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# VOLATILE COMPONENTS IN *SIDERITIS SCARDICA* GRISEB. CULTIVAR

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**Abstract**

Volatile components of nine *Sideritis scardica* samples from cultivar planted at different ecological conditions were studied by GC and GC/MS. Thirty-eight individual components in concentration exceeding 0.2% were used for comparison of the studied samples. They belong to mono-, sesqui- and diterpenes, and their oxygenated derivatives, aromatic and aliphatic compounds. Domination of terpene hydrocarbons could be noted as their common feature. The essential oil composition showed significant chemical polymorphism of the *S. scardica* samples. A relation between the oil profiles, altitude and climatic zones was discussed briefly.

**Key words:** *Sideritis scardica*, cultivar, volatile compounds, terpenoids

**Introduction.** *S. scardica* Griseb. is an alpine endemic plant for the Balkan Peninsula [1] distributed in Greece, Albania, Bulgaria, the Republic of Macedonia and Turkey. It is used in some countries either as a herb or flavouring agent as well as for medicinal purposes. Few reports on the essential oils from wild growing taxa showed significant variability in the chemical composition. Thus $\alpha$-pinene (52%), $\beta$-pinene (13%) and myrcene (13%) were the principal components in the oil from Greek taxon [2]. $\beta$-Pinene (17.9%) and carvacrol (14.8%) were dominant constituents in the plant of Turkish origin [3]. $\alpha$-Cadinol was a major compound (20.0%) of *S. scardica* from Macedonia [4], while diterpenes (21.7–31.0%) and octadecenol (23.0–25.3%) were characteristic for this species growing

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in Bulgaria [4]. According to the recently reported data [5], in another Macedonian *S. scardica* taxon the most abundant compound was hexadecanoic acid (12.9%). It was the only component exceeding 10%. Oxygenated mono- and sesquiterpenes constituted significant percentages (30.0 and 25.5% respectively) in this oil.

The distribution of *S. scardica* in Bulgaria is quite limited because of its intensive gathering from the natural habitats for years. Thus the species is included in the Red Data Book of Bulgaria and classified as endangered [6]. The cultivation is an approach to protect destruction of the native populations. Being a high mountainous plant, cultivation of *S. scardica* is difficult and its adaptation to lower altitude was achieved by selection of this species. As a result, cultivar "Sofia2" was obtained [7, 8].

The present study aims to evaluate the influence of the environmental conditions on essential oil composition of cultivar planted in different experimental fields.

**Materials and methods.** ***Plant material.*** Cultivar "Sofia2" was planted in 9 experimental fields in Bulgaria. The samples are described on Table 1. Voucher specimens are deposited in the Herbarium of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences.

Table 1

Plant samples

| Sample | Voucher | Floristic region | Locality | Altitude [m] | CZ* |
|---|---|---|---|---|---|
| S | Co 1128 | Sofia | Sofia | 560 | C |
| VT | Co 1131 | Stara Planina Mt. | Vlado Trichkov | 650 | C |
| Rm | Co 1132 | Znepole | Radomir | 690 | C |
| V | Co 1133 | Vitosha Mt. | Vitosha | 1600 | C |
| GD | Co 1134 | Mesta Valley | Gotse Delchev | 550 | A |
| Tsh | Co 1135 | Pirin Mt. | Teshovo | 1000 | A |
| Tg | Co 1130 | Rhodope Mts | Trigrad | 1240 | A |
| J | Co 1129 | Rhodope Mts | Jundola | 1400 | A |
| Bg | Co 1127 | Rhodope Mts | Beglika | 1500 | B |

*CZ: Climatic zone [13]; A – Continental Mediterranean climatic zone; B – Temperate Continental climatic zone; C – Transitional Continental climatic subzone of the European Continental zone

***Preparation and analysis of essential oils.*** Micro-steam distillation-extraction of the air-dried flowering plant parts (5 g) was carried out in modified Likens–Nickerson apparatus [9] for 3 h using diethyl ether as a solvent. Gas chromatography (GC) and gas chromatography – mass spectrometry (GC–MS) analyses were carried under the experimental conditions reported earlier [10]. The oil components were identified by comparison of their RI and MS, with those published in the literature [11, 12] and presented in NIST 2008 as well as a library developed by us. Mass spectra (EI, 70 eV) $m/z$ (%) of the unidentified diterpenoids are given bellow:

**RI 1980**: 272 (19), 57 (21), 229 (47), 201 (17), 189 (30), 161 (50),147 (50), 133 (45), 133 (45), 93 (100), 81 (95), 69 (60), 67 (63);

**RI 2325**: 288 (20), 270 (100), 255 (49), 242 (10), 199 (14), 187 (20), 145 (15), 131 (20), 107 (20), 105 (20), 94 (43), 79 (20), 69 (16);

**RI 2349**: 288 (29), 273 (15), 257 (30), 161 (47), 145 (30), 133 (30), 131 (30), 119 (50), 105 (100), 91 (70), 81 (20), 79 (22), 67 (25).

**Results and discussion.** Volatile components of *S. scardica* cultivar from 9 experimental fields located at altitude from 550 to 1600 m a.s.l. in 3 different climatic zones (Table 1) were studied.

Analyses of the oils were performed by GC and GC–MS and the oil compositions are given on Table 2. The comparison of the studied essential oils was based on 38 individual components in concentration exceeding 0.2% in the oils. All of the oils were characterized by high concentrations of mono- and sesquiterpene hydrocarbons (up to 58.8 and 39.7% respectively). Diterpenoids, aromatic and aliphatic components varied in wide ranges of 1.4–14.1%, 1.2–14.2% and 0.8–11.3%, respectively.

Table 2

Volatile components (%) of *S. scardica* cultivar from different experimental fields

| RI* | Compounds | S | VT | Bg | V | GD | Rm | Tg | J | Tsh |
|---|---|---|---|---|---|---|---|---|---|---|
| 932 | $\alpha$-Pinene | 4.6 | 6.2 | 9.4 | 8.9 | 14.6 | 4.9 | 18.6 | 14.6 | 11.7 |
| 959 | Benzaldehyde | t | 0.2 | t | t | 0.6 | t | t | t | 0.6 |
| 970 | Sabinene | t | 1.0 | 2.5 | t | t | 1.7 | t | t | 1.4 |
| 976 | $\beta$-Pinene | 18.4 | 19.4 | 24.4 | 24.8 | 6.9 | 4.5 | 33.2 | 37.5 | 32.0 |
| 980 | 1-Octen-3-ol | 0.4 | 1.7 | 3.7 | 4.9 | 10.2 | 6.8 | t | t | 2.4 |
| 990 | Myrcene | 0.6 | 0.7 | 0.9 | 0.6 | t | t | 1.1 | 0.9 | 1.2 |
| 1026 | Limonene | 2.4 | 2.5 | 5.1 | 1.9 | 1.7 | 5.4 | 3.4 | 3.6 | 6.0 |
| 1040 | Phenylacet-aldehyde | 1.1 | 2.3 | 0.7 | 0.9 | 2.0 | 1.5 | 1.9 | 0.7 | t |
| 1061 | Octen-1-ol (2E) | 0.5 | 0.5 | 0.5 | t | t | 1.4 | 0.8 | 0.6 | t |
| 1088 | Terpinolene | 0.5 | 0.4 | 0.5 | t | t | t | 0.5 | 0.4 | 0.5 |
| 1101 | Nonanal | t | 0.5 | t | t | t | 1.3 | t | 1.1 | t |
| 1110 | Phenylethyl alcohol | 0.7 | 2.6 | 2.2 | 0.7 | 0.8 | 3.1 | 1.8 | 0.6 | 1.3 |
| 1137 | *E*-Pinocarveol | 1.7 | 0.6 | t | 4.9 | 2.9 | 4.7 | 0.9 | 0.5 | t |
| 1141 | *E*-Verbenol | 1.0 | 0.4 | t | 2.2 | t | 4.6 | 0.6 | t | t |
| 1163 | Pinocarvone | 1.2 | 0.5 | t | 3.8 | t | 1.5 | 0.9 | t | t |
| 1194 | Myrtenal | 1.1 | 0.3 | t | 3.7 | t | 2.3 | 0.7 | t | t |
| 1196 | Myrtenol | 0.5 | 0.2 | t | 2.4 | t | 1.2 | 0.6 | 0.3 | t |
| 1206 | Verbenone | t | 0.2 | t | 1.1 | 1.7 | 1.1 | 0.5 | 0.2 | t |
| 1358 | Eugenol | t | 1.5 | 0.5 | t | 7.8 | 3.4 | t | t | t |
| 1376 | $\alpha$-Copaene | 2.1 | 2.6 | t | 2.2 | t | 2.1 | 1.6 | 1.2 | 1.9 |
| 1390 | $\beta$-Elemene | 0.6 | 0.5 | t | t | t | t | t | 0.6 | t |

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|---|---|---|---|---|---|---|---|---|---|---|
| 1419 | β-Caryophyllene | 4.1 | 0.6 | t | t | 3.2 | 2.1 | t | 0.6 | 0.5 |
| 1456 | E-β-Farnesene | 11.0 | 6.9 | 12.8 | 2.7 | 1.3 | 2.1 | 4.5 | 4.1 | 5.2 |
| 1482 | Germacrene D | 16.1 | 11.9 | 8.2 | 3.6 | 0.8 | 3.8 | 4.1 | 5.4 | 4.2 |
| 1500 | Bicyclogermacrene | 2.1 | 2.4 | 3.0 | 0.8 | 2.0 | 2.0 | 1.0 | 1.2 | 4.0 |
| 1508 | β-Bisabolene | 1.1 | 2.5 | 0.4 | 1.2 | t | 6.3 | 1.3 | 0.8 | 0.8 |
| 1525 | δ-Cadinene | 2.6 | 3.2 | 0.4 | 1.9 | t | 2.9 | 2.4 | 1.8 | 3.1 |
| 1578 | Spathulenol | t | 0.5 | 4.0 | t | t | t | t | t | t |
| 1585 | Caryophyllene oxide | 0.7 | 0.6 | t | t | t | t | 0.9 | t | t |
| 1762 | Benzylbenzoate | 0.8 | 1.9 | 1.4 | 0.5 | 3.0 | 2.3 | 1.2 | 1.1 | t |
| 1845 | Hexahydrofarnesyl acetone | 1.0 | t | t | t | t | 1.8 | t | t | t |
| 1857 | Phenylethyl benzoate | 0.5 | 0.4 | 0.8 | 0.7 | t | t | t | t | 0.4 |
| 1897 | 2-Heptadecanone | 0.6 | 0.7 | 0.7 | 0.8 | t | 1.8 | t | t | t |
| 1980 | M = 272 | 3.1 | 4.2 | 5.4 | 4.3 | 7.1 | 2.1 | 1.7 | 2.4 | 1.6 |
| 2237 | 7α-Hydroxy-manool | 0.2 | t | t | t | 5.0 | 2.9 | t | t | 1.4 |
| 2269 | Sandaracopi-maradiene-3β-ol | 0.8 | t | t | t | 1.8 | 1.1 | t | 0.8 | t |
| 2325 | M = 288 | 0.9 | 0.7 | t | 0.2 | 2.7 | 0.7 | t | 0.4 | 1.5 |
| 2349 | M = 288 | 0.8 | 0.5 | t | 0.4 | 4.4 | 1.5 | t | t | 1.5 |
| | | | | | | | | | | |
| | Monoterpene hydrocarbons | 26.5 | 29.8 | 42.3 | 36.2 | 23.2 | 16.5 | 56.8 | 57.0 | 52.3 |
| | Oxygenated monoterepenes | 5.5 | 2.2 | t | 18.1 | 4.6 | 15.4 | 4.2 | 1.0 | t |
| | Sesquiterpene hydrocarbons | 39.7 | 30.6 | 24.8 | 12.4 | 7.3 | 21.3 | 14.9 | 15.7 | 19.7 |
| | Oxygenated sesquiterpenes | 1.7 | 1.1 | 4.0 | t | t | 1.8 | 0.9 | t | t |
| | Diterpenoids | 5.8 | 5.4 | 5.4 | 4.9 | 21.0 | 8.3 | 1.7 | 3.6 | 6.0 |
| | Aromatic compounds | 3.1 | 8.7 | 5.6 | 2.8 | 14.2 | 10.3 | 4.9 | 2.4 | 2.3 |
| | Aliphatic compounds | 1.5 | 3.4 | 4.9 | 5.7 | 10.2 | 11.3 | 0.8 | 1.7 | 2.4 |
| | Total | 83.8 | 81.2 | 87.5 | 80.1 | 80.5 | 84.9 | 84.2 | 81.4 | 82.7 |

*RI: Retention indices relative to $C_8$-$C_{25}$ $n-$alkanes on HP5-MS column; t: traces (< 0.2%)

As it can be seen from the essential oil profiles (Table 2), the studied samples could be divided into 3 groups (A, B and C). The samples in group A (Sofia – S, Vlado Trichkov – VT, Beglika – Bg, and Vitosha – V) were characterized by significant concentrations of β-pinene and α-pinene (18.4–24.8% and 4.6–9.4 respectively). The samples in group A could be separated in subgroup $\underline{A}^1$ (S and VT) in which sesquiterpenes dominated over monoterpenes, and subgroup $\underline{A}^2$ (Bg and V) in which this relation was reversed. Group B consisted of two samples (Gotse Delchev – GD and Radomir – Rm). Their essential oils contained lower concentration of $\beta$-pinene, higher content of 1-octen-3-ol and the highest amount of aromatic and aliphatic compounds (above 10%) in comparison with the other oils. On the other hand, these two oils differed considerably among themselves in the quantity of sesquiterpenes which were 3 times lower in GD sample.

The rest of the studied samples (Trigrad – Tg, Jundola – J and Teshovo – Tsh) formed group C. $\beta$-Pinene (more than 32%) and $\alpha$-pinene (more than 11%) were the principal constituents in these oils. Only in this group, monoterpenes exceeded 50% and sesquiterpenes were in the range of 15–20%.

It should be noted that a qualitative difference in the composition of the analyzed 9 essential oils was not observed. Besides, the common features in the groups (A, B, C) discussed above, the concentration of the oil components displayed a high variability although the origin of the starting material was the same. It is known that the quantitative and qualitative characteristics of essential oils are very sensitive to the environmental impact. Thus the chemical composition of the oils from S and VT (Table 2) both located at 560–650 m a.s.l. and the same climatic zone (transitional continental) showed significant similarity. Continental Mediterranean climatic zone and altitude of 1000–1400 m a.s.l. characterized the samples of group C, which differed significantly from A and B with an extremely high concentration of monoterpenes (over 50%). Although Gotse Delchev (GD) is located in the same climatic zone as the cultivation fields included in group C, the oil composition of *S. scardica* originating from it is similar to that of Rm (group B), probably because of the influence of the quite lower altitude. On the other hand, the climate and altitude of Rm are similar to the samples of $A^1$ group (S and VT) but different in oil profile. It should be noted that Mediterranean air masses invade Bulgaria through Struma and Mesta valleys and affect GD and Rm locations. Further, samples differing in climate and altitude (S, VT, Bg, V) were combined in one group (A). It is worth to note that all of the studied samples originated from a cultivar planted at different ecological factors. Obviously, the oil composition is strongly related to the altitude and climate. In addition, many other ecological factors (microclimate, soil fertility and pH, humidity, light intensity, daily temperature, exposure, etc.) also affect the biosynthesis and accumulation of secondary metabolites, resulting in the high chemical polymorphism of the studied *S. scardica* samples.

On the other hand, compared to the previously reported essential oil composition of two wild growing *S. scardica* taxa from Bulgaria [4], the results discussed above differed considerably. Wild populations were rich in diterpenoids, while the cultivars were rich in mono- and sesquiterpene hydrocarbons. $\alpha$-Pinene and $\beta$-Pinene were main components in the cultivated plants, but they were not detected even in traces in native populations. Further, phenylacetaldehyde, phenylethyl alcohol, eugenol, 1-octen-3-ol, E-pinocarveol, E-verbenol, pinocarvone, myrtenal, myrtenol, etc. found in the studied samples were not described previously as constituents of the *S. scardica* essential oils. The presence of $\alpha$-bisabolol,

$\alpha$-cadinene, isopropyl miristate, octadecenol differentiated the wild taxa from the cultivar. The method of preparation (by Clevenger [4] or Likens–Nickerson [in this study]) of the analyzed samples could result in some qualitative differences but the observed significant deviation in oil composition is probably due to the cultivation process.

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