Fatty acid composition of the cypselae of two endemic Centaurea species (Asteraceae)

Pedja Janačković1*, Milan Gavrilović1, Ljubodrag Vujisić2, Vlado Matevski3, Petar D. Marin1

1 University of Belgrade - Faculty of Biology, Institute of Botany and Botanical Garden “Jevremovac”, Studentski trg 16, Belgrade 11000, Serbia
2 University of Belgrade - Faculty of Chemistry, Studentski trg 12-16, Belgrade 11000, Serbia
3 Faculty of Natural Sciences and Mathematics, Saints Cyril and Methodius University in Skopje, Blvd. Goce Delcev 9, Skopje 1000, Republic of Macedonia

ABSTRACT: The fatty acid composition of cypselae of two endemic species from Macedonia, Centaurea galicicae and C. tomorosii, is analysed for the first time, using GC/MS (gas chromatography/mass spectrometry). In the cypselae of C. galicicae, 11 fatty acids were identified, palmitic (hexadecanoic) acid (32.5%) being the most dominant. Other fatty acids were elaidic [(E)-octadec-9-enoic] acid (13.9%), stearic (octadecanoic) acid (12.8%) and linoleic [(9Z,12Z)-9,12-octadecadienoic] acid (10.6%). Of the 11 identified fatty acids, seven were saturated fatty acids, which represented 41.5% of total fatty acids, while unsaturated fatty acids altogether constituted 58.5%. In the cypselae of C. tomorosii, five fatty acids were identified. The major fatty acid was linolelaidic [(9E,12E)-octadeca-9,12-dienoic] acid (48.8%). The second most dominant fatty acid was oleic [(9Z)-octadec-9-enoic] acid (34.2%). Thus, unsaturated fatty acids were present with 83%. The other three fatty acids identified were saturated fatty acids, which represented 17% of total fatty acids. As a minor fatty acid, levulinic (4-oxopentanoic) acid was determined in both C. galicicae and C. tomorosii (0.3% and 3.2%, respectively). The obtained results differ from published data on dominant fatty acids in the cypselae of other species belonging to the same section as the species investigated in the present paper (section Arenariae, subgenus Acrolophus, genus Centaurea). They also, differ from published data referable to other genera belonging to the same tribe (Cardueae). The general chemotaxonomic significance of fatty acids is discussed.

Keywords: fatty acids, Centaurea, Arenariae, Compositae, chemotaxonomy

INTRODUCTION

The genus Centaurea L., one of the largest genera of the Asteraceae (Compositae) family, comprises between 400 and 700 species (Dittrich 1977; Bremer 1994; Wagenitz & Hellwig 1996; Garcia-Jacas et al. 2001). It belongs to the tribe Cardueae Cass. and subtribe Centaureinae (Cass.) Dumort. The genus is distributed in the Mediterranean region and in South-East Asia. On the Balkan Peninsula, there are about 146 species (Lovrić 1990). Taxonomy of the genus is somewhat vague: sections are still being revised, and interrelationships are not well resolved (Garcia-Jacas et al. 2006).

Fatty acids are distributed in all plant organs and occur as free fatty acids or in the form of esters. From the chemotaxonomic point of view, the most important are fatty acids from seeds and fruits (cypselae in Asteraceae) because of their conservative nature. The cypselae can be defined as a dry, indehiscent, unilocular fruit with a single seed not adnate to the pericarp (linked only

*correspondence: pjanackovic@bio.bg.ac.rs © 2017 Institute of Botany and Botanical Garden Jevremovac, Belgrade
by the funicle), originating from an inferior ovary and with the pericarp formed by the real pericarp (cells from the ovarian wall) and extracarpellar tissues from the receptacle (Marzinek et al. 2008). Seed fatty acids can be good taxonomic markers at the intra- and interspecies levels and can indicate hybridisation between related taxa or even reflect the phylogeny of certain plant groups (Aitzetmüller 1996; Janačković 2001; Ozcan et al. 2000; Coutinho et al. 2015). In many plants, unusual fatty acids have been recorded that are also considered important as taxonomic characters (Aitzetmüller 1993). Unusual fatty acids can sometimes be used as a fingerprint in delimitation of taxa (Aitzetmüller 1996; Aitzetmüller 1997; Tsevegsüren et al. 1996). The composition of seed fatty acids is considered to be a good taxonomic character at the subfamily level of the Lamiaceae family (Marin et al. 1991). As a rule, the oils from seeds of the family Asteraceae are rich in linoleic acid and contain smaller amounts of palmitic and oleic acid, stearic and linolenic acids being minor fatty acids (Shorland 1963). Some Asteraceae taxa possess unusual C18 unsaturated acids as dominant, i.e., > 10% of total seed oil fatty acids (Shorland 1963; Hegnauer 1964; Hilditch & Williams 1964). Seed oils of some Cardueae taxa contain hydroxy- and epoxy-fatty acids with conjugated double bonds. Such fatty acids are found in Calendula, Artemisia, Helianthus, Cosmos, Vernonnia, Tragopogon, Osteospermum and Dimorphoteca species (Wagner 1977). An unusual fatty acid, γ-linolenic, was found in oil of the seeds of some Saussurea species (Tsevegsüren et al. 1997). Oil of the seeds of some Mongolian Compositae contains unusual trans-fatty acids (Tsevegsüren et al. 2000).

There are a number of phytochemical investigations of lactones and flavonoids in members of the genus Centaurea (Youssef & Frahm 1995; Fortuna et al. 2001; Janačković et al. 2004a; López-Rodríguez et al. 2009). The genus has also been the subject of some phytochemical research on the fatty acid composition of the aerial part of the plant (Tekeli et al. 2010; Zengin et al. 2010; Aktumsek et al. 2011; Zengin et al. 2011a; Zengin et al. 2011b; Erdogan et al. 2014).

The endemic species C. galicicae Micevski is a perennial herbaceous plant with purple flowers and a cypsela 2.5 mm long with a pappus twice as long as the cypsela. It grows in the southwestern part of the Former Yugoslav Republic (FYR) of Macedonia, in the foothills of Mt. Galičica in the Galičica National Park (Micevski 1985). (Fig. 1a, b, c). A new record for the flora of Albania was confirmed in the Prespa National Park (Shuka & Tan 2013). The endemic species C. tomorosii Micevski is a perennial herbaceous plant with pale yellow flowers and a cypsela 4 mm long with a short pappus 3 mm long. It grows among calcareous rocks, on Mt. Galičica: [Tomoros, 1450 m a.s.l. (Micevski 1985) (Fig. 1d, e, f)]. Both species, C. galicicae and C. tomorosii, belong to the section Arenariae (Hayek) Dostál of the subgenus Acrolophus (Cass.) Dobrocz of the genus Centaurea L. (Micevski 1985). Phytotochemical study of these two species is scarce. There is only one study dealing with secondary metabolites of three endemic species, including C. galicicae and C. tomorosii, where the lactone cinicin and seven flavonoids were isolated and identified (Tešević et al. 2014). No previous phytochemical study dealing with the fatty acids of these species has been reported. The present study therefore aims to investigate fatty acid composition of the cypsela of C. galicicae and C. tomorosii in order to further elucidate phytochemistry of the genus Centaurea. In addition to this, we here discuss the general usefulness of fatty acid composition as a taxonomic marker.

**MATERIAL AND METHODS**

**Plant Material.** Mature cypselae of C. galicicae were collected during the flowering period on July 7, 2012 at Konjsko, close to lake Prespa, FYR Macedonia (N 40.93504, E 20.93702201). Mature cypselae of C. tomorosii were collected during the flowering period on July 7, 2012 at a locality on Mt. Galičica, FYR Macedonia (N 40.9819000, E 20.86370006). Voucher specimens (accession numbers BEOU 38477 and BEOU 38474) were deposited in the Herbarium of the Institute of Botany and Botanical Garden “Jevremovac”, University of Belgrade (BEOU)—Faculty of Biology, Serbia.

**Isolation of Fatty Acids.** Mature cypselae (100 mg) of the investigated species were separately boiled for 10 min at 80°C in 1 mL of 2-propanol (iPrOH) (Kurata et al. 2005; Sokovic et al. 2009). The samples were then homogenised with 8 mL of undecanoic acid (conc. 0.2 mg/mL CH2Cl2) used as an internal standard. The homogenised samples were stored at 4°C for 24 h (lipid extraction), after which 2 mL 0.7% NaCl in H2O was added. The lower phase was taken from the funnel, transferred into a test tube and dried with anhydrous Na2SO4. The samples were then evaporated, after which 6 mL of a mixture of 1% H2SO4 in MeOH was added and the material heated for 2 h. A saturated solution of NaHCO3 was then added to the samples. After neutralisation, the samples were extracted with dichlormethane (CH2Cl2), and the lower phase from the funnel was taken for analysis.

**Gas Chromatography/Mass Spectrometry (GC/MS) Analysis.** An Agilent 7890A GC instrument equipped with two detectors, a flame ionisation detector (FID) and an Agilent 5975C mass selective detector (MSD), was used for GC and GC/MS measurements. The analyses were performed with a DB-23 fused silica capillary column (60 m × 250 μm × 0.25 μm). The carrier gas was helium at an initial rate of 4.1 mL/min (in constant pressure mode). The injection volume was 1 μL, the mode was splitless and the injector temperature was 220°C.
The oven temperature was programmed as follows: 50°C for 1 min; 50–175°C, 25°C/min; 175–235°C, 4°C/min; and 235°C for 5 min. The FID temperature was 300°C and total run time was 26 min. The MS transfer line was heated at 250°C. Mass spectra were collected in the electron ionisation mode (EI energy 70eV). The ion source was heated at 230°C and the quadrupole at 150°C. The mass range was 33-550 m/z.

Identification of methyl esters of fatty acids was done by comparison of mass spectra and retention times with standard Supelco® 37 Component FAME Mix and with the NIST11 and Wiley 7 commercial mass spectral libraries.

Quantification methyl esters of fatty acids was done using the internal standard mass and relative percentage of GC in relation to each component. Fatty acids are expressed as the percentage of total fatty acids and as the fatty acid mass (mg) in 100 mg of mature cypselae.

RESULTS AND DISCUSSION

In this work the fatty acid composition of cypselae of two endemic species, *C. galicicae* and *C. tomorosii*, was analysed using GC/MS (Table 1). In the cypselae of *C. galicicae*, 11 fatty acids were identified. The major fatty acid was palmitic (hexadecanoic) acid (32.5%). Other fatty acids with a high percentage were elaidic (E-octadec-9-enoic) (13.9%), stearic (octadecanoic) (12.8%) and linoleic (9Z,12Z)-9,12-octadecadienoic (10.6%) acids. Levulinic (4-oxopentanoic) acid was a minor fatty acid (0.3%). Out of the 11 identified fatty acids, seven were saturated, and they constituted 41.5% of total fatty acids. On the other hand, monounsaturated fatty acids were present with 33.9% and polyunsaturated fatty acids with 24.6% of total fatty acids. We identified five isomers of C18:2 fatty acids with an undetermined position of
the double bond. In total, unsaturated fatty acids were present with 58.5% of total fatty acids. In the cypselae of *C. tomorosii*, five fatty acids were identified. The major fatty acid was linolelaidic [(9\(E\),12\(E\))-octadeca-9,12-dienoic] acid (48.8%). The second most dominant fatty acid was oleic [(9\(Z\))-octadec-9-enoic] acid (34.2%). Thus, unsaturated fatty acids were present with 83%. Out of the five identified fatty acids, three were saturated, and they constituted 17% of total fatty acids. Levulinic (4-oxopentanoic) acid was present as a minor fatty acid (3.2%), the same as in *C. galicicae*.

In the present work, moreover, two free amino acids were identified in the cypselae of *C. galicicae* (L-aspartic and L-glutamic acid) that were not detected in the cypselae of *C. tomorosii*. This finding could be of importance because it has been shown that seed free amino acid composition can be used as a taxonomic marker (Bhunia & Mondal 2014).

Eleven fatty acids were identified in the cypselae of *C. galicicae*, as compared with five in *C. tomorosii*. The results showed that in both investigated species unsaturated fatty acids were present in greater amounts than saturated fatty acids. However, the two species differed with respect to the most dominant fatty acid, which in *C. galicicae* was palmitic (32.5%), a saturated fatty acid, while in *C. tomorosii* it was linolelaidic (48.8%), a polyunsaturated fatty acid. Palmitic acid was also identified in the cypselae of *C. tomorosii*, but in a smaller percentage (10%), while the percentage of linolelaidic acid in *C. galicicae* was only 2.1%. With respect to the dominant fatty acids in their cypselae, the investigated species differed from other species of the section to which they belong (section

**Table 1. Fatty acid composition of investigated Centaurea species.**

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Centaurea galicicae</th>
<th>Centaurea tomorosii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systematic name</td>
<td>Trivial name</td>
<td>Rt, min</td>
</tr>
<tr>
<td>4-oxopentanoic acid (C5:0)</td>
<td>levulinic acid</td>
<td>7.298</td>
</tr>
<tr>
<td>tetradecanoic acid (C14:0)</td>
<td>myristic acid</td>
<td>9.766</td>
</tr>
<tr>
<td>pentadecanoic acid (C15:0)</td>
<td>pentadecylic acid</td>
<td>10.637</td>
</tr>
<tr>
<td>hexadecanoic acid (C16:0)</td>
<td>palmitic acid</td>
<td>11.639</td>
</tr>
<tr>
<td>heptadecanoic acid (C17:0)</td>
<td>margaric acid</td>
<td>12.740</td>
</tr>
<tr>
<td>octadecanoic acid (C18:0)</td>
<td>stearic acid</td>
<td>13.967</td>
</tr>
<tr>
<td>(E)-octadec-9-enoic acid (C18:1n9E)</td>
<td>elaidic acid</td>
<td>14.173</td>
</tr>
<tr>
<td>(9Z)-octadec-9-enoic acid (C18:1n9Z)</td>
<td>oleic acid</td>
<td>14.341</td>
</tr>
<tr>
<td>C18:2 *</td>
<td>-</td>
<td>14.596</td>
</tr>
<tr>
<td>(9E,12E)-octa deca-9,12-dienoic acid</td>
<td>linolelaidic acid</td>
<td>14.647</td>
</tr>
<tr>
<td>C18:2 *</td>
<td>-</td>
<td>14.720</td>
</tr>
<tr>
<td>(9Z,12Z)-9,12-octadecadienoic acid</td>
<td>linoleic acid</td>
<td>15.034</td>
</tr>
<tr>
<td>C18:2 *</td>
<td>-</td>
<td>15.341</td>
</tr>
<tr>
<td>C18:2 *</td>
<td>-</td>
<td>15.421</td>
</tr>
<tr>
<td>icosanoic acid (C20:0)</td>
<td>arachidic acid</td>
<td>16.634</td>
</tr>
</tbody>
</table>

* isomers of c18:2 fatty acid with an undetermined position of the double bond; Rt, min - retention time, minutes.


**REFERENCES**


Powell RG, Smith CR & Wolff JA. 1967. cis-5,cis-9,cis-12-Octadecatrienoic acid and some unusual oxygenated acids in Xeranthemum annuum seed oil. Lipids 2: 172-177.


Zengin G, Guler GO, Cakmak YS & Aktumsek A. 2011b. Antioxidant capacity and fatty acid profile of Centaurea kotchyi (Boiss. & Heldr.) Hayek var. persica (Boiss.) Wagenitz from Turkey. Grasas y Aceites 62: 90–95.
Sastav masnih kiselina cipsela dve endemične vrste Centaurea (Asteraceae)

Pedja Janačković, Milan Gavrilović, Ljubodrag Vujisić, Vlado Matevski i Petar D. Marin

U ovom radu je po prvi put analiziran sastav masnih kiselina cipsela dve endemične vrste Centaurea galicicae i C. tomorosii iz Makedonije, upotrebom GH/MS (gasne hromatografije i masene spektrometrije). U cipselama vrste C. galicicae identifikovano je 11 masnih kiselina, među kojima je dominantna palmitinska kiselina (32,5%). Ostale visoko zastupljene masne kiseline su elaidinska (13,9%), stearinska (12,8%) i linolna (10,6%). Od prisutnih, 7 su zasićene masne kiseline i predstavljaju 41,5% od ukupnih masnih kiselina, dok su nezasićene masne kiseline zastupljene sa 58,5%. U cipselama vrste C. tomorosii identifikovano je 5 masnih kiselina. Među njima su dominantne linolelaidinska (48,8%) i oleinska masna kiselina (34,2%). Nezasićene masne kiseline predstavljaju 83%, dok zasićene predstavljaju 17% od ukupnih masnih kiselina. Najmanje zastupljena masna kiselina je levulinska kod C. galicicae (0,3%) kao i kod C. tomorosii (3,2%). Naši rezultati pokazali su razliku u poređenju sa literaturnim podacima u odnosu na prisustvo dominantnih masnih kiselina u cipselama drugih ranije istraživanih vrsta koje, takođe, pripadaju sekciji Arenariae podrodu Acrolophus rodu Centaurea. Takođe, uočene su razlike u prisustvu dominantnih masnih kiselina cipsela u odnosu na druge rodove iz istog tribusa Cardueae. Taksonomski značaj masnih kiselina je diskutovan.

Ključne reči: masne kiseline, Centaurea, Arenariae, Compositae, hemotaksonomija