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**RESEARCH ARTICLE**

# Chemical composition of the essential oil of *Stachys menthifolia Vis.*

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**Abstract**

*Stachys menthifolia* Vis. (Lamiaceae) is an endemic species from the Balkan Peninsula spread throughout Albania, Greece, Montenegro, and Croatia. This article presents the first investigation of the essential oil composition of this species from Croatia. Aerial parts of the plant were collected from three different natural habitats in the region of Biokovo Mountain. The studied populations showed similarity in qualitative, but not in quantitative, composition of their essential oils. Hydrodistilled volatile oil obtained from the plant material of *S. menthifolia* was subjected to gas chromatographic analysis coupled to mass spectrometry. More than 100 compounds were identified in the three samples, representing 86.8–90.8% of the total oil. The terpene profile of *S. menthifolia* is characterized by a high content of oxygenated sesquiterpenes (48.4–58.9%) and diterpene hydrocarbons (3.5–25.2%), with 8-α-acetoxyelemol (6.9–21.3%), abietatriene (3.5–21.1%), and 4′-methoxyacetophenone (4.5–17.0%) as the main constituents.

**Keywords:** Stachys menthifolia *Vis.; 8-α-acetoxyelemol; abietatriene; essential oil composition; GC-MS*



## Introduction

*Stachys* L. (Lamiaceae) is a large genus of herbs and shrubs comprising about 300 species, distributed in temperate and tropical regions of the world (Mabberley, 1997). Plants of this genus have been used in folk medicine for centuries to treat genital tumors, sclerosis of the spleen, inflammatory diseases, cough, ulcers, and infected wounds (Hartwell, 1982). Like most plants used for medicinal purposes, *Stachys* taxa have been submitted to several investigations in order to determine the content of biologically active compounds. As many other representatives of the family Lamiaceae, *Stachys* species also produce essential oils, but in spite of the large size of this genus, the composition of volatile compounds is known only in a small number of species (Maly, 1985; Cakir et al., 1997; Mariotti et al., 1997; Pélissier et al., 1999; Chalchat et al., 2000, 2001; Skaltsa at al., 2003; Tirillini et al., 2004; Bilusic Vundac et al., 2006; Radulovic et al., 2007).

More than 175 years ago, Roberto Visiani described the endemic species *Stachys menthifolia* Vis. of the West Balkan region at the locality of Ascrivium – the classical name for Kotor (Montenegro). This species was also found in Croatia, in the Biokovo area (Šilić & Šolić, 2002).

This article presents the first investigation of the essential oil composition of *S. menthifolia* collected from three localities in the Biokovo area, which represents the only certain and verified place where this plant is found in Croatia.

## Material and methods

### *Plant material*

Three specimens of *S. menthifolia* were gathered from three natural habitats on the eastern slopes of the Biokovo area. Details of the collection localities are presented in Table 1. All samples were collected in

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**Table 1.** Origin of plant material of *Stachys menthifolia* Vis.

| Sample | Voucher specimen (MAKAR) | Locality | Altitude[a] (m s. m.) | Slope (°) | Exposure |
|---|---|---|---|---|---|
| 1 | KU-2309 | Vrgorac | 180 | 60–90 | E-SE |
| 2 | KU-2310 | Vrgorac | 30–130 | 0–30 | S |
| 3 | KU-2311 | Ploče | 60 | 30 | NW |

[a]m s. m. - meter 'supra mare' (lat.)

the course of flowering on the very same day in June 2005. Voucher specimens of each population were identified by Dr. Marija Edita Šolić, and deposited in the scientific collection of the Institute "Mountain and Sea," in the Herbarium of the Biokovo region (MAKAR) in Makarska, Croatia, and at the Department of Chemistry, Faculty of Science, University of Sarajevo, Bosnia and Herzegovina.

### *Reagents*

All applied reagents were of the highest purity available and purchased from the Sigma-Aldrich Chemical Co.

### *Isolation of volatile oil*

Essential oils from the air-dried parts of *S. menthifolia* were isolated by hydrodistillation for 2 h. The oils were extracted with dichloromethane and dried over anhydrous sodium sulfate and stored at 4°C in the dark.

### *Gas chromatography-mass spectrometry*

Gas chromatography-mass spectrometry (GC-MS) was carried out on a Hewlett-Packard (HP) 6890 Series II gas chromatograph fitted with a fused silica HP-5 (5% phenyl methyl siloxane) capillary column (30 m × 0. 252 mm, 0.25 μm film thickness), coupled to a HP 6890 Series II mass selective detector (MSD). The column temperature was programmed from 60 to 240°C at 3°C $min^{-1}$, and helium was used as the carrier gas. Other operating conditions were as follows: inlet pressure 9.43 psi, injector temperature 250°C, detector temperature 280°C, split ratio 1:25, injection volume 1 μL. Ionization of the sample components was performed in electron ionization (EI) mode (70 eV), with scan range 20–555 amu, and scan time 1. 60 s.

### *Qualitative and quantitative analysis*

The linear retention indices (RIs) for all compounds were determined by co-injection of hexane solution containing the homologous series of $C_8$–$C_{26}$ *n*-alkanes (Van Del Dool & Kratz, 1963). Identification of the essential oil constituents was accomplished by visual interpretation, comparing their RIs and mass spectra with literature data (Adams, 2007) from computer library search (HP Chemstation computer library NBS75K.L, NIST/EPA/NIH Mass Spectral Library 2.0, and Mass Finder 3 Computer Software and Terpenoids Library), and the laboratory's own database. Semiquantitative analysis was carried out directly from peak areas in the GC profile.

## Results and discussion

The essential oils were extracted by hydrodistillation from the aerial parts of *S. menthifolia*. The content of essential oil based on the dry weight of the plant material was 0.07, 0.14, and 0.13% for sample 1, 2, and 3, respectively.

The total ion chromatograms of essential oils are displayed in Figure 1. The figure shows the heterogeneity of the mixtures, and also similarity in qualitative, but not in quantitative, composition of the samples. The components identified in *S. menthifolia*, their retention indices, and percentage compositions are summarized in Table 2, where all compounds are arranged in order of their elution on the HP-5 column.

Ninety-eight compounds were identified in sample 1, representing 87.0% of the total oil. The most abundant compounds were 8-α-acetoxyelemol (21.3%), 4'-methoxyacetophenone (17.0%), and cryptomeridiol (6.7%). One hundred-eleven chemical constituents were identified in sample 2, representing 86.8% of the total volatiles. Major compounds in this sample were abietatriene, 4'-methoxyacetophenone, and 8-α-acetoxyelemol, with percentages of 11.8, 7.3, and 6.9%, respectively. Ninety-eight identified components in sample 3 represented 90.8% of the total essential oil. The study of chemical composition of this sample revealed richness in abietatriene (21.1%), 8-α-acetoxyelemol (12.8%), and caryophyllene oxide (5.2%).

The distribution of the terpenoid compounds in the studied *S. menthifolia* essential oils is given in Table 3. Oxygenated sesquiterpenes were predominant in all samples, with a content of 58.9% in sample 1 and the same relative amount of 48.4% in samples 2 and 3. The next most abundant class of compounds in sample 1 were aromatic compounds, representing 17.0% of the total oil, while samples 2 and 3 showed a high abundance of diterpene hydrocarbons, 12.6 and 25.2%, respectively.

It is highly possible that the observed differences in content and composition of the essential oils of three *S. menthifolia* populations, collected from the fissures of limestone or open, limy, rocky ground in a relatively small area of Biokovo Mountain, are the result of different geographical parameters, such as altitude, slope, and exposure (Table 1). To be more precise, the highest altitude and solar exposure might have had some influence on the total amount of oxygen-containing compounds in

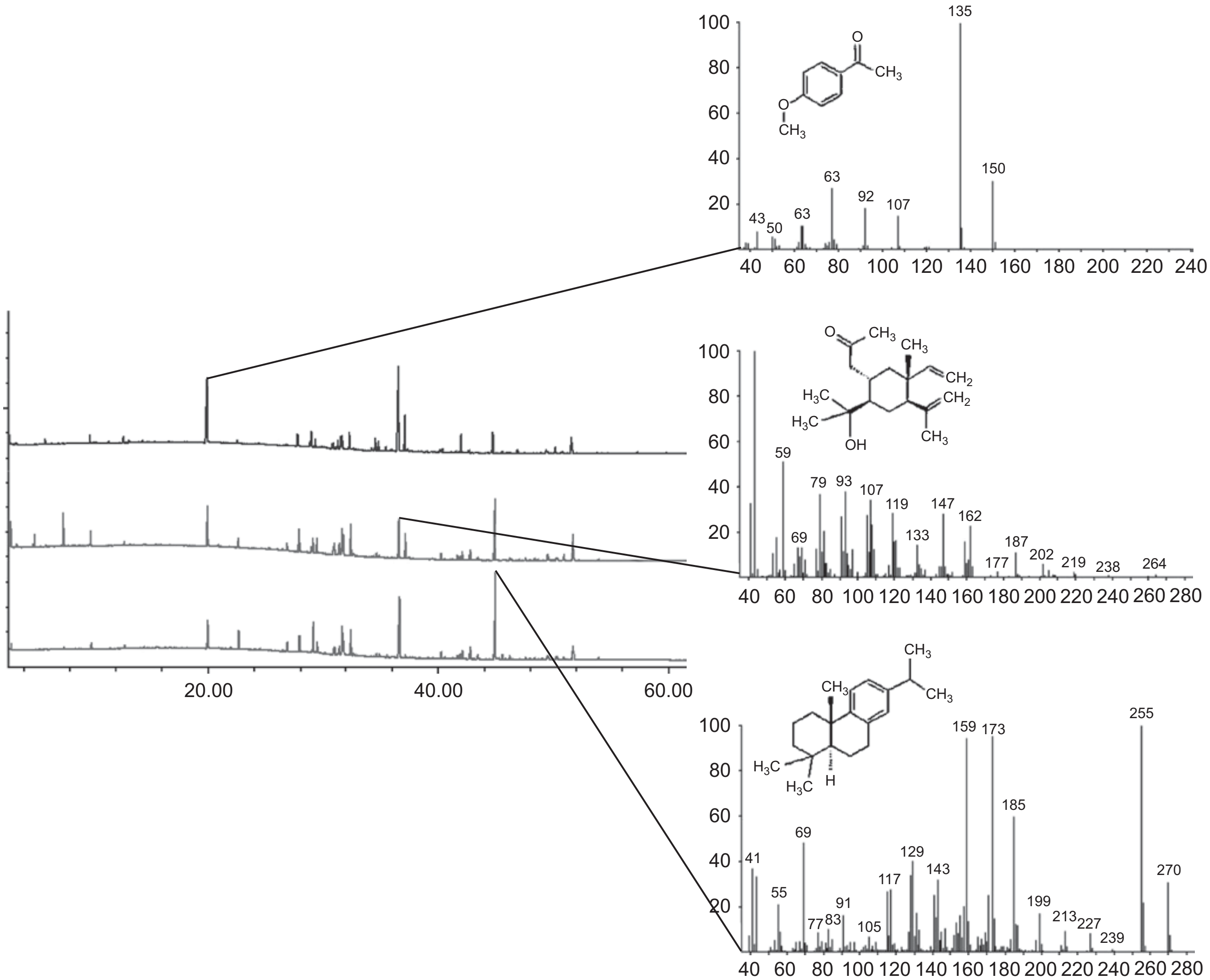


**Figure 1.** Total ion chromatogram (TIC) of the essential oils of *Stachys menthifolia* Vis. with mass spectra of the major constituents.

**Table 2.** Essential oil composition of *Stachys menthifolia* Vis.

| No. | RI | Compound | Sample 1 RA (%) | Sample 2 RA (%) | Sample 3 RA (%) |
|---|---|---|---|---|---|
| 1 | 801 | Hexanal | 0.2 | 0.2 | t |
| 2 | 848 | (2*E*)-Hexenal | 0.1 | 0.2 | t |
| 3 | 901 | Heptanal | t | t | t |
| 4 | 921 | α-Thujene | — | 0.2 | t |
| 5 | 927 | α-Pinene | — | 1.0 | t |
| 6 | 943 | (3*Z*)-Heptenal | t | t | — |
| 7 | 945 | (2*E*)-Heptenal | t | t | — |
| 8 | 952 | Thuja-2,4(10)-diene | t | — | t |
| 9 | 953 | Benzaldehyde | — | t | t |
| 10 | 975 | 1-Octen-3-ol | 0.5 | 0.2 | t |
| 11 | 982 | 6-Methyl-5-hepten-2-one | t | t | t |
| 12 | 991 | β-Myrcene | — | t | t |
| 13 | 994 | 3-Octanol | 0.1 | t | t |
| 14 | 997 | (2*E*, 4*E*)-Heptadienal | t | t | t |
| 15 | 1020 | *p*-Cymene | t | 0.2 | t |
| 16 | 1024 | Limonene | 0.1 | 3.4 | 0.3 |
| 17 | 1029 | 1,8-Cineole | t | t | — |

Table 2. continued on next page

**Table 2.** Continued.

| No. | RI | Compound | Sample 1 RA (%) | Sample 2 RA (%) | Sample 3 RA (%) |
|---|---|---|---|---|---|
| 18 | 1031 | (3*E*)-Octen-2-one | t | t | — |
| 19 | 1039 | Benzene acetaldehyde | t | 0.1 | t |
| 20 | 1053 | γ-Terpinene | — | t | t |
| 21 | 1068 | *cis*-Linalool oxide (furanoid) | t | t | — |
| 22 | 1085 | *trans*-Linalool oxide (furanoid) | t | t | — |
| 23 | 1096 | Linalool | 0.9 | 1.7 | 0.7 |
| 24 | 1102 | *n*-Nonanal | t | t | — |
| 25 | 1111 | Phenyl ethyl alcohol | t | t | — |
| 26 | 1113 | 6-Camphenol | t | t | — |
| 27 | 1122 | α-Campholenal | t | t | t |
| 28 | 1133 | *cis*-*p*-Mentha-2,8-dien-1-ol | t | t | — |
| 29 | 1136 | *trans*-Pinocarveol | t | t | — |
| 30 | 1140 | *trans*-Verbenol | 0.3 | t | 0.2 |
| 31 | 1173 | Terpinen-4-ol | 0.9 | 0.5 | 0.4 |
| 32 | 1184 | *p*-Cymen-8-ol | t | t | t |
| 33 | 1186 | *cis*-Pinocarveol | t | t | — |
| 34 | 1187 | α-Terpineol | 0.3 | 0.2 | t |
| 35 | 1189 | Methyl salycilate | t | t | — |
| 36 | 1193 | Myrtenol | t | t | — |
| 37 | 1194 | Myrtenal | t | t | t |
| 38 | 1197 | *cis*-Piperitol | t | t | — |
| 39 | 1208 | Verbenone | t | — | t |
| 40 | 1216 | *trans*-Carveol | 0.3 | t | 0.2 |
| 41 | 1240 | Carvone | 0.2 | t | 0.2 |
| 42 | 1270 | Pregeijerene B | — | t | 0.3 |
| 43 | 1350 | 4’-Methoxyacetophenone | 17.0 | 7.3 | 4.5 |
| 44 | 1352 | Thymol acetate | t | t | t |
| 45 | 1372 | α-Copaene | t | 0.3 | 0.2 |
| 46 | 1381 | β-Bourbonene | t | t | — |
| 47 | 1405 | Longifolene | — | t | t |
| 48 | 1415 | β-Caryophyllene | 0.5 | 1.4 | 2.8 |
| 49 | 1436 | β-Guaiene | — | t | t |
| 50 | 1449 | α-Humulene | t | t | 0.2 |
| 51 | 1451 | Geranyl acetone | — | t | t |
| 52 | 1462 | *cis*-Cadina-1,6(4)-diene | 0.3 | t | 0.2 |
| 53 | 1481 | *ar*-Curcumene | t | 0.2 | t |
| 54 | 1485 | (*E*)-β-Ionone | t | — | t |
| 55 | 1492 | 10,11-Epoxy-calamenene | t | t | t |
| 56 | 1497 | α-Muurolene | t | 0.2 | 0.2 |
| 57 | 1507 | α-Bisabolene | — | t | 0.1 |
| 58 | 1511 | γ-Cadinene | t | 0.4 | 0.4 |
| 59 | 1514 | *endo*-1-Bourbonanol | t | 0.2 | 0.3 |
| 60 | 1521 | δ-Cadinene | 0.4 | 1.5 | 1.6 |
| 61 | 1524 | Dihydroactinidiolide | 0.4 | t | t |
| 62 | 1534 | α-Cadinene | t | t | 0.2 |
| 63 | 1540 | α-Calacorene | 0.2 | 0.2 | 0.2 |
| 64 | 1547 | Elemol | 2.2 | 3.6 | 2.8 |
| 65 | 1563 | Palustrol | t | 0.2 | t |
| 66 | 1566 | β-Calacorene | t | t | t |
| 67 | 1568 | (3*Z*)-Hexenyl benzoate | t | t | t |
| 68 | 1574 | Spathulenol | 1.5 | 1.7 | 0.7 |
| 69 | 1579 | Caryophyllene oxide | 2.8 | 2.4 | 5.2 |
| 70 | 1588 | Globulol | 1.5 | 2.4 | 1.9 |
| 71 | 1594 | Viridiflorol | t | t | t |

Table 2. continued on next page

**Table 2.** Continued.

| No. | RI | Compound | Sample 1 RA (%) | Sample 2 RA (%) | Sample 3 RA (%) |
|---|---|---|---|---|---|
| 72 | 1600 | Guaiol | 0.3 | — | t |
| 73 | 1605 | 5-*epi*-7-*epi*-α-Eudesmol | t | 0.3 | 0.4 |
| 74 | 1608 | β-Oplopenone | t | t | t |
| 75 | 1615 | 10-*epi*-γ-Eudesmol | t | t | t |
| 76 | 1617 | 1,10-*di*-*epi*-Cubenol | — | t | t |
| 77 | 1626 | *trans*-Isolongifolanone | 1.2 | 1.6 | 1.2 |
| 78 | 1628 | γ-Eudesmol | 1.1 | 1.9 | 1.4 |
| 79 | 1633 | Caryophylla-4(12),8(13)-dien-5-α-ol | 0.4 | t | 0.3 |
| 80 | 1640 | *epi*-α-Muurolol | 1.8 | 2.6 | 2.0 |
| 81 | 1643 | Hinesol | t | t | t |
| 82 | 1647 | β-Eudesmol | 2.4 | 4.8 | 5.0 |
| 83 | 1650 | α-Eudesmol | 1.4 | 3.2 | 3.3 |
| 84 | 1652 | α-Cadinol | 2.3 | 3.1 | 2.0 |
| 85 | 1664 | 14-Hydroxy-β-caryophyllene | t | t | t |
| 86 | 1668 | Valeranone | 3.4 | 5.7 | 4.6 |
| 87 | 1762 | (*Z*)-Lanceol | t | t | t |
| 88 | 1674 | *n*-Tetradecanol | — | 0.6 | 0.5 |
| 89 | 1675 | Khusinol | 0.4 | — | — |
| 90 | 1682 | Germacra-4(15),5,10(14)-tien-1-α-ol | 0.4 | 0.3 | t |
| 91 | 1688 | Eudesma-4(15),7-dien-1-β-ol | 0.3 | t | 0.2 |
| 92 | 1699 | Amorpha-4,9-dien-2-ol | t | 0.2 | 0.5 |
| 93 | 1734 | Oplopanone | 2.3 | 0.8 | 0.5 |
| 94 | 1742 | 8-α-11-Elemodiol | 1.9 | 0.3 | 0.5 |
| 95 | 1760 | Cadalene | 0.8 | 0.2 | 0.4 |
| 96 | 1772 | α-Costol | 0.8 | 0.2 | — |
| 97 | 1792 | 8-α-Acetoxyelemol | 21.3 | 6.9 | 12.8 |
| 98 | 1806 | Eudesm-11-en-4-α,6-α-diol | 0.6 | — | — |
| 99 | 1811 | Cryptomeridiol | 6.7 | 4.3 | 0.5 |
| 100 | 1824 | Khusinol acetate | — | t | t |
| 101 | 1902 | Isopimara-9(11),15-diene | 0.5 | 0.9 | 1.1 |
| 102 | 1953 | Pimaradiene | 0.2 | 0.6 | 0.8 |
| 103 | 1983 | Manool oxide | 0.7 | 2.3 | — |
| 104 | 2002 | (*E*)-Labda-7,12,14-triene | — | 0.5 | 2.7 |
| 105 | 2005 | 13-*epi*-Dolabradien | — | 0.3 | 0.6 |
| 106 | 2050 | Abietatriene | 3.5 | 11.8 | 21.1 |
| 107 | 2072 | Abietadiene | — | t | 0.8 |
| 108 | 2141 | Abienol | — | 0.9 | 0.7 |
| 109 | 2206 | 2-Keto-manool oxide | 0.7 | 1.2 | 0.7 |
| 110 | 2225 | 7-α-Hydroxy-manool | — | 0.3 | 0.2 |
| 111 | 2237 | n.i. 1 | 1.5 | 0.9 | 0.9 |
| 112 | 2258 | Larixol | 0.4 | 1.1 | 0.7 |
| 113 | 2284 | n.i. 2 | 4.3 | 6.2 | 2.6 |
| 114 | 2287 | Dehydroabietal | — | — | 1.5 |
| 115 | 2298 | *n*-Tricosane | — | t | t |
| 116 | 2398 | *n*-Tetracosane | 0.1 | t | t |
| 117 | 2499 | *n*-Pentacosane | 0.2 | t | t |
| 118 | 2598 | *n*-Hexacosane | 0.2 | t | t |
| | | Total identified | 87.0 | 86.8 | 90.8 |

RI, retention indices calculated on HP-5 column; RA, relative area; t, traces (<0.1%); n.i., not identified; n.i. 1: $m/z$ (%) = 27 (12), 43 (100), 55 (64), 67 (46), 81 (55), 95 (45), 107 (37), 121 (36), 135 (53), 147 (25), 161 (13), 175 (37), 190 (33), 207 (21), 218 (3), 227 (1), 236 (2), 243 (4), 255 (50), 273 (7), 291 (67); n.i. 2: $m/z$ (%) = 27 (19), 43 (100), 55 (75), 69 (63), 81 (46), 95 (47), 109 (38), 123 (70), 137 (29), 149 (28), 159 (21), 177 (21), 187 (16), 205 (39), 215 (16), 225 (2), 233 (7), 243 (17), 255 (5), 261 (19), 271 (7), 291 (5), 304 (2).

**Table 3.** Classes of compounds identified in essential oil of *Stachys menthifolia* Vis.

| Class of compounds | Sample 1 (%) | Sample 2 (%) | Sample 3 (%) |
|---|---|---|---|
| Aliphatic compounds | 1.4 | 1.2 | 0.5 |
| Aromatic compounds | 17.0 | 7.6 | 4.5 |
| Monoterpene hydrocarbons | 0.1 | 4.6 | 0.3 |
| Oxygenated monoterpenes | 2.9 | 2.4 | 1.7 |
| Sesquiterpene hydrocarbons | 1.4 | 4.2 | 6.4 |
| Oxygenated sesquiterpenes | 58.9 | 48.4 | 48.4 |
| Diterpene hydrocarbons | 3.5 | 12.6 | 25.2 |
| Oxygenated diterpenes | 1.8 | 5.8 | 3.8 |
| Total | 87.0 | 86.8 | 90.8 |

sample 1 being extremely high (80.6%). In contrast, the content of terpenoid hydrocarbons in this sample (5.0%) was significantly lower than in essential oil obtained from sample 2, collected from a lower altitude and more southern exposure (21.4%). In addition, essential oil from sample 3, originating from a natural habitat found near the sea-coast, at low altitude, had the highest percentage of terpenoid hydrocarbons (31.9%), probably due to the north-western, less solar exposure.

The literature survey on studies related to essential oils obtained from plants belonging to the *Stachy*s genus from the Balkan Peninsula showed significant variability in their chemical compositions depending on the location and stage of plant development. Essential oil obtained from *Stachys milanii* Petrovic (Palic et al., 2006) had borneol and terpinen-4-ol as the most abundant compounds, while the major constituents of essential oil from Bosnian *Stachys alpina* L. spp. *dinarica* Murb. were β-caryophyllene and germacrene D (Kukic et al., 2006). Essential oil obtained from *Stachys officinalis* L. (Trevis) possessed a similar chemical constitution, with a mixture of isocaryophyllene and β-caryophyllene as the principal components (Chalchat et al., 2001). Dehydroabietatriene was the predominant compound in the essential oil of *Stachys plumose* Griseb. (Petrovic et al., 2006). Sesquiterpene hydrocarbons were the main group of constituents in Croatian *Stachys* species (*S. officinalis*, *S. palustris*, *S. recta*, and *S. salviifolia*) except in *S. alpina*, which had a higher content of oxygenated sesquiterpenes (Bilusic Vundac et al., 2006).

According to the presence and quantity of dominant compounds, the essential oil of investigated populations of *S. menthifolia* significantly differs from the only previously published data concerning this species (Skaltsa et al., 2003). Essential oil obtained from *S. menthifolia* of Greek origin had abietatriene (13.7%), kaurene (9.0%), and 13-*epi*-manoyl oxide (7.5%) as the most abundant compounds. In general, this oil was characterized by a high content of terpenoid hydrocarbons (42.3%), while Croatian samples of this species had a smaller amount of this class of compounds (5.0–31.9%).

Although previously examined, *S. menthifolia* from Greece had 13-*epi*-manoyl oxide as one of the principal constituents of its oil, the total amount of oxygen-containing compounds was significantly low (28.2%) in comparison to Croatian specimens (58.4–80.6%). Moreover, the main components of essential oil from Greek *S. menthifolia* were not found in Croatian specimens, with the exception of abietatriene, found as one of the major constituents of the essential oil investigated in the present work. In conclusion, the observed differences in qualitative and quantitative composition of the essential oils obtained from these two geographically isolated populations of *S. menthifolia* confirm the influence of environmental conditions on the nature of the plant chemical composition.

Based on the profile of the principal terpene constituents, the studied populations of Croatian *S. menthifolia* showed a unique essential oil composition among species of *Stachys* genus. In general, all samples were characterized by a high content of oxygenated sesquiterpenes. This study presents the first report of an essential oil with high percentage of 8-α-acetoxyelemol, obtained from a plant that belongs to the Lamiaceae family. Up to now, this oxygenated sesquiterpene has been known as one of the main characteristics of essential oils of the *Juniperus* genus (Cupressaceae), (Adams, 2004; Adams et al., 2006).

To summarize, GC-MS analysis of the volatile constituents of three *S. menthifolia* populations growing wild in the Biokovo area of Croatia indicated similarities in qualitative, but differences in quantitative, composition of their essential oils. From the presented results it can be concluded that two of the three populations clearly belong to the abietatriene chemotype, while the third population appears to belong to a new 8-α-acetoxyelemol chemotype.

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