



*J. Serb. Chem. Soc.* 85 (12) 1565–1575 (2020)  
JSCS–5393

SHORT COMMUNICATION

**Composition of essential oils and headspace constituents of  
*Artemisia annua* L. and *A. scoparia* Waldst. et Kit.**

JOVANA D. ICKOVSKI, KATARINA D. STEPIĆ and GORDANA S. STOJANOVIĆ<sup>#\*</sup>

*Department of Chemistry, Faculty of Science and Mathematics, University of Niš,  
Višegradska 33, 18000 Niš, Serbia*

(Received 27 July, revised 22 September, accepted 26 September 2020)

**Abstract:** Headspace volatiles (HS) and hydrodistilled essential oils (EO) of fresh aerial parts of *Artemisia annua* L. and *A. scoparia* Waldst. et Kit., were analyzed by GC–MS/FID. Artemisia ketone was found to be the most abundant component among the EO volatiles (55.8 %), as well as among HS (52.1 %) of *A. annua*. Additionally, in both *A. annua* samples, EO and HS,  $\alpha$ -pinene (12.7 and 24.2 %, respectively) was found in high percentage. On the other hand, it has been determined that the dominant components of *A. scoparia* EO and HS were different; in the essential oil capillene (63.8 %) was found as the main constituent, while  $\beta$ -pinene (26.1 %), (*Z*)- $\beta$ -ocimene (23.8 %) and limonene (10.7 %) were the major components among the HS. This is the first report on the composition of HS volatiles of the *A. annua* and *A. scoparia* obtained by direct static headspace.

**Keywords:** gas chromatography–mass spectrometry; artemisia ketone; capillene; pinene.

INTRODUCTION

Static headspace is an analytical technique for the easy and effective extraction of multifarious types of compounds, from various types of samples.<sup>1–3</sup> Even though headspace GC is extensively used a limited number of papers is focused on the analysis of headspace volatiles obtained directly from the plant material under static conditions.<sup>4–11</sup> More papers are related to HS-SPME analysis some of which refer to *A. scoparia*<sup>12</sup> and *A. annua*.<sup>13–17</sup>

Static headspace analysis of plants is a very fast and inexpensive method. Also, no special sample preparation is required, it could be performed even without a solvent and the conditions of analysis are not vigorous, so degradation of the components of the sample is minimized and the loss of the most volatile com-

\* Corresponding author. E-mail: gocast@pmf.ni.ac.rs

<sup>#</sup> Serbian Chemical Society member.

<https://doi.org/10.2298/JSC200727061I>

ponents is greatly reduced. Additionally, the plant material amount that is used for the analysis is only a few grams (or less), so it is useful in cases when not enough plant material could be provided.

If those fact are considered, it is unclear why there is so little data on this type of direct headspace analysis of volatile organic components. All of the above-mentioned is exactly the main reason why we have decided to supplement the existing data on headspace volatiles and compare the chemical composition of the essential oils (EO) and the headspace samples (HS) of two representatives of the genus *Artemisia*. For the purpose of this research, we selected species that were not previously investigated from the point of view of the direct static headspace gas chromatography–mass spectrometry (GC–MS) technique: *Artemisia annua* L. (sweet wormwood) and *Artemisia scoparia* Waldst. et Kit. (virgate wormwood, capillary wormwood, redstem wormwood). The selected species are significant in terms of the biological activity of their essential oils and volatile components, as it has been shown for both to have, among other potentials, a high fumigant and repellent activity.<sup>18–20</sup>

#### EXPERIMENTAL

*Plant material.* The aerial parts (flowers, leaves and stems) of *Artemisia annua* L. and *Artemisia scoparia* Waldst. et Kit. were collected in Niška Banja, near Niš, southeast Serbia, in September 2018 in the full-blooming stage. Both species were harvested from the same site. The voucher specimens were deposited in the Herbarium Moesiacum Niš (HMN), Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš, under the acquisition number No. 13813 for *A. annua* and acquisition number No. 13815 for *A. scoparia*.

*Sample preparation for HS and EO isolation.* 550 g of fresh plant material (each species separately) was ground in a mixer. Further, for the distillation of the essential oil 500 g of this mass was taken. 1500 mg of the rest of the plant material was taken and divided into three parts and used for three separate headspace analysis.

*Essential oil isolation.* Fresh plant biomass samples (500 g each) were hydrodistilled in Clevenger-type glass apparatus for 2.5 h. The essential oil samples were dried over anhydrous magnesium sulfate and analyzed by GC and GC–MS. The essential oil yields (%) (g of the essential oil/g biomass) were calculated on a fresh weight basis. For *A. annua* the oil yield was determined to be 0.9 % and for *A. scoparia* 0.3 %. For the GC–MS/FID analysis both essential oil solutions in hexane (1:100) were put in three vials each.

*GC–MS/FID analysis and identification.* The samples (three repetitions) were analyzed by a 7890/7000B GC/MS/MS triple quadrupole system in MS1 scan mode (Agilent Technologies, USA) equipped with a Combi PAL sampler and Headspace for G6501B/G6509B. The fused silica capillary column HP-5 MS (5 % phenylmethylsiloxane, 30 m×0.25 mm, film thickness 0.25 µm) was used. The injector and interface operated at 230 and 300°C, respectively. Temperature program: from 45 to 290 °C at a heating rate of 4 °C/min. The carrier gas was He with a flow of 1.0 mL/min. For the essential oil solution injection volume was 1 µL and split ratio was adjusted at 40:1. For the HS volatiles 500 mg of milled plant material was put into 20 mL HS vial and then soaked with 2 mL of distilled water. The sample was heated at 80 °C for 20 min with the following mixing program: shaking for 5 s, pause for 2 s. The aliquot of vapor generated from the samples (500 µL) was drawn out from the vial using a

gas-tight syringe (90 °C) and injected directly in the chromatographic column via a transfer line (75 °C). The split ratio was set to 10:1. Post run: back flash for 1.89 min, at 280 °C, with helium pressure of 50 psi. MS conditions were as follows: ionization voltage of 70 eV, acquisition mass range 40–440 Da, scan time 0.32 s. The GC analysis was carried out under the same experimental conditions using the same column as described for the GC–MS. The percentage composition was computed from the GC peak areas without any corrections and was given as mean  $\pm$  standard deviation.

*Identification of volatile compounds.* Components were identified by comparison of their mass spectra with those of Wiley 6, Adams (2007) and NIST 11 libraries, applied on Agilent Mass Hunter Workstation (B.06.00) and AMDIS (2.1, DTRA/NIST, 2011) software and confirmed by comparing of calculated retention indexes (relative to C<sub>8</sub>–C<sub>40</sub> *n*-alkanes) with the literary values of the retention indices.

## RESULTS AND DISCUSSION

The GC and GC–MS analysis resulted in the identification of 64 components in the *A. annua* EO, representing 98.6 % of the essential oil and 40 components in the *A. annua* HS, representing 99.2 % of the HS sample (Table I). All of the HS components were ingredients of EO, except *n*-hexanol and propyl isobutyrate. The more volatile components such as  $\alpha$ -pinene and 1,8-cineole were present in a higher percentage in HS (24.2 and 6.6 %, respectively), than in EO (12.7 and 2.7 %, respectively). The dominant component artemisia ketone (55.8 % in the oil and 52.1 % in the HS) was represented approximately equally in the EO and HS. The most dominant class of compounds in EO and HS of *A. annua* were oxygenated monoterpenes (78.4 and 67.5 %, respectively). Additionally, the ratio of oxygenated monoterpenes to hydrocarbon monoterpenes in the essential oil of *A. annua* was approximately 5, while the ratio of oxygenated monoterpenes to hydrocarbon monoterpenes in the headspace samples was approximately 2.5. None of the sesquiterpenes was present in HS sample of *A. annua*.

The number of identified components of the *A. scoparia* essential oil and HS (36 in EO, representing 99.1 % of the essential oil and 28 in HS, representing 99.4 % of the HS sample) was less than in *A. annua* EO and HS. Hexanal, (2*E*)-hexenal, (3*Z*)-hexenol and *n*-hexanol (which are known as green leaf volatiles GLV, the components which are released when the plant is under attack/stress and by which a plant communicate with other plants and insects in its surroundings) were identified only in HS of *A. scoparia*. The most dominant components of *A. scoparia* EO were phenyldiacetylenes: capillene (63.8 %) and 2,4-pentadiynyl-benzene (10.0 %), while in the HS monoterpenes:  $\beta$ -pinene (26.1 %) and (*Z*)- $\beta$ -ocimene (23.8 %) were the most abundant. The difference in the composition of *A. scoparia* essential oil and HS volatiles is a presumably consequence of their volatility. Namely, capillene boiling point is 140.00 to 143.00 °C at 10.00 mm Hg\* while the boiling point of  $\beta$ -pinene is 163.00 to 166.00 °C at 760.00 mm Hg.

\* 760 mm Hg = 101.325 Pa

TABLE I. Chemical composition of the essential oil and headspace volatiles of *A. annua* and *A. scoparia* (the results of this study are presented in parallel with the obtained literature data); \* – identified by NIST Chemistry WebBook Retention indices; tr: traces (<0.1 %); –: not detected. Components represented by more than 5 % at least in one of the samples are given in bold

| Compound                       | RL   | RI <sup>a</sup> | Content <sup>b</sup> , % |                  |          |                  |                    |                  |          |                  | Class |    |
|--------------------------------|------|-----------------|--------------------------|------------------|----------|------------------|--------------------|------------------|----------|------------------|-------|----|
|                                |      |                 | <i>A. annua</i>          |                  |          |                  | <i>A. scoparia</i> |                  |          |                  |       |    |
|                                |      |                 | EO                       | EO <sup>13</sup> | HS       | HS <sup>13</sup> | EO                 | EO <sup>12</sup> | HS       | HS <sup>12</sup> |       |    |
| Methyl 2-methylbutyrate        | 783  | 783*            | t                        |                  | 0.2±0.1  |                  |                    | –                |          | –                |       | CD |
| Hexanal                        | 801  | 801             | t                        |                  | t        |                  |                    | –                |          | 0.1±0.0          |       | O  |
| Ethyl 2-methylbutyrate         | 845  | 846*            | 0.2±0.1                  |                  | 2.1±0.3  |                  |                    | –                |          | –                |       | CD |
| Ethyl isovalerate              | 849  | 849             | t                        |                  | 0.2±0.1  |                  |                    | –                |          | –                |       | CD |
| (2 <i>E</i> )-Hexenal          | 851  | 846             | –                        |                  | –        | 3.05             |                    | –                |          | t                |       | O  |
| (3 <i>Z</i> )-Hexenol          | 849  | 850             | –                        |                  | –        |                  |                    | –                |          | t                |       | O  |
| Propyl isobutyrate             | 853  | 853             | –                        |                  | 0.1±0.0  |                  |                    | –                |          | –                |       | CD |
| <i>n</i> -Hexanol              | 866  | 863             | –                        |                  | t        |                  |                    | –                |          | t                |       | O  |
| Santolina triene               | 905  | 906             | 0.1±0.0                  |                  | 0.2±0.1  |                  |                    | –                |          | –                |       | M  |
| Tricyclene                     | 920  | 921             | t                        |                  | t        |                  |                    | –                |          | –                |       | M  |
| $\alpha$ -Thujene              | 925  | 924             | t                        |                  | t        |                  |                    | –                |          | –                |       | M  |
| $\alpha$ -Pinene               | 932  | 932             | 12.7±0.8                 | 4.79             | 24.2±0.9 | 12.03            | 0.9±0.2            | 0.8              | 6.8±1.0  | 4.6              |       | M  |
| Propyl 2-methylbutyrate        | 943  | 944*            | 0.2±0.1                  |                  | 0.6±0.2  |                  |                    | –                |          | –                |       | CD |
| Camphene                       | 947  | 946             | 0.3±0.1                  | 2.74             | 0.7±0.2  | 4.90             | t                  |                  |          | t                |       | M  |
| Thuja-2,4(10)-diene            | 952  | 953             | t                        |                  | t        |                  |                    | –                |          | –                |       | M  |
| Sabinene                       | 972  | 969             | 0.3±0.1                  |                  | 0.6±0.2  |                  | 0.1±0.0            | 0.2              | 1.3±0.2  | 1.4              |       | M  |
| $\beta$ -Pinene                | 975  | 974             | 1.1±0.3                  | 1.25             | 1.8±0.3  | 2.74             | 3.5±0.4            | 3.3              | 26.1±1.3 | 20.8             |       | M  |
| Myrcene                        | 988  | 988             | 0.1±0.0                  |                  | 0.1±0.0  |                  | 0.9±0.2            | 1.0              | 8.0±0.5  | 12.8             |       | M  |
| Yomogi alcohol                 | 997  | 999             | 0.9±0.2                  |                  | 0.8±0.3  |                  | –                  |                  | –        | –                |       | MO |
| $\alpha$ -Terpinene            | 1015 | 1014            | t                        |                  | 0.2±0.1  |                  | t                  |                  | 0.2±0.0  |                  |       | M  |
| <i>o</i> -Cymene               | 1023 | 1022            | t                        |                  | 0.1±0.0  |                  | 0.5±0.1            |                  | 3.4±0.3  |                  |       | M  |
| Limonene                       | 1027 | 1024            | 0.1±0.0                  |                  | t        | 0.99             | 1.7±0.2            | 1.3              | 10.7±0.5 | 11.0             |       | M  |
| 1,8-Cineole                    | 1030 | 1026            | 2.7±0.2                  | 4.38             | 6.6±0.5  | 6.76             | 0.1±0.0            | 0.2              | 0.5±0.2  | 0.9              |       | MO |
| Santolina alcohol              | 1034 | 1034            | 0.2±0.1                  |                  | 0.1±0.0  |                  | –                  |                  | –        | –                |       | MO |
| ( <i>Z</i> )- $\beta$ -Ocimene | 1035 | 1032            | t                        |                  | –        |                  | 4.3±0.3            | 1.0              | 23.8±0.8 | 16.4             |       | M  |
| ( <i>E</i> )- $\beta$ -Ocimene | 1046 | 1044            | –                        |                  | –        |                  | 0.3±0.1            |                  | 0.6±0.2  | 0.4              |       | M  |
| $\gamma$ -Terpinene            | 1057 | 1054            | 0.2±0.0                  |                  | 0.1±0.0  |                  | 4.0±0.3            | 0.2              | 7.0±0.4  | 3.8              |       | M  |
| Artemisia ketone               | 1060 | 1056            | 55.8±1.5                 | 8.79             | 52.1±1.3 | 11.24            | 0.3±0.1            |                  | –        | –                |       | MO |
| <i>cis</i> -Sabinene hydrate   | 1067 | 1065            | 0.5±0.2                  |                  | 0.4±0.2  |                  | –                  |                  | –        | –                |       | MO |
| Artemisia alcohol              | 1082 | 1080            | 3.1±0.4                  | 2.61             | 2.0±0.3  | 3.34             | t                  |                  | –        | –                |       | MO |
| Terpinolene                    | 1087 | 1086            | t                        |                  | t        |                  | –                  |                  | –        | –                |       | M  |
| $\alpha$ -Pinene oxide         | 1089 | 1099            | t                        |                  | –        |                  | –                  |                  | –        | –                |       | MO |
| <i>trans</i> -Sabinene hydrate | 1097 | 1098            | 0.1±0.0                  |                  | –        |                  | –                  |                  | –        | –                |       | MO |
| <i>n</i> -Nonanal              | 1102 | 1100            | –                        |                  | –        |                  | t                  |                  | –        | –                |       | O  |

TABLE I. Continued

| Compound                           | RL   | RI <sup>a</sup> | Content <sup>b</sup> , % |                  |         |                  |                    |                  |         |                  | Class |    |
|------------------------------------|------|-----------------|--------------------------|------------------|---------|------------------|--------------------|------------------|---------|------------------|-------|----|
|                                    |      |                 | <i>A. annua</i>          |                  |         |                  | <i>A. scoparia</i> |                  |         |                  |       |    |
|                                    |      |                 | EO                       | EO <sup>13</sup> | HS      | HS <sup>13</sup> | EO                 | EO <sup>12</sup> | HS      | HS <sup>12</sup> |       |    |
| 2-Methylbutyl-2-methylbutyrate     | 1003 | 1100            | t                        |                  | t       |                  |                    | –                |         | –                |       | CD |
| 3-Methyl-3-butenyl isovalerate     | 1111 | 1112            | 0.2±0.0                  |                  | 0.2±0.1 |                  |                    | –                |         | –                |       | CD |
| <i>trans</i> -Pinene hydrate       | 1121 | 1119            | t                        |                  | –       |                  |                    | –                |         | –                |       | MO |
| $\alpha$ -Campholenal              | 1125 | 1122            | 0.5±0.2                  |                  | 0.2±0.1 |                  |                    | –                |         | –                |       | MO |
| <i>allo</i> -Ocimene               | 1127 | 1128            | –                        |                  | –       |                  |                    | t                |         | t                |       | M  |
| <i>cis-p</i> -Mentha-2,8-dien-1-ol | 1134 | 1133            | t                        |                  | –       |                  |                    | –                |         | –                |       | MO |
| <i>trans</i> -Pinocarveol          | 1139 | 1135            | 6.4±0.5                  |                  | 2.1±0.4 |                  |                    | t                |         | –                |       | MO |
| Camphor                            | 1145 | 1141            | 0.3±0.1                  | 16.30            | 0.2±0.0 | 11.37            |                    | –                |         | –                |       | MO |
| Sabina ketone                      | 1156 | 1154            | 0.2±0.1                  |                  | –       |                  |                    | –                |         | –                |       | MO |
| Pinocarvone                        | 1163 | 1160            | 5.6±0.4                  | 1.73             | 2.6±0.4 | 1.23             |                    | t                |         | –                |       | MO |
| Borneol                            | 1166 | 1165            | 0.4±0.2                  | 1.77             | t       | 1.21             |                    | –                |         | –                |       | MO |
| Lavandulol                         | 1166 | 1165            | –                        |                  | –       |                  | 0.4±0.1            |                  | 0.1±0.0 |                  |       | MO |
| Terpinen-4-ol                      | 1177 | 1174            | 0.3±0.1                  | 1.34             | 0.1±0.0 |                  |                    | t                |         | –                |       | MO |
| $\alpha$ -Terpineol                | 1190 | 1186            | t                        |                  | t       |                  |                    | t                |         | –                |       | MO |
| Myrtenal                           | 1198 | 1195            | 0.8±0.2                  |                  | 0.2±0.0 |                  |                    | –                |         | –                |       | MO |
| <i>trans</i> -Carveol              | 1218 | 1215            | 0.1±0.0                  |                  | –       |                  |                    | –                |         | –                |       | MO |
| (3Z)-Hexenyl 2-methylbutyrate      | 1230 | 1229            | t                        |                  | 0.1±0.0 |                  |                    | t                |         | t                |       | CD |
| (3Z)-Hexenyl 3-methylbutyrate      | 1234 | 1232            | –                        |                  | –       |                  |                    | t                |         | t                |       | CD |
| Hexyl-2-methylbutyrate             | 1234 | 1233            | t                        |                  | t       |                  |                    | –                |         | –                |       | CD |
| Hexyl 3-methylbutyrate             | 1240 | 1241            | –                        |                  | t       |                  |                    | –                |         | –                |       | CD |
| Carvone                            | 1244 | 1239            | t                        |                  | –       |                  |                    | –                |         | –                |       | MO |
| 2,4-pentadienyl-Benzene            | 1294 | 1298            | –                        |                  | –       |                  | 10.0±0.6           |                  | 7.7±0.4 |                  |       | P  |
| <i>cis</i> -Pinocarvyl acetate     | 1307 | 1311            | 0.1±0.0                  |                  | –       |                  |                    | –                |         | –                |       | MO |
| (3Z)-Hexenyl tiglate               | 1323 | 1319            | –                        |                  | –       |                  | 0.2±0.1            |                  | t       |                  |       | CD |
| Eugenol                            | 1357 | 1356            | t                        |                  | –       | 1.94             | 2.0±0.2            | 0.1              | 0.3±0.1 |                  |       | PP |
| $\alpha$ -Copaene                  | 1378 | 1374            | 0.3±0.1                  |                  | –       |                  | –                  |                  | –       |                  |       | S  |
| Benzyl isovalerate                 | 1386 | 1385            | 0.1±0.0                  |                  | –       |                  | –                  |                  | –       |                  |       | CD |
| $\alpha$ -Isocomene                | 1390 | 1387            | –                        |                  | –       |                  |                    | t                |         | –                |       | S  |
| $\beta$ -Cubebene                  | 1392 | 1387            | t                        |                  | –       |                  | –                  | –                | –       | –                |       | S  |
| $\beta$ -Elemene                   | 1393 | 1389            | t                        |                  | –       |                  | –                  | –                | –       | –                |       | S  |
| ( <i>E</i> )-Caryophyllene         | 1423 | 1417            | 0.8±0.2                  | 3.82             | –       | 0.65             | 2.2±0.3            | 9.6              | 0.3±0.1 | 16.4             |       | S  |
| ( <i>E</i> )- $\beta$ -Farnesene   | 1455 | 1454            | t                        | 2.32             | –       | 2.67             | –                  | –                | –       | –                |       | S  |
| $\alpha$ -Humulene                 | 1457 | 1452            | t                        |                  | –       |                  | 0.2±0.1            | 0.7              | t       | 0.4              |       | S  |
| $\gamma$ -Curcumene                | 1480 | 1481            | –                        |                  | –       |                  | 0.3±0.2            | 0.3              | t       | 0.3              |       | S  |

TABLE I. Continued

| Compound                           | RL   | RI <sup>a</sup> | Content <sup>b</sup> , % |                  |             |                  |                    |                  |             |                  | Class |
|------------------------------------|------|-----------------|--------------------------|------------------|-------------|------------------|--------------------|------------------|-------------|------------------|-------|
|                                    |      |                 | <i>A. annua</i>          |                  |             |                  | <i>A. scoparia</i> |                  |             |                  |       |
|                                    |      |                 | EO                       | EO <sup>13</sup> | HS          | HS <sup>13</sup> | EO                 | EO <sup>12</sup> | HS          | HS <sup>12</sup> |       |
| Germacrene D                       | 1485 | 1484            | 1.1±0.3                  | 7.14             | –           | 0.7              | 0.3±0.1            | 0.5              | –           | 0.4              | S     |
| $\beta$ -Selinene                  | 1490 | 1489            | 0.1±0.0                  | 10.41            | –           | –                | –                  | –                | –           | –                | S     |
| Capillene                          | 1498 | 1493            | –                        | –                | –           | –                | 63.8±1.6           | 53.1             | 2.2±0.2     | 3.2              | P     |
| Bicyclogermacrene                  | 1500 | 1500            | 0.4±0.1                  | –                | –           | –                | –                  | 4.2              | t           | 4.9              | S     |
| $\delta$ -Cadinene                 | 1525 | 1522            | t                        | –                | –           | –                | t                  | –                | –           | –                | S     |
| Spathulenol                        | 1576 | 1577            | t                        | –                | –           | –                | 0.9±0.2            | 6.5              | –           | –                | SO    |
| Viridiflorol                       | 1585 | 1592            | 0.1±0.0                  | –                | –           | –                | –                  | –                | –           | –                | SO    |
| Caryophyllene oxide                | 1588 | 1582            | 0.2±0.0                  | 1.65             | –           | –                | 0.5±0.2            | 2.6              | –           | –                | SO    |
| <i>allo</i> -Aromadendrene epoxide | 1630 | 1639            | 0.2±0.1                  | –                | –           | –                | –                  | –                | –           | –                | SO    |
| Selina-3,11-di-en-6- $\alpha$ -ol  | 1635 | 1642            | 0.2±0.1                  | –                | –           | –                | –                  | –                | –           | –                | SO    |
| $\beta$ -Eudesmol                  | 1656 | 1649            | –                        | –                | –           | –                | 1.4±0.3            | –                | –           | –                | SO    |
| Amorpha-4,9-di-en-2-ol             | 1691 | 1700            | 0.2±0.1                  | –                | –           | –                | –                  | –                | –           | –                | SO    |
| Number of components               |      |                 | <b>64</b>                |                  | <b>40</b>   |                  | <b>36</b>          |                  | <b>28</b>   |                  |       |
|                                    |      |                 | Class of compounds       |                  |             |                  |                    |                  |             |                  |       |
|                                    |      |                 | <b>93.6</b>              |                  | <b>95.7</b> |                  | <b>17.2</b>        |                  | <b>88.6</b> |                  |       |
| Monoterpenes                       |      |                 |                          |                  |             |                  |                    |                  |             |                  |       |
| Hydrocarbons (M)                   |      |                 | 15.2                     |                  | 28.2        |                  | 16.3               |                  | 88.0        |                  |       |
| Oxygenated (MO)                    |      |                 | 78.4                     |                  | 67.5        |                  | 0.9                |                  | 0.6         |                  |       |
| Sesquiterpenes                     |      |                 | <b>4.1</b>               |                  | –           |                  | <b>5.8</b>         |                  | <b>0.4</b>  |                  |       |
| Hydrocarbons (S)                   |      |                 | 3.1                      |                  | –           |                  | 3.0                |                  | 0.4         |                  |       |
| Oxygenated (SO)                    |      |                 | 1.0                      |                  | –           |                  | 2.8                |                  | –           |                  |       |
| Carbonic acid derivatives (CD)     |      |                 | 0.8                      |                  | 3.4         |                  | 0.3                |                  | t           |                  |       |
| Phenyldiacetylenes (P)             |      |                 | –                        |                  | –           |                  | <b>73.8</b>        |                  | <b>9.9</b>  |                  |       |
| Phenylpropenes (PP)                |      |                 | t                        |                  | –           |                  | 2.0                |                  | 0.3         |                  |       |
| Other (O)                          |      |                 | 0.1                      |                  | 0.1         |                  | t                  |                  | 0.2         |                  |       |
| Total identified                   |      |                 | <b>98.6</b>              |                  | <b>99.2</b> |                  | <b>99.1</b>        |                  | <b>99.4</b> |                  |       |

<sup>a</sup>Compounds are listed in order of elution from a HP-5 MS column; *RL*: literature retention indices; *RI*: experimental retention indices relative to C<sub>8</sub>–C<sub>40</sub> *n*-alkanes; <sup>b</sup>the percentage composition of the individual components is given as the mean ± standard deviation, and for the classes of compounds as the mean

The situation with *A. annua* EO and HS is different. The main component, artemisia ketone, is the same in both, EO and HS. This is because its boiling point (181.00 °C at 760.00 mm Hg) is low enough to evaporate even under HS analysis conditions. In general, more volatile components were present to a greater extent in HS samples of *A. scoparia* than in essential oil, just like in the

case of *A. annua*. The predominant class of compounds in HS were hydrocarbon monoterpenes (88.0 %), while oxygenated monoterpenes were present in percentage less than one in both samples of *A. scoparia*. Also, hydrocarbon sesquiterpenes were determined only in traces, while none of the oxygenated sesquiterpenes were found.

In the case of *A. annua* both methods applied yielded volatiles with a very similar composition in monoterpene fraction. Likewise, this was the case for the *A. scoparia*, for which the two analyses showed minor qualitative variations in monoterpenes. Quantitative differences of the volatiles in HS sample and essential oil in *A. annua* were noticeable, but not overly expressed (*e.g.*, the percentage of artemisia ketone in both samples was almost equal; the percentage of  $\alpha$ -pinene in the HS sample was less than 2 times higher than that in the essential oil). On the contrary, the differences in the quantity of the components present in both, the EO and the HS sample of *A. scoparia* were significant (*e.g.*, the percentage of capillene in the EO sample was 29 times higher than that in the HS sample; the percentage of myrcene in the HS sample was almost 9 times higher than that in the EO sample).

The chemical composition of the EO obtained from both plants was the subject of several scientific papers, but we have concentrated here only on those papers that deal with these plants in the same developmental phase as ours and whose essential oil was obtained from fresh aboveground parts of the plant. Apart from same developmental phase, the volatile chemical composition has been very diverse depending on a large number of factors, such as geographic locations, weather conditions, soil type, and others.

Composition of the essential oil of *A. annua* at the blooming stage determined by Rana *et al.*<sup>21</sup> showed that camphor was represented with 28.6 %, 1,8-cineole with 12.9 % and limonene with 4.5 %. The *A. annua* essential oil obtained from species growing wild in Bulgaria was analyzed by Tzenkova *et al.*<sup>22</sup> and the results showed the presence of  $\alpha$ -caryophyllene (24.73 %),  $\alpha$ -cubebene (13.53 %),  $\alpha$ -copaene (7.42 %),  $\alpha$ -selinene (8.21 %) and artemisia ketone (8.45 %). The chemical composition of the essential oils with 85 compounds from *A. annua* from Hungary was determined by Héthelyi *et al.*<sup>23</sup> The main components of the essential oil obtained from fresh flowering shoots were artemisia ketone (33-75 %) and artemisia alcohol (15-56 %). Artemisia ketone (52.9 %), 1,8-cineole (8.4 %) and camphor (6.0 %) were the major constituents of the essential oil from Northern India, obtained by Jain *et al.*<sup>24</sup> The principal components of the Indian *A. annua* essential oil determined by Rao *et al.*<sup>25</sup> were 1,8-cineole (11.1 %), camphor (36.6 %),  $\beta$ -caryophyllene (5.7 %) and germacrene D (5.9 %). 1,8-cineole (15.1 %) was prominent component followed by  $\alpha$ -terpineole (14 %), *p*-cymene (12.9 %), carvone (12 %),  $\gamma$ -elemene (6.2 %) and *Z*- $\alpha$ -bisabolene (5.4 %) according to Mukhtar *et al.*<sup>26</sup>

The major components of the essential oil from Iran determined by Rasooli *et al.*<sup>27</sup> were artemisia ketone (24.2 %),  $\alpha$ -pinene (12.1 %), 1,8-cineole (9.8 %), camphor (8.4 %),  $\alpha$ -selinene (7.5 %) and borneol (6.0 %). Nekoei *et al.*<sup>13</sup> also examined samples from Iran and compared the composition of the EO and the volatiles obtained by headspace-solid phase microextraction (HS-SPME) from the fresh aerial parts of *A. annua* L. Compounds represented by more than 10 % in at least one sample were camphor (EO 16.30 %; HS-SPME 11.37 %),  $\beta$ -selinene (EO 10.41 %),  $\alpha$ -pinene (HS-SPME 12.03 %) and artemisia ketone (HS-SPME 11.24 %). Compared to the sample examined here, a similarity in the qualitative composition could be noticed, which could not be said for the quantitative composition for both, EO and HS volatiles. The difference in content is especially pronounced for artemisia ketone, camphor, germacrene D and  $\beta$ -selinene (Table I).

The major constituents of the essential oil from mature leaves of Indian *A. scoparia* detected by Singh *et al.*<sup>29</sup> were *p*-cymene (27.06 %), acenaphthalene (24.4 %),  $\beta$ -myrcene (20.89 %) and (+)-limonene (12.63 %).<sup>28</sup> The essential oil analyzed by Singh *et al.*<sup>29</sup> was rich in  $\beta$ -myrcene (29.27 %), followed by (+)-limonene (13.3 %), (*Z*)- $\beta$ -ocimene (13.37 %) and  $\gamma$ -terpinene (9.51 %). The most abundant volatile constituents from another Indian *A. scoparia* essential oil were  $\gamma$ -terpinene (21.8 %), eugenol (20.4 %), eugenyl valerate (5.5 %), limonene (5.0 %) and *p*-cymene (4.6 %) according to Ali *et al.*<sup>30</sup> The major components of the essential oil from Iranian plant analyzed by Morteza-Semnani and Akbarzadeh<sup>31</sup> were camphor (37.9 %), 1,8-cineole (27.8 %) and borneol (21.1 %). Acenaphthalene (36.86 %) was the major component of *A. scoparia* from India, while *p*-cymene (20.5 %) was the major monoterpene constituent, followed by  $\beta$ -myrcene (13.95 %) and (+)-limonene (12.53 %) according to Kaur *et al.*<sup>32</sup> The essential oil of *A. scoparia* from Korea was rich in 1,8-cineole (21.5 %), camphor (11.0 %) and  $\beta$ -caryophyllene (6.8 %) as the major compounds according to Cha *et al.*<sup>33</sup> Contrary to the above references, the composition of *A. scoparia* EO and HS-SPME volatiles from Turkey examined by Demirci *et al.*<sup>12</sup> was very similar to the composition of *A. scoparia* EO and HS examined through our work, except for 2,4-pentadiynyl-benzene. In the sample analyzed here, the content of 2,4-pentadiynyl-benzene was 10.0 % in EO and 7.7 % in HS volatiles, while in the sample from Turkey it was not detected at all. In the volatile components obtained by direct HS and HS-SPME, the higher percentage (16.4 %) of (*E*)-caryophyllene could be noticed in HS-SPME volatiles, compared to those directly obtained HS components (0.3 %).

#### CONCLUSION

This is the first report on the chemical volatile composition of the samples of *A. annua* and *A. scoparia* obtained by direct static headspace. After extensive



research and comparative analysis of the essential oil and HS samples, the following could be concluded:

– *Artemisia* ketone was found to be the most abundant component in EO and HS sample of *A. annua*, while  $\alpha$ -pinene was found to be the second most abundant.

– On the contrary, the dominant components in *A. scoparia* samples were different by two methods; in EO capillene was the main constituent, while in HS sample it was  $\beta$ -pinene probably due to their volatility.

– For *A. annua* and *A. scoparia*, both applied methods yielded volatiles with a very similar composition in monoterpene fraction.

– Quantitative differences of the volatiles in HS and EO samples of *A. annua* and *A. scoparia* were noticeable, wherein these differences are more pronounced in *A. scoparia*.

– Comparing the composition of volatile components obtained by direct HS and HS-SPME, for both examined species, a similarity in the qualitative composition could be noticed, which could not be said for the quantitative composition.

– Direct headspace is an easy and fast method that could be used for analyzing the monoterpene fraction of the samples and it is less demanding than HS-SPME which includes adsorption and desorption from a suitable adsorbent. However, both of these methods could not replace an essential oil analysis, which gives a much more complete picture of the plant's volatile profile.

*Acknowledgment.* Financial support of the Ministry of Education, Science and Technological Development of Serbia (Project No. 451-03-68/2020-14/200124) is gratefully acknowledged.

#### ИЗВОД

САСТАВ ЕТАРСКОГ УЉА И "HEADSPACE" САСТОЈЦИ *Artemisia annua* L.

И *A. scoparia* Waldst. et Kit

ЈОВАНА Д. ИЦКОВСКИ, КАТАРИНА Д. СТЕПИЋ И ГОРДАНА С. СТОЈАНОВИЋ

*Департаман за хемију, Природно-математички факултет, Универзитет у Нишу, Вишеградска 33,  
18000 Ниш*

"Headspace" испарљива једињења и састојци хидродестилованих етарских уља свежих надземних делова *Artemisia annua* L. и *Artemisia scoparia* Waldst. et Kit., анализирани су GC-MS/FID методом. Артемизија-кетон (55,8 %) је најзаступљенији састојак етарског уља, као и испарљивих "headspace" једињења (52,1 %) узорка *A. annua*. Поред тога, у оба узорка *A. annua*, у етарском уљу и "headspace" узорку,  $\alpha$ -пинен (12,7 и 24,2 %, редом) нађен је у високом проценту. С друге стране, утврђено је да су доминантне компоненте *A. scoparia* у етарском уљу и "headspace" узорку биле различите; у етарском уљу је као главна компонента одређен капилен са 63,8 %, док су  $\beta$ -пинен (26,1 %), (*Z*)- $\beta$ -оцимен (23,8 %) и лимонен (10,7 %) главне компоненте у "headspace" узорку. Ово су први резултати о саставу испарљивих компонента *A. annua* и *A. scoparia* добијени директним статичким "headspace" техником.

(Примљено 27. јула, ревидирано 22. септембра, прихваћено 26. септембра 2020)

## REFERENCES

1. N. H. Snow, G.C. Slack, *TRAC - Trends Anal. Chem.* **21** (2002) 608 ([https://doi.org/10.1016/S0165-9936\(02\)00802-6](https://doi.org/10.1016/S0165-9936(02)00802-6))
2. B. Kolb, L.S. Ettre, *Static Headspace Gas Chromatography: Theory and Practice*, Wiley-VCH, New York, 1997, ISBN: 0-471-19238-4
3. B. V. Ioffe, A. G. Vitenberg, *Head-Space Analysis and Related Methods in Gas Chromatography* (translated by I. A. Mamantov), Wiley, New York, 1984, ISBN: 978-0-471-06507-4
4. G. Stojanović, O. Jovanović, G. Petrović, V. Mitić, V. Jovanović, *Nat. Vol. Essent. Oils* **1** (2014) 60 (<https://dergipark.org.tr/tr/pub/nveo/issue/19770/211762>)
5. J. Rzepa, L. Wojtal, D. Staszek, G. Grygierczyk, K. Labe, M. Hajnos, T. Kowalska, M. Waksmundzka-Hajnos, *J. Chromatogr. Sci.* **47** (2009) 575 (<https://doi.org/10.1093/chromsci/47.7.575>)
6. G. M. Petrovic, J. G. Stamenkovic, O. P. Jovanovic, G. S. Stojanovic, *Nat. Prod. Commun.* **14** (2019) (<https://doi.org/10.1177/1934578X19850691>)
7. G. M. Petrovic, J. G. Stamenkovic, G. S. Stojanovic, V. D. Mitic, B. K. Zlatkovic, *Nat. Prod. Commun.* **12** (2017) 1513 (<https://doi.org/10.1177/1934578X1701200932>)
8. I. R. Kostevski, G. M. Petrovic, G. S. Stojanovic, J. G. Stamenkovic, B. K. Zlatkovic, *Nat. Prod. Commun.* **11** (2016) 543 (<https://doi.org/10.1177/1934578X1601100431>)
9. S. C. Jovanovic, O. P. Jovanovic, G. M. Petrovic, G. S. Stojanovic, *Nat. Prod. Commun.* **10** (2015) 661 (<https://doi.org/10.1177/1934578X1501000434>)
10. O. P. Jovanovic, B. K. Zlatkovic, S. C. Jovanovic, G. M. Petrovic, G. S. Stojanovic, *J. Essent. Oil Res.* **27** (2015) 182 (<https://doi.org/10.1080/10412905.2015.1014119>)
11. O. P. Jovanović, G. S. Stojanović, *Facta Univ. Ser. Phys Chem. Tech.* **12** (2014) 41 (DOI:10.2298/FUPCT1401041J)
12. B. Demirci, F. Demirci, K. H. C. Başer, *Flavour Fragr. J.* **20** (2005) 395 (<https://doi.org/10.1002/ffj.1426>)
13. M. Nekoei, M. Mohammadhosseini, H. Akhlaghi, *J. Essent. Oil-Bear. Plants* **15** (2012) 926 (<https://doi.org/10.1080/0972060X.2012.10662595>)
14. A. R. Jassbi, R. Miri, Ian T. Baldwin, *Chem. Nat. Compd.* **49** (2014) 1148 (<https://doi.org/10.1007/s10600-014-0846-7>)
15. E. M. Lopes, F. Guimarães-Dias, T. S. S. Gama, A. L. Macedo, A. L. Valverde, M. C. Moraes, A. C. A. Aguiar-Dias, H. R. Bizzo, M. Alves-Ferreira, E. S. Tavares, A. F. Macedo, *Plant Cell Rep.* **39** (2020) 101 (<https://doi.org/10.1007/s00299-019-02476-0>)
16. S. Reale, P. Fasciani, L. Pace, F. Angelis, G. Marcozzi, *Rapid Commun. Mass Spectrom.* **25** (2011) 2511 (<https://doi.org/10.1002/rcm.5155>)
17. H. Sun, F. Zhang, S. Chen, Z. Guan, J. Jiang, W. Fang, F. Chen, *Biochem. Sys. Ecol.* **60** (2015) 225 (<https://doi.org/10.1016/j.bse.2015.04.023>)
18. D. Goel, R. Goel, V. Singh, M. Ali, G. Mallavarapu, K. Sushil, *J. Nat. Med.* **61** (2007) 458 (<https://doi.org/10.1007/s11418-007-0175-2>)
19. N. Zhang, L. Tang, W. Hu, K. Wang, Y. Zhou, H. Li, C. Huang, J. Chun, Z. Zhang, *J. Insect Sci.* **14** (2014) 241 (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5634023/>)
20. M. Negahban, S. Moharramipour, F. Sefidkon, *J. Asia-Pac. Entomol.* **9** (2006) 381 ([https://doi.org/10.1016/S1226-8615\(08\)60318-0](https://doi.org/10.1016/S1226-8615(08)60318-0))
21. V. S. Rana, K. Abirami, M. A. Blázquez, S. Maiti, *J. Spices Aromat. Crops* **22** (2013) 181 (<https://updatepublishing.com/journal/index.php/josac/article/view/5031>)
22. R. Tzenkova, Z. Kamenarska, A. Draganov, A. Atanassov, *Biotechnol. Biotechnol. Equip.* **24** (2010) 1833 (<https://doi.org/10.2478/V10133-010-0030-6>)

23. E. B. Héthelyi, I. B. Cseko, M. Grósz, G. Márk, J. J. Palinkás, *J. Essent. Oil Res.* **7** (1995) 45 (<https://doi.org/10.1080/10412905.1995.9698460>)
24. N. Jain, S. K. Srivastava, K. K. Aggarwal, S. Kumar, K. V. Syamasundar, *J. Essent. Oil Res.* **14** (2002) 305 (<https://doi.org/10.1080/10412905.2002.9699863>)
25. B. R. Rao, K. V. Syamasundar, R. P. Patel, *J. Essent. Oil Res.* **26** (2014) 486 (<https://doi.org/10.1080/10412905.2014.949881>)
26. H. M. Mukhtar, S. H. Ansari, M. Ali, S. R. Mir, M. Z. Abdin, P. Singh, *J. Essent. Oil Res. Plants* **10** (2007) 168 (<https://doi.org/10.1080/0972060X.2007.10643537>)
27. I. Rasooli, M. B. Rezaee, M. L. Moosavi, K. Jaimand, *J. Essent. Oil Res.* **15** (2003) 59 (<https://doi.org/10.1080/10412905.2003.9712268>)
28. H. P. Singh, S. Kaur, S. Mittal, D. R. Batish, R. K. Kohli, *Food Chem. Toxicol.* **48** (2010) 1040 (<https://doi.org/10.1016/j.fct.2010.01.017>)
29. H. P. Singh, S. Kaur, S. Mittal, D. R. Batish, R. K. Kohli, *J. Chem. Ecol.* **35** (2009) 154 (<https://link.springer.com/article/10.1007/s10886-009-9595-7>)
30. M. Ali, A. Chaudhari, A. Velasco-Negueruela, M. J. Pérez-Alonso, *J. Essent. Oil Res.* **12** (2000) 64 (<https://doi.org/10.1080/10412905.2000.9712043>)
31. K. Morteza-Semnani, M. Akbarzadeh, *J. Essent. Oil Res.* **17** (2005) 321 (<https://doi.org/10.1080/10412905.2005.9698918>)
32. S. Kaur, H. P. Singh, S. Mittal, D. R. Batish, R. K. Kohli, *Ind. Crops Prod.* **32** (2010) 54 (<https://doi.org/10.1016/j.indcrop.2010.03.007>)
33. J. D. Cha, M. R. Jeong, S. I. Jeong, S. E. Moon, J. Y. Kim, B. S. Kil, Y. H. Song, *Planta Med.* **71** (2005) 186 (<https://www.thieme-connect.de/products/ejournals/abstract/10.1055/s-2005-837790>).