



Fatty acids of oil and antioxidant capacity of phenolics from fruits of 11 Cardueae (Carduoideae, Asteraceae) taxa from northeast Anatolia (Turkey)

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ABSTRACT: Members of the tribe Cardueae have become a subject of major interest due to problems of their taxonomy and phylogeny, their possession of biologically active metabolites and their use in traditional medicine. The present study was conducted on 11 taxa of the tribe Cardueae collected from natural habitats in Turkey. In it we investigated the oil content of cypselae, fatty acid composition of the oil and the antioxidant capacity of phenolics from the cypselae. The results showed that the total oil content ranged from 1.45 to 9.28%. The main fatty acid was linoleic acid (C18:2n6; 44.36–70.49%), followed by oleic acid (C18:1n9; 11.41–23.71%), a situation which varied significantly among the taxa, as did the concentrations of different sums of fatty acids (PUFAs, 45.21–78.82%; SFAs, 6.53–14.06%; MUFAs, 12.21–41.40%). The total content of phenolic compounds (TPC; 428.17–752.14 mg/100 g of dry weight) and total flavonoid content (TF; 132.19–336.41 mg/100 g of dry weight) were in strong positive correlation with antioxidant capacity (range; micromol/g of dry weight) determined using DPPH (65.94–147.9), FRAP (32.32–86.42), CUPRAC (41.04–92.91) and ORAC (22.11–51.24) assays. The data demonstrated that a higher content of phenolic compounds resulted in a higher antioxidant capacity, while a lower content resulted in a low antioxidant capacity. Relative proportions and quantities of fatty acids can be used as additional biochemical markers in taxonomy of the tribe. The present findings suggest that consumption of cypselae of those species that are rich in phenolic compounds and fatty acids may potentially be beneficial to human health by preventing chronic diseases caused by oxidative stress.

KEYWORDS: chemotaxonomy, cypselae, *Arctium*, Cardueae, *Carthamus*, *Cirsium*, *Echinops*.

Received: 11 January 2019

Revision accepted: 22 March 2019

UDC: 582.998.1:631.576:[547.56+577.115.3]:66.094.3.097.8(560)

DOI: <https://doi.org/10.2298/BOTSERB1901031K>

INTRODUCTION

The tribe Cardueae contains more than 2360 species from 73 genera (BREMER 1994; SUSANNA & GARCIA-JACAS 2007), including some of the largest genera of the subfamily Carduoideae, family Asteraceae (STEVENS 2001).

According to recent reports, the tribe in Turkey consists of approximately 500 taxa, belonging to 39 genera (DAVIS 1975; GUNER *et al.* 2012). Representing 22 genera, nearly 100 taxa are naturally distributed in Northeast Anatolia (Turkey). Taxonomic conflicts with the phylogeny of this tribe have been addressed several times, and much

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phylogenetic research is still being conducted on it. The tribal delimitation of Cardueae is controversial, and the traditional classification has fluctuated widely (GARCIA-JACAS *et al.* 2002). The tribe Cardueae was initially divided into four subtribes, viz., Echinopinae, Carlinae, Carduinae and Centaureinae (BENTHAM 1873; HOFFMANN 1890-1894). However, CASSINI (1819) noted an affinity between the latter two groups. The tribes were subsequently converted to subtribes of one large tribe, Cynareae, by DUMORTIER (1829). BENTHAM (1873) and HOFFMANN (1890-1894) re-established Cassini's classification, describing the Echinopeae and Carlineae as separate tribes, and uniting the Carduinae and Centaureinae in the Cardueae s. str. PETIT (1997) proposed uniting the Carduinae and Centaureinae in his Carduinae series. DITTRICH (1970, 1977) suggests groups of genera based on cypselae (achene) morphology, but offers no phylogenetic treatment. Only a few Carduinae genera were included in the phylogenetic analyses of the Cardueae by SUSANNA *et al.* (1995) and HELLWIG (1996) based on DNA sequencing data. PETIT (1997) investigated representatives of the entire tribe Cardueae and included 14 genera of the Carduinae resolved on the basis of 22 phylogenetically informative binary characters in his morphological analysis. HÄFFNER & HELLWIG (1999) determined DNA sequences in representatives of 17 Carduinae genera. The tribe Cardueae contains many plants generally known as thistles. This tribe, especially the subtribe Carduinae, is predominantly distributed in the northern hemisphere of the Old World, where its representatives are found in large parts of Asia, Europe and North Africa. Only a few groups extend to tropical East Africa (*Carduus*, *Cirsium*), North and Central America (*Cirsium*, *Saussurea*) and Australia (HÄFFNER 2000).

The genera *Cirsium* Miller and *Centaurea* L., the two largest in this tribe, are closely related and exhibit a complex taxonomy (CHARADZE 1998). Species of *Cirsium* also display affinities with *Carduus* L., *Cnicus* L. and *Centaurea* with respect to some of their morphological characteristics, such as structure of the florets and achene, leaf shape and spiny habitus (DAVIS & PARRIS 1975). Earlier LINNAEUS (1753) considered the *Cirsium* species as members of the genus *Cnicus*, but CHARADZE (1998) considered uniting *Cirsium* with *Carduus*. The separation of *Carduus* from *Cirsium* is mainly based on a single character, and it is often regarded as artificial (BREMER 1994). The genus *Centaurea* also exhibits morphological similarity with other genera in the tribe Cardueae, such as *Cirsium* and *Jurinea* Cass. The genus *Centaurea* was subsequently divided into four genera, viz., *Centaurea* L., *Rhaponticoides* Vaill., *Psephellus* Cass. and *Cyanus* Mill. (WAGENITZ & HELLWIG 2000; GREUTER 2003a, b) in the light of recent investigations.

Differences in fatty acid profiles among taxa at different hierarchical levels have long been regarded as valuable characteristic patterns for a phylogenetic ap-

proach (ÖZCAN 2008; ÖZCAN *et al.* 2016; AYAZ *et al.* 2017). In addition to the importance of these biochemical markers in solving phylogenetic problems, their chemopreventive effects deriving from their biologically active compounds have also received wide attention in the treatment of various chronic or age-related degenerative diseases in humans (ROS & HU 2013). As the storage organs, in addition to their reproductive function in plants, seeds represent a unique nutritional source of essential fatty acids that the human body is unable to synthesise (KERMODE 2011; SARWAR *et al.* 2013). Seeds have also been considered as a potential source of oil-based ethanol (CHHETRI *et al.* 2008). It has frequently been postulated that diets rich in monounsaturated fatty acids (MUFAs, C18:1n9; oleic acid, OA) and some polyunsaturated fatty acids (PUFAs, C18:2n6; linoleic acid, LA, C18:3n3; linolenic acid, LN) can reduce or inhibit cardiovascular diseases, while diets rich in saturated fatty acids (SFAs) increase or trigger the risks of such diseases by raising serum concentrations of low-density lipoprotein (LDL) (RUXTON *et al.* 2004; DAMUDE & KINNEY 2008; SARWAR *et al.* 2013). Efforts have therefore been made to introduce new oil seed sources with high nutritional and pharmaceutical values against such chronic and degenerative diseases (SARWAR *et al.* 2013). Many edible plants are capable of producing natural chemopreventive compounds which have no synthetic counterparts and which play a protective role in the maintenance of human health (WU *et al.* 2017).

Members of the tribe Cardueae have attracted major interest in traditional medicine in a number of countries due to their biologically active metabolites (POJAR & MAC KINNON 1994; MOERMAN 2001; GÜRHAN & EZER 2004). Several studies have recently focused on the antioxidant properties of seed and seed fatty acid profiles in various species from the *Centaurea* group, one of the largest groups of the tribe Cardueae (Asteraceae). In that context, 16 species of the group's genera have been examined for their biological activities (YILDIRIM *et al.* 2009; AKTUMSEK *et al.* 2011, 2013; ERDOĞAN *et al.* 2014; AYAZ *et al.* 2017). The data obtained showed that the composition of fatty acids in the studied taxa differed considerably in the various genera.

In the present study, the fatty acid profile, antioxidant capacity and total phenolic compound (TPC) or total flavonoid (TF) content were determined in representative taxa (especially *Cirsium* and *Centaurea*) of the tribe Cardueae. Taxa selected from different sections were investigated in order to identify diagnostic characters at the tribal level. Here fatty acid profiles were examined in the cypselae of 11 taxa, including 14 populations (belonging to nine genera), some of which have not been previously reported. The data were evaluated from chemotaxonomic perspectives in order to develop an approach to solving taxonomic and phylogenetic

problems in the tribe, and in terms of antioxidant activity in order to be able to judge whether use of their biologically active compounds can be recommended in the prevention of chronic or degenerative diseases.

MATERIALS AND METHODS

Plant samples. The plant materials consisted of 11 Cardueae taxa (including nine genera and 14 accessions) growing naturally in NE Anatolia in Turkey. Plant materials (cypselae) were collected during the flowering period from different localities in Turkey during July, August and September from 2007 to 2013. The genera and taxa were arranged in phylogenetic order. Information concerning the collection, localities and voucher numbers of the 11 studied Cardueae taxa is provided in Table 1. The samples were identified following the taxonomy described by DAVIS (1975) and GÜNER *et al.* (2012).

Vouchers were deposited in the Artvin Coruh University Herbarium (ARTH).

Extraction of seed oils and total lipids. Mature cypselae (0.5 g DW) from at least three specimens per accession from air-dried (in shade) plants were pulverised and extracted with the aid of a Soxhlet extractor using *n*-hexane (3 × 30 ml, 2 h) and chloroform : methanol (2:1, v/v) to yield oils and total lipids, following the method described by FOLCH *et al.* (1957) with some minor modifications. The extracts were combined, concentrated under reduced pressure, diluted with chloroform and stored at -20°C until further analysis.

Fatty acid methyl ester (FAME) analysis. The method described by ICHIHARA *et al.* (1996) was followed to prepare fatty acid methyl esters (FAME) using transmethyl-lation. In brief, 10 mg of extracted oil was dissolved

Table 1. Collection data for the investigated Cardueae taxa.

Taxon	Locality	Voucher
<i>Arctium platylepis</i> (Boiss. & Bal.) Sosn. Grossh.	Trabzon; Araklı, Dağbaşı, above Çatak, roadsides, 920 m a.s.l., 22 September 2013	M. Ozcan 667
<i>Carthamus lanatus</i> L.	Artvin: Seyitler village, near university campus, wasted areas, 524 m, 06 August 2013	M. Ozcan 635
<i>Cirsium rhizocephalum</i> subsp. <i>sinuatum</i> (Boiss.) P.H.Davis & Parris	Trabzon/Gümüşhane: near Limon suyu, 2369 m a.s.l., 22 September 2013	M. Ozcan 665
	Rize: Cimil, high plateau, alpine grasses, 2650 m a.s.l., 16 September 2011	M. Ozcan 487
<i>C. simplex</i> C.A. Meyer subsp. <i>armenum</i> (DC.) Petr.	Rize: Çamlıhemşin Yukarı Kavrun high plateau, 2280 m a.s.l., 05 August 2011	M. Ozcan 452
	Trabzon: Bayburt - Trabzon boundary, near Soğanlı Pass, stream banks, 2290 m a.s.l., 18 August 2011	M. Ozcan 461
<i>C. subinerme</i> Fisch. & C.A. Mey.	Gümüşhane: Gezge village, stream banks, 1804 m a.s.l., 22 September 2013	M. Ozcan 666
	Bayburt, Kop Dağı, humid areas, among grass, 2438 m a.s.l., 20 August 2011	M. Ozcan 465
<i>Echinops orientalis</i> Trautv.	Gümüşhane: Torul, near Torul bridge, steppe area, roadsides, 931 m a.s.l., 28 August 2013	M. Ozcan 643
<i>Jurinea alpigena</i> K. Koch	Gümüşhane: Vauk mountain, roadsides, 1874 m a.s.l., 28 August 2013	M. Ozcan 645
<i>Onopordum turcicum</i> Danin	Gümüşhane: Vauk mountain, Kılıçören road, Sarıçiçek village, 2054 m a.s.l., 26 August 2007	M. Ozcan 152b
<i>Picnomon acarna</i> (L.) Cass.	Artvin: Seyitler village, near university campus, wasted areas, 530 m a.s.l., 06 August 2013	M. Ozcan 639
<i>Ptilostemon afer</i> (Jacq.) Greuter subsp. <i>eburneus</i> Greuter	Gümüşhane; near Kale, roadsides, 1374 m a.s.l., 15 September 2011	M. Ozcan 472
<i>Rhaponticum repens</i> (L.) Hidalgo	Artvin: Ardanuç, near hospital, roadsides, 526 m a.s.l., 14 July 2009	M. Ozcan 293

in 2 mL of hexane, followed by 4 mL of 2 M KOH in pure methanol. The mixture was then vortexed gently for at least 2 min at room temperature. After centrifugation (4000 rpm for 5 min), the hexane layer was taken for gas chromatography (GC) analyses. The GC running conditions of FAME followed the method that we recently published elsewhere (OZCAN *et al.* 2016; AYAZ *et al.* 2017). A Clarus 500 GC instrument (Perkin-Elmer, USA) equipped with an autosampler, a flame ionisation detector and a fused silica capillary SGE column (30 m × 0.32 mm ID 0.25 lm BP20 0.25 UM, USA) was used. The initial oven temperature was 140°C. It was held for 5 min, subsequently increased to 200°C at a rate of 2°C/min, and then increased to 220°C at a rate of 1°C/min. The injector and detector temperatures were set at 220 and 280°C, respectively. The sample size was 1 µL, and the carrier gas was controlled at 16 psi. The split ratio was 1:100. Fatty acid peaks were identified by comparing the retention times of FAME with the standard 37 components of the FAME mixture. Three replicate GC analyses were performed, and the results were expressed in GC area % as mean values ± standard deviation.

Extraction. Phenolic compounds were extracted by the same method recently employed by us with some minor modifications (OZCAN *et al.* 2016; AYAZ *et al.* 2017). In brief, 0.5 g of a cypselas sample was homogenised with 10 mL of methanol (80% v/v), and the homogenate was then fractionated with chloroform. The chloroform and methanol phases were then concentrated separately using a rotary evaporator below 40°C under reduced pressure. The aqueous residue was dried using a lyophiliser (Christ, Alpha 1-2LD plus, Germany). The methanol residue was dissolved in 1 mL of methanol, and the chloroform residue was dissolved in 1 mL of toluene and stored at -20°C for further analysis.

Determination of total phenolic compound (TPC) content and total flavonoid (TF) content. The method described by SLINKARD & SINGLETON (1977) was employed to measure TPC content in the methanol extract (500 µL) mixed with 2N Folin-Ciocalteu (FC) reagent (25 µL) and 2% Na₂CO₃ (975 µL). The reaction mixture was then kept in the dark for 30 min, and absorbance of the resulting blue colour was measured at 750 nm using a double-beam UV-vis spectrophotometer (Thermo, Evolution 100, England) against a gallic acid calibration curve. The result was expressed as mg of gallic acid equivalents (GAE)/100 g of dry weight (dw).

The method described by HUANG *et al.* (2004) was used to determine total flavonoid (TF) content of the methanol extract of a reaction mixture containing 500 µL of a sample and 500 µL of AlCl₃ (2%, w/v). After incubation, the mixture was kept in the dark for 30 min at room temperature, and the absorbance was measured at 415 nm with the same UV-vis spectrophotometer

against a calibration curve using quercetin. The result was expressed as mg of quercetin equivalents (QE) per 100 g of dw.

Measurement of extract antioxidant capacities. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity was measured using DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals according to a method based on the one described by BLOIS (1958), with slight modifications. One milliliter of DPPH in a methanol solution was mixed with 100 µL of methanol extract, and the reaction mixture was incubated at room temperature in the dark. The reaction mixture's absorbance was measured at 520 nm after 30 min, and the DPPH radical-scavenging activity was expressed as µmol of Trolox equivalents-100 g⁻¹ of dw.

The FRAP value of the methanol extract was determined according to the method described by BENZIE & STRAIN (1999), with slight modifications. Briefly, a total volume of the FRAP reagent (3000 µL)-consisting of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) and 20 mM FeCl₃·6H₂O in proportions of 10:1:1 (v/v/v) were shaken vigorously and incubated at 37°C for 30 min. The absorbance of the mixture was read at 593 nm against a blank and a calibration prepared with Trolox, and activity was expressed as µmol of Trolox equivalents (TE) 100 g⁻¹ of dw.

The cupric ion-reducing antioxidant capacity (CUPRAC) of the extract was determined using a modification of the method described by ARAK *et al.* (2004). Absorbance of the reaction mixture (4 mL; 10 mM neocuproine, 7.5 mM CuCl₂, 1 mM acetate buffer-pH 7.0, and sample extract) was read at 450 nm using a UV-VIS spectrophotometer, and activity was expressed as µmol of TE g⁻¹ of dw.

Oxygen radical absorbance capacity (ORAC) was determined as described by OU *et al.* (2001). A reaction mixture containing 100 µL of 500 nmol/L fluorescein and 25 µL of diluted extracts pipetted into each working well of a microplate was mixed with 25 µL of 250 mmol/L AAPH. After shaking the microplate for 5 s, the fluorescence (excitation and emission wavelengths of 485 and 510 nm, respectively) was read every 3 min for 90 min using a Multiskan Ascent device (Labsystems, Helsinki, Finland) under a net area of the curve used to calculate antioxidant capacity expressed as TE g⁻¹ of dw.

Statistical analysis. All extractions and analyses were performed in triplicate ($n = 3$), and the data are presented as the mean ± the pooled standard deviation. Data were analysed using one-way ANOVA and Duncan's multiple range test (IBM SPSS Statistics Ver. 22.0) for comparison of means at a significance level of $P < 0.05$. A statistical software package was employed to perform principal component analysis (PCA) and cluster analysis

using agglomerative hierarchical clustering (AHC) (XLSTAT statistical and data analysis solution, Addinsoft, 2019, Long Island, NY, USA).

RESULTS AND DISCUSSION

Phenolic compound content and antioxidant capacity of Cardueae taxa. The antioxidant capacity values TPC and TF content in both methanol and oil extracts are given in Table 2. They differed significantly ($P < 0.05$) among the studied taxa. The TPC content in the taxa ranged from 752.14 to 428.17 mg/100 g of dw, while the TF content ranged from 336.41 to 132.17 mg/100 g of dw, being highest in *Cirsium simplex* subsp. *armenum* and lowest in *Carthamus lanatus*. The highest ($\mu\text{mol/g}$ of dw) DPPH (147.9) and FRAP (86.42), values were determined for *C. simplex* subsp. *armenum* and *C. subinerme*, respectively, and the highest CUPRAC (92.21) and ORAC (51.24) values for *C. rhizocephalum* subsp. *sinuatum*, while *Carthamus lanatus* and *Arctium platylepis* exhibited the lowest DPPH (65.94), ORAC (22.1) and FRAP (32.32) values (Table 2).

Highly significant and strong correlations and linear regressions were found between the values of phenolic compound content and antioxidant capacity recorded in methanol extracts of cypselae of the various studied Cardueae taxa Fig. 1A-D. Thus, antioxidant capacity values in the methanol extract were in strong positive correlation with TPC (range; $r = 0.897 - 0.947$, $P < 0.05$) and TF (range; $r = 0.871 - 0.942$, $P < 0.05$) content and exhibited moderate linear relationships with FRAP ($R^2 = 0.594$), CUPRAC ($R^2 = 0.627$) and ORAC ($R^2 = 0.574$) and high linear relationships with DPPH ($R^2 = 0.757$). The TF content exhibited strong linear relationships with CUPRAC ($R^2 = 0.749$), FRAP ($R^2 = 0.851$) and ORAC ($R^2 = 0.849$), and high correlation with DPPH ($R^2 = 0.877$) (Fig. 1A-D).

The content of phenolic compounds (TPC or TF) and DPPH values in oil extract also differed significantly ($P < 0.05$) among the studied taxa (Table 2). *Cirsium simplex* subsp. *armenum* exhibited the highest concentrations of both TPC (198.74 mg/100 g of dw) and TF (88.21 mg/100 g of dw), while *Arctium platylepis* and *Carthamus lanatus* had the lowest concentrations (TPC: 105.41, TF: 35.14 mg/100 g of dw). Similarly, the lowest and highest DPPH values were in *C. lanatus* and *C. rhizocephalum* subsp. *sinuatum*, respectively, ranging from 38.44 to 76.14 mmol of TE/g of dw.

The relationship (R^2) between DPPH values and TPC or TF content in the oil extract of different Cardueae taxa is given in Fig. 1E. The average DPPH values of oil extracted from the examined Cardueae taxa were in strong positive correlation with TPC ($r = 0.917$, $P < 0.05$) and TF ($r = 0.898$, $P < 0.05$) content. Moreover, TPC content exhibited a strong linear relationship with DPPH values ($R^2 = 0.806$), while TF content exhibited a moderate linear relationship with DPPH value ($R^2 = 0.532$) (Fig. 1E).

Principal component analysis (PCA) was employed in order to better visualise potential differences in the content of phenolic compounds and antioxidant capacity values in methanol extract as compared with oil extracts obtained from cypselae of all of the studied taxa and to easily characterise their distribution. This reduced the active variables (TPC, TF, DPPH, FRAP, CUPRAC and ORAC) of methanol and oil extracts of the six taxa (sin, arm, ala, pcn, o.tr and pti, see Fig. 1F) in the upper and lower right-hand quadrants of PC1 (88.47% of the total variance), with highly significant strong correlations (range; $r = 0.730$ to 0.967 , $P < 0.05$, Fig. 1F). The remaining five taxa in the upper and lower left-hand quadrants were negatively loaded on PC2 with a correspondingly low variance (5.32% of the total variance), and they were not associated or correlated with any of the active variables. The conducted PCA showed that phenolic compound content and antioxidant capacity of the taxa *Cirsium rhizocephalum* subsp. *sinuatum* (sin), *C. simplex* subsp. *armenum* (arm), *Onopordum turcicum* (o.tr), *Picnomon acarna* ("pcn") and *Echinops orientalis* ("ech") are unique (Fig. 1F).

Fatty acid profiles of Cardueae taxa. The fatty acid profiles of the investigated Cardueae taxa are shown in Table 3. The concentrations differed significantly ($P < 0.05$) among them. The main unsaturated fatty acid was linoleic acid (C18:2n6), followed by oleic acid (C18:1n9) and palmitic acid (C16:0). The average linoleic acid content was 62.32%, ranging between 70.49% (*Onopordum turcicum*) and 44.36% (*Jurinea alpigena*). The oleic acid concentration averaged 18.44%, ranging from 23.71% (*Rhaponticum repens*) to 11.41% (*Cirsium simplex* subsp. *armenum*). The palmitic acid concentration among the taxa varied between 4.51% (*Onopordum turcicum*) and 12.22% (*C. simplex* subsp. *armenum*), averaging 8.46% (Table 3).

Intra- and interspecies differences in the concentrations of fatty acids and their different sums were also determined in all of the investigated Cardueae taxa (11 taxa, 14 accessions). Oil content in two accessions of *Cirsium rhizocephalum* subsp. *sinuatum* differed significantly (Trabzon: $7.43 \pm 0.62\%$, Rize: $3.14 \pm 0.45\%$), being 2.4 times greater in the former than in the latter, while the levels of oil content in *C. simplex* subsp. *armenum* (Rize: $5.97 \pm 0.72\%$, Trabzon: $6.16 \pm 0.84\%$) and *C. subinerme* (Gümüşhane: $6.25 \pm 0.83\%$, Bayburt: $7.45 \pm 0.75\%$) were almost identical (Table 3). However, the concentration of linoleic acid in the Trabzon accession of *C. rhizocephalum* subsp. *sinuatum* was ~1.3 times greater than in the Rize accession and ~1.1 times greater in the Trabzon accession of *C. simplex* subsp. *armenum* than in the Rize accession. On the other hand, the difference of fatty acid concentrations in *C. subinerme* collected from the Gümüşhane and Bayburt accessions was not as great as that recorded in the two *Cirsium* species. Oleic acid

Table 2. Values of antioxidant capacity and phenolic compound content in methanol and oil extracts of cypselae of some Cardueae taxa. An analysis of variance (SPSS version 22, one-way ANOVA) was used for comparison of the means. Means in the same column followed by different letters in superscript are significantly different ($P < 0.05$).

Taxon	Methanol extract						Oil extract					
	TPC	TF	DPPH	FRAP	CUPRAC	ORAC	TPC	TF	TPC	TF	DPPH	
<i>Arctium platylepis</i>	428.65 ± 0.52 ^a	178.88 ± 0.95 ^d	87.55 ± 0.88 ^b	32.32 ± 0.03 ^a	41.04 ± 1.35 ^a	24.57 ± 0.57 ^b	105.41 ± 0.27 ^a	46.40 ± 0.81 ^c	105.41 ± 0.27 ^a	46.40 ± 0.81 ^c	38.71 ± 0.23 ^a	
<i>Carthamus lanatus</i>	428.17 ± 0.36 ^a	132.19 ± 1.11 ^a	65.94 ± 0.06 ^a	38.23 ± 0.35 ^b	42.28 ± 0.87 ^a	22.11 ± 0.02 ^a	112.90 ± 0.10 ^b	35.14 ± 0.17 ^a	112.90 ± 0.10 ^b	35.14 ± 0.17 ^a	38.44 ± 0.31 ^a	
<i>Cirsium rhizocephalum</i> subsp. <i>sinuatum</i> ^T	681.86 ± 0.86 ^b	265.86 ± 1.70 ^g	134.18 ± 0.95 ^f	78.51 ± 1.01 ^h	73.18 ± 0.36 ^d	40.46 ± 1.28 ^f	180.80 ± 0.60 ^b	75.40 ± 0.39 ^g	180.80 ± 0.60 ^b	75.40 ± 0.39 ^g	65.52 ± 0.33 ^h	
<i>C. rhizocephalum</i> subsp. <i>sinuatum</i> ^R	712.76 ± 0.38 ⁱ	273.52 ± 2.41 ^h	143.11 ± 0.31 ^h	85.23 ± 1.41 ^k	92.21 ± 0.57 ^h	51.24 ± 1.11 ^j	188.13 ± 0.26 ⁱ	85.24 ± 0.24 ^h	188.13 ± 0.26 ⁱ	85.24 ± 0.24 ^h	76.14 ± 0.41 ^k	
<i>C. simplex</i> subsp. <i>armenum</i> ^R	750.23 ± 4.36 ^j	333.12 ± 2.01 ^m	147.90 ± 1.67 ⁱ	83.49 ± 0.59 ^j	83.73 ± 0.87 ^f	49.74 ± 1.22 ⁱ	196.54 ± 1.01 ^j	87.14 ± 0.16 ⁱ	196.54 ± 1.01 ^j	87.14 ± 0.16 ⁱ	68.18 ± 0.41 ^j	
<i>C. simplex</i> subsp. <i>armenum</i> ^T	752.14 ± 3.41 ^j	336.41 ± 3.12 ^m	147.81 ± 0.84 ⁱ	83.74 ± 0.81 ^j	82.68 ± 0.88 ^f	47.11 ± 1.53 ^h	198.74 ± 1.14 ^j	88.21 ± 0.25 ⁱ	198.74 ± 1.14 ^j	88.21 ± 0.25 ⁱ	68.78 ± 0.57 ^j	
<i>C. subinermis</i> ^G	588.61 ± 1.13 ^e	250.79 ± 0.55 ^f	126.15 ± 0.77 ^d	73.46 ± 0.86 ^f	75.77 ± 0.53 ^e	27.39 ± 0.14 ^c	152.58 ± 0.77 ^d	66.03 ± 0.14 ^c	152.58 ± 0.77 ^d	66.03 ± 0.14 ^c	50.49 ± 0.33 ^e	
<i>C. subinermis</i> ^B	675.12 ± 0.76 ^g	283.12 ± 0.24 ⁱ	138.64 ± 0.24 ^g	86.42 ± 1.21 ^k	87.12 ± 0.48 ^g	38.12 ± 0.24 ^c	173.62 ± 0.72 ^g	73.44 ± 0.12 ^f	173.62 ± 0.72 ^g	73.44 ± 0.12 ^f	67.52 ± 0.11 ⁱ	
<i>Echinops orientalis</i>	545.75 ± 1.04 ^d	236.19 ± 0.92 ^e	132.19 ± 0.08 ^e	66.89 ± 0.05 ^e	52.42 ± 0.32 ^b	33.43 ± 0.88 ^d	113.38 ± 0.36 ^b	53.40 ± 0.32 ^d	113.38 ± 0.36 ^b	53.40 ± 0.32 ^d	41.55 ± 0.32 ^b	
<i>Jurinea alpigena</i>	487.81 ± 0.06 ^c	166.99 ± 0.01 ^c	86.16 ± 0.75 ^b	51.16 ± 0.03 ^d	63.82 ± 0.3 ^c	26.16 ± 0.25 ^c	113.48 ± 0.50 ^b	45.00 ± 0.36 ^c	113.48 ± 0.50 ^b	45.00 ± 0.36 ^c	47.64 ± 0.18 ^d	
<i>Onopordium turcicum</i>	647.38 ± 1.50 ^f	303.96 ± 0.40 ⁱ	138.86 ± 0.98 ^h	75.87 ± 0.02 ^g	86.53 ± 0.54 ^g	43.52 ± 0.01 ^g	157.58 ± 0.53 ^c	75.48 ± 0.55 ^g	157.58 ± 0.53 ^c	75.48 ± 0.55 ^g	53.55 ± 0.20 ^f	
<i>Picnomon acarna</i>	675.38 ± 0.5 ^g	287.16 ± 0.01 ^k	144.28 ± 0.16 ^h	81.57 ± 0.09 ⁱ	85.57 ± 0.88 ^g	46.91 ± 0.38 ^h	160.81 ± 0.30 ^f	73.13 ± 0.27 ^f	160.81 ± 0.30 ^f	73.13 ± 0.27 ^f	63.34 ± 0.35 ^g	
<i>Philostemon ajfer</i> subsp. <i>eburneus</i>	588.93 ± 1.06 ^e	281.55 ± 0.32 ⁱ	125.55 ± 0.20 ^h	76.02 ± 0.01 ^g	73.99 ± 0.05 ^d	39.10 ± 0.9 ^e	144.69 ± 0.71 ^c	54.44 ± 0.47 ^d	144.69 ± 0.71 ^c	54.44 ± 0.47 ^d	45.58 ± 0.29 ^c	
<i>Rhaponticum repens</i>	460.94 ± 0.61 ^b	156.54 ± 0.46 ^b	97.17 ± 0.40 ^c	43.39 ± 0.16 ^c	52.33 ± 0.34 ^b	23.78 ± 0.78 ^a	143.02 ± 0.82 ^c	41.25 ± 0.45 ^b	143.02 ± 0.82 ^c	41.25 ± 0.45 ^b	45.56 ± 0.32 ^c	

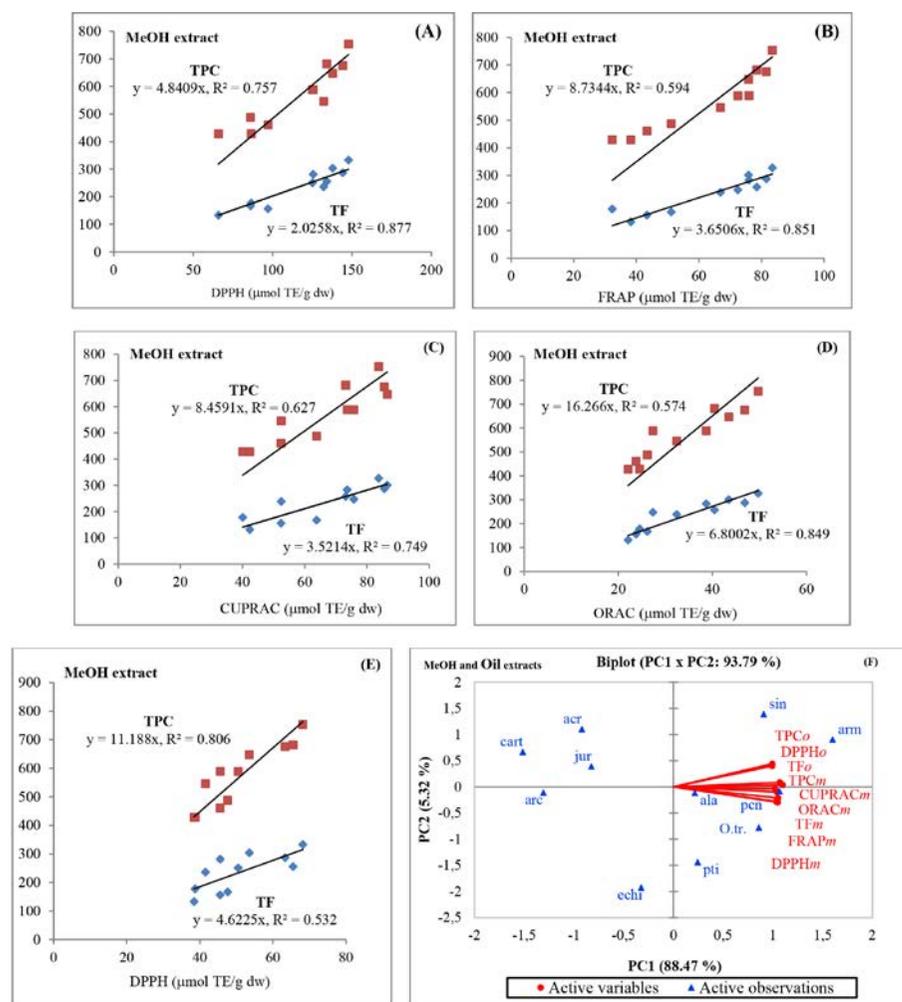


Fig. 1. Relationships between antioxidant capacity and total phenolic compound (TPC) or total flavonoid (TF) content in methanol extract of cypsela from some Cardueae taxa (A-E); and principal component analysis (PCA) of some Cardueae taxa based on antioxidant capacity and total phenolic compound (TPC) or total flavonoid (TF) content of methanol and oil extracts (F). Each code represents a taxon. Taxa are coded as follows: arc = *Arctium platylepis*; arm = *Cirsium simplex* subsp. *armenum*; crt = *Carthamus lanatus*; ech = *Echinops orientalis*; jur = *Jurinea alpigena*. o.tr = *Onopordum turcicum*; pcn = *Picnomon acarna*; pti = *Ptilostemon afer* subsp. *eburneus*; rrp = *Rhaponticum repens*; sin = *Cirsium rhozocephalum* subsp. *sinuatum*; sub = *C. subinermis*. m: methanol extract; o: oil extract. Values differ from 0 with an alpha significance level = 0.05.

concentrations in the accessions were ~1.8-, 1.5- and 1.2 times higher, respectively. However, the palmitic acid concentrations were almost identical (~1-fold) (Table 3). A similar situation was also observed with respect to the concentrations of total saturated and unsaturated fatty acids in different accessions. While the ratios of total PUFAs (1.3, 1.1 and 1.1, respectively) and SFAs (1.3, 1.1 and 1.1, respectively) were almost the same, the ratio total content of MUFAs was noticeably higher (1.8, 1.5 and 1.2, respectively).

Multivariate analyses. Principal component analysis (PCA) of fatty acid composition revealed that the first two PC factors accounted for approximately 60.04% of the total variance. The first principal component (PC1) explained 36.51% of the variance, and the second (PC2) explained 23.53%. Two distinct groups were separated along the the first two axes. The taxa coded as crt, arm, sin, sub and arc were separated as one group. The four taxa coded as pcn, ech, pti and rrp comprised the second

group, and the remaining two taxa (o.tr and jur) were established as the third and fourth groups, respectively (Fig. 2A, B). Only the main fatty acids (18:2 and 18:1) of the studied taxa were significantly high and strongly correlated (negatively or positively, $\alpha = 0.05$) with total fatty acid content (e.g., MUFA: $r = -0.841$ and 0.708 , PUFA: $r = 0.910$ and -0.677 , respectively), as were the ratios of n3/n6 or n6/n3 fatty acids with 18:3 ($r = 0.638$ and 0.991 or -0.828 and -0.842).

In the bi-plot, only oleic acid (C18:1n9) was located on the right side of the figure, whereas all the other fatty acids were situated on the left side. Linolenic acid and palmitic acid were in the lower left-hand part of the figure. As can be seen, C16:1, C16:0, C18:3n3, C18:0 and C18:2n6 exhibited negative loading on PC1. *Cirsium* taxa, *Arctium platylepis* and *Jurinea alpigena* were located together on the negative side, while *Carthamus lanatus*, *Picnomon acarna*, *Echinops orientalis*, *Ptilostemon afer* subsp. *eburneus* and *Rhaponticum repens* were positioned on the positive side (Fig. 2A, B).

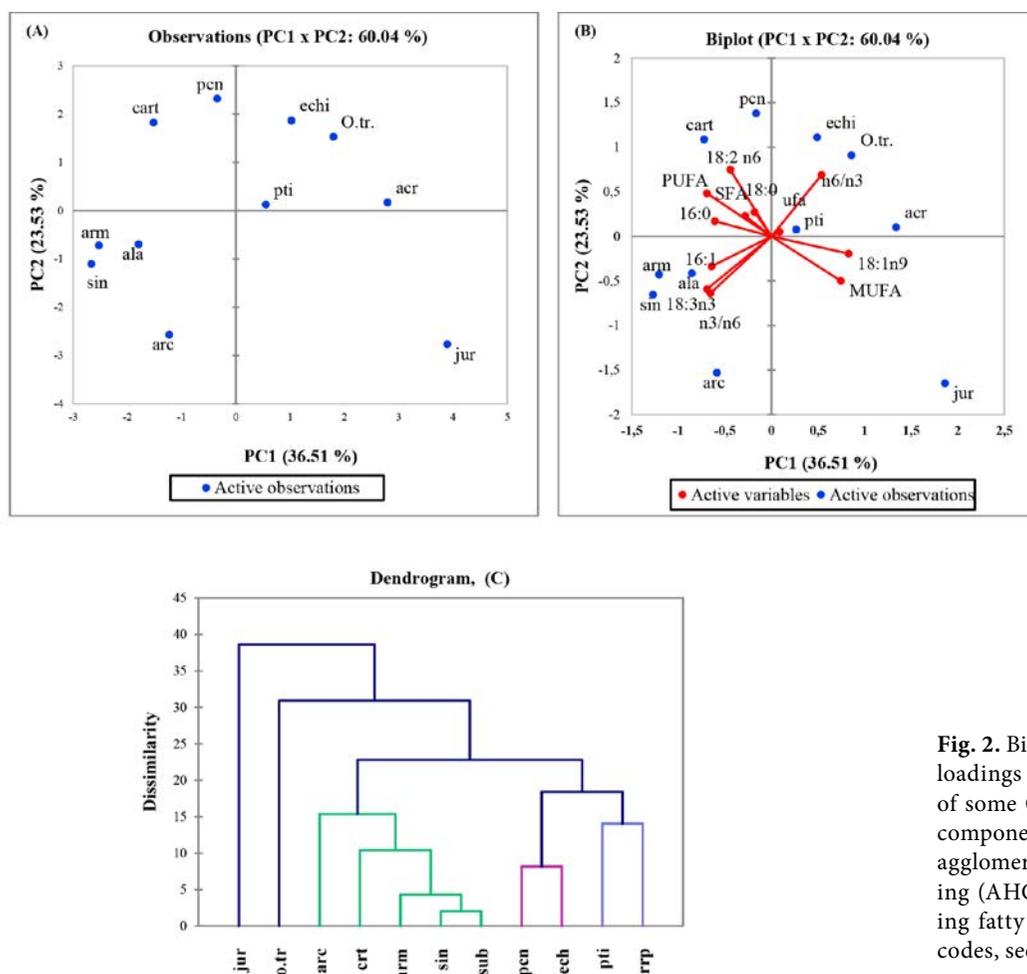


Fig. 2. Biplot (PC1 x PC2) of scores and loadings of fatty acids of the cypselae of some Cardueae taxa from principal component analysis (PCA) (A, B); and agglomerative hierarchical clustering (AHC) in some Cardueae taxa using fatty acid characters (C). For taxa codes, see Fig.1

The bi-plot also reveals which components of taxa are correlated and responded similarly to the PCs. Three main groups emerged based on the PC axes: group 1 included the taxa coded jur, rrp, pti, o.tr and pti; group 2 included pcn and crt; and group 3 included the three *Cirsium* taxa (arm, sin, sub) and arc. The components C18:3n3 and C16:1 were capable of identifying the taxa present in the genus *Cirsium* and *Arctium platylepis*, while C18:2n6, C18:0 and C16:0 identified *Carthamus lanatus* and *Picnomon acarna*. However, only C18:1n9 alone can be used to differentiate the other plants in the tribe Cardueae.

For cluster analysis (UPGMA, unweighted pair group method with arithmetic mean), 10 fatty acid traits of 11 taxa (14 accessions) were analysed and their interspecific relationships were observed (Tables 3-4; Fig. 2C). The phenogram shows that three major groups were separated from all other taxa in distinct positions. The delimitation of these groups is mainly based on the occurrence of oleic acid and linoleic acid ($P < 0.05$). *Jurinea alpigena* was separated at the top level (Euclidean 39.0) from the other taxa, indicating the distinctive nature of *J. alpigena*. The shortest distance between *Cirsium*

taxa was recorded between *C. subinerme* and *C. simplex* subsp. *armenum*, where the distance coefficient was approximately 3.0. This result is in accordance with their morphological characteristics.

The taxa were divided into three major groups on the dendrogram (Fig. 2C). The first group included nine taxa and was divided into two major clusters with a dissimilarity level of approximately 23.0. The first of these clusters consisted of two sub-clusters with dissimilarity level of approximately 17.0. The first sub-cluster was the more heterogeneous and consisted of *Ptilostemon afer* subsp. *eburneus* and *Rhaponticum repens* with a distance coefficient of 13.0, while the second sub-cluster included *Picnomon acarna* and *Echinops orientalis* at a dissimilarity level of almost 8.0. The second cluster was divided into three sub-clusters, the first consisting of *Arctium platylepis* and the second of *Carthamus lanatus*. The former species (*Arctium platylepis*) of this cluster was split off from the other four taxa with a distance coefficient of approximately 15.0. The third subcluster included only *Cirsium* taxa, *C. simplex* subsp. *armenum*, *C. rhizocephalum* subsp. *sinuatum* and *C. subinerme*, which indicated their high similarity. These three taxa were very similar

Table 3. Fatty acid composition (% dw) of cypselae of the Cardueae taxa examined.

Taxon	Palmitic acid	Palmitoleic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Σ main fatty acid	Σ other fatty acid
<i>Arctium platylepis</i>	6.03±0.01 ^b	0.78±0.01 ^f	2.30±0.02 ^d	20.26±0.02 ^h	59.34±0.14 ^e	6.20±0.01 ^j	95.45	4.55
<i>Carthamus lanatus</i>	8.46±0.03 ^f	0.41±0.02 ^c	2.53±0.06 ^c	13.66±0.04 ^e	69.39±0.01 ^j	0.92±0.04 ^d	98.22	1.78
<i>Cirsium rhizocephalum</i> subsp. <i>sinuatum</i> ^T	8.06±0.02 ^e	0.52±0.04 ^{de}	1.93±0.02 ^b	12.16±0.02 ^b	68.65±0.05 ⁱ	6.49±0.01 ^k	98.61	1.39
<i>C. rhizocephalum</i> subsp. <i>sinuatum</i> ^R	10.51±0.44 ^d	0.59±0.17 ^e	3.42±0.18 ^s	22.35±0.06 ^f	54.46±0.39 ^b	4.38±0.11 ^s	95.71	4.29
<i>C. simplex</i> subsp. <i>armenum</i> ^R	9.33±0.18 ^h	0.57±0.09 ^e	3.24±0.21 ^{ab}	17.78±0.23 ^f	61.98±0.18 ^f	4.64±0.14 ^b	97.54	2.46
<i>C. simplex</i> subsp. <i>armenum</i> ^T	12.22±0.09 ^j	0.81±0.03 ^f	1.84±0.07 ^f	11.41±0.12 ^a	69.39±0.35 ^f	2.04±0.03 ^e	97.71	2.29
<i>C. subinermis</i> ^G	9.54±0.02 ⁱ	0.42±0.07 ^{cd}	2.43±0.01 ^{de}	16.24±0.01 ^e	63.60±0.07 ^s	4.84±0.03 ⁱ	97.24	2.76
<i>C. subinermis</i> ^B	9.28±0.05 ^h	0.53±0.06 ^e	2.33±0.12 ^d	20.21±0.18 ^h	62.10±0.25 ^f	3.41±0.04 ^f	97.86	2.14
<i>Echinops orientalis</i>	8.93±0.05 ^s	0.38±0.02 ^c	2.46±0.07 ^{de}	20.74±0.04 ^f	66.30±0.02 ^h	0.24±0.01 ^a	99.18	0.82
<i>Jurinea alpigena</i>	6.66±0.08 ^c	0.22±0.03 ^{ab}	2.42±0.10 ^{de}	22.54±0.35 ^f	44.36±0.70 ^a	0.85±0.01 ^d	97.38	2.62
<i>Onopordum turcicum</i>	4.51±0.10 ^d	0.20±0.01 ^{bc}	2.02±0.02 ^a	19.99±0.11 ^d	70.49±0.44 ^f	0.43±0.21 ^c	97.64	2.36
<i>Picnomon acarna</i>	10.29±0.02 ^j	0.37±0.01 ^c	3.29±0.01 ^{fg}	17.98±0.02 ^f	66.25±0.01 ^h	0.34±0.00 ^b	99.15	0.85
<i>Ptilostemon ajfer</i> subsp. <i>eburneus</i>	9.01±0.03 ^s	0.71±0.03 ^f	2.10±0.09 ^c	19.23±0.08 ^s	58.52±0.10 ^d	0.30±0.02 ^{ab}	89.87	10.13
<i>Rhaponticum repens</i>	5.70±0.09 ^a	0.19±0.01 ^a	1.89±0.02 ^b	23.71±0.10 ^k	57.70±0.32 ^c	0.29±0.00 ^{ab}	95.14	4.86

An analysis of variance (SPSS version 22, one-way ANOVA) was used for comparison of the means. Values with the same letter within a column are not significantly different at $P < 0.05$. Accessions: T: Trabzon (2369 m a.s.l), R: Rize (2650 m a.s.l), G: Gümüşhane (1804 m a.s.l), B: Bayburt (2438 m a.s.l).

Table 4. Total amounts of fatty acids (% dw) and some ratios in cypselae of the Cardueae taxa examined*.

Taxon	ΣSFAs	ΣMUFA	ΣPUFA	ΣUFA	ω 3/ω6	ω 6/ω3	Total lipids
<i>Arctium platylepis</i>	9.13±0.02 ^a	21.04±0.02 ⁱ	65.54±0.05 ^d	86.58±0.15 ^g	0.10±0.00 ^j	9.57±0.02 ^a	8.34 ± 0.38
<i>Carthamus lanatus</i>	13.84±0.05 ^h	14.08±0.06 ^c	70.31±0.03 ^h	84.39±0.09 ^d	0.001±0.00 ^e	75.25±3.22 ^e	4.62 ± 0.11
<i>Cirsium rhizocephalum</i> subsp. <i>sinuatum</i> ^T	10.79±0.03 ^b	12.68±0.06 ^b	75.14±0.04 ^j	87.82±0.08 ^b	0.09±0.00 ⁱ	10.58±0.02 ^a	7.43 ± 0.62
<i>C. rhizocephalum</i> subsp. <i>sinuatum</i> ^R	13.93±0.62 ^h	22.95±0.22 ^j	58.84±0.48 ^c	81.79±0.28 ^b	0.08±0.00 ^h	12.44±0.23 ^{ab}	3.14 ± 0.45
<i>C. simplex</i> subsp. <i>armenum</i> ^R	12.57±0.57 ⁱ	18.35±0.32 ^f	66.63±0.20 ^e	84.98±0.22 ^f	0.07±0.00 ^g	13.36±0.40 ^{ab}	5.97 ± 0.72
<i>C. simplex</i> subsp. <i>armenum</i> ^T	14.06±0.16 ^h	12.21±0.14 ^a	71.42±0.38 ⁱ	83.64±0.48 ^c	0.03±0.00 ^e	34.07±0.37 ^c	6.16 ± 0.84
<i>C. subinerm</i> ^G	12.14±0.02 ^e	16.66±0.06 ^e	68.43±0.09 ^f	85.09±0.08 ^f	0.08±0.00 ^g	13.15±0.08 ^{ab}	6.25 ± 0.83
<i>C. subinerm</i> ^B	11.61±0.12 ^d	20.74±0.15 ^h	65.51±0.23 ^d	86.25±0.33 ^g	0.05±0.00 ^f	18.21±0.24 ^b	7.45 ± 0.75
<i>Echinops orientalis</i>	11.52±0.11 ^d	21.12±0.04 ⁱ	66.54±0.03 ^e	87.66±0.03 ^h	0.003±0.00 ^a	272.57±6.31 ^h	8.02 ± 0.13
<i>Jurinea alpigena</i>	10.77±0.18 ^b	41.40±0.26 ⁱ	45.21±0.71 ^a	86.61±0.56 ^g	0.02±0.00 ^d	51.98±0.66 ^d	7.30 ± 0.51
<i>Onopordium turcicum</i>	6.53±0.12 ^{cd}	20.18±0.11 ^d	70.92±0.47 ^g	91.10±0.54 ^{de}	0.01±0.00 ^b	210.58±26.25 ^f	7.33 ± 0.66
<i>Picnomon acarna</i>	14.22±0.03 ^b	18.35±0.02 ^f	78.82±10.59 ^e	84.94±0.02 ^{ef}	0.005±0.00 ^a	194.86±0.02 ^g	8.32 ± 0.22
<i>Ptilostemon ajfer</i> subsp. <i>eburneus</i>	11.11±0.11 ^{bc}	19.94±0.10 ^g	58.81±0.11 ^c	78.75±0.13 ^a	0.01±0.00 ^a	197.59±10.04 ^g	9.28 ± 0.11
<i>Rhaponticum repens</i>	13.25±0.10 ^g	23.89±0.10 ^k	57.99±0.32 ^b	81.88±0.41 ^b	0.005±0.00 ^a	198.97±1.11 ^g	1.45 ± 0.04

Analysis of variance (SPSS version 22, one-way ANOVA) was used for comparison of the means. Values with the same letter within a column are not significantly different at $P < 0.05$. Accessions: T: Trabzon (2369 m a.s.l.); R: Rize (2650 m a.s.l.); G: Gümüşhane (1804 m a.s.l.); B: Bayburt (2438 m a.s.l.).

to each other in terms of morphological properties. The second and third groups included only one taxon each, *Onopordum turcicum* and *Jurinea alpigena*, respectively.

For all of the investigated taxa, the fatty acid composition and antioxidant activity of fruits are presented here for the first time, except in the case of the fatty acid composition of *Carthamus lanatus*. MURTHY & ANJANI (2007) previously reported fruit oil compositions of seven *Carthamus* species from India. The four main fatty acids identified were palmitic acid (5.7 to 9.7%), stearic acid (1.6 to 6.3%), oleic acid (11 to 68%) and linoleic acid (61 to 82%). A high percentage of linoleic acid (64.4 to 76.8%) was determined in *C. lanatus*, which also exhibited the highest oleic acid content (23.2%), followed by palmitic acid (9.7%). Another report gave an average saturated fatty acid content of 10.1% and an average unsaturated fatty acid content of 89.0% (ARSLAN & TARIKAHYA-HACIOGLU 2018) for plants from Turkey, and suggested that the section *Carthamus* and section *Atractylis* of the genus *Carthamus* can be differentiated on the basis of their fatty acid ratios. Our findings are in good agreement with those in previously published literature. There have also been some previous reports that treated the fatty acid compositions of genera from the tribe Cardueae, such as *Centaurea* (AKTUMSEK *et al.* 2013; TEKELI *et al.* 2013; AYAZ *et al.* 2017) and *Cirsium* (OZCAN *et al.* 2016). Similar to our results, oleic and linoleic acids were the dominant fatty acid among the examined taxa. JOHNSON & FRITSCH (2012) reported that oleic and linoleic acids possess the ability to lower blood cholesterol levels, and consumption of these fatty acids is highly recommended by nutritionists and health professionals. In addition, PARIKH *et al.* (2005) reported that oleic acid has the ability to reduce levels of LDL and possibly increase those of HDL. Linolenic acid exhibits a protective effect against heart disease and has been shown to play a role in development of the brain and retina (CONNOR 1999). AKTUMSEK *et al.* (2013) proposed that *Centaurea* extracts and oils might be used as a source of natural antioxidants and unsaturated fatty acids for nutritional and pharmacological purposes. In addition to their consumption as food, plant oils have also been used in industrial products such as plastics, pharmaceutical products, inks, adhesives, coatings, etc. (SALIMON *et al.* 2012). AYAZ *et al.* (2017) asserted that relative proportions and quantities of fatty acids can be used as additional biochemical markers in the taxonomy of *Centaurea*. Fatty acids have been regarded as a highly important chemo-taxonomical marker in the systematic classification of plant species, and they have been used in many plant groups (STUESSY 2009; ZHANG *et al.* 2015; OZCAN *et al.* 2016). AKTUMSEK *et al.* (2013) examined four *Centaurea* taxa and determined a significant relationship between antioxidant capacity and total phenolic components. Large amounts of phenolic compounds and flavonoids were found in these *Centaurea* taxa. Sim-

ilarly, AYAZ *et al.* (2017) investigated various *Centaurea* taxa and determined high TPC and TF content among them. We investigated 11 Cardueae taxa, and high TPC and TF values were detected among the taxa, ranging from 105.41 to 196.54 and from 35.14 to 87.14, respectively (Table 2).

Significant high variations were observed in antioxidant capacity values and the content of phenolic or flavonoid compounds among the studied taxa, regardless of whether collected from the same or different geographical regions or at different altitudes. Altitude and temperature have been identified as two of the major environmental factors affecting plant chemical composition despite genetic differences (LIU *et al.* 2016). For instance, *Cirsium rhizocephalum* subsp. *sinuatum* collected from a higher altitude (2650 m a.s.l. from Rize) and specimens of the same species from a lower altitude (2369 m a.s.l. from Trabzon) differed significantly with respect to oleic acid (22.35 and 12.6%), linoleic acid (54.46 and 68.65%) and the sums of MUFAs (22.95 and 12.68%) and PUFAs (58.84 and 75.14%), respectively. In like manner, despite being collected from similar altitudes, oleic acid (17.78 and 11.41%) and linoleic acid (61.98 and 69.39%) content in *C. simplex* subsp. *armenum* also differed, as did the sum of MUFAs (18.35 and 12.21%) and PUFAs (66.63 and 71.42%), between altitudes of 2280 m (Rize) and 2290 m (Trabzon), respectively. In addition, concentrations of the same fatty acids also varied in samples of *C. subinermis* collected from the Gümüşhane (1804 m a.s.l.) and Bayburt (2438 m a.s.l.) provinces (see Tables 3-4 for greater detail).

Altitude and geography or region significantly affected antioxidant capacity values and total phenolic compound (TPC and TF) content. For *Cirsium rhizocephalum* subsp. *sinuatum* and *C. subinermis*, the antioxidant capacity values of phenolic compounds in methanol and oil extracts were higher in specimens collected at high altitudes in Rize (2650 m a.s.l.) and Bayburt (2438 m a.s.l.) than in specimens collected at lower altitudes (2369 and 1804 m a.s.l.). However, the data for total phenolic compound content (TPC and TF) and antioxidant capacity values in *C. simplex* subsp. *armenum* collected from the Trabzon and Rize provinces at approximately the same altitudes (2280 and 2290 m a.s.l.) were not significantly different and were even slightly higher in samples from the Trabzon province (see Table 2 for greater detail). Two *Tripleurospermum rosellum* var. *album* locations at 220 m in the province of Çanakkale and at 1800-1850 m in the province of Rize in the far western and eastern regions of Turkey were also recently reported to differ with respect to antioxidant capacity and TPC and TF content. The specimen collected from the higher altitude exhibited higher phenolic content and greater antioxidant capacity (COLAK *et al.* 2017). A varietal difference of antioxidant capacity and TPC or TF content in the capitulum was also determined between *T. oreades* var.

tchihatchewii growing at 2185 m in the Artvin (Şavşat) province and *T. oreades* var. *oreades* growing at 1719 m in the Giresun (Kümbet) province (COLAK *et al.* 2017). Similarly, noticeable differences of phenolic compound content and antioxidant capacity were observed within and between subspecies in the taxa examined in the present study. As has recently been emphasised, this increase in content of phenolics and antioxidant capacity can be attributed to differences between altitudes with respect to ultraviolet radiation and to low temperatures at high altitudes [see GHARIBI *et al.* (2013) and COLAK *et al.* (2017)].

CONCLUSIONS

Cypselas fatty acid profiles, antioxidant capacity and TPC and TF content of the oil and methanol extracts exhibited significant variations among the examined 11 taxa (14 accessions) of the tribe Cardueae. The findings for fatty acids are in good agreement with the data reported to date and suggest that their profiles can be used as supplementary distinguishing characteristics for taxonomic separations based on morphology. The investigated taxa contained considerable amounts of PUFAs (with linoleic acid showing the highest content) and MUFAs (with oleic acid showing the highest content). Based on their recorded concentrations, those two were the main fatty acids present. Strong correlations and relationships were determined between antioxidant capacity values and TPC or TF concentrations depending on the sampling altitudes. The data obtained in the present study also revealed a regional difference in the content of chemical components. However, these findings should still be considered preliminary. Results of the present study suggest that the phenolics and oil from cypselas can be used as a potential source of natural antioxidants and unsaturated fatty acids for nutritional and pharmacological applications and as ingredients in the formulation of functional foods. However, further studies are needed to isolate and characterise the active phenolic compounds responsible for the recorded antioxidant capacities. Further research is also required to verify the presence of individual phenolic compounds. Several additional taxa with populations ranging over wider geographical locations, as well as more genera in the subfamily Carduoideae, should be included in future research.

Acknowledgements – We are most grateful to Mr. Uzeyir Özcan for his help with the collection of plant materials during the fieldwork. This study was partly supported by the Research Fund of Artvin Coruh University (Project No: 2016.F10.01.08).

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Botanica SERBICA



REZIME

Masne kiseline i antioksidativni kapacitet ulja i fenola iz plodova jedanaest taksona iz tribusa *Cardueae* (*Carduoideae*, *Asteraceae*) iz SI Anatolije (Turska)

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Predstavnici tribusa *Cardueae* postali su predmet pažnje zbog svog taksonomskog konflikta sa filogenijom, biološki aktivnih metabolita i upotrebe u tradicionalnoj medicini. U radu se 11 taksona tribusa *Cardueae*, sakupljenih sa prirodnih staništa u Turskoj istražuje sa aspekta sastava masnih kiselina, antioksidativnog kapaciteta fenola i ulja iz cipsela. Rezultati pokazuju da je ukupni sadržaj ulja u opsegu od 1.45% do 9.28%. Glavna masna kiselina je linoleinska (C18:2n6; 44.36–70.49%), za kojom sledi oleinska (C18:1n9; 11.41–23.71%), koja značajno varira među taksonima, kao i koncentracije različitih suma masnih kiselina (PUFA; 45.21–78.82%, SFA, 6.53–14.06% MUFA; 12.21–41.40%). Ukupni sadržaj fenola (TPC; 428.18 – 753.23 mg/100 g dry weight) i ukupni sadržaj flavonoida (TF; 133.19–333.12 mg/100 g suve mase) su pozitivno jako korelisani sa antioksidativnim kapacitetom contents were positively strongly correlated with antioxidant capacity (opseg; mmol/g suve mase) određenog pomoći DPPH (65.94–147.9), FRAP (32.3–83.49), CUPRAC (42.28–86.52) i ORAC (22.1–49.74) eseja. Podaci pokazuju da veći sadržaj fenolnih jedinjenja rezultuje višim oksidativnim kapacitetom, dok redukcija sadržaja rezultuje smanjenim oksidativnim kapacitetom. Relativni udeo i količina masnih kiselina se može koristiti kao dodatan biohemijski pokazatelj u taksonomiji tribusa. Ova saznanja sugerišu da potencijalno korišćenje cipsela ovih vrsta koje su bogate fenolnim jedinjenjima i masnim kiselinama može biti korisno za ljudsko zdravlje u prevenciji hroničnih oboljenja izazvanih oksidativnim stresom.

KLJUČNE REČI: hemotaksonomija, cipsela, *Arctium*, *Cardueae*, *Carthamus*, *Cirsium*, *Echinops*

