, and the Mount Rudnik (RUD) population of menthone.

Our findings align with previously published studies on the content and quality of essential oil within this taxon (Ševarda *et al.* 1987; Hanlidou *et al.* 1991; Kitić *et al.* 2001; Mimica-Dukić *et al.* 2004; Karousou *et al.* 2012).

When comparing our findings with previously published research on *C. menthifolium* subsp. *ascendens*, distinct differences in the quantity and quality of the essential oil are evident. Castilho *et al.* (2007) reported oil content higher than 1.8% in *C. menthifolium* subsp. *ascendens* with a predominance of isopulegone, while Debbabi *et al.* (2021) observed oil content lower than 0.3% with a dominance of pulegone, piperitenone, and

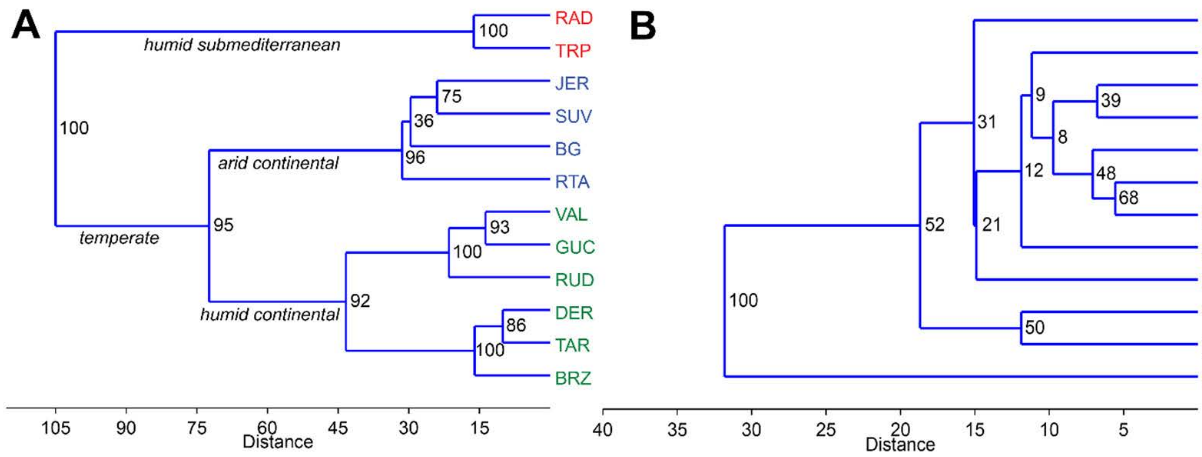

Fig. 2. A – Cluster analysis of the *Clinopodium menthifolium* subsp. *menthifolium* sample localities constructed based on 36 climatic parameters; B – Cluster analysis of the chemical composition of the essential oils of *Clinopodium menthifolium* subsp. *menthifolium* constructed based on all the identified compounds using the unweighted pair-group method (UPGMA) with the Euclidean similarity index. All acronyms of the samples are given in Table 1. Blue font colour indicates the samples growing in arid continental, green indicates the samples growing in humid continental and red the samples growing in humid sub-Mediterranean climate variants. The numbers on the clusters indicate the statistical support for branching.

Table 2. Yield and chemical composition (%) of the essential oils of the *Clinopodium menthifolium* subsp. *menthifolium* samples[a]

ACRONYM	RI_{exp}[b]	RTA[c]	BRZ	TRP	RAD	GUC	TAR	JER	DER	RUD	BG2	BG3	BG1	VAL	SUV
Yield (%, V/w)		1.0	1.8	1.3	1.5	0. 5	0.7	0.7	0.5	0.6	0.9	1.3	0.7	1.1	1.1
Compounds															
1. *α*-Thujene	927	0.2	-	-	-	0.1	-	-	-	-	0.5	0.5	0.5	0.8	-
2. *α*-Pinene	935	1.2	0.9	0.6	0.7	0.7	0.3	1.0	0.4	0.1	1.2	0.6	1.2	1.7	1.3
3. Sabinene	974	1.3	0.7	t[d]	0.6	0.5	0.4	0.9	0.3	0.1	0.9	0.6	0.7	t	0.9
4. *β*-Pinene	979	1.7	1.2	1.3	0.6	0.8	0.4	1.1	0.5	0.2	1.5	0.7	1.1	2.6	1.8
5. Myrcene	992	1.4	0.9	0.6	0.5	0.7	0.3	0.7	0.3	0.2	1.2	1	1	2.3	1.5
6. 3-Octanol	996	1.3	0.2	t	0.3	0.6	0.6	1.7	1.1	0.4	0.2	-	0.2	0.5	0.2
7. *α*-Terpinene	1022	0.6	-	t	0.5	-	0.8	0.7	-	-	0.6	0.4	-	0.3	0.1
8. *ρ*-Cymene	1026	1.5	1.2	t	t	0.2	t	0.5	1.4	0.7	1.4	0.5	4.4	0.4	0.2
9. **Limonene**	1030	**10.5**[e]	8.5	7.6	4.0	5.0	2.4	4.0	2.8	0.8	**19.0**	**12.5**	**15.6**	**13.5**	**24.5**
10. (*Z*)-*β*-Ocimene	1038	1.6	0.4	1.3	0.6	0.4	0.7	0.8	-	0.2	0.8	0.4	0.2	0.6	0.8
11. (*E*)-*β*-Ocimene	1049	-	-	t	t	-	t	t	-	-	0.1	-	-	0.2	0.4
12. *γ*-Terpinene	1059	1.6	0.3	2.0	1.2	0.2	1.6	1.3	-	0.2	3.7	5.6	0.3	4.9	0.1
13. *cis*-Sabinene hydrate	1069	5.3	5.8	9.0	4.3	4.6	4.0	**12.0**	-	1.7	2.3	2.9	-	-	0.4
14. Terpinolene	1090	0.4	-	t	0.3	-	0.4	0.3	-	-	0.2	-	-	0.4	0.1
15. Linalool	1099	-	0.6	-	-	0.2	-	-	-	-	-	-	-	-	0.3
16. *tans*-Sabinene hydrate	1101	0.6	-	t	0.4	-	4	0.6	-	0.5	0.2	0.4	-	-	-
17. *cis*-Thujone	1105	-	-	0.9	-	-	-	-	-	-	-	-	-	-	-
18. *cis*-*p*-Menth-2-en-1-ol	1122	-	0.6	-	t	-	0.3	-	-	-	0.4	-	-	-	0.1
19. **Menthone**	1154	-	-	-	-	2.2	t	t	-	**10.8**	0.7	0.6	0.5	-	-
20. Isomenthone	1164	-	-	-	-	2.1	-	-	t	-	-	-	t	-	-
21. Borneol	1174	-	0.4	t	t	-	t	-	-	-	-	-	-	-	0.1
22. Terpinen-4-ol	1181	3.4	0.7	1.2	2.6	0.5	4.3	1.3	6.2	3.1	2.1	1.4	3.2	1.2	0.3
23. Isomenthol	1189	-	-	-	-	-	-	-	-	3.1	-	-	-	-	-
24. *α*-Terpineol	1194	0.4	0.5	-	t	0.3	0.4	-	0.4	0.4	0.3	0.3	0.3	-	0.3
25. *cis*-Carveol	1230	1.0	0.3	-	-	0.4	-	-	-	-	-	-	-	-	0.1

26. Carvone	1245	-	-	-	-	-	-	-	-	-	-	-	-	1.4	0.1
27. **Piperitone epoxide**	1256	**60.8**	**60.4**	**63.0**	**65.6**	**69.5**	**59.2**	**56.9**	**71.9**	**63.3**	**54.9**	**54.8**	**61.2**	**46.6**	**41.8**
28. Geranial	1270	-	-	-	-	0.5	-	-	-	-	-	-	-	-	-
29. Isopiperitenone	1281	-	-	t	0.7	-	t	-	-	-	-	-	-	-	-
30. Dihydro edulan II	1289	0.6	-	-	1.1	-	1.4	-	-	0.3	-	-	-	-	-
31. Thymol	1295	-	0.8	0.8	1.1	0.6	1.1	2.8	0.6	1.8	0.7	1.7	0.8	3.4	0.3
32. Carvacrol	1305	-	-	-	-	-	-	-	-	0.2	-	0.4	0.2	1.2	-
33. 2-Hydroxy-Piperitone	1310	-	-	-	0.5	-	0.4	-	-	-	-	-	-	-	-
34. Isomenthyl acetate	1311	-	-	-	-	0.1	-	-	-	1.1	-	-	0.4	-	-
35. Dihydro carveol acetate	1316	-	-	-	-	0.3	-	-	-	0.6	-	-	-	-	-
36. Neoiso-isopulegyl acetate	1322	0.7	1.9	-	t	-	0.4	-	0.4	-	0.6	0.3	0.2	-	-
37. Piperitenone	1341	-	-	-	t	-	t	-	-	-	-	-	-	-	9.1
38. **Piperitenone oxide**	1367	-	2.5	t	0.6	0.8	0.6	0.4	-	2.4	1.4	0.6	0.6	1.3	**12.1**
39. *trans-p*-Menth-6-ene-2,8-diol	1372	-	-	-	-	0.2	-	-	-	-	-	-	-	-	-
40. *α*-Copaene	1375	-	-	t	t	-	t	t	-	-	-	-	-	-	0.2
41. *β*-Bourbonene	1388	-	0.3	t	0.4	0.2	0.5	t	0.2	0.2	-	0.4	0.2	0.7	0.1
42. (*E*)-Caryophyllene	1417	2.0	2.9	5.2	6.2	3.2	7.5	2.6	1.2	3.1	1.6	3.8	0.8	1.0	0.3
43. *α*-Humulene	1452	0.4	0.7	0.7	1.8	0.8	1.9	0.7	0.4	1.2	0.1	0.4	0.2	-	-
44. (*E*)-*β*-Farnesene	1458	-	0.3	t	0.4	0.3	0.5	t	0.3	-	-	0.3	0.1	0.4	-
45. Germacrene D	1484	0.4	1.3	2.4	2.6	1.6	4.9	0.8	-	1.6	-	2.2	-	2.6	0.1
46. Bicyclogermacrene	1504	-	-	t	t	-	t	t	-	-	0.1	0.2	-	0.3	-
47. Spathulenol	1581	-	0.5	-	-	-	-	-	-	-	-	-	-	0.5	-
48. Caryophyllene oxide	1587	-	0.9	t	0.6	0.2	0.8	3.5	2.9	0.5	-	0.2	0.7	-	-
49. Viridiflorol	1596	-	-	t	t	-	t	0.6	-	-	-	-	-	-	-
50. Humulene epoxide II	1612	-	-	-	-	-	-	0.7	0.7	-	-	-	-	-	-
Monoterpenes		94.8	88.6	88.3	85.9	90.7	83.4	85.0	85.2	91.8	94.7	86.2	92.4	82.8	96.7
Monoterpene hydrocarbons		22.0	14.1	13.4	9.0	8.6	7.3	11.3	5.7	2.5	31.1	22.8	25.0	27.7	31.7
Oxygenated monoterpenes		72.8	74.5	74.9	76.9	82.1	76.1	73.7	79.5	89.3	63.6	63.4	67.4	55.1	65.0
Oxygenated monoterpenes of menthane class		**60.8**	**63.5**	**63.0**	**67.4**	**74.7**	**60.5**	**57.3**	**71.9**	**80.7**	**57.4**	**56.0**	**62.7**	**47.9**	**63.1**
Sesquiterpenes		2.8	6.9	8.3	12.0	6.3	16.1	8.9	5.7	6.6	1.8	7.5	2.0	5.5	0.7
Sesquiterpene hydrocarbons		2.8	5.5	8.3	11.4	6.1	15.3	4.1	2.1	6.1	1.8	7.3	1.3	5.0	0.7
Oxygenated sesquiterpenes		-	1.4	-	0.6	0.2	0.8	4.8	3.6	0.5	-	0.2	0.7	0.5	-
Other		1.3	0.2	-	0.3	0.6	0.6	1.7	1.1	0.4	0.2	-	0.2	0.5	0.2
Total:		98.9	95.7	96.6	98.2	97.6	100	95.6	92.0	98.8	96.7	93.7	94.6	88.8	97.6

[a] The arrangement of the samples is in accordance with the results of the cluster analysis (Fig. 2B); [b] Retention indices relative to C_8-C_{40} *n*-alkanes experimentally determined on the HP-5MS column; [c] The acronyms of the sample localities: RTA (Rtanj), BRZ (Beli Rzav) TRP (Trpejca), RAD (Radožda), GUČ (Gučevo), TAR (Tara), JER (Jerma), DER (Derventa), RUD (Rudnik), BG2 (Belgrade, plant material collected in 2015), BG3 (Belgrade, plant material collected in 2021), BG1 (Belgrade, plant material collected in 2014), VAL (Valjevo), SUV (Suvodol); [d] trace (< 0.1%); [e] dominant constituents in bold font

piperitone epoxide. Moreover, upon comparison with other *Clinopodium* (Syn. *Calamintha*) species (Ševarda *et al.* 1987; Akgül *et al.* 1991; Ristorcelli *et al.* 1996, Kitić *et al.* 2002a, b, 2005; Dobravalskytė *et al.* 2012; Karousou *et al.* 2012; Milenković *et al.* 2018) our taxon displays a distinct trait characterised by the prevalence of piperitone epoxide in the oil and the absence of pulegone. Additionally, a similar significant content of piperitone epoxide and the absence of pulegone were noted in the oil of endemic Cretan species *Clinopodium creticum* (L.) Kuntze (Karousou *et al.* 1996). The similarity in the essential oil composition between these two

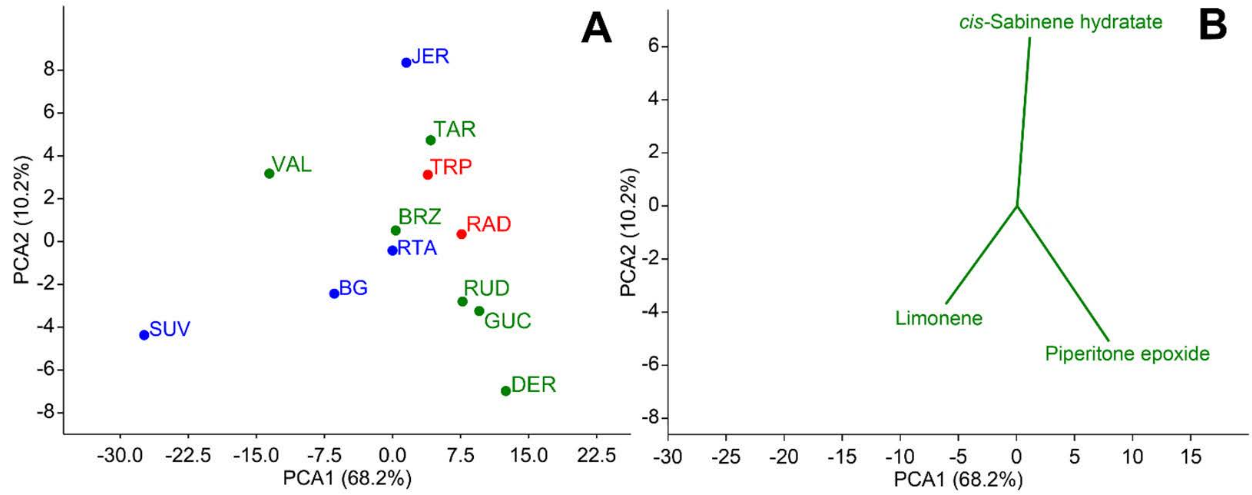

Fig. 3. Principal component analysis (PCA) of the chemical composition of the essential oils of *Clinopodium menthifolium* subsp. *menthifolium* with the components which contribute most to the shown variability. Blue markings – arid continental climate; green markings – humid continental climate; red markings – humid sub-Mediterranean climate. All the acronyms of the samples are given in Table 1.

taxa is notably greater than among the subspecies of *C. menthifolium*.

In the research conducted by Ševarda *et al.* (1987), the essential oil composition of taxa *C. nepeta* subsp. *glandulosum* (Req.) Govaerts, *C. vardarense* and *C. menthifolium* was found to be correlated with the ecological conditions of their habitats. Namely, the mesophytic *C. menthifolium* exhibited oil dominated by piperitenone epoxide, while xerophytes *C. nepeta* subsp. *glandulosum* and *C. vardarense* contained oils predominantly comprising pulegone. Ševarda (1993) proposed a thesis suggesting that as the climate becomes more continental (with a decrease in the average annual temperature and annual precipitation), the content of epoxides in oils increases. In contrast, our study revealed that the plants growing in humid continental climates had higher piperitone epoxide content (46.6%–71.9%) than those in dry continental regions (41.8%–61.2%). The plants from the humid sub-Mediterranean areas also displayed high piperitone epoxide content in their oil (63.0%–65.6%). These findings suggest a potential correlation between humidity and piperitone epoxide content.

According to Kimbaris *et al.* (2017), this metabolite exhibits nematicidal activity. Therefore, it could be inferred that the relatively uniform composition of the essential oils in all the populations of *C. menthifolium* subsp. *menthifolium* with a predominance of piperitone epoxide probably represents a chemical adaptation developed over the course of evolution in response to the presence of certain plant pests.

Multivariate analysis. Principal component analysis (PCA) showed significant variability in the essential oil samples. The first and second axes explained 78.4%, 68.2% and 10.2% of the total variability respectively. Only the Suvodol (SUV) and Valjevo (VAL) samples along the first axis (PCA1) and the Derventa Canyon (DER) and Jerma Gorge (JER) samples along the second axis (PCA2) are more or less separated from the others (Fig. 3). The compounds which contribute most to the observed structure of variability are *cis*-sabinene hydrate, piperitone epoxide, limonene, (*E*)-caryophyllene and germacrene D.

Cluster analysis revealed two major differentiated clades (Fig 2B). Each clade displays specificity in terms of the content of particular compounds. The most distant was the sample from Suvodol (SUV). The distinctive feature of the oil extracted from the plants in this population lies in the lowest quantity of piperitone epoxide (41.8%), and the highest quantities of limonene (24.5%), piperitenone (9.1%) and piperitenone oxide (12.1%). In contrast, in all the other samples piperitenone was either absent or present in trace amounts, while piperitenone oxide was either absent or present in low quantities (0.4%–2.5%).

The second major clade was divided into two groups. The first comprises samples from Valjevo (VAL) and Belgrade (BG). The essential oils of these populations are characterised by a lower content of piperitone epoxide (46.6%–61.2%) and a higher content of limonene (12.5%–19.0%) compared to the samples from the second group. The samples from Belgrade which were collected at a seven-year interval exhibit substantial similarities in terms of the composition of the essential oil (Table 2). The sample from Valjevo is characterised by the lowest quantity of piperitone epoxide in the essential oil (46.6%) compared to the other populations within this group.

Consequently, the second group comprised nine samples characterised by a concentration range of piperitone epoxide between 56.9% and 71.9% and limonene

Table 3. Pearson correlation matrix between nine compounds present at a minimum of 5% in at least one sample and ten bioclimatic parameters correlated with at least one compound (-0.5 < r > 0.5). The statistically supported r-values are in bold ($p < 0.05$).

	bio2[a]	bio3	bio4	bio7	bio8	bio10	bio12	bio13	bio16	bio19
Limonene	-0.08	-0.33	**0.66**	**0.54**	0.45	**0.54**	**-0.6**	-0.52	**-0.6**	-0.47
α-Terpinene	-0.06	-0.15	0.28	0.19	0.2	0.41	-0.26	-0.13	-0.19	-0.14
cis-Sabinene hydrate	0.52	**0.54**	-0.41	0.08	-0.43	-0.19	-0.09	-0.07	-0.09	0.12
Menthone	-0.35	-0.31	0.06	-0.2	0.08	-0.16	0.05	0.13	0.17	-0.12
Terpinen-4-ol	-0.52	-0.31	-0.15	**-0.58**	-0.08	-0.36	0.35	0.30	0.36	0.16
Piperitone epoxide	0.16	0.32	-0.4	-0.33	-0.31	-0.13	0.50	0.52	0.50	0.50
Piperitenone	-0.01	-0.12	0.21	0.23	0.1	-0.02	-0.31	-0.33	-0.32	-0.31
Piperitenone oxide	-0.08	-0.19	0.23	0.22	0.16	-0.04	-0.25	-0.31	-0.28	-0.31
(*E*)-Caryophyllene	0.16	0.41	**-0.6**	-0.5	**-0.6**	-0.18	0.46	**0.62**	**0.57**	**0.62**

[a] The acronyms of the bioclimatic parameters: bio2 - monthly temperature range, bio3 - isothermality (2/7) (* 100), bio4 - temperature seasonality (STD * 100), bio7 - annual temperature range, bio8 - mean temperature of the wettest quarter, bio10 - mean temperature of the warmest quarter, bio12 - annual precipitation, bio13 - precipitation of the wettest month, bio16 - precipitation of the wettest quarter, bio19 - precipitation of the coldest quarter

ranging from 0.8% to 10.5%. Within this heterogeneous group, the Mount Rudnik (RUD) sample stands out due to its high quantity of piperitone epoxide (63.3%), the highest content of menthone (10.8%) and the lowest quantity of limonene (0.8%). Conversely, the sample from Derventa Canyon (DER) stands apart from other samples due to the highest content of piperitone epoxide (71.9%) and terpinen-4-ol (6.2%) in its oil.

The remaining part of the second group includes samples from Rtanj (RTA), Beli Rzav Canyon (BRZ), Trpejca (TRP), Radožda (RAD), Gučevo (GUČ), Tara (TAR), and Jerma Gorge (JER). The populations from Rtanj mountain and Beli Rzav Canyon exhibit close proximity, characterised by similarities in the content of piperitone epoxide (60.8%, 60.4%) and *cis*-sabinene hydrate (5.3%, 5.8%). The Trpejca population, while connected at some distance with these populations, displays higher quantities of piperitone epoxide (63%) and *cis*-sabinene hydrate (9.0%). The Radožda and Gučevo populations are distinguished by higher quantities of piperitone epoxide (65.6%, 69.5%), and lower quantities (4.3%, 4.6%) of *cis*-sabinene hydrate. The population from Tara stands out from the other populations due to a lower quantity of piperitone epoxide (59.2%) and the highest content of *(E)*-caryophyllene (7.5%) in its essential oil. Finally, the population from Jerma Gorge exhibits a short-distance connection with the other populations, marked by the lowest quantity of piperitone epoxide (56.9%) and the highest quantity of *cis*-sabinene hydrate (12.0%) in its essential oil.

Univariate analysis. The analysis of variance (ANOVA) revealed that five compounds (myrcene, F = 28.6, p = 0.03; *trans*-sabinene hydrate, F = 31.9, p = 0.03; menthone, F = 971.5, p = 0.001; isomenthone, F = 11160.1, p = 0.000; piperitenone oxide, F = 53.3, p = 0.01) were responsible for the differentiation of all the samples. The content of three compounds was shown to be significantly different between climatic groups (sabinene, F = 7.39, p = 0.009; *cis*-thujone, F = 4.71, p = 0.03; isopiperitenone, F = 4.97, p = 0.02), while small differences between altitudinal and biogeographic groups were due to differences in the concentration of three (α-thujene, F = 6.78, p = 0.02; limonene, F = 4.99, p = 0.04; *cis*-sabinene hydrate, F = 6.67, p = 0.02) and four compounds (sabinene, F = 4.97, p = 0.02; (*Z*)-β-ocimene, F = 5.53, p = 0.02; *cis*-thujone, F = 4.71, p = 0.03; isopiperitenone, F = 4.97, p = 0.029), respectively. Regardless of the fact that most of the mentioned components made some contribution to group separation, their total content in the studied samples is insignificantly low. Therefore, the fluctuations in the compounds primarily defining the essential oils were shown in the box-and-whiskers plots. Moderate distinction among the analysed *a priori* groups was evident concerning limonene and (*E*)-caryophyllene, while little differentiation among the climatic groups was observed based on the most important compound, piperitone epoxide (Fig. S2).

Correlation and regressions. A pairwise Pearson correlation of the nine main compounds and 19 bioclimatic parameters was performed. None of the compared compounds were strongly correlated with the bioclimatic parameters (r > 0.8). Most compounds were not correlated, while four compounds were slightly to moderately correlated (r = 0.5–0.8; $p < 0.05$) with the bioclimatic parameters (limonene, *cis*-sabinene hydrate, terpinen-4-ol and (*E*)-caryophyllene). Limonene and (*E*)-caryophyllene are correlated with most of the bioclimatic parameters (limonene - bio4, bio7, bio10, bio12, bio16; (*E*)-caryophyllene - bio4, bio8, bio13, bio16, bio19), while *cis*-sabinene hydrate and terpinen-4-ol are correlated with one bioclimatic parameter, bio3 and bio7 respectively. The dominant compound, piperitone epoxide, showed a slight correlation with bio12, bio13, bio16 and bio19, with low statistical significance ($p > 0.05$). The bioclimatic parameters which influence the presence of a larger number of compounds are annual temperature range (bio7), the precipitation of the wettest month (bio13) and the precipitation of the wettest quarter (bio16) (Table 3).

Overall, based on significant correlation coefficients, the bioclimatic parameters related to precipitation have a slightly greater influence on compound content than the temperature parameters. On the other hand, the temperature seasonality (bio4) and the annual temperature range (bio7) have a significant influence on the content of limonene and (*E*)-caryophyllene, although there are two different trends. With increasing temperature seasonality (bio4), annual temperature range (bio7) and the mean temperature of the wettest quarter (bio8), the content of limonene increases, while the content of (*E*)-caryophyllene decreases. In addition, the content of terpinen-4-ol decreases with increasing annual temperature range (bio7) and *cis*-sabinene hydrate decreases with the mean temperature of the wettest quarter (bio8), following the same pattern as (*E*)-caryophyllene. For the bioclimatic factors of precipitation (bio12, bio13, bio16 and bio19), opposite trends are observed for the content of limonene and (*E*)-caryophyllene, namely with increasing annual precipitation (bio12), precipitation in the wettest month (bio13), precipitation in the wettest quarter (bio16) and precipitation in the coldest quarter (bio19), the limonene content increases and the (*E*)-caryophyllene content decreases. The dominant compound, piperitone epoxide, shows a slight correlation with the bioclimatic factors of precipitation (bio12, bio13, bio16, bio19). In all cases, piperitone epoxide shows the same tendency - an increase in the mentioned precipitation factors causes an increase in its concentration. In general, the bioclimatic parameters affecting the concentration of compounds showed a high degree of collinearity. Both the temperature and precipitation parameters show the same paths of compound variations (Fig. 4).

Overall, in the case of *C. menthifolium* subsp. *menthifolium*, bioclimatic parameters are not a dominant factor in determining the composition and quantity of compounds, in contrast to *Rosmarinus officinalis* in the Balkans (Lakušić *et al.* 2012), where most bioclimatic factors strongly influence the composition of essential oils. Regardless of the fact that climatic conditions can influence yields and essential oil composition (Llorens *et al.* 2014; Aboukhalid *et al.* 2017), *C. menthifolium* subsp. *menthifolium* has stable oil composition with a low dependence on climatic fluctuations. Although most compounds are not affected by temperature and precipitation factors, limonene and (*E*)-caryophyllene show a moderate dependence on them. Three out of four samples (with the exception of Jerma) from the arid climate variant have a significantly higher content of limonene (10.5%–24.5%) compared to the samples from humid climate variants (0.8%–13.5%). Several studies confirm the influence of climate on the content of limonene (Lakušić *et al.* 2011; Llorens *et al* 2014; Melito *et al* 2016; Dodoš *et al.* 2019). In the case of the Balkan endemic *Satureja horvatii*, no limonene was detected in the samples from natural habitats with extreme humidity (Mount Lovćen and Mount Orjen), in contrast to the samples from the garden in Belgrade (arid climate variant), where limonene was detected in every sample (Lakušić *et al.* 2011). Similarly, the content of limonene in the genus *Satureja* from the Central Balkans was significantly higher in the sample from the driest climate (Slavkovska *et al.* 1998; Dodoš *et al.* 2019). In addition, samples from locations with less precipitation show a lower concentration of limonene in *Thymus richardii* from the Mediterranean region (Llorens *et al.* 2014) and *Helichrysum italicum* subsp. *microphyllum* from Sardinia (Melito *et al.* 2016). Although (*E*)-caryophyllene showed a dependence on both temperature and precipitation parameters, there are no studies confirming similar patterns.

The distances between the locations in the cluster analysis based on the climatic data are significantly larger than those within the cluster based on the phytochemical properties of the essential oil. Although there are obvious climatic differences between the locations where the analysed plants grow, these differences were not reflected in the composition of the essential oil. Thus, the Trpejca (TRP) sample is phytochemically closest to the samples from the Beli Rzav Canyon (BRZ) and Rtanj mountain (RTA), although it grows under the influence of different climatic conditions. This also applies to the samples from Radožda (RAD) and Gučevo (GUC).

On the other hand, the difference between phytochemical and climatic diversification is observed in the samples from Valjevo (VAL) and Gučevo (GUC), which grow under very similar climatic conditions but differ phytochemically.

Our results lead us to conclude that climatic conditions have an insignificant influence on the composition of the essential oils of the studied taxon. However, to fully explain the patterns of variation in the essential oil constituents, it is necessary to sample this species over as wide a range as possible, covering a variety of habitats from different climatic zones. If this research is continued with samples from across the range, different correlations between bioclimatic parameters and essential oil constituents could be discovered. In addition, a com-

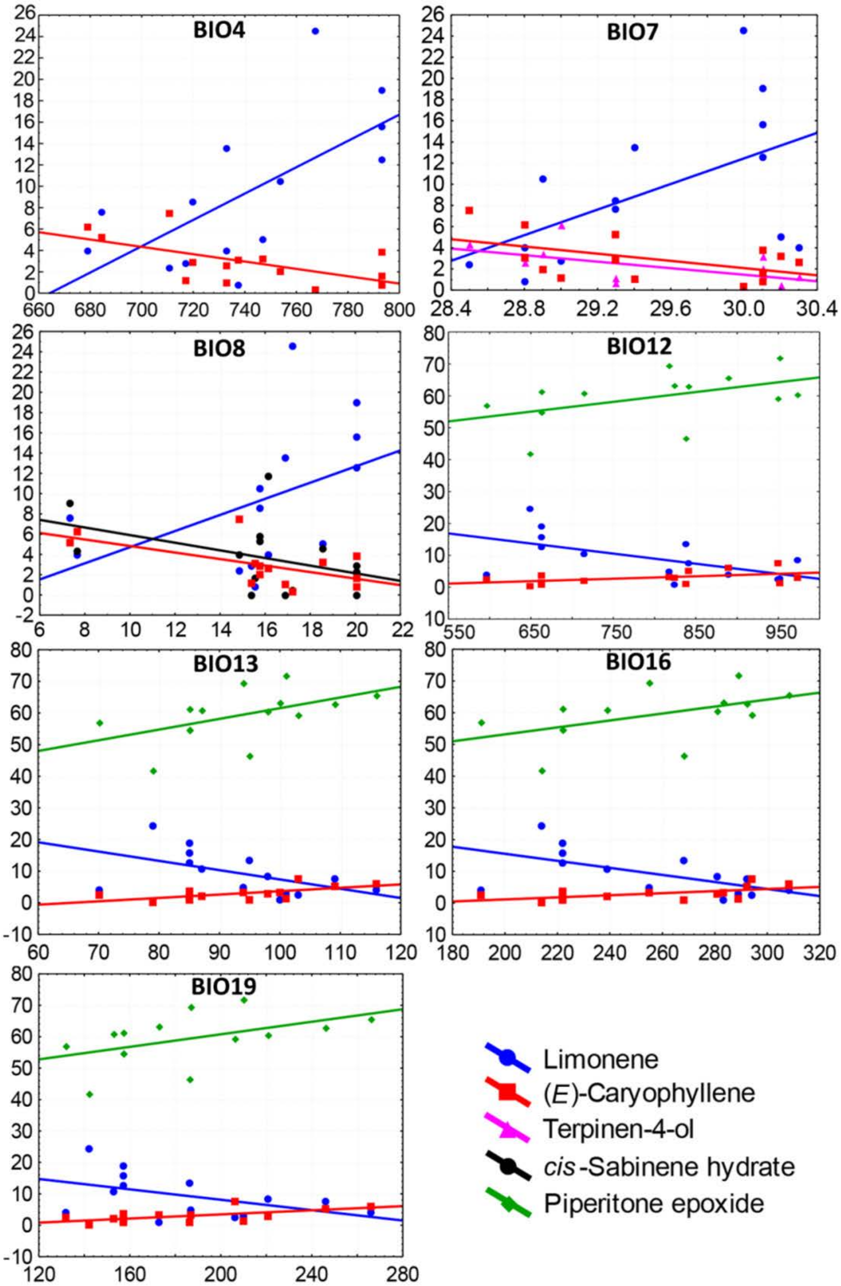

Fig. 4. The relationship between the selected correlated bioclimatic factors and five selected compounds. The acronyms of the bioclimatic parameters: BIO4 - temperature seasonality (STD * 100); BIO7 - annual temperature range; BIO8 - mean temperature of the wettest quarter; BIO12 - annual precipitation; BIO13 - precipitation of the wettest month; BIO16 - precipitation of the wettest quarter; BIO19 - precipitation of the coldest quarter.

parative analysis of chemotypes and genotypes could provide a clearer understanding of essential oil variation patterns.

CONCLUSIONS

Although the analysed plants live in different climatic zones, altitudes and biogeographical regions, they showed no significant differences in the amount and composition of their essential oils. All the samples had an essential oil yield of ≥ 0.5%, V/w. The content of monoterpenes in the oils was much higher than that of sesquiterpenes. Among the monoterpenes, the oxygenated C-3 monoterpenes of the menthane class were dominant. Piperitone epoxide was the major compound in the essential oils of all the samples, indicating that the analysed plants belong to the piperitone epoxide chemotype. Of all the compounds identified, only limonene and (*E*)-caryophyllene showed dependence on the variation of bioclimatic parameters. The relative stability of the quantity and quality of the essential oils of *C. menthifolium* subsp. *menthifolium* suggests that these traits are likely to be subject to natural selection and have adaptive significance. Consequently, these traits could potentially serve as the chemotaxonomic features of this taxon.

Acknowledgments – This research was funded by the Ministry of Science, Technological Development and Innovation, Republic of Serbia through Grant Agreement with the University of Belgrade-Faculty of Pharmacy No 451-03-65/2024-03/ 200161 and No: 451-03-66/2024-03/ 200161. We express our gratitude to Prof. Branislava Lakušić and Prof. Dmitar Lakušić for providing the plant material from its natural habitat and for cultivating it in their private botanical garden.

REFERENCES

Aboukhalid K, Al Faiz C, Douaik A, Bakha M, Kursa K, Agacka-Mołdoch M & Lamiri A. 2017. Influence of environmental factors on essential oil variability in *Origanum compactum* Benth. growing wild in Morocco. *Chemistry & Biodiversity* **14**(9): e1700158.

Adams RP. 2017. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*. 4th ed. Allured Publishing Corporation Illinois, USA.

Akgül A, de Pooter LH & de Büyck FL. 1991. The essential oils of *Calamintha nepeta* subsp. *glandulosa* and *Ziziphora clinopodioides* from Turkey. *Journal of Essential Oil Research* **3**: 7–10.

Ball PW & Getliffe FM. 1972. *Calamintha* Miller. In: Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM & Webb DA (eds.), *Flora Europaea, vol. 3*, pp. 166–167, Cambridge University Press, Cambridge.

Bentham G. 1848. *Labiatae*. In: de Candolle AP (ed.), *Prodromus Systematis Universalis Regni Vegetabilis*, *vol. 12*, pp. 212–226, Treuttel & Würtz, Paris.

Castilho P, Liu K, Rodrigues AI, Feio S, Tomi F & Casanova J. 2007. Composition and antimicrobial activity of the essential oil of *Clinopodium ascendens* (Jordan) Sampaio from Madeira. *Flavour and Fragrance Journal* **22**(2): 139–144.

Debbabi H, El Mokni R, Nardoni S, Chaieb I, Maggi F, Kamgang Nzekoue F, Caprioli G & Hammami S. 2021. Chemical diversity and biological activities of essential oils from native populations of *Clinopodium menthifolium* subsp. *ascendens* (Jord.) Govaerts. *Environmental Science & Pollution Research* **28**(11): 13624–13633.

Dobravalskytė D, Venskutonis PR & Talou T. 2012. Antioxidant properties and essential oil composition of *Calamintha grandiflora* L. *Food Chemistry* **135**(3): 1539–1546.

Dodoš T, Rajčević N, Janaćković P, Vujisić L & Marin PD. 2019. Essential oil profile in relation to geographic origin and plant organ of *Satureja kitaibelii* Wierzb. ex Heuff. *Industrial Crops and Products* **139**: 111549.

Doroszenko MA. 1986. Taxonomic studies of *Satureja* complex (*Labiatae*). PhD Thesis, University of Edinburgh. Edinburgh.

Euro+Med 2006+ [continuously updated]: Euro+Med PlantBase - the information resource for Euro-Mediterranean plant diversity. Published at http://www.europlusmed.org [Accessed 31 October 2023]

Hammer Ø, Harper DAT & Rayan PD. 2001. PAST: Paleontological Statistics software package for education and data analysis. *Paleontologia Electronica* **4**(1): 1.

Hanlidou E, Kokkini S, Bosabalidis MA & Bessière M. 1991. Glandular trichomes and essential oil constituents of *Calamintha menthifolia* (*Lamiaceae*). *Plant Systematics and Evolution* **177**(1–2): 17–26.

Hijmans RJ, Guarino L, Mathur P. 2012. DIVA-GIS. Version 7.5. Available at: www.diva-gis.org.

Karousou R, Hanlidou E & Lazari D. 2012. Essential-oil diversity of three *Calamintha* species from Greece. *Chemistry & Biodiversity* **9**: 1364–1372.

Karousou R, Kokkini S, Bessière J M & Vokou D. 1996. *Calamintha cretica* (Lamiaceae), a Cretan endemic: Distribution and essential oil composition. *Nordic Journal of Botany* **16**(3): 247–252.

Kimbaris AC, González-Coloma A, Andrés MF, Vidali VP, Polissiou MG & Santana-Méridas O. 2017. Biocidal compounds from *Mentha* sp. essential oils and their structure–activity relationships. *Chemistry & Biodiversity* **14**(3): e1600270.

Kitić D, Jovanović T, Ristić M, Palić R & Stojanović G. 2002a. Chemical composition and antimicrobial activity of the essential oil of *Calamintha nepeta* (L.) Savi ssp. *glandulosa* (Req.) P.W. Ball from Montenegro. *Journal of Essential Oil Research* **14**: 150–152.

Kitić D, Palić R, Ristić M, Stojanović G & Jovanović T. 2001. The volatile constituents of *Calamintha sylvatica* Bromf. subsp. *sylvatica*. *Flavour and Fragrance Journal* **16**(4): 257–258.

Kitić D, Palić R, Stojanović G & Ristić M. 2002b. Composition of the essential oil of *Calamintha vardarensis* (Greuter et Burdet) Šilić. *Journal of Essential Oil Research* **14:** 58–59.

Kitić D, Stojanović G, Palić R & Ranđelović V. 2005. Chemical Composition and Microbial Activity of the Essential Oil of *Calamintha nepeta* (L.) Savi ssp. *nepeta* var. *subisodonda* (Borb.) Hayek from Serbia. *Journal of Essential Oil Research* **17**: 701–703.

Lakušić B, Lakušić D, Ristić M, Marčetić M & Slavkovska V. 2014. Seasonal variations in the composition of the essential oils of *Lavandula angustifolia* (Lamiacae). *Natural Product Communications* **9**(6): 859–862.

Lakušić B, Ristić M, Slavkovska V, Milenković M & Lakušić D. 2011. Environmental and seasonal impacts on the chemical composition of *Satureja horvatii* Šilić (Lamiaceae) essential oils. *Chemistry & Biodiversity* **8**: 483–493.

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**(2): 127–139.

Lakušić D, Ristić M, Slavkovska V, Šinžar-Sekulić J & Lakušić B. 2012. Environment-related variations of the composition of the essential oils of rosemary (*Rosmarinus officinalis* L.) in the Balkan Peninsula. *Chemistry & Biodiversity* **9**: 1286–1302.

Li Y & Zidorn C. 2022. Seasonal variations of natural products in European herbs. *Phytochemistry Reviews* **21**(5): 1549–1575.

Llorens L, Llorens-Molina JA, Agnello S & Boira H. 2014. Geographical and environment-related variations of essential oils in isolated populations of *Thymus richardii* Pers. in the Mediterranean basin. *Biochemical Systematics and Ecology* **56**: 246–254.

Melito S, Petretto GL, Podani J, Foddai M, Maldini M, Chessa M & Pintore G. 2016. Altitude and climate influence *Helichrysum italicum* subsp. *microphyllum* essential oils composition. *Industrial Crops and Products* **80**: 242–250.

Meusel H, Jäger E & Weinert E. 1965. *Vergleichende Chorologie der Zentraleuropäischen Flora*. Bd. 1. Gustav Fischer Verlag Jena.

Milenković M, Stošović J & Slavkovska V. 2018. Synergy between essential oils of *Calamintha* species (Lamiaceae) and antibiotics. *Natural Product Communications* **13**(3): 371–374.

Mimica-Dukić N, Couladis M, Tzakou O, Jančić R & Slavkovska V. 2004. Essential oil of *Calamintha sylvatica* Bromf. and *Calmintha vardarensis* Šilić. *The Journal of Essential Oil Research* **16**: 219-222.

Ph. Eur. 2020. *European Pharmacopoeia*, 10th Edition. Council of Europe, Strasbourg, France.

Ristorcelli D, Tomi F & Casanova J. 1996. Essential oils of *Calamintha nepeta* subsp. *nepeta* and subsp. *glandulosa* from Corsica (France). *Journal of Essential Oil Research* **8**: 363-366.

Slavkovska V, Jančić R, Živanović P, Bajić D, Milosavljević S, Đoković D & Tešević V. 1998. Examination of the variability of the composition of the essential oils of *Satureja montana* L. and *Satureja kitaibelii* Wierzb. Ex Heuff. (Lamiaceae). *Ekologija* **33**: 145-159.

StatSoft, Inc. 2007. STATISTICA (Data Analysis Software System). Version 7.0. StatSoft Inc., Tulsa (OKLA).

Ševarda A. 1993. *Structural and functional features of the terpenogen complex in some plants of the Eastern Mediterranean (Northwestern part of the Balkan Peninsula)*. PhD dissertation. Komarov Botanical Institute of the Russian Academy of Sciences, Saint Petersburg.

Ševarda LA, Kuznjecova AG, Pavlović S, Živanović P, Jančić R & Vujčić S. 1987. Comparative studies of the composition of the essential oil of species *Calamintha glandulosa* (Reg.) Bentham, *C. vardarensis* Šilić and *C. sylvatica* Bromf. *Acta Pharmaceutica Jugoslavica* **37**: 103-106.

Šilić Č. 1979. *Monografija rodova Satureja L., Calamintha Miller, Micromeria Bentham, Acinos Miller i Clinopodium L. u flori Jugoslavije,* pp. 117-172, Zemaljski muzej BiH, Sarajevo.

Zbiljić M, Lakušić B, Kuzmanović N, Stojanović D & Lakušić D. 2023. Morphological diversification of *Teucrium montanum sensu lato* on the Balkan Peninsula. *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology* **157**(3): 670-687.

REZIME

Botanica SERBICA

Clinopodium menthifolium subsp. *menthifolium* centralnog dela Balkanskog poluostrva – sastav etarskog ulja u odnosu na klimatske uslove

Violeta Slavkovska, Miloš Zbiljić i Danilo Stojanović

Uticaj ekoloških faktora na količinu i sastav etarskog ulja *Clinopodium menthifolium* subsp. *menthifolium* do sada nije istraživan. Da bismo ostvarili ovaj cilj, ispitivali smo količinu i sastav etarskog ulja biljaka sakupljanih iz 11 prirodnih populacija centralnog dela Balkanskog poluostrva i jedne gajene biljke. U cilju utvrđivanja korelacije između varijacija etarskog ulja i ekoloških uslova, svako stanište je okarakterisano sa 36 klimatskih i 19 bioklimatskih parametara. Uprkos tome što rastu u različitim klimatskim zonama, biogeografskim regionima i na različitim nadmorskim visinama, značajne razlike u sadržaju, kvalitativnom i kvantitativnom sastavu etarskih ulja među analiziranim biljkama nisu zapažene. Svi uzorci su imali ≥ 0,5%, V/w etarskog ulja, pri čemu je glavni sastojak bio piperiton epoksid. Među identifikovanim jedinjenjima, zavisnost od bioklimatskih parametara pokazali su jedino limonen i (*E*)-kariofilen. Bioklimatski parametri koji utiču na količinu većeg broja jedinjenja su godišnji opseg temperature i padavine najvlažnijeg kvartala. Ujednačena količina i sastav etarskog ulja *C. menthifolium* subsp. *menthifolium* ukazuju na njihov verovatni adaptivni značaj i mogu poslužiti kao hemotaksonomske odlike ovog taksona.

Ključne reči: *Calamintha sylvatica,* isparljiva jedinjenja, bioklimatski parametri, Centralni Balkan, piperiton epoksid, limonen

ORCID:

Violeta Slavkovska https://orcid.org/0000-0003-4789-8121
Miloš Zbiljić https://orcid.org/0000-0001-8620-7480
Danilo Stojanović https://orcid.org/0000-0001-6165-6511