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*Original Scientific Paper*

# Chemical composition and chemosystematic evaluation of the fruit and root headspace fractions of selected *Heracleum* taxa from Southeastern Europe

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**ABSTRACT:**
This work is focused on nine *Heracleum* taxa (Apiaceae): *H. sphondylium*, *H. sibiricum*, *H. montanum*, *H. ternatum*, *H. pyrenaicum* subsp. *pollinianum*, *H. pyrenaicum* subsp. *orsinii* and *H. verticillatum*, belonging to the *H. sphondylium* group, and *H. orphanidis*, all from sect. *Heracleum*, as well as *H. austriacum* subsp. *siifolium* from sect. *Wendia*. The aim was to isolate the headspace (HS) fractions from 17 fruit samples (collected from all nine *Heracleum* taxa) and 13 root samples (collected from all eight taxa belonging to sect. *Heracleum*), to investigate their composition and chemosystematic significance, as well as to compare these results with those previously obtained for the essential oils isolated by hydrodistillation from the same fruit and root samples. The HS fractions were isolated using an automatic static HS sampler and analysed by GC-FID and GC-MS. The fruit HS fractions were dominated by octyl acetate and/or α-pinene (the taxa from the sect. *Heracleum*), or *n*-octanol (*H. austriacum*), whereas the root HS fractions mainly contained β-pinene and/or (*Z*)-β-ocimene (the taxa from the *H. sphondylium* group), or *n*-nonane (*H. orphanidis*). The chemosystematic significance was evaluated using multivariate statistical methods: principal component analysis (PCA), non-metric multidimensional scaling (nMDS) and agglomerative hierarchical clustering based on the unweighted pair-group arithmetic average (UPGMA) algorithm. As in the case of previously investigated essential oils, the statistical analysis of the fruit and root HS fractions generally resulted in the grouping of the investigated *H. sphondylium* group representatives, and within this group, the grouping of morphologically related *H. sphondylium* and *H. montanum* and the isolated position of *H. verticillatum*. The statistical analysis of the root HS fractions resulted in better separation of the taxa (even compared to previous analysis of the essential oils), i.e. isolated positions of *H. ternatum* and *H. pyrenaicum* were observed.

**Keywords:**
Apiaceae, GC-FID, GC-MS, PCA, nMDS, UPGMA

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## INTRODUCTION

The members of the genus *Heracleum* L. (Apiaceae), commonly known as cow parsnips or hogweeds, are aromatic plants mostly distributed in the Northern Hemisphere. This is a taxonomically complex genus, with approximately 120 taxa divided into eight sections (Mandenova 1951; Logacheva *et al.* 2007). In Southeastern Europe 12 taxa are autochthonous. Ten of them belong to the section *Heracleum* (including eight members of the *H. sphondylium* group), while the remaining two are representatives of the section *Wendia* (Hoffm.) DC. (Don 1834; Mandenova 1951; Brummitt 1968; Hartvig 1986; Tonascia 1992; Logacheva *et al.* 2007).

The focus of our study includes nine *Heracleum* taxa from Southeastern Europe. Eight of them, including seven members of the *H. sphondylium* group [*H. sphondylium* L., *H. sibiricum* L., *H. montanum* Schleich. ex Gaudin, *H. ternatum* Velen., *H. pyrenaicum* subsp. *pollinianum* (Bertol.) F. Pedrotti & Pignatti, *H. pyrenaicum* subsp. *orsinii* (Guss.) F. Pedrotti & Pignatti and *H. verticillatum* Pančić] and *H. orphanidis* Boiss. belong to the section *Heracleum*, whereas *H. austriacum* subsp. *siifolium* (Scop.) Nyman is a representative of the section *Wendia*. The main morphological differences and distribution of these taxa were previously summarized (Ušjak *et al.* 2018). In traditional classifications (Brummitt 1968 followed by Hand 2011 and The Plant List 2013), the investigated taxa from the *H. sphondylium* group are considered subspecies of *H. sphondylium*. However, the majority of them are regarded as independent species in the recent studies carried out by Jogan (2001) (*H. montanum*), Assyov *et al.* (2012) (*H. sibiricum, H. ternatum* and *H. verticillatum*), Niketić (2014) (*H. verticillatum*) and Bartolucci *et al.* (2018) (*H. pyrenaicum* subsp. *pollinianum*), as well as on the Plants of the World Online - POWO website (2021) (*H. sibiricum*). The names of these taxa according to different sources are given in Supplementary Table 1. In previous research, we investigated the chemosystematic significance of the constituents of the essential oils obtained by hydrodistillation from the roots (or roots and rhizomes in the case of *H. austriacum* subsp. *siifolium*), fruits, leaves and flowers of these taxa, as well as of the furanocoumarins present in the roots and fruits (Ušjak *et al.* 2018, 2020). In this study, the headspace (HS) fractions of the roots and fruits of these *Heracleum* taxa are studied.

Static HS extraction is a quick, non-destructive and solvent-free method, which can be applied in the analysis of plant volatile constituents and demands only small amounts of material (Kusano *et al.* 2016; Raber *et al.* 2021). It implies placing a sample in an airtight container in which its volatile components equilibrate with the air (at a predetermined temperature) and then the defined volume of this mixture is injected into a gas chromatograph (Siddiquee 2014; Kubeczka 2020). Compared to essential oils isolated by hydrodistillation, HS fractions obtained by static sampling contain significantly higher quantities of the oils' most volatile constituents, while trace components are often absent (Kubeczka 2020). However, like essential oils, HS fractions were shown to have potential in chemosystematic investigations (Raber *et al.* 2021).

The aim of the current study was to investigate the chemical composition of the HS fractions extracted from 17 fruit samples (from all nine *Heracleum* taxa) and 13 root samples (from all eight taxa belonging to sect. *Heracleum*), to evaluate their chemosystematic significance and to compare these results with those previously obtained for essential oils isolated by hydrodistillation from the same fruit and root samples of the investigated *Heracleum* taxa.

## MATERIALS AND METHODS

**Plant material.** Information about the acronyms and localities (with GPS coordinates) of the collected root and fruit samples of the investigated *Heracleum* taxa, as well as the voucher numbers of the specimens deposited in the Herbarium of the Natural History Museum, Belgrade (BEO) are presented in Table 1; the samples were authenticated by Dr. Marjan Niketić (curator/botanist of the BEO). The fruits and roots were collected from 10-15 individuals of each taxon, dried at room temperature and kept in sealed paper bags in a cold, dry, dark place. Immediately prior to the analysis the plant material was ground; most of it was hydrodistilled (Ušjak *et al.* 2018) and corresponding amounts were extracted by a static HS sampler.

**Static HS extraction.** The dried and ground roots (4 g) and fruits (3 g) were hermetically sealed in HS vials. For the extraction, an Agilent G1888 automatic HS sampler coupled with an Agilent 6890N Gas Chromatograph was used (single extraction was performed for each sample); experimental conditions (Arsenijević *et al.* 2013): oven temperature 90°C, loop temperature 100°C, transfer line 110°C, equilibration time 30 min, shaking low; pressurization time 0.08, carrier gas He, in vial pressure 15 psi, loop fill 0.5, loop equilibration 0.05, inject time 1.00.

**GC-FID and GC-MS analysis.** An Agilent 6890N Gas Chromatograph was equipped with a split/splitless injector (200°C), a FID detector and an Agilent HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness), and coupled with an Agilent 5975C mass selective detector (MSD), operating in the EI mode (70 eV); FID and MSD transfer line temperatures: 300 and 250°C, carrier gas: He (flow 1.0 mL/min), temperature program (linear): 60 to 280°C at 3°C/min, split ratio: 1:10. The linear retention indices (RIs) of the volatiles were determined in relation to the homologue series of *n*-alkanes ($C_8$-$C_{40}$) (Fluka, Buchs, Switzerland) run under the same operating conditions. The identification of the volatiles was based on the comparison of their retention indices (RI), retention times (RT), and mass spectra with those from the NIST/NBS 05, Wiley libraries 8th edition, and the literature (Adams 2007). The relative percentages of the volatiles were calculated based on the peak areas from the FID data.

**Statistical analysis.** The chemosystematic evaluation of the HS fractions was performed using multivariate statistical methods: principal component analysis (PCA), non-metric multidimensional scaling (nMDS) and agglomerative hierarchical clustering based on the

**Table 1.** Acronyms, localities (with GPS coordinates) and voucher numbers of the collected *Heracleum* samples.

| Taxa / acronym | Locality | GPS coordinates |  | Voucher number (BEO) | Collected organs |
|---|---|---|---|---|---|
|  |  | Latitude | Longitude |  |  |
| *H. sphondylium* / |  |  |  |  |  |
| *sph* | Krško (SVN) | 45.985859 | 15.466234 | 20150704/01 | fruits and roots |
| *H. sibiricum* / |  |  |  |  |  |
| *sib1* | Niš (SRB) | 43.407869 | 21.827686 | 20140717/01 | fruits |
| *sib2* | Niš (SRB) | 43.407869 | 21.827686 | 20140717/01 | roots |
| *sib3* | Niš (SRB) | 43.410655 | 21.832809 | 20140717/01 | roots |
| *sib4* | Niš (SRB) | 43.412524 | 21.835369 | 20140717/01 | fruits |
| *sib5* | Žarkova čuka, Mts Stara Planina (SRB) | 43.385688 | 22.631045 | 20120904/01 | fruits and roots |
| *sib6* | Arbinje, Mts Stara Planina (SRB) | 43.262601 | 22.773324 | 20140722/01 | fruits and roots |
| *sib7* | Mt Veliki Jastrebac (SRB) | 43.384808 | 21.451424 | 20110904/04 | fruits |
| *H. montanum* / |  |  |  |  |  |
| *mon* | Kamnik-Savinja Alps (SVN) | 46.295374 | 14.523282 | 20150707/01 | fruits and roots |
| *H. ternatum* / |  |  |  |  |  |
| *ter1* | Mt Durmitor (MNE) | 43.1553 | 19.120968 | 20130807/14 | fruits and roots |
| *ter2* | Mt Durmitor (MNE) | 43.1553 | 19.120968 | 20130807/14 | fruits |
| *H. pyrenaicum* subsp. *pollinianum* / |  |  |  |  |  |
| *pol1* | Mt Jablanica (MKD) | 41.224697 | 20.540559 | 20090801/32 | fruits |
| *pol2* | Mt Jablanica (MKD) | 41.230413 | 20.527751 | 20090801/32 | roots |
| *pol3* | Mt Bistra (MKD) | 41.605862 | 20.684781 | 20100702/01 | roots |
| *pol4* | Mt Galičica (MKD) | 40.944101 | 20.826265 | 20120716/01 | fruits |
| *H. pyrenaicum* subsp. *orsinii* / |  |  |  |  |  |
| *ors1* | Mt Durmitor (MNE) | 43.125166 | 19.072264 | 20110804/01 | fruits |
| *ors2* | Mt Durmitor (MNE) | 43.125166 | 19.072264 | 20110804/01 | fruits and roots |
| *H. verticillatum* / |  |  |  |  |  |
| *ver1* | Arbinje, Mts Stara Planina (SRB) | 43.301482 | 22.780801 | 20140722/02 | fruits and roots |
| *ver2* | Arbinje, Mts Stara Planina (SRB) | 43.301482 | 22.780801 | 20140722/02 | fruits |
| *ver3* | Arbinje, Mts Stara Planina (SRB) | 43.301482 | 22.780801 | 20140722/02 | roots |
| *H. orphanidis* / |  |  |  |  |  |
| *orp1* | Mt Baba Planina (MKD) | 41.033486 | 21.218105 | 20120706/01 | fruits |
| *orp2* | Mt Baba Planina (MKD) | 41.033486 | 21.218105 | 20120706/01 | roots |
| *H. austriacum* subsp. *siifolium* / |  |  |  |  |  |
| *aus* | Kamnik-Savinja Alps (SVN) | 46.295374 | 14.523282 | 20150713/01 | fruits |

unweighted pair-group arithmetic average (UPGMA) algorithm (in further text UPGMA). The raw data (the quantities of the volatiles expressed as relative percentages) were transformed prior to statistical analysis using arcsine and log transformations, as well as coding (Milutinović *et al.* 2018), in order to reduce the large differences between them. The best output was produced using coding, when the values were assigned in the following way: value 1 for 0% (i.e. when the constituents were not detected), value 2 for traces (< 0.1%), value 3 for quantities ≥ 0.1% and < 1%, value 4 for quantities ≥ 1% and < 5%, value 5 for quantities ≥ 5% and < 10%, value 6 for quantities ≥ 10% and < 20%, value 7 for quantities ≥ 20% and < 40%, value 8 for quantities ≥ 40% and < 60%, value 9 for quantities ≥ 60% and < 80%, and value 10 for quantities ≥ 80%. Thus, only the analysis based on these code values is presented in this study. The nMDS and UPGMA were based on the Bray-Curtis pairwise

**Table 2.** Composition of the HS fractions of the fruits of the investigated *Heracleum* taxa (%)

| RI$_{exp}$[a] | RI$_{lit}$[b] | Constituent[c] | *sph*[d] | *sib1* | *sib4* | *sib5* | *sib6* | *sib7* | *mon* | *ter1* | *ter2* | *pol1* | *pol4* | *ors1* | *ors2* | *ver1* | *ver2* | *orp1* | *aus* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 888 | 880 | Isopropyl 2-methyl butanoate | 1.4[e] | tr | 1.9 | 7.9 | tr | 9.2 | 2.2 | 2.2 | 4.3 | 3.1 | - | 3.2 | 4.8 | - | tr | - | - |
| 894 | - | Isopropyl isovalerate | 1.0 | 2.4 | tr | 10.9 | 2.8 | 13.9 | 2.3 | 8.2 | 6.4 | 3.4 | - | 10.2 | 15.3 | - | - | - | - |
| 899 | 900 | *n*-Nonane | 1.3 | - | 1.9 | - | - | - | 1.1 | - | - | - | - | - | - | 10.9 | 10.0 | - | - |
| 916 | 908 | Isobutyl isobutanoate | - | - | - | - | - | 1.7 | 0.4 | 0.4 | 1.1 | 0.7 | tr | 2.0 | 1.2 | 6.3 | 5.7 | - | - |
| 931 | 924 | *α*-Thujene | - | - | - | - | - | - | - | - | - | 0.6 | - | - | - | - | - | - | - |
| 940 | 932 | ***α***-Pinene | 1.9 | 0.3 | 1.0 | 1.7 | 12.0 | 4.5 | 3.1 | 23.7 | 33.9 | 29.3 | 15.6 | 27.3 | 21.3 | tr | tr | 0.1 | - |
| 955 | 946 | Camphene | 0.4 | - | 0.1 | - | 1.9 | 1.1 | 0.7 | 2.5 | 2.9 | 2.3 | - | 4.3 | 4.7 | - | - | - | - |
| 979 | 969 | Sabinene | - | - | - | - | - | - | - | - | - | 0.2 | - | tr | tr | - | - | - | - |
| 987 | 974 | ***β***-Pinene | tr | - | - | - | 1.0 | 2.3 | tr | 1.0 | 2.1 | 1.3 | 2.6 | 2.5 | 5.7 | 1.6 | 1.6 | - | - |
| 994 | 988 | Myrcene | - | 0.4 | - | - | 3.2 | - | - | tr | - | - | 0.7 | tr | 7.1 | - | - | - | - |
| 1004 | - | Isobutyl 2-methyl butanoate | - | - | - | - | - | - | - | 3.1 | 2.3 | 2.2 | 5.0 | 4.0 | 3.2 | - | 1.2 | - | - |
| 1004 | 998 | *n*-Octanal | 3.9 | 2.5 | 8.1 | 5.9 | tr | tr | 2.3 | - | - | - | - | - | - | 12.2 | tr | 9.9 | 1.1 |
| 1006 | - | Isobutyl isovalerate | - | tr | tr | tr | tr | 8.6 | 1.9 | 3.0 | 2.0 | 1.6 | - | 4.0 | 3.2 | - | 1.1 | - | - |
| 1015 | 1007 | Isoamyl isobutanoate | - | - | - | tr | - | - | - | - | - | 0.6 | - | - | - | - | - | - | - |
| 1017 | - | 2-Methyl butyl isobutanoate | - | tr | - | tr | 1.2 | 3.0 | 0.8 | 1.6 | 1.3 | 1.0 | 0.8 | 3.7 | 2.5 | 1.8 | 1.8 | - | - |
| 1017 | 1007 | Hexyl acetate | 0.4 | - | - | - | - | - | 0.4 | - | - | - | - | - | - | - | - | - | - |
| 1028 | 1020 | *p*-Cymene | - | - | tr | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 1036 | 1024 | Limonene | 0.4 | - | 0.4 | 0.7 | 1.9 | 0.5 | 0.5 | 1.7 | 2.1 | 1.7 | 0.8 | 3.9 | 4.1 | tr | tr | - | - |
| 1043 | 1032 | (*Z*)-***β***-Ocimene | - | 1.0 | 1.8 | 6.2 | 12.2 | 1.9 | - | 0.7 | tr | 0.6 | 2.1 | tr | 5.0 | - | - | - | - |
| 1044 | - | Butyl 2-methyl butanoate | tr | - | - | - | - | - | tr | tr | 0.2 | 0.5 | tr | 1.6 | tr | - | - | - | - |
| 1049 | - | Butyl isovalerate | tr | - | - | - | - | - | tr | - | tr | 0.6 | - | 0.9 | tr | - | - | - | - |
| 1052 | 1044 | (*E*)-*β*-Ocimene | - | - | - | - | 2.8 | - | - | - | - | - | 0.9 | - | tr | tr | - | - | - |
| 1072 | 1063 | *n*-Octanol | 22.1 | 5.5 | 17.0 | 4.4 | 5.2 | 11.5 | 15.8 | 7.3 | 4.8 | 3.9 | 5.9 | 0.8 | tr | 5.2 | 1.2 | 3.5 | 48.5 |
| 1082 | - | Isobutyl 3-methyl 2-butenoate | - | - | - | - | - | - | - | - | - | - | - | 0.4 | tr | 2.8 | 1.4 | - | - |
| 1092 | 1086 | Terpinolene | - | - | - | - | - | - | - | - | - | 0.9 | - | - | - | - | - | - | - |
| 1100 | 1100 | Isopentyl 2-methyl butanoate | - | 0.3 | 0.5 | 0.7 | 0.5 | 0.3 | - | tr | tr | 0.4 | 0.3 | 0.3 | tr | - | - | - | - |
| 1104 | 1100 | 2-Methyl butyl 2-methyl butanoate | - | 1.0 | 1.1 | - | 1.5 | 2.7 | - | 1.2 | 1.2 | tr | 1.8 | 2.4 | 2.1 | tr | tr | - | - |
| 1106 | 1102 | Isopentyl isovalerate | - | - | - | - | - | - | 0.9 | - | - | 1.1 | tr | - | - | - | - | - | - |
| 1109 | 1103 | 2-Methyl butyl isovalerate | - | 1.5 | 0.5 | 1.6 | 1.4 | 0.3 | 1.6 | 1.2 | 0.8 | 1.5 | 1.5 | 2.1 | 2.2 | 0.5 | 0.3 | - | - |
| 1151 | 1147 | Hexyl isobutanoate | tr | 0.4 | 0.5 | tr | tr | 0.4 | tr | 0.7 | 0.4 | 0.5 | 0.6 | 0.4 | 0.5 | 0.5 | 0.6 | - | - |
| 1216 | 1211 | Octyl acetate | 65.7 | 77.0 | 58.4 | 57.0 | 45.6 | 36.1 | 63.3 | 36.9 | 29.4 | 40.4 | 52.3 | 21.5 | 14.0 | 47.8 | 65.2 | 85.9 | 2.0 |
| 1239 | 1233 | Hexyl 2-methyl butanoate | - | 0.6 | 0.8 | 0.3 | - | - | - | tr | tr | - | 0.8 | 0.2 | - | - | - | - | tr |
| 1244 | 1241 | Hexyl isovalerate | - | 1.5 | 0.8 | tr | tr | tr | - | tr | tr | tr | 0.6 | tr | 0.7 | - | - | - | - |
| 1289 | 1287 | Bornyl acetate | - | - | - | - | - | - | - | - | - | tr | - | tr | tr | - | - | - | - |
| 1303 | - | Octyl propanoate | - | - | - | - | - | - | - | - | - | - | tr | - | - | - | - | - | - |
| 1346 | - | Octyl isobutanoate | - | - | - | tr | tr | tr | - | - | tr | tr | 2.8 | - | - | 4.9 | 5.2 | - | 11.0 |
| 1389 | - | Octyl butanoate | tr | 2.0 | 0.3 | tr | 0.4 | 0.2 | tr | - | - | - | - | 0.1 | tr | - | - | 0.1 | 1.4 |
| 1410 | 1407 | Decyl acetate | - | - | - | - | tr | - | - | - | - | - | - | - | - | - | - | 0.1 | - |
| 1421 | 1417 | (*E*)-Caryophyllene | - | - | - | - | tr | - | - | - | - | - | - | - | tr | - | - | - | - |
| 1435 | - | Octyl 2-methyl butanoate | - | - | - | tr | tr | tr | - | - | tr | - | 0.8 | - | - | 2.3 | 3.2 | - | 22.8 |
| 1437 | 1432 | *α*-*trans*-Bergamotene | - | - | - | - | 1.1 | - | - | - | - | - | - | - | - | - | - | - | - |
| 1439 | - | Octyl isovalerate | - | - | - | - | - | - | - | - | - | - | 0.7 | - | - | 0.9 | tr | - | - |
| 1461 | 1454 | (*E*)-*β*-Farnesene | - | - | - | - | tr | - | - | - | - | - | - | - | - | - | - | - | - |
| 1486 | 1484 | Germacrene D | - | - | - | - | tr | - | - | - | - | - | - | - | - | - | - | - | - |
| 1512 | 1505 | *β*-Bisabolene | - | tr | - | - | tr | - | - | - | - | - | - | - | - | - | - | - | - |
| 1559 | 1555 | Elemicin | - | - | - | - | tr | - | - | - | - | - | - | - | - | - | - | - | - |
| 1575 | 1568 | (*Z*)-Isoelemicin | - | - | - | - | tr | - | - | - | - | - | - | - | - | - | - | - | - |
| 1583 | - | Octyl hexanoate | tr | tr | - | - | tr | - | tr | 0.2 | tr | tr | tr | 0.2 | 0.5 | - | - | 0.2 | 9.8 |
|  |  | Monoterpene hydrocarbons | 2.7 | 1.7 | 3.2 | 8.6 | 35.1 | 10.3 | 4.3 | 29.5 | 41.1 | 37.1 | 22.7 | 38.0 | 47.9 | 1.6 | 1.6 | 0.1 | - |
|  |  | Other aliphatic hydrocarbons | 1.3 | tr | 1.9 | - | 1.1 | - | 1.1 | - | - | - | - | - | tr | 10.9 | 10.0 | - | - |
|  |  | Aliphatic esters | 68.6 | 86.7 | 64.7 | 78.4 | 53.4 | 76.4 | 73.8 | 58.6 | 49.6 | 57.6 | 68.0 | 57.1 | 50.2 | 67.7 | 85.5 | 86.3 | 47.0 |
|  |  | Aliphatic alcohols | 22.1 | 5.5 | 17.0 | 4.4 | 5.2 | 11.5 | 15.8 | 7.3 | 4.8 | 3.9 | 5.9 | 0.8 | tr | 5.2 | 1.2 | 3.5 | 48.5 |
|  |  | Aliphatic aldehydes | 3.9 | 2.5 | 8.1 | 5.9 | tr | tr | 2.3 | - | - | - | - | - | - | 12.2 | tr | 9.9 | 1.1 |
|  |  | Total identified | 98.6 | 96.4 | 94.9 | 97.3 | 94.8 | 98.1 | 97.2 | 95.4 | 95.4 | 98.6 | 96.6 | 95.9 | 98.0 | 97.5 | 98.3 | 99.8 | 96.5 |
|  |  | № of compounds | 16 | 19 | 19 | 19 | 31 | 21 | 21 | 21 | 24 | 28 | 24 | 27 | 28 | 17 | 19 | 7 | 8 |

[a]RI$_{exp}$ - retention indices on the HP-5MS column relative to $C_8$-$C_{40}$ *n*-alkanes. [b]RI$_{lit}$ - retention indices obtained from the literature (ADAMS 2007). [c]The constituents are listed in order of elution on the HP-5MS column. [d]The acronyms are explained in Table 1. [e]The relative percentages of the compounds were calculated from FID data; tr, trace (<0.1%); -, not detected

**Table 3.** Composition of the HS fractions of the roots of the investigated *Heracleum* taxa (%)

| $\text{RI}_{\text{exp}}$[a] | $\text{RI}_{\text{lit}}$[b] | Constituent[c] | *sph*[d] | *sib2* | *sib3* | *sib5* | *sib6* | *mon* | *ter1* | *pol2* | *pol3* | *ors2* | *ver1* | *ver3* | *orp2* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 865 | - | 2-Methyl octane | 3.5[e] | 0.1 | - | - | - | 5.0 | 1.1 | 0.4 | tr | 2.3 | - | - | - |
| 899 | 900 | *n*-Nonane | - | - | - | - | - | 3.7 | 1.0 | - | - | 1.9 | - | - | 89.8 |
| 904 | 901 | Heptanal | 1.4 | - | - | - | - | - | - | - | - | - | - | - | - |
| 915 | - | 4-Nonene + 3-nonene | - | 0.4 | 0.4 | - | - | - | - | - | - | - | 6.8 | 10.6 | - |
| 916 | - | 2-Nonene | - | - | - | - | 0.3 | - | - | 0.6 | tr | - | 1.8 | 2.0 | - |
| 931 | 924 | **α**-Thujene | tr | 2.0 | 2.3 | 2.7 | 7.5 | tr | - | tr | 4.1 | tr | - | tr | - |
| 940 | 932 | **α**-Pinene | 4.4 | 14.2 | 16.1 | 19.5 | 18.9 | 3.6 | 12.4 | 13.9 | 19.3 | 14.6 | 2.0 | 3.5 | 0.5 |
| 955 | 946 | Camphene | tr | 2.2 | 2.5 | 2.7 | 3.1 | tr | tr | 2.5 | 1.7 | 1.4 | - | - | - |
| 979 | 969 | Sabinene | - | 0.1 | - | - | - | - | - | - | - | tr | - | - | - |
| 987 | 974 | **β**-Pinene | 49.5 | 63.4 | 63.1 | 49.6 | 52.6 | 12.0 | 79.7 | 49.7 | 55.3 | 58.6 | 44.0 | 60.4 | 3.7 |
| 994 | 988 | Myrcene | 6.0 | 6.4 | 7.0 | 7.4 | 4.3 | 3.8 | - | 6.5 | 3.5 | 5.9 | - | 2.0 | - |
| 995 | 984 | 2-Pentyl furan | - | - | - | - | - | - | - | - | - | - | - | - | 0.4 |
| 1004 | 998 | *n*-Octanal | - | - | - | - | - | - | - | - | - | - | - | - | 0.6 |
| 1021 | 1014 | *α*-Terpinene | - | - | - | - | 0.2 | - | - | - | tr | - | - | - | - |
| 1028 | 1020 | *p*-Cymene | - | tr | - | - | - | - | - | - | - | tr | - | - | 0.2 |
| 1036 | 1024 | Limonene | 2.4 | 4.8 | 5.0 | 9.5 | 7.4 | 1.1 | 1.3 | 3.0 | 3.0 | 2.1 | 21.3 | 9.9 | 0.3 |
| 1043 | 1032 | (*Z*)-**β**-Ocimene | 28.4 | 5.6 | 2.8 | 7.8 | 5.3 | 65.4 | 3.6 | 7.8 | 7.8 | 11.4 | 4.2 | 1.5 | 1.2 |
| 1052 | 1044 | (*E*)-*β*-Ocimene | 2.1 | - | - | - | - | 2.4 | tr | - | - | - | 2.8 | 1.2 | 0.6 |
| 1063 | 1054 | *γ*-Terpinene | - | tr | 0.1 | - | 0.4 | - | - | - | 0.4 | - | - | - | tr |
| 1092 | 1086 | Terpinolene | - | 0.6 | 0.5 | 0.8 | tr | - | 0.8 | 15.7 | 4.9 | 1.8 | 15.1 | 6.0 | 1.8 |
| 1100 | 1100 | *n*-Undecane | - | - | - | - | - | - | - | - | - | - | - | - | 0.7 |
| 1109 | 1103 | 2-Methyl butyl isovalerate | - | - | - | - | - | - | - | - | - | - | 0.2 | tr | - |
| 1131 | 1128 | *allo*-Ocimene | tr | - | - | tr | tr | 0.7 | - | 0.1 | - | - | - | - | - |
| 1150 | - | 4,8-Epoxy-*p*-menth-1-ene | - | - | - | - | - | - | - | - | - | - | 0.2 | - | - |
| 1167 | 1160 | Pinocarvone | - | - | - | - | tr | - | - | - | - | - | - | - | - |
| 1192 | 1187 | 1-Dodecene | - | - | - | - | - | 0.2 | - | - | - | - | - | - | - |
| 1200 | 1195 | Methyl chavicol | - | - | - | tr | - | - | - | - | - | - | - | - | - |
| 1216 | 1211 | Octyl acetate | - | tr | - | - | - | - | tr | - | tr | - | 0.3 | - | tr |
| 1289 | 1287 | Bornyl acetate | - | - | - | tr | tr | - | - | - | - | - | - | - | - |
| 1298 | 1300 | *n*-Tridecane | - | - | - | - | - | - | - | tr | - | - | - | - | - |
| 1346 | - | Octyl isobutanoate | - | - | - | - | - | - | - | - | - | - | 0.1 | - | - |
| 1381 | 1380 | Daucene | - | - | - | - | tr | - | - | tr | - | - | - | - | - |
| 1404 | 1403 | Methyl eugenol | - | 0.1 | 0.1 | tr | - | - | - | - | - | - | - | - | - |
| 1435 | - | Octyl 2-methyl butanoate | - | - | - | - | - | - | - | - | - | - | 0.1 | - | - |
| 1437 | 1432 | *α*-*trans*-Bergamotene | 0.5 | tr | tr | - | tr | tr | - | tr | - | - | - | tr | - |
| 1439 | - | Octyl isovalerate | - | - | - | - | - | - | - | - | - | - | tr | - | - |
| 1498 | 1500 | Bicyclogermacrene | - | - | - | - | - | - | tr | tr | - | - | - | - | - |
| 1500 | 1500 | Isodaucene | tr | - | - | - | tr | - | - | tr | - | - | - | - | - |
| 1512 | 1505 | *β*-Bisabolene | tr | - | - | - | tr | tr | - | - | - | - | - | tr | tr |
| 1518 | 1514 | (*Z*)-*γ*-Bisabolene | tr | - | - | - | - | tr | - | - | - | - | - | - | - |
| 1532 | 1529 | Kessane | - | - | - | - | tr | - | - | - | - | - | - | - | - |
| 1559 | 1555 | Elemicin | - | tr | 0.1 | tr | - | - | - | - | - | - | - | - | - |
|  |  | Monoterpene hydrocarbons | 92.8 | 99.3 | 99.4 | 100.0 | 99.7 | 89.0 | 97.8 | 99.0 | 100.0 | 95.8 | 89.2 | 84.5 | 8.1 |
|  |  | Other aliphatic hydrocarbons | 3.5 | 0.6 | 0.4 | tr | 0.3 | 8.9 | 2.2 | 1.0 | tr | 4.2 | 8.6 | 12.6 | 90.5 |
|  |  | Other | 1,9 | 0,1 | 0,2 | tr | tr | - | tr | - | tr | - | 0,9 | 0,1 | 1,1 |
|  |  | Total identified | 98.2 | 100.0 | 100.0 | 100.0 | 100.0 | 97.9 | 100.0 | 100.0 | 100.0 | 100.0 | 98.7 | 97.2 | 99.7 |
|  |  | № of compounds | 15 | 17 | 13 | 13 | 19 | 15 | 11 | 16 | 13 | 12 | 14 | 13 | 14 |

[a] $\text{RI}_{\text{exp}}$ - retention indices on the HP-5MS column relative to $C_8$-$C_{40}$ *n*-alkanes. [b] $\text{RI}_{\text{lit}}$ - retention indices obtained from the literature (Adams 2007). [c] The constituents are listed in order of elution on the HP-5MS column. [d] The acronyms are explained in Table 1. [e] The relative percentages of the compounds were calculated from FID data; tr, trace (<0.1%); -, not detected.

distance matrix, and included those volatiles which were present in at least one HS fraction in the quantity ≥ 1% (arbitrary threshold). The PCA included the volatiles present in at least one HS fraction in the quantity ≥ 5% (arbitrary threshold), analogously to the previous analysis of the essential oils, which was performed using the stated threshold in order to avoid overlapping of the number of variables in the PCA plot (Ušjak *et al.* 2018, 2020). The analysis was performed by means of Statistica 6.0 software (Statsoft Inc., Tulsa, OK, USA).

## RESULTS AND DISCUSSION

**The composition of the *Heracleum* HS fractions.** The HS volatiles of 17 fruit samples from the nine studied *Heracleum* taxa are given in Table 2. The number of the identified volatiles ranged from 7 (*orp1*; the acronyms are given in Table 1) to 31 (*sib6*), comprising 94.8% (*sib6*) to 99.8% (*orp1*) of the total amount of detected compounds. Like the previously analysed essential oils of these fruit samples (Ušjak *et al.* 2018), the HS fractions were rich in aliphatic esters (47.0-86.7%). These compounds were dominant in all the HS fractions of the fruit samples from the section *Heracleum*, with octyl acetate (36.1-85.9%) being the most abundant in the majority of them (in 13 out of 16; with the exception of the *ter2*, *ors1* and *ors2* samples which were dominated by α-pinene; 21.3-33.9%). On the other hand, in the HS fraction of the *aus* fruit sample (the only investigated sample from the section *Wendia*) free aliphatic alcohol *n*-octanol prevailed (48.5%), whereas among the aliphatic esters, octyl 2-methylbutanoate (22.8%) was predominant. Octyl acetate was also the most abundant component of the essential oils in these same samples from the section *Heracleum* (30.3-84.5%), while in the essential oil from the *aus* sample, instead of *n*-octanol and octyl 2-methylbutanoate (48.5 and 22.8% in the HS fraction; 5.4 and 11.0% in the essential oil), a less volatile ester, octyl hexanoate (49.8% in the essential oil; 9.8% in the HS fraction), prevailed (Ušjak *et al.* 2018). Additionally, all the *H. ternatum* and *H. pyrenaicum* fruit HS fractions and one *H. sibiricum* HS fraction (*ter1*, *ter2*, *pol1*, *pol4*, *ors1*, *ors2* and *sib6*) contained significant amounts of monoterpenes (22.7-47.9%), mainly α-pinene (12.0-33.9%). This is in accordance with the composition of the essential oils in these samples and with the volatility of the monoterpenes. Namely, although all previously studied fruit essential oils had a generally low monoterpene content, in the essential oils of the aforementioned samples their amounts were higher (0.7-4.1%) in relation to the other taxa (maximum 0.3%) (Ušjak *et al.* 2018). Significantly higher amounts of monoterpenes in the HS fractions compared to the essential oils are also expected due to the fact that monoterpenes are among their most volatile components (i.e. they are more volatile than most of the identified aliphatic esters).

The composition of the HS fractions of 13 root samples of the eight studied taxa from the section *Heracleum* is presented in Table 3. The number of identified volatile components ranged from 11 (*ter1*) to 19 (*sib6*), comprising 97.2% (*ver3*) to 100% (eight samples) of the total amount of detected compounds. The dominant HS volatiles in the root samples of the investigated *H. sphondylium* group members were monoterpenes (84.5-100.0%), and in the majority of them (except in the *mon* sample), β-pinene was the most abundant (44.0-79.7%). (*Z*)-β-Ocimene was the predominant component of the HS fraction of the *mon* root sample (65.4%) and the second most abundant in that of the *sph* root sample (28.4%). Some other monoterpenes were also present in significant amounts in the root HS fractions of the studied *H. sphondylium* group members: α-pinene in the case of all of the *H. sibiricum* (14.2-19.5%), *H. ternatum* (12.4%) and *H. pyrenaicum* (13.9-19.3%) samples, limonene in the case of both *H. verticillatum* samples (9.9-21.3%), and terpinolene in the case of all of the *H. pyrenaicum* subsp. *pollinianum* (4.9-15.7%) and *H. verticillatum* (6.0-15.1%) samples. The composition of the root HS fractions of the investigated members of the *H. sphondylium* group was similar to that of previously analysed essential oils of the same root samples (Ušjak *et al.* 2018), because the dominant components of the essential oils were at the same time their most volatile constituents [i.e. β-pinene (16.0-47.3%) was the most abundant in all the oils of these root samples, with the exception of the *sph* and *mon* oils

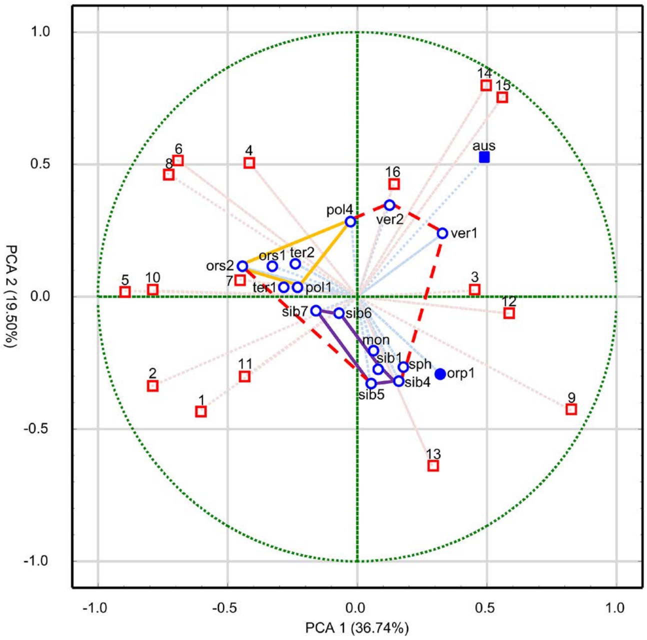

**Fig. 1.** PCA analysis of the composition of the fruit HS fractions of the investigated *Heracleum* taxa. The acronyms are given in Table 1. The circles represent the samples of the taxa of the section *Heracleum*: the empty ones stand for the taxa of the *H. sphondylium* group and the coloured one stands for *H. orphanidis*; the coloured square represents *H. austriacum* subsp. *siifolium*; the dashed line groups the samples of the taxa of the *H. sphondylium* group, and the full lines group the samples of individual species *H. sibiricum* (violet; *sib1*, *sib4*-*sib7*) and *H. pyrenaicum* (yellow; *pol1*, *pol4*, *ors1*, *ors2*). The compounds are represented by empty squares and are numbered as follows: isopropyl 2-methyl butanoate (1), isopropyl isovalerate (2), *n*-nonane (3), isobutyl isobutanoate (4), α-pinene (5), β-pinene (6), myrcene (7), isobutyl 2-methyl butanoate (8), *n*-octanal (9), isobutyl isovalerate (10), (Z)-β-ocimene (11), *n*-octanol (12), octyl acetate (13), octyl isobutanoate (14), octyl 2-methyl butanoate (15) and octyl hexanoate (16).

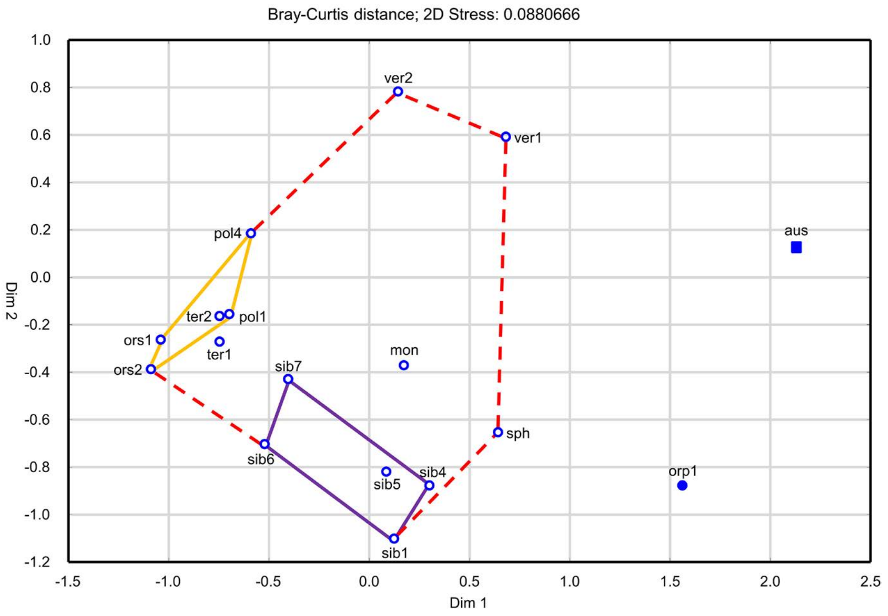

**Fig. 2.** nMDS analysis of the composition of the fruit HS fractions of the investigated *Heracleum* taxa. The acronyms are given in Table 1. The circles represent the samples of the taxa of the section *Heracleum*: the empty ones stand for the taxa of the *H. sphondylium* group and the coloured one for *H. orphanidis*; the coloured square represents *H. austriacum* subsp. *siifolium*; the dashed line groups the samples of the taxa of the *H. sphondylium* group, and the full lines group the samples of individual species *H. sibiricum* (violet; *sib1*, *sib4-sib7*) and *H. pyrenaicum* (yellow; *pol1*, *pol4*, *ors1*, *ors2*).

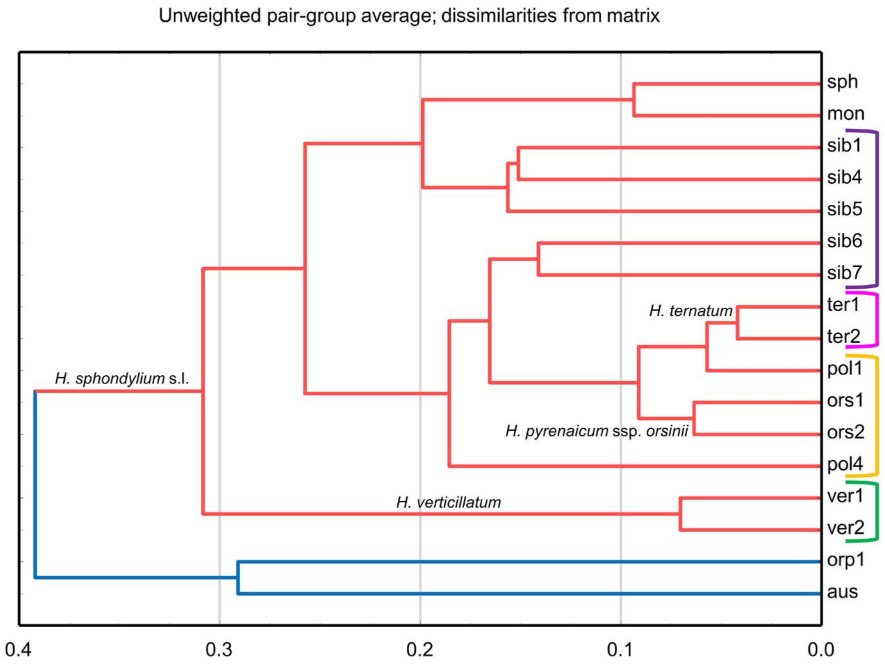

**Fig. 3.** UPGMA analysis of the composition of the fruit HS fractions of the investigated *Heracleum* taxa. The acronyms are given in Table 1.

where ($Z$)-β-ocimene prevailed (28.9 and 20.4%); furthermore, notable amounts of limonene were present in the *H. verticillatum* oils (16.0-19.2%) and terpinolene in the *H. pyrenaicum* subsp. *pollinianum* (8.6-22.5%) and *H. verticillatum* (9.0-9.2%) oils]. On the other hand, the dominant component of the HS fraction of the *H. orphanidis* roots was highly volatile *n*-nonane (89.8%), which was present only in small amounts (1.8%) in the essential oil of this sample. This was expected since the most abundant component in the *H. orphanidis* root essential oil was (Z)-falcarinol (80.0%) (a compound of lower volatility) (Ušjak *et al.* 2018).

**The chemosystematic significance of the *Heracleum* HS fractions.** The PCA analysis of the fruit HS fractions showed the clear distinction of the *H. austriacum* subsp. *siifolium* sample from the investigated samples of the section *Heracleum* (Fig. 1). The position of the *H. austriacum* subsp. *siifolium* sample was mostly influenced by octyl isobutanoate, octyl 2-methylbutanoate, isopropyl isovalerate and isobutyl isovalerate. Namely, isopropyl isovalerate and isobutyl isovalerate, with high negative factor loadings (in both cases -0.79), significantly contributed to the separation along the first principal axis (which explained 36.74% of the variation). Both these

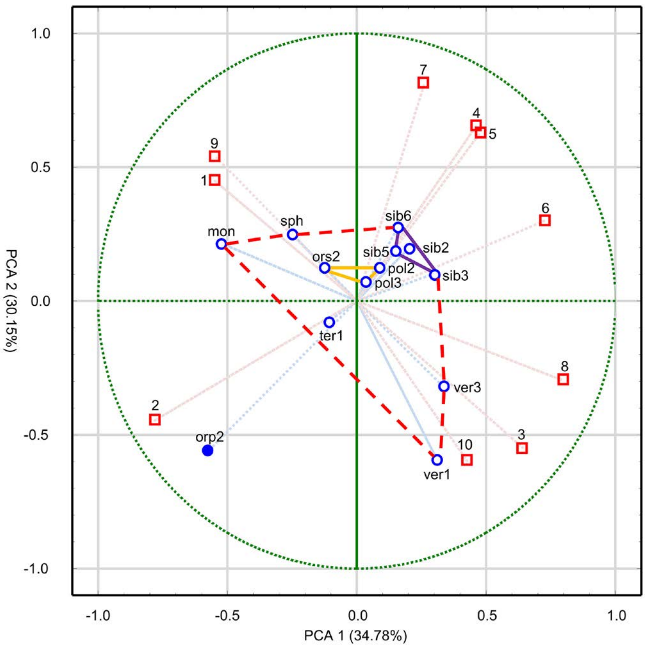

**Fig. 4.** PCA analysis of the composition of the root HS fractions of the investigated taxa of the sect. *Heracleum*. The acronyms are given in Table 1. The empty circles stand for the taxa of the *H. sphondylium* group and the coloured one for *H. orphanidis*; the dashed line groups the samples of the taxa of the *H. sphondylium* group, and the full lines group the samples of individual species *H. sibiricum* (violet; *sib2*, *sib3*, *sib5*, *sib6*) and *H. pyrenaicum* (yellow; *pol2*, *pol3*, *ors2*). The compounds are represented by empty squares and are numbered as follows: 2-methyl octane (1), *n*-nonane (2), 4-nonene+3-nonene (3), α-thujene (4), α-pinene (5), β-pinene (6), myrcene (7), limonene (8), (*Z*)-β-ocimene (9) and terpinolene (10).

esters of isovaleric acid were characteristically absent from the *H. austriacum* subsp. *siifolium* fruit HS fraction. Octyl isobutanoate and octyl 2-methylbutanoate, present in significant amounts in the *H. austriacum* subsp. *siifolium* fruit HS fraction, contributed the most to the separation of the samples along the second principal axis (which explained 19.50% of the variation), with high positive factor loadings (0.80 and 0.76).

In addition to the separation of the *H. austriacum* subsp. *siifolium* sample, the nMDS analysis of the fruit HS fractions (Fig. 2) also resulted in the clear distinction of the *H. orphanidis* sample from the investigated *H. sphondylium* group representatives, as well as in the isolated position of the *H. verticillatum* samples within this group. In both the PCA and nMDS analyses, morphologically closely related *H. sphondylium* and *H. montanum* (e.g. both have mostly white flowers, among which the outer ones are radiate; Brummitt 1968) were located near each other. The same relations were observed in the UPGMA analysis of these fractions (Fig. 3), i.e. all the investigated *H. sphondylium* group members formed a single cluster and the *H. verticillatum* samples, as well as the *H. sphondylium* and *H. montanum* samples, were located in the two separate sub-clusters.

However, in all three statistical methods, the complete separation of the *H. ternatum* samples from the *H. pyrenaicum* samples was not achieved, and in the UPGMA analysis, two *H. sibiricum* samples (*sib6* and *sib7*) were also grouped together with these *H. pyrenaicum* and *H. ternatum* samples. It should be noted that *H. sibiricum* and *H. ternatum* are morphologically closely related (e.g. both have mostly greenish flowers, among which the outer ones are not radiate or only slightly radiate; Brummitt 1968). Similar relations among the investigated taxa, including the overlapping of the samples of these three species were also observed in previous PCA, nMDS and UPGMA analyses of their fruit essential oils (Ušjak *et al.* 2018).

The PCA analysis of the root HS fractions, which included only the members of the sect. *Heracleum*, resulted in the clear distinction of the *H. orphanidis* sample from the samples of the *H. sphondylium* group members (Fig. 4). Furthermore, within this group, the isolated positions of the *H. verticillatum* samples and the proximity of the *H. sphondylium* and *H. montanum* samples were also observed. In addition, the separation of the *H. ternatum* sample from the *H. pyrenaicum* samples was noticed (which was not the case in the statistical analysis of the fruit HS fractions). The separation along the first principal axis (which explained 34.78% of the variation) was mainly influenced by limonene (0.79) and β-pinene (0.73) with high positive, and *n*-nonan (-0.78) with high negative factor loadings. These three components were among the dominant volatiles in the studied root HS fractions (β-pinene in the majority of the samples of the *H. sphondylium* group, limonene in both *H. verticillatum* samples, and *n*-nonane in the *H. orphanidis* sample). Along the second principal axis (which explained 30.15% of the variation), myrcene (0.81) with a high positive factor loading contributed the most to the separation. Myrcene was not among the dominant HS volatiles, but its absence from the *H. ternatum* and *H. orphanidis* fractions could be considered significant [e.g. it was present in varying amounts (3.5-6.5%) in the *H. pyrenaicum* samples].

The relations among the investigated taxa revealed in the PCA analysis of the root HS fractions were also observed in the nMDS and UPGMA analyses (Figs. 5 & 6). In the UPGMA clustering, all the investigated *H. sphondylium* group members formed a single cluster, as in the case of the fruit HS fraction analysis. Additionally, in the UPGMA analysis of the root HS fractions, the *H. sibiricum*, *H. pyrenaicum*, *H. ternatum*, as well as *H. sphondylium* and *H. montanum* samples formed separate sub-clusters.

Similar relations among the investigated taxa were also shown in the PCA, nMDS and UPGMA analyses of their root essential oils (Ušjak *et al.* 2018). Moreover, the analyses of the root HS fractions resulted in even bet-

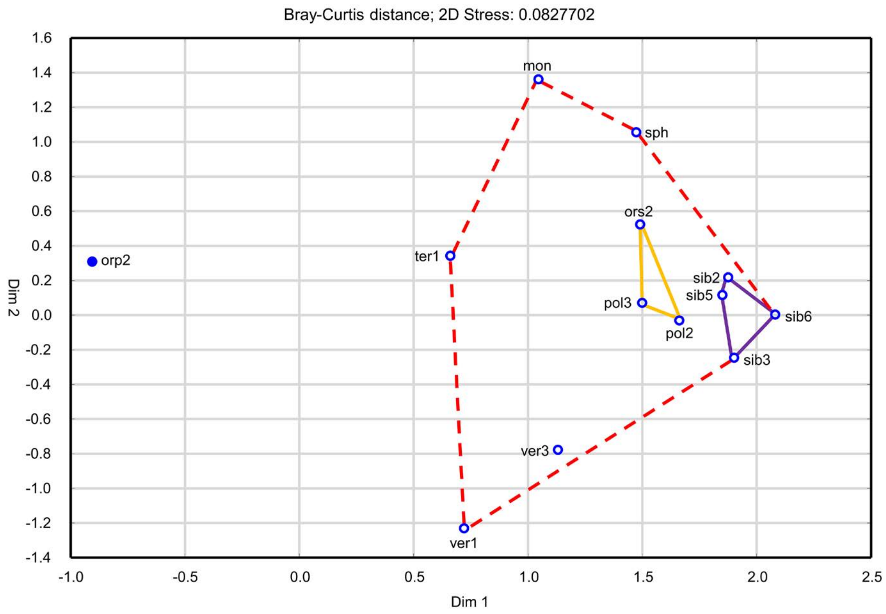

**Fig. 5.** nMDS analysis of the composition of the root HS fractions of the investigated taxa of the sect. *Heracleum*. The acronyms are given in Table 1. The empty circles stand for the taxa of the *H. sphondylium* group and the coloured one for *H. orphanidis*; the dashed line groups the samples of the taxa of the *H. sphondylium* group, and the full lines group the samples of individual species *H. sibiricum* (violet; *sib2*, *sib3*, *sib5*, *sib6*) and *H. pyrenaicum* (yellow; *pol2*, *pol3*, *ors2*).

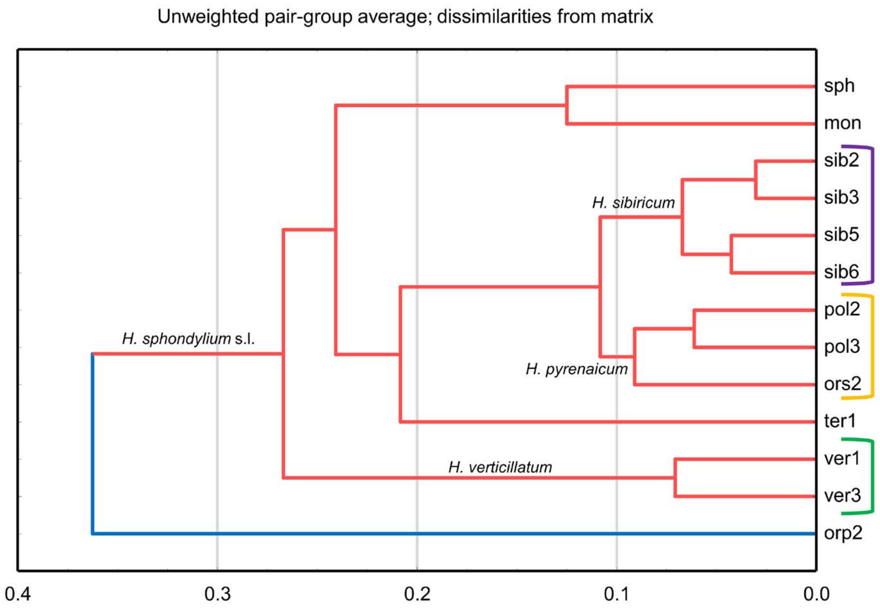

**Fig. 6.** UPGMA analysis of the composition of the root HS fractions of the investigated taxa of the sect. *Heracleum*. The acronyms are given in Table 1

ter separation. Namely, in the case of the root essential oils, the *H. ternatum* sample was not completely separated from the *H. pyrenaicum* samples, i.e. it was located close to the *H. pyrenaicum* subsp. *orsinii* sample (Ušjak *et al.* 2018). After the separation of the *H. ternatum* and *H. pyrenaicum* subsp. *orsinii* samples (in the root HS fraction analysis), it is clear that only the close positions of the two *H. pyrenaicum* subspecies, as well as of *H. sphondylium* and *H. montanum* were observed in all the statistical analyses of the volatiles [essential oils (Ušjak *et al.* 2018, 2020) and HS fractions] of the *H. sphondylium* group members. Thus, it can be concluded that, based on the analyses of their volatiles, *H. montanum* should rather be treated as a subspecies of *H. sphondylium*, i.e. *H. sphondylium* subsp. *elegans* (Crantz) Schübl. & G. Martens (Hand 2011), than as an independent species. On the other hand, the isolated positions of the remaining six taxa from the *H. sphondylium* group were confirmed, as was also the case in our previous investigations of the essential oils (Ušjak *et al.* 2018, 2020).

Static HS sampling was also used recently by Raber *et al.* (2021) to demonstrate the variation of intra- and interspecific terpene profiles of *Picea pungens* Engelm. and *P. abies* (L.) H. Karst. (Pinaceae) branches. As in the case of the *Heracleum* root samples investigated in the current study, monoterpenes (i.e. α-pinene, β-pinene and limonene) were the dominant HS volatiles. The compounds belonging to this class of terpenes were also the most abundant in several more similar studies, which were conducted to show intraspecific variations in *Pinus mugo* Turra (Pinaceae) needles (α-pinene and δ-3-carene; Mitić *et al.* 2021), *Picea omorika* (Pančić) Purk. twigs (α-pinene, β-pinene and myrcene; Nikolić *et al.* 2020), *Origanum microphyllum* (Benth.) Vogel (Lamiaceae) leaves (sabinene, *cis*-sabinene-hydrate and *trans*-sabinene-hydrate; Gotsiou *et al.* 2002), *Ocimum basilicum* L. (Lamiaceae) leaves and flowers (linalool and 1,8-cineole; Ćavar Zeljković *et al.* 2020) and *Lavandula angustifolia* Mill. (Lamiaceae) flowers (linalool, linalyl acetate and lavandulyl acetate; Zhu *et al.* 2018).

## CONCLUSION

In this study, the chemical analysis of the fruit and root HS fractions of nine *Heracleum* taxa was carried out for the first time. By means of multivariate statistical analysis, the chemosystematic significance of the studied HS fractions was demonstrated. Moreover, in the case of the root HS fractions, the statistical analysis resulted in the even better separation of the taxa compared to the analysis of the essential oils. Thus, static HS extraction has proven to be a good option for the evaluation of the chemosystematic significance of the volatiles of *Heracleum* taxa.

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## REZIME

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# Hemijski sastav i hemosistematski značaj *headspace* frakcija plodova i korena odabranih taksona roda *Heracleum* iz jugoistočne Evrope

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Fokus ovog rada bio je na devet taksona roda *Heracleum* (Apiaceae): *H. sphondylium*, *H. sibiricum*, *H. montanum*, *H. ternatum*, *H. pyrenaicum* subsp. *pollinianum*, *H. pyrenaicum* subsp. *orsinii* i *H. verticillatum* iz grupe *H. sphondylium*, i *H. orphanidis*, svi iz sect. *Heracleum*, kao i *H. austriacum* subsp. *siifolium* iz sect. *Wendia*. Cilj rada je bila izolacija *headspace* (HS) frakcija iz 17 uzoraka plodova (prikupljenih od svih devet ispitivanih taksona roda *Heracleum*) i 13 uzoraka korena (prikupljenih od svih osam ispitivanih taksona iz sect. *Heracleum*), ispitivanje njihovog sastava i hemosistematskog značaja, kao i poređenje ovih rezultata sa prethodno dobijenim rezultatima za etarska ulja izolovana destilacijom vodenom parom ovih istih uzoraka plodova i korena. Za izolovanje HS frakcija korišćen je automatski statički HS *sampler*, a za njihovu analizu gasna hromatografija sa FID i MS detekcijom. U HS frakcijama plodova dominirali su oktilacetat i/ili α-pinen (taksoni iz sect. *Heracleum*), ili *n*-oktanol (*H. austriacum*), a u HS frakcijama korena β-pinen i/ili (*Z*)-β-ocimen (taksoni iz grupe *H. sphondylium*), ili *n*-nonan (*H. orphanidis*). Hemosistematski značaj je procenjen primenom metoda multivarijantne statističke analize: analize glavnih komponenti (PCA), nemetričkog multidimenzionalnog skaliranja (nMDS) i aglomerativne hijerarhijske klaster analize zasnovane na *unweighted pair-group arithmetic averages* (UPGMA) algoritmu. Kao i u slučaju prethodno ispitivanih etarskih ulja, u statističkoj analizi HS frakcija plodova i korena generalno je primećeno grupisanje ispitivanih predstavnika grupe *H. sphondylium*, kao i grupisanje morfološki srodnih *H. sphondylium* i *H. montanum* i izolovan položaj *H. verticillatum* u okviru ove grupe. Statistička analiza HS frakcija korena rezultovala je boljim razdvajanjem taksona (čak i u poređenju sa prethodnom analizom etarskih ulja), tj. primećene su izolovane pozicije *H. ternatum* i *H. pyrenaicum*.

**Ključne reči:** Apiaceae, GC-FID, GC-MS, PCA, nMDS, UPGMA