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Fatty acid composition and antioxidant capacity of cypselas in *Centaurea* s.l. taxa (Asteraceae, Cardueae) from NE Anatolia

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ABSTRACT

Fatty acid composition, antioxidant capacity of phenolics and oil in cypselas of 10 *Centaurea* and two related taxa collected from various natural habitats in Turkey were investigated. Total oil (1.88–19.13%) and concentration of the major fatty acids which are palmitic acid: C16:0 (5.22–12.06%), oleic acid: C18:1n9 (8.57–30.29%) and linoleic acid: C18:2n6 (49.15–79.15%) varied significantly ($P < 0.05$) among the taxa. Polyunsaturated (PUFA, 49.15–79.15%), saturated (SFA, 7.24–19.06%) and monounsaturated (MUFA, 9.02–31.52%) fatty acids also differed significantly ($P < 0.05$). Concentrations (mg/100 g dw) of total phenolic compounds (TPC; 761.25–352.71 and total flavonoids (TF; 291.90–117.74) were strongly associated and correlated with antioxidant capacity (μmol/g dw) tested by using DPPH (55.52–144.61), FRAP (27.44–86.32) and CUPRAC (27.02–94.38). A higher content of TPC or TF reflected a higher antioxidant capacity, and a reduction in TPC indicated low antioxidant capacity. Relative proportions and quantities of fatty acids can be used as additional biochemical markers in the taxonomy of *Centaurea*. The current study also revealed that the consumption of these species that is rich in total phenolics and antioxidants would have several beneficial effects in terms of prevention of diseases caused by oxidative stress.

1. Introduction

The genus *Centaurea* L. (Asteraceae) is mainly distributed in SW Asia and the Mediterranean area and is represented by approximately 600 annual, biennial and perennial grassy species (Wagenitz and Hellwig, 1996; Brummitt, 2004). In recent research, combining morphological with molecular studies, this genus has been divided into four genera that are *Centaurea* L., *Rhaponticoides* Vaill., *Psephellus* Cass. and *Cyanus* Mill. (Wagenitz and Hellwig, 2000; Greuter, 2003a, 2003b). In Turkey, the genus *Centaurea* is represented by 195 species, excluding the species included in the genera *Cyanus*, *Psephellus* and *Rhaponticoides* (Uzunhisarcıklı et al., 2007; Daşkın and Yilmaz, 2009; Uysal, 2012; Uysal et al., 2017). This is the third largest genus after *Astragalus* L. and *Verbascum* L., and most of the taxa are endemics (Davis et al., 1988; Duran and Duman, 2002). The endemism ratio is 54% (Wagenitz, 1975; Guner, 2000; Uysal, 2012), which shows that Turkey is one of the gene centers of this genus (Wagenitz, 1986; Uzunhisarcıklı et al., 2007; Martin et al., 2009).

Centaurea, with more than 100 endemic species, is one of the largest genera of the tribe *Cardueae* Cass. It possesses characteristics of a complex taxonomy, and further study is needed. The problem of reasonable and natural divisions of this vast genus still remains to be resolved. Species delimitation is problematic in many sections, and there are numerous intermediate species (Wagenitz, 1975; Davis et al., 1988). Many *Centaurea* species have been traditionally used for their antidiabetic, antidiarrheal, antirheumatic, anti-inflammatory, choleretic, digestive, stomachic, diuretic, menstrual, astringent, hypotensive, antipyretic, cytotoxic and antibacterial properties. Most of them have previously been investigated in terms of chemical compositions, ecological, biological properties, and fatty acid composition and antimicrobial activity (Oksuz and Serin, 1997; Orallo et al., 1998; Tekeli et al., 2010). There is still growing worldwide interest in the fatty acids and antioxidant capacity of phenolics of the genus.

A great deal of work has emphasized the importance of seed fatty acid compositions within and among species as a valuable chemotaxonomical marker for determining a phylogenetic relationship. In general, linoleic acid (LA, C18:2n6) and α- and γ-linolenic acids (α-LA and γ-LA, C18:3n3 and C18:3n6) are the most common fatty acids among them. An important contribution to solving taxonomical problems as valuable data at different hierarchical levels has been established for members of some taxa of Rosaceae (*Alchemilla* L.; Ayaz et al., 1999), Cyperaceae (*Carex* L.; Ayaz and Olgun, 2000), Boraginaceae (*Cordia* L., *Heliotropium* L., *Myosotis* L., *Cynoglossum* Miller, *Lithospermum* L., *Echium* L., *Onosma* L., *Symphytum* L., *Trachystemon*

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L.G.Don., *Anchusa* L., *Nonea* Medicus, *Alkanna* Tausch, *Borago* L.; Özcan, 2008), Asteraceae, Onagraceae, Primulaceae, Ranunculaceae, Saxifragaceae, Scrophulariaceae (Gunstone, 1992), and Asteraceae (Zhang et al., 2015; Ozcan et al., 2016).

Apart from the importance of fatty acids in resolving phylogenetic problems, they also contribute an essential part of the human diet as food which provides energy and plays very significant biological roles (Kuna and Achinna, 2016; Ozcan et al., 2016). The beneficial or detrimental effects of dietary fats from seeds as the storage organ and vegetables of plants on human health and well-being largely depend on their fatty acid composition, which mostly are polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs). Therefore, PUFAs and MUFAs that are beneficial have been studied intensively as nutrients that protect against cardiovascular diseases (CVDs) (Ros and Mataix, 2006; Sarwar et al., 2013; Kuna and Achinna, 2016). Research has already revealed that diets rich in saturated fatty acid (SFA) are a major risk factor for CVDs due to their raising of serum low density lipoprotein (LDL) cholesterol concentrations. In contrast, it has been suggested that diets rich in MUFAs and some PUFAs with a substantial positive impact on human health, especially oleic acid (OA, C18:1n9), linoleic acid (LA, C18:2n6) and linolenic acid (α-LA and γ-LA), reduce or inhibit the possibility of such CVDs (as reviewed by Ozcan et al., 2016). Current guidelines recommend that no more than 25–35% of total daily calories be consumed from fat, with most coming from sources heavily endowed with MUFA and PUFA and no more than 10% of calories deriving from SFA (Kuna and Achinna, 2016).

The human body is unable to synthesize essential FAs (LA, α-LA and γ-LA) endogenously, while plants have the ability to synthesize C18:3n3 acid de novo (Damude and Kinney, 2008; Sarwar et al., 2013). In this respect, plant species with potential seed oil containing significant amounts of these FAs have been examined for their nutrient and industrial purposes to discover alternative new sources for many human age-related degenerative diseases (Ozcan et al., 2016). These essential FAs need to be supplied by dietary components such as seed oil (safflower, sunflower, corn, pine nuts, almond, sesame, flaxseed, hazelnut, walnut, soybean, canola, etc.), or fish (whale, seal, walrus, cod-liver, herring, salmon, etc.) (Ros and Mataix, 2006; Sarwar et al., 2013). Although seeds have potential uses for FAs, the scientific research continues to introduce new oil seeds sources of high nutritional and pharmaceutical value for the human diet.

The members of *Cardueae* have become a popular subject of current interest in medicine due to their biologically active metabolites. Recently, a number of studies have focused on antioxidant activities in seven taxa of the tribe *Cardueae* (Jeong et al., 2008). Total phenolic content and antioxidant properties of leaf (Nazaruk, 2008; Nazaruk et al., 2008), flavonoid compounds of flower heads (Nazaruk, 2009; Nazaruk et al., 2012) and cypsela fatty acid profiles in some *Cirsium* taxa (Ozcan et al., 2016) and also *Centaurea* species (two of the largest groups of *Cardueae*) have been reported. In that context, 16 species of *Centaurea* have been studied for their biological activities (Aktumsek et al., 2011; Zengin et al., 2011; Aktumsek et al., 2013; Tekeli et al., 2013; Erdogan et al., 2014). The fatty acid composition of members of *Cardueae* differs among the genera.

There are four main purposes of the present study which were (*1*) to identify fatty acid composition in cypsela samples of 12 *Centaurea* s.l. taxa (14 populations) collected from various natural habitats in NE Anatolia, Turkey, (*2*) to determine antioxidant capacity and total phenolic compounds (TPC) or total flavonoid (TF) contents of methanol extracts and oil fractions of cypselas, *(3)* to assess whether this species may represent a potential source of natural antioxidants for pharmaceutical applications or as ingredients for the formulation of functional foods and *(4)* to generate an approach aimed at solving phylogenetic problems of the genus. Thus, this is the first report about total phenolics or flavonoids in conjunction with antioxidant capacity of all taxa and fatty acid profiles of nine out of 12 taxa.

2. Materials and methods

2.1. Plant material

Twelve taxa (14 accessions) were investigated for the present study. The plant cypselas were collected from NE Anatolia (Turkey) (Fig. 1) during the flowering periods between 2007 and 2013. The taxa were arranged in alphabetical order, and specimen voucher information is provided in Table 1. The plant samples were identified by Dr. Melahat OZCAN and the voucher specimens have been deposited in the Artvin Coruh University Herbarium (ARTH).

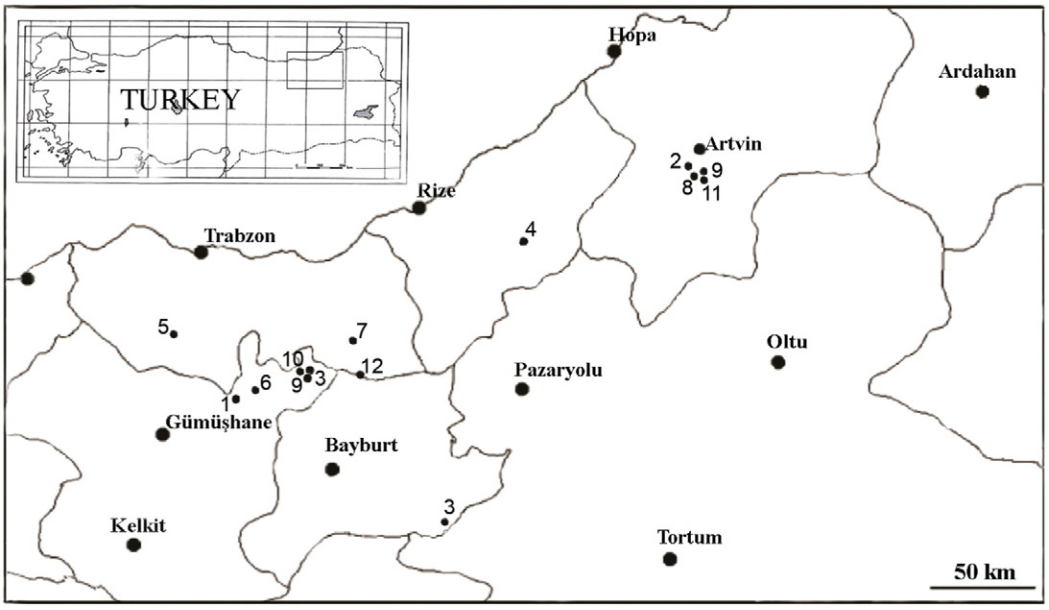


Fig. 1. Distribution map of studied *Centaurea* s.l. taxa 1: *Centaurea aggregata*, 2: *C. calcitrapa*, 3: *C. glastifolia*, 4: *C. helenioides*, 5: *C. jacea*, 6: *C. pseudreflexa*, 7: *C. salicifolia*, 8: *C. solstitialis*, 9: *C. urvillei* ssp. *stepposa*, 10: *C. virgata*, 11: *Cyanus woronowii*, 12: *Psephellus hypoleucus*.

Table 1
Collection data of studied *Centaurea* s.l. taxa.

Taxon	Locality	Coordinates	Voucher
Centaurea aggregata Fisch. & C.A. Mey.	Gümüşhane: Torul, above Gülaçar Village, among grass, 2500 m	39°08′41.8″E, 40°23′29.9″N	M. Ozcan 590
C. calcitrapa L.	Artvin: Seyitler Village, near University campus, wasted areas, 531 m	41°51′05.1″E, 41°11′52.4″N	M. Ozcan 637
C. glastifolia L.	Gümüşhane: Vauk Mountain, Kılıçören road, Sarıçiçek Village, 2020 m	39°51′05.1″E, 40°23′49.8″N	M. Ozcan 152a
	Bayburt: Kop Mountain, among alpine grass, 2415 m	40°30′43.0″E, 40°02′13.6″N	M. Ozcan 655
C. helenioides Boiss.	Rize: Cimil High Plateau, roadsides, among grass, 2220 m	40°49′34.0″E, 40°43′36.1″N	M. Ozcan 484
Centaurea jacea L.	Trabzon: near Zigana pass, Hamsiköy road, 1407 m	39°27′16.4″E, 40°41′25.4″N	M. Ozcan 642
C. pseudreflexa Hayek	Gümüşhane; Yağmurdere, alpine slopes, 1624 m	39°45′05.1″E, 40°26′23.7″N	M. Ozcan 92
C. salicifolia M. Bieb. ex. Willd.	Trabzon: Araklı, Ayvadere Village, roadsides, among grass, 312 m	40°00′01.8″E, 40°53′01.2″N	M. Ozcan 641
C. solstitialis L.	Artvin: Seyitler Village, near University campus, wasted areas, 531 m	41°51′07.8″E, 41°11′51.3″N	M. Ozcan 638
C. urvillei DC ssp. *stepposa* Wagenitz	Artvin: Seyitler Village, above University campus, wasted areas, 568 m	41°51′0.06″E, 41°11′59.2″N	M. Ozcan 369
	Gümüşhane: Vauk Mountain, roadsides, 1814 m	39°50′59.2″E, 40°26′23.7″N	M. Ozcan 406
Centaurea virgata Lam.	Gümüşhane: Vauk Mountain, 1874 m	39°50′37.6″E, 40°21′59.4″N	M. Ozcan 646
Cyanus woronowii (Bornm. ex Sosn.) Soják	Artvin: Seyitler Village; roadsides, 588 m	41°51′49.7″E, 41°12′35.1″N	M. Ozcan 347
Psephellus hypoleucus (DC.) Boiss.	Gümüşhane: Gezge Village, eroded rocky areas, 1824 m	40°03′26.7″E, 40°33′42.9″N	M. Ozcan 420

2.2. Extraction of seed oils and total lipids

Air-dried (in shade) mature cypselas were transferred to an oven set at 35 °C for one week. After that period, all achene samples were kept in a desiccator until reaching a constant weight. No changes were observed in the weights following measurement at 3-day intervals. These samples were pulverized 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 the oils and total lipids following the method described by Folch et al. (1957) with minor modifications. The combined extracts were concentrated on the thickness under reduced pressure and diluted with chloroform and stored at −20 °C for further analysis.

2.3. Fatty acid methyl ester (FAME) analysis

Mature cypselas for fatty acid analysis were taken from at least three specimens per accession, depending on their availability. These were dried and ground in a grinder with a 2 mm diameter-mesh. Lipid extraction was performed according to the Folch et al. (1957). Methyl esters were prepared by *trans*-methylation using 2 M KOH in methanol and *n*-hexane according to the method described by Ichihara et al. (1996) with minor modification; 10 mg of extracted oil was dissolved in 2 ml hexane, followed by 4 ml of 2 M methanolic KOH. The tube was then vortexed for 2 min at room temperature. After centrifugation at 4000 rpm for 10 min, the hexane layer was taken for gas chromatography (GC) analyses.

2.4. Gas chromatographic conditions

The fatty acid profile was analyzed using a Clarus 500 GC device with an autosampler (Perkin-Elmer, USA) equipped with a flame ionization detector and a fused silica capillary SGE column (30 m × 0.32 mm ID 0.25 lm BP20 0.25 UM, USA). The oven temperature was 140 °C, held for 5 min, after which this was increased to 200 °C at a rate of 2 °C/min and held at 220 °C at a rate of 1 °C/min. The injector and the detector temperatures were set at 220 and 280 °C, respectively. The sample size was 1 μl, and the carrier gas was controlled at 16 ps. The split ratio was 1:100. Fatty acid peaks were identified by comparing the retention times of FAME with the standard 37 component of the FAME mixture. Three replicate GC analyses were performed, and the results were expressed in GC area % as mean values ± standard deviation.

2.5. Extraction

Approximately 0.5 g of plant sample was homogenized with 20 mL methanol (80% v/v). The homogenate was then fractionated with 20 ml of chloroform for 1 day and shaken at specific time intervals at room temperature. The chloroform and methanolic phases were then concentrated using a rotary evaporator (Heidolph Instruments GmbH & Co. KG, Germany) at 35 °C under reduced pressure. The aqueous residue was dried using a lyophilizator (Christ, Alpha 1-2LD plus, Germany). The methanolic residue was dissolved with 2 mL methanol, and the chloroform residue was dissolved with toluene.

2.6. Determination of total phenolic (TPC) and total flavonoid (TF) contents

Total phenolic content (TPC) in each extract was measured with Folin–Ciocalteu (FC) reagent using the method described by Slinkard and Singleton (1977) with minor modifications. The extract (0.5 ml) was added to FC reagent (2 N, 25 μl) and mixed. Na_2CO_3 (2% w/v, 975 μl) was added to the mixture, and the reaction mixture was then kept in the dark for 30 min. The absorbance of the resulting blue color was measured at 750 nm using a double beam UV–VIS spectrophotometer (Thermo, Evolution 100, England). The content of total phenolic compound was expressed as mg gallic acid equivalents (GAE)/100 g dry weight (dw).

Total flavonoid (TF) content was determined based on a method described by Huang et al. (2004), with some modifications. The methanolic sample extract (500 μl) and 2% (w/v) 500 μl $AlCl_3$ in methanol were mixed well in a 2 ml test tube, and the mixture was then incubated in the dark for 30 min at room temperature. The absorbance of the reaction mixture was measured at 415 nm, and the content was expressed as mg of quercetin equivalent per 100 g dw.

2.7. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

Scavenging activity was measured using DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical based on the method described by Blois (1958), with slight modifications. One milliliter of DPPH in methanol solution was mixed with 100 μl methanolic extract, and the reaction mixture was incubated at room temperature in the dark. The absorbance of the reaction mixture was measured at 520 nm after 30 min, and the DPPH radical scavenging activity was expressed as μmol Trolox equivalent 100 g^{-1} dw.

2.8. Ferric reducing antioxidant power (FRAP)

FRAP of the methanolic extract was determined using the method described by Benzie and Strain (1996), with slight modification. Briefly, the FRAP reagent (total volume 3000 μl) consisting of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) and 20 mM $FeCl.6H_2O$ in proportions of 10:1:1 (v/v/v) was shaken vigorously with 100 μl of each sample. After incubation at 37 °C for 30 min, the absorbance of the mixture was determined at 593 nm against a blank. Trolox was used for calibration, and FRAP values were expressed as μmol Trolox 100 g^{-1} dw.

2.9. Cupric ion reducing antioxidant capacity (CUPRAC)

Determination of the cupric ion reducing antioxidant capacity (CUPRAC) in the extract was performed using a modified version of the method described by Apak et al. (2004). The assay was performed with 10 mM neocuproine, 7.5 mM $CuCl_2$, 1 mM acetate buffer (pH 7.0) and sample extract (total volume of mixture 4 mL). The mixture was kept at room temperature for 30 min, and absorbance at 450 nm was then measured. The CUPRAC value of an extract was expressed as μmol Trolox g^{-1} dw.

2.10. Statistical analysis

Multivariate analyses were performed to evaluate the fatty acid characters on SPSS version 19.0 (SPSS, Chicago, Illinois, USA) software. Fatty acid compounds (6 quantitative with mean value) of the taxa and their grouping were determined using the clustering analysis method (UPGMA, dissimilarity, standardized variable) as well as ordination based on principle component analysis (PCA). In addition, the percentages of fatty acids were tested using analysis of variance (ANOVA), and comparisons between means were performed using Duncan's test. Differences among means were considered significant at $P < 0.05$.

3. Results and discussion

3.1. Cypselas oil content and fatty acid concentration changes in the studied taxa

Table 1 shows the number of taxa, their localities, coordinates and voucher numbers. Fig. 1 also shows the distribution of the studied 12 taxa collected from different localities in four cities in NE Anatolia (Turkey). The data reveal significant differences ($P < 0.05$) among the 12 taxa in terms of seed oil content (Table 2), ranging from 1.88 to 19.13% in *P. hypoleucus* and *C. aggregata*, respectively. Oil content for two accessions of *C. glastifolia* (7.83 ± 0.72%, 7.59 ± 0.58%, Gümüşhane, Bayburt, ave. 7.71%) and *C. urvillei* ssp. *stepposa* (8.82 ± 0.28%, 7.62 ± 0.68%, Artvin, Gümüşhane, ave. 8.22) collected from different locations was almost identical.

An interspecies difference in the fatty acid concentrations was also determined in the 12 taxa (10 *Centaurea*, 1 *Psephellus* and 1 *Cyanus*) (Table 2). In general, all taxa were represented by six fatty acids, two saturated (C16:0 and C18:0) and four unsaturated (C18:2n6 and C18:1n9). These taxa had slightly lower palmitic acid (C16:0; 5.22–12.06%) and stearic acid (C18:0; 0.81–3.23%) levels and a higher content of linoleic acid (C18:2n6; 49.15 – 79.15%) and oleic acid (C18:1n9, 8.57–30.29%). Additionally, a very low concentration of the remaining two fatty acids was determined as palmitoleic acid (C16:1; 0.04–2.63%) and linolenic acid (0.36–8.47%) (Table 2). Among the taxa, *P. hypoleucus*, *C. helenoides*, *C. solstitialis* and *C. woronowii* represented the highest content of C16:0 (12.06%), C18:0 (3.23%), C18:2n6 (79.15%) and C18:1n9 (30.29%), respectively.

Total fatty acid concentrations were almost identical in most of the taxa, ranging from 95.26 to 99.14%, although they differed slightly in a few number of species. A locational difference was also observed for total monounsaturated fatty acids (MUFAs; 13.65–15.85%, 18.51–28.97%) and polyunsaturated fatty acids (PUFAs; 73.71–75.98%, 56.80–70.87%), as well as unsaturated fatty acids (UFAs; 87.35–91.82%, 85.76–89.38%) (Table 3). The ω6/ω3 ratio exhibited a highly significant ($P < 0.05$) interspecies difference among the studied taxa, ranging from the lowest level of 7.97 in *C. glastifolia* to the highest level of 177.72 in *C. helenoides*. A significant ($P < 0.05$) difference, albeit not as marked as the ω6/ω3 ratio, was also seen in the ratio of ω3/ω6 (Table 3).

A survey of the literature concluded that the major fatty acids of *Centaurea* are C61:0, C18:1 and C18:2, followed in part by C18:3 (Barros et al., 2010; Aktumsek et al., 2011; Zengin et al., 2011; Aktumsek et al., 2013; Erdogan et al., 2014). These recent findings showed considerable variations among the four major fatty acids, ranging from 16.6 to 37.8% (ave. 25.3%) for C16:0, 3.4 to 25.1% (ave. 12.1%) for C18:1, 0.4 to 35.9% (ave. 18.4%) for C18:2 and 1.1 to 25.3% (ave. 12.1%) for C18:3. The findings obtained from the present study do not concur with data giving in the above literatures comparing some *Centaurea* taxa having average of 7.54% C16:0, 17.5% C18:1, 66.3% C18:2 and 1.4% C18:3. Compared to the data from the above citations, our taxa had ~3.4-fold lower C16:0, ~1.4- and 3.6-fold higher C18:1 and C18:2 and ~8.7-fold lower C18:3 fatty acids. In addition, noticeable variations in the concentrations of the major fatty acids, in particular C18:2 (~4–14.3%) and C18:1 (2.1–3.4%), were found between Gümüşhane, Bayburt and Artvin accessions of *C. glastifolia* and *C. urvillei* subsp. *stepposa* (abbreviated as G, B and A in the Figures and Tables). These findings clearly show that the difference in part in the total oil content and marked variations in the fatty acid profiles among the reported and the studied taxa may be due to several factors including temperature, varieties, genotype, growing conditions, and other environmental factors (as reviewed by Aktumsek et al., 2013; Ozcan et al., 2016).

3.1.1. Multivariate and principal component analysis (PCA) of fatty acids

The UPGMA and PCA phenograms showing the two major clusters (Figs. 2 and 3) confirm the separations of *Psephellus* and *Cyanus* from

Table 2
Fatty acid composition (%, mean ± SD, n = 6) in cypselas of *Centaurea* s.l. taxa investigated. Analysis of variance (ONE-WAY ANOVA) was used for comparisons. Means in the same column followed by different letters at superscript are significant at $P = 0.05$.

Taxon	Palmitic acid (C16:0)	Palmitoleic acid (C16:1)	Stearic acid (C18:0)	Oleic acid (C18:1n9)	Linoleic acid (C18:2n6)	Linolenic acid (C18:3n3)	∑ major fatty acid	∑ other fatty acid	Total lipid
C. aggregata	8.08 ± 0.04^{h}	0.41 ± 0.02^{d}	2.20 ± 0.02^{d}	11.25 ± 0.14^{b}	62.78 ± 0.16^{d}	0.69 ± 0.06^{d}	96.37	3.63	19.13
C. calcitrapa	5.22 ± 0.02^{a}	0.22 ± 0.04^{b}	2.50 ± 0.02^{fg}	12.01 ± 0.01^{c}	77.44 ± 0.04^{j}	0.72 ± 0.05^{de}	99.12	0.88	5.57
C. glastifolia[G]	6.10 ± 0.09^{c}	0.25 ± 0.02^{bc}	2.78 ± 0.07^{hi}	$13.39 \pm 0.79_{d}$	71.37 ± 0.26^{h}	2.30 ± 2.33^{f}	96.19	3.81	7.83
C. glastifolia[B]	5.70 ± 0.02^{b}	0.32 ± 0.04^{c}	0.81 ± 0.02^{a}	15.53 ± 0.01^{e}	67.51 ± 0.01^{f}	8.47 ± 0.01^{i}	99.07	0.93	7.59
C. helenioides	8.34 ± 0.08^{i}	0.33 ± 0.06^{c}	3.23 ± 0.09^{j}	23.51 ± 0.46^{h}	63.00 ± 0.13^{d}	0.36 ± 0.06^{a}	98.77	1.23	10.47
C. jacea	6.14 ± 0.01^{c}	0.60 ± 0.00^{gh}	2.45 ± 0.02^{ef}	13.43 ± 0.03^{d}	74.76 ± 0.03^{i}	0.42 ± 0.01^{ab}	99.11	0.89	11.24
C. pseudreflexa	7.04 ± 0.04^{f}	0.66 ± 0.08^{h}	1.95 ± 0.29^{c}	20.82 ± 0.76^{g}	64.24 ± 0.30^{e}	0.55 ± 0.04^{c}	95.26	4.74	9.40
C. salicifolia	6.80 ± 0.10^{e}	0.42 ± 0.09^{d}	2.18 ± 0.05^{d}	13.23 ± 0.15^{d}	74.81 ± 0.01^{i}	0.78 ± 0.01^{e}	98.44	1.56	6.09
C. solstitialis	7.52 ± 0.02^{g}	0.45 ± 0.01^{de}	2.31 ± 0.03^{de}	8.57 ± 0.04^{a}	79.15 ± 0.05^{k}	0.45 ± 0.01^{b}	99.14	0.86	8.33
C. urvillei ssp. *stepposa*[A]	8.52 ± 0.13^{j}	0.52 ± 0.03^{ef}	2.59 ± 0.12^{fg}	28.45 ± 0.42^{i}	56.15 ± 0.12^{c}	0.64 ± 0.07^{d}	96.87	3.13	8.82
C. urvillei ssp. *stepposa*[G]	6.40 ± 0.02^{d}	0.54 ± 0.01^{fg}	1.70 ± 0.02^{b}	17.97 ± 0.05^{f}	70.41 ± 0.01^{g}	0.46 ± 0.01^{b}	98.28	1.72	7.62
C. virgata	5.75 ± 0.04^{b}	0.04 ± 0.01^{a}	2.65 ± 0.03^{gh}	18.40 ± 0.17^{f}	62.99 ± 0.03^{d}	0.49 ± 0.04^{bc}	99.07	0.93	6.66
Cyanus woronowii	11.17 ± 0.16^{k}	1.23 ± 0.06^{i}	2.77 ± 0.15^{hi}	30.29 ± 0.08^{j}	49.15 ± 0.18^{a}	2.94 ± 0.05^{g}	97.55	2.45	7.41
Psephellus hypoleucus	12.06 ± 0.01^{l}	2.63 ± 0.03^{j}	2.91 ± 0.01^{i}	17.93 ± 0.04^{f}	55.06 ± 0.11^{b}	3.12 ± 0.02^{h}	97.80	2.20	1.88

[G] Gümüşhane accession (see Table 1).
[B] Bayburt accession (see Table 1).
[A] Artvin accession (see Table 1).

Table 3
Total oil and total amounts of fatty acids (%) in cypselas of *Centaurea* and related taxa investigated. Analysis of variance (One-way ANOVA) was used for comparisons. Means in the columns followed by different letters at superscript are significant at $P = 0.05$.

Taxon	∑SFA	∑MUFA	∑PUFA	∑UFA	ω3/ω6	ω6/ω3
Centaurea aggregata	12.19 ± 0.08^{i}	20.69 ± 0.11^{f}	63.47 ± 0.22^{d}	84.16 ± 0.33^{b}	0.01 ± 0.00^{e}	90.99 ± 7.82^{c}
C. calcitrapa	8.73 ± 0.04^{b}	12.22 ± 0.03^{b}	78.16 ± 0.07^{k}	90.39 ± 0.10^{g}	0.009 ± 0.00^{cd}	107.94 ± 6.03^{cd}
C. glastifolia^{B}	7.24 ± 0.02^{a}	15.85 ± 0.03^{d}	75.98 ± 0.011^{j}	91.82 ± 0.02^{h}	0.13 ± 0.00^{i}	7.97 ± 0.009^{a}
C. glastifolia^{G}	8.88 ± 0.16^{bc}	13.65 ± 0.80^{c}	73.71 ± 0.27^{g}	87.35 ± 0.82^{d}	0.03 ± 0.00^{f}	30.59 ± 0.46^{b}
C. solstitialis	10.52 ± 0.05^{f}	9.02 ± 0.03^{a}	79.59 ± 0.05^{l}	88.62 ± 0.08^{e}	0.01 ± 0.00^{a}	177.21 ± 2.30^{g}
C. helenioides	11.57 ± 0.16^{h}	23.83 ± 0.49^{h}	63.36 ± 0.18^{d}	87.20 ± 0.65^{d}	0.01 ± 0.00^{a}	177.72 ± 26.44^{g}
C. jacea	9.89 ± 0.02^{e}	14.03 ± 0.03^{c}	75.18 ± 0.04^{h}	89.21 ± 0.03^{ef}	0.01 ± 0.00^{a}	176.61 ± 2.32^{g}
C. pseudreflexa	9.00 ± 0.28^{cd}	21.49 ± 0.77^{g}	64.79 ± 0.26^{e}	86.28 ± 0.54^{c}	0.01 ± 0.00^{bc}	116.54 ± 8.97^{de}
C. salicifolia	9.21 ± 0.15^{d}	13.65 ± 0.23^{c}	75.59 ± 0.01^{i}	89.24 ± 0.24^{ef}	0.01 ± 0.00^{de}	96.33 ± 0.73^{c}
C. urvillei ssp. *stepposa*^{A}	11.12 ± 0.18^{g}	28.97 ± 0.40^{j}	56.80 ± 0.06^{b}	85.76 ± 0.34^{c}	0.01 ± 0.00^{e}	88.01 ± 9.97^{c}
C. urvillei ssp. *stepposa*^{G}	8.80 ± 0.02^{bc}	18.51 ± 0.06^{e}	70.87 ± 0.07^{f}	89.38 ± 0.12^{f}	0.007 ± 0.00^{a}	155.79 ± 25.10^{f}
C. virgata	9.97 ± 0.09^{e}	25.62 ± 0.12^{i}	63.48 ± 0.07^{d}	89.10 ± 0.17^{ef}	0.01 ± 0.00^{b}	128.26 ± 10.68^{e}
Cyanus woronowii	13.94 ± 0.23^{j}	31.52 ± 0.02^{k}	52.09 ± 0.22^{a}	83.61 ± 0.23^{b}	0.06 ± 0.00^{h}	16.74 ± 0.20^{ab}
Psephellus hypoleucus	19.06 ± 0.03^{k}	20.57 ± 0.04^{f}	58.17 ± 0.11^{c}	78.74 ± 0.14^{a}	0.06 ± 0.00^{g}	17.67 ± 0.09^{ab}

^{G} Gümüşhane accession (see Table 1).
^{B} Bayburt accession (see Table 1).
^{A} Artvin accession (see Table 1).

Centaurea (Wagenitz and Hellwig, 2000). Ten *Centaurea* taxa are closely positioned and distinctly separated from other species, *Psephellus hypoleucus* and *Cyanus woronowii*. The species coded as agr - vir, urv - psr, sal – jac and slt - cal are morphologically very similar to each other (Fig. 2). Wagenitz (1975) classified these species in the sections *Acrolopus* (Cass.) DC., *Acrocentron* (Cass.) DC., *Jacea* (Mill.) DC., and *Mesocentron* (Cass.) DC., respectively. In addition, *C. glastifolia* (coded as gla) and *C. helenioides* (coded as hel) resemble other *Centaurea* species at a slightly distant position. These two species appear in the Flora of Turkey in the sections of *Chartolepis* (Cass.) DC. and *Grossheimia* (Sons. & Takht.). M. Dittrich, respectively. Our data for the fatty acid contents of 10 taxa are in accordance with their sectional delimitations in the genus *Centaurea* based on morphological similarity (Wagenitz, 1975) and also support dividing this genus into four different genera (Wagenitz and Hellwig, 2000; Greuter, 2003a, 2003b).

Ten of the taxa investigated were assigned to the genus *Centaurea* in the second cluster (Figs. 2 and 3). The remaining two taxa, *P. hypoleucus* and *C. woronowii*, were separated as a single cluster. α-Linolenic acid (C18:2n6) was the major contributor to the separation of *Centaurea* species from others. This polyunsaturated acid was determined at a higher level in *Centaurea* taxa, ranging from 56.15 to 79.15%, than the others, with a range between 49.15% (*C. woronowii*) and 55.06% (*P. hypoleucus*) (Table 2).

As a final point, the fatty acid profiles among the taxa are in accordance with their generic and also species delimitations presented in the Flora of Turkey. The present data suggest that fatty acids can be used as distinguishing characters and are useful for additional characters to their morphologies.

3.2. Multivariate analyses

For the cluster analysis (UPGMA, unweighted pair group method with arithmetic mean), six fatty acid characteristics of 12 taxa (14 accessions) were analyzed and their interspecific relationships observed (Tables 1 & 2, Fig. 1). The phenogram shows that two distinct major clusters were separated from all taxa. The delimitation of these groups is mainly based on the occurrence of linoleic acid and stearic acid ($P < 0.05$). In the dendrogram (Fig. 2), 12 taxa were divided into two major clusters at a distance coefficient of approximately 31.0. The first cluster included two taxa (*P. hypoleucus* and *C. woronowii*) at a dissimilarity level of approximately 4.0.

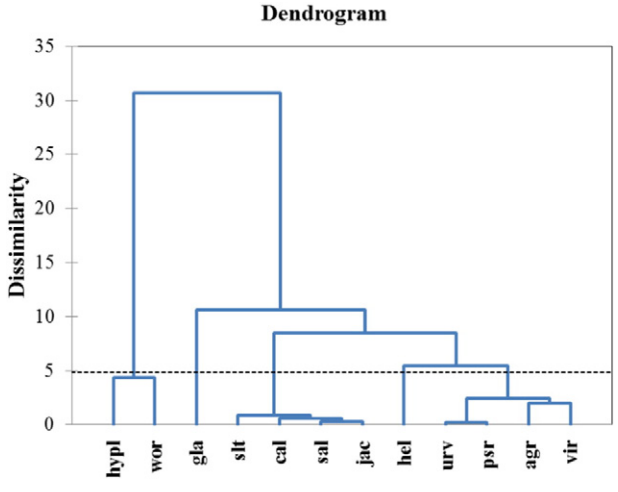


Fig. 2. UPGMA Cluster analysis using the fatty acid characters of investigated taxa. Each code represents a taxon. Taxa codes; agr; *C. aggregata*, cal; *C. calcitrapa*, gla; *C. glastifolia*, hel; *C. helenioides*, hypl: *Psephellus hypoleucus*, jac; *C. jacea*, psr: *C. pseudreflexa*, sal; *C. salicifolia*, slt; *C. solstitialis*, urv; *C. urvillei* subsp. *stepposa*, vir; *C. virgata,* wor; *Cyanus woronowii.*

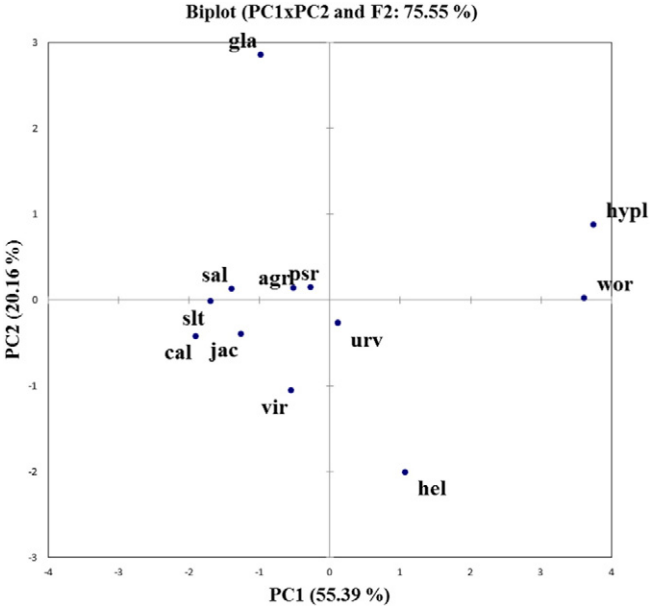


Fig. 3. Principle Component Analysis (PCA) of some *Centaurea* s.l. taxa based on fatty acid profile. Each code represents a taxon and related group is indicated by different symbol.

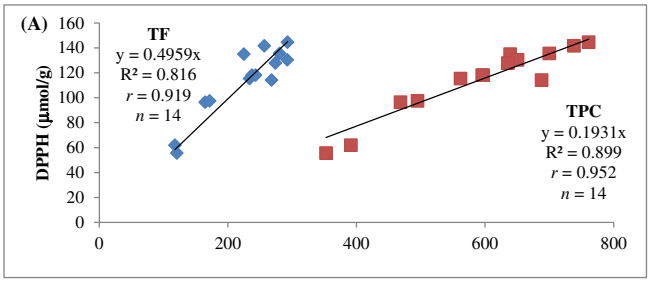
The second cluster (*Centaurea* taxa only) was divided into four sub-clusters, the first of which consisted of *C. glastifolia*. The second sub-cluster was split into two groups at a dissimilarity level of approximately 11.0: one group consisted of *C. solstitialis*, *C. calcitrapa*, *C. salicifolia* and *C. jacea*, and the second group included five taxa. The subgroups were separated from each other at a distance coefficient of approximately 8.0. The third sub-cluster was also split into two groups at a dissimilarity level of approximately 6.0: one group consisted of *C. helenioides*, and the second group included four taxa. The fourth sub-cluster was split into two groups at a dissimilarity level of approximately 3.0: one consisted of two taxa; *C. urvillei* ssp. *stepposa* and *C. pseudreflexa*, and the second of *C. aggregata* and *C. virgata*. The clustering of the two taxa (*C. urvillei* ssp. *stepposa* and *C. pseudreflexa*) at a low distance coefficient (almost 1.0), together with two species (*C. aggregata* and *C. virgata*) at a distance coefficient (almost 3.0), indicates an underlying morphological resemblance between them. These four taxa were also very similar to each other in terms of morphological properties.

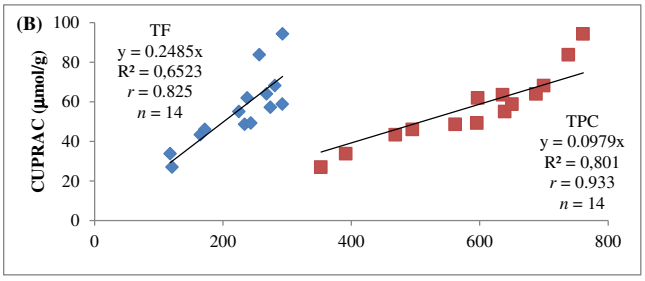
Principal component analysis (PCA) of fatty acid compositions revealed that the first two PC factors accounted for approximately 75.55% of the total variance. The variance explained by the first principal component (PC1) was 55.39%, while the second (PC2) explained 20.16%. Two distinct groups were separated, according to the first two axes. The taxa coded as gla, cal, slt, jac, agr, psr, vir, urv and hel were separated as one group, while the remaining two taxa (hypl; *Psephellus hypoleucus* and wor; *Cyanus woronowii*) were delimited as a second group (Fig. 4).

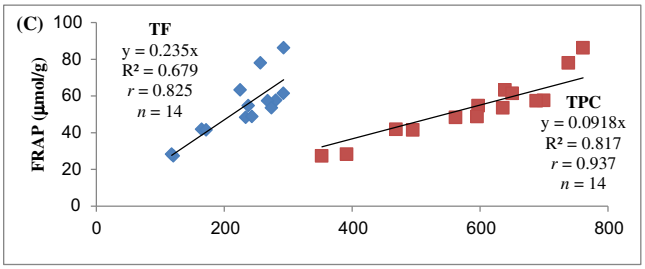
The first two principal components (PCs) were used to visualize the structure of the data in a PCA bi-plot accounting for 72.53% of the total variance. In the bi-plot, linoleic acid (C18:2n6) was located singularly at the left side of the figure, whereas all other fatty acids were situated on the right side. Linolenic acid was at the upper right side. C16:1, C16:0, C18:3n3, C18:0 and C18:1n9 exhibited positive loading on PC1 that correlated with the separation of taxa in the genera *Psephellus* and *Cyanus*, whereas C18:2n6 was negatively loaded. In terms of PC2, four components were located on the positive side and two C18:0 and C18:1n9 on the negative side (Table 4). The bi-plot also reveals which components of taxa are correlated and responded similarly to the PCs. Two main groups occurred according to the PC axes; group 1 included the taxa coded gla, sal, agr, psr, slt, cal, jac, vir, urv and hel within the genus *Centaurea*, and group 2 included 2 taxa (hypl, wor) within the other two genera, *Psephellus* and *Cyanus*. The components C18:2n6 and C18:0 were able to identify the taxa present in the genus *Centaurea*. Similarly, C18:3n3 C16:0, C16:1, C18:1n9 and C18:0 were used to differentiate plants included in the *genera Psephellus* and *Cyanus*.

3.3. Total phenolics (TPC), flavonoids (TF) and antioxidant capacity changes in the studied taxa

TPC and TF contents in the *Centaurea* taxa are shown in Table 4. TPCs estimated included all flavonoids, anthocyanins and non-flavonoid phenolic compounds. *Centaurea urvillei* ssp. *stepposa* had the highest average concentration of TPC (761.25 mg/100 g dw), followed by *C. jacea* (738.25 mg/100 g dw), while *C. aggregate* had the lowest (352.71 mg/100 g dw). Similarly, *C. urvillei* ssp. *stepposa* also had the highest average concentration of TF (292.90 mg/100 g dw), followed by *C. virgata* (292.67 mg/100 g dw), while *C. solstitialis* had the lowest (117.74 mg/100 g dw). Plants produce diverse compounds known as secondary metabolites that exhibit different antioxidant capacities. Various phytochemical compounds, including the flavonoids, phenylpropanoids, and phenolic acids in the metabolite class, are known to be responsible for antioxidant capacity in fruits and vegetables (Aktumsek et al., 2013). Among the studied taxa in this study, the values (μmol TE/100 g dw, lowest and highest) were in the range of 55.52 to 144.61 for DPPH, 27.44 to 86.32 for FRAP and 27.02 to 94.38 for CUPRAC, being the lowest in *C. aggregata* and highest in *C. urvillei* ssp. *stepposa* (Table 4).







Correlation matrix (Pearson, *r*) between phenolic compounds contents and antioxidant capacity values

Variables	TPC	TF	DPPH	CUPRAC	FRAP
TPC	-	0.939*	0.952*	0.933*	0.937*
TF	-	-	0.919*	0.825*	0.825*
DPPH	-	-	-	0.863*	0.907*
CUPRAC	-	-	-	-	0.957*
FRAP	-	-	-	-	-

Fig. 4. Correlation (Pearson) and relationships between antioxidant capacity and total phenolic compounds (TPC) or total flavonoid (TF) contents in cypselas of *Centaurea* s.l. taxa. Average values were used for the plots. *Values are different 0 with a significance level alpha = 0.05.

The correlations between antioxidant capacity (DPPH, CUPRAC and FRAP) and TPC or TF contents of different *Centaurea* taxa are presented in Fig. 4. The average values for DPPH ($r = 0.952, 0.919$), CUPRAC ($r = 0.933, 0.825$) and FRAP ($r = 0.937, 0.825$) exhibited strong positive correlations and high linear relationships ($R^2 = 0.899, 0.816$; $R^2 = 0.801, 0.652$; $R^2 = 0.817, 0.679$, respectively) with TPC and TF contents in the samples.

The average antioxidant capacity values of oil extracted from cypselas of *Centaurea* measured using DPPH were positively strongly correlated ($r = 0.981$) with PUFA values (Table 3) and there was a high level of relationship between them ($R^2 = 0.814$). The average antioxidant capacity values for *Centaurea*, *Psephellus* and *Cyanus* were 113.81, 96.4 and 130.45 for DPPH, 53.56, 41.88 and 61.48 for FRAP and 57.98, 43.30 and 58.82 for CUPRAC, respectively. Additionally, the average values for TPC and TF contents were 596.37, 468.66 and 650.54, and 227.86, 164.77 and 280.81 mg/100 g dw, respectively. Positive strong correlations were found between the average values for TPC and the antioxidant capacity values for *Centaurea* ($r = 0.952$, 0.933 and 0.937, DPPH, CUPRAC and FRAP), as well as between TF

Table 4
Antioxidant capacity (DPPH, FRAP and CUPRAC) values and total phenolics (TPC) or total flavonoid (TF) contents in methanol extract and DPPH value in oil extract of cypselas of *Centaurea* and related taxa. Values are expressed as means ± SD (n = 3). Analysis of variance (ONE-WAY ANOVA) was used for comparisons. Means in the same column followed by different letters at superscript are significantly different ($P < 0.05$). GE; Gallic equivalents, TE; Trolox equivalents and dw; dry weight.

Taxon	Methanol extract					Oil
	DPPH**	FRAP**	CUPRAC**	TPC*	TF*	DPPH**
Centaurea aggregata	55.52 ± 0.50^{a}	27.44 ± 0.38^{a}	27.02 ± 0.43^{a}	352.71 ± 17.23^{a}	120.67 ± 0.57^{b}	53.44 ± 0.31^{f}
C. calcitrapa	115.37 ± 0.63^{d}	48.37 ± 0.27^{e}	48.58 ± 1.27^{de}	561.92 ± 13.70^{c}	233.86 ± 0.25^{f}	62.21 ± 0.47^{c}
C. glastifolia^{G}	114.26 ± 0.87^{d}	57.44 ± 0.36^{h}	64.00 ± 1.10^{h}	688.0 ± 28.72^{fg}	268.08 ± 0.83^{j}	58.01 ± 0.35^{e}
C. glastifolia^{B}	97.79 ± 0.64^{c}	41.51 ± 0.42^{c}	46.07 ± 2.12^{cde}	495.15 ± 35.40^{c}	171.81 ± 0.32^{d}	67.11 ± 0.32^{a}
C. helenioides	118.08 ± 0.52^{e}	45.84 ± 0.45^{d}	49.28 ± 2.70^{e}	595.71 ± 34.34^{cd}	243.37 ± 0.64^{h}	57.53 ± 0.26^{e}
C. jacea	141.71 ± 0.61^{i}	78.06 ± 0.37	83.83 ± 0.18^{j}	738.25 ± 21.15^{hi}	256.67 ± 0.58^{i}	65.38 ± 0.56^{b}
C. pseudreflexa	127.78 ± 0.70^{f}	53.57 ± 0.59^{f}	63.59 ± 0.81^{h}	635.88 ± 36.75^{de}	273.88 ± 0.20^{k}	65.18 ± 0.15^{b}
C. salicifolia	135.03 ± 2.05^{h}	63.39 ± 0.36^{j}	55.08 ± 1.19^{f}	639.01 ± 20.49^{de}	224.93 ± 0.13^{e}	59.56 ± 0.81^{c}
C. solstitialis	61.83 ± 0.48^{b}	28.33 ± 0.67^{b}	33.75 ± 1.51^{b}	391.53 ± 25.70^{a}	117.74 ± 0.44^{a}	57.37 ± 0.33^{e}
C. urvillei ssp. *stepposa*^{A}	144.61 ± 0.61^{j}	86.32 ± 0.60^{l}	94.38 ± 3.80^{k}	761.25 ± 18.15^{i}	292.90 ± 0.17^{n}	45.09 ± 0.37^{g}
C. urvillei ssp. *stepposa*^{G}	118.17 ± 0.84^{e}	54.81 ± 0.69^{g}	61.94 ± 3.24^{h}	597.14 ± 12.91^{cd}	237.77 ± 0.39^{g}	43.27 ± 0.37^{h}
C. virgata	135.56 ± 0.48^{h}	57.63 ± 0.24^{h}	68.19 ± 0.74^{i}	699.86 ± 15.35^{gh}	292.67 ± 0.57^{m}	68.35 ± 0.21^{a}
Cyanus woronowii	130.45 ± 0.59^{g}	61.48 ± 0.50^{i}	58.82 ± 0.61^{g}	650.54 ± 23.82^{ef}	280.81 ± 0.33^{l}	65.25 ± 0.47^{b}
Psephellus hypoleucus	96.47 ± 1.94^{c}	41.88 ± 0.41^{c}	43.30 ± 0.95^{c}	468.66 ± 20.49^{b}	164.77 ± 0.39^{c}	57.29 ± 0.41^{e}

* (mg GE/100 g dw).
** (μmol TE/100 g dw.
^{G} Gümüşhane accession (see Table 1).
^{B} Bayburt accession (see Table 1).
^{A} Artvin accession (see Table 1).

contents and antioxidant capacity (see Fig. 4) which had a high correlation. These values indicated that total antioxidant capacity was strongly related to TPC and TF contents (Fig. 4).

In the present study, noticeable variation was observed in antioxidant values and TPC or TF concentrations within and among the taxa. Two accessions of *C. glastifolia* and *C. urvillei* ssp. *stepposa* collected from the three different geographical regions exhibited 192.9 mg and 92 mg differences in concentrations of TPC and TF, and antioxidant capacities ranging between 16.8 and 18 μmol, respectively. In brief, higher content of TPC or TF reflected higher antioxidant capacity values, and a decrease in TPC lessened antioxidant capacity in the studied taxa of *Centaurea*. Similarly, considerable variations in antioxidant capacity values and TPC or TF concentrations among 10 *Centaurea* species/taxa were reported by Barros et al. (2010) and Aktumsek et al. (2011, 2013). Their data seem remarkably higher than those of the present study. They reported their values as mg/g extract. Both the study groups and our data supported the idea that a higher content of TPC or TF reflected higher antioxidant capacity, and a reduction in TPC content reflected decreased antioxidant capacity based on species difference.

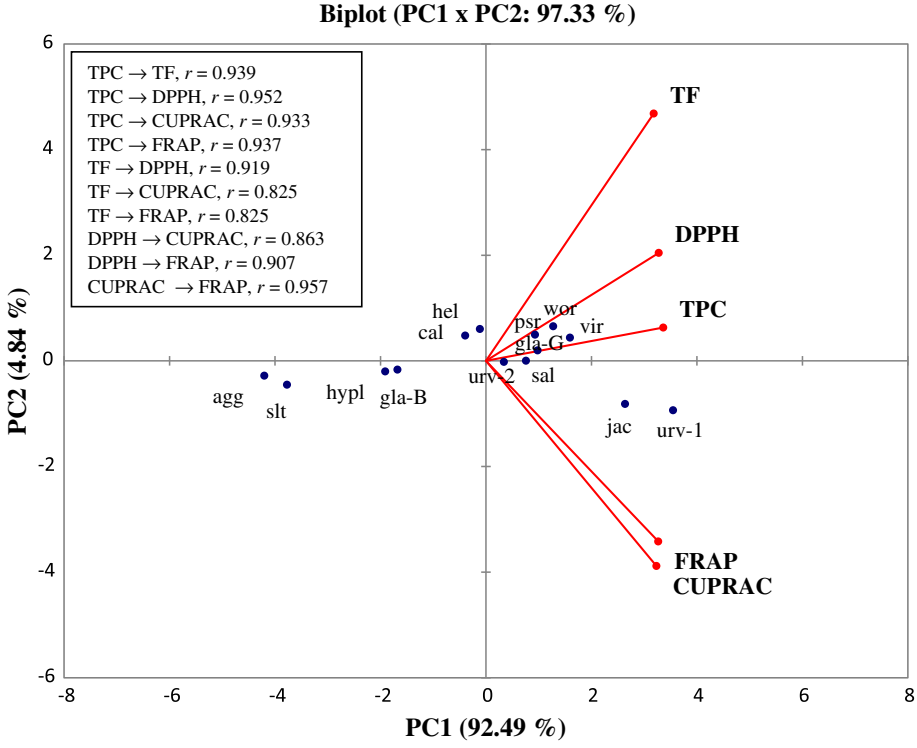


Fig. 5. Principle component analysis (PCA) of some *Centaurea* s.l. taxa based on antioxidant capacity and total phenolics (TPC) or total flavonoid (TF) contents. ^{G}Gümüşhane, ^{B}Bayburt and ^{A}Artvin accessions (see Table 1). The small box at the left upper plan shows correlation matrix (Pearson correlation, *r*) of the data.

3.4. Principal component analysis (PCA)

PCA of antioxidant capacity values, measured using the three assays (DPPH, CUPRAC, FRAP) and TPC or TF explained 97.33% of the total variation in PC1, accounting for 92.49% of the variance, and PC2 for 4.84% (Fig. 5). PC1 separated urv-1, jac, sal and urv-2 situated at the right lower and gla, psr, vir and wor situated at the rigt upper quadrant, on which they were strongly associated and correlated with antioxidant capacity values and TPC or TF (see Fig. 4). The taxa urv-2 and sal were situated along the axis on PC1. There was association or correlation between antioxidant capacity test and TPC or TF contents with the taxa (agg, slt, hypl, gla-B and cal and hel) situated at the lower and upper quadrants on PC2.

The use of chemical characters in biochemical systematics or chemotaxonomy as a distinctive and developed field of study is still the subject of phytochemistry research. The content of chemical compounds from plants' homologous organs based on primary or secondary metabolisms makes a great contribution to solving taxonomical problems for delimitation of taxa. In this respect, one of the chemo-taxonomical markers of great importance in the systematic classification of plant species is fatty acids, which have been used in many plant groups (Stuessy, 2009; Zhang et al., 2015; Ozcan et al., 2016).

The industrial use of agricultural commodities has generated significant interest. As the cost of petroleum-derived products increases, the need to change to a more bio-based economy is obvious (Salimon et al., 2012). In addition to food usages, plant oils have been used into industrial products such as the plastics, pharmaceutical, inks, adhesives, coatings, and many other industrial products. As the author reviewed (Salimon et al., 2012), the advantages of plant oil-derived industrial products can be proven by several of the 12 principles of green chemistry, including the call for the use of renewable feedstock, the minimization of hazards, and the generation of substances with as little toxicity as possible. Due to their bio-based nature, products formed from plant oil are often biodegradable, and because the CO_2 generated from their degradation can be incorporated into the next year's crop, they can be nearly CO_2 neutral (as reviewed by Salimon et al., 2012). With ~68% PUFA content, the oil of the present taxa may form part of the industrial products in question in the related sector.

4. Conclusions

The present study indicated noticeable significant variations in the concentrations of fatty acids, antioxidant capacity of the oil extracted and TPC or TF contents among the taxa of *Centaurea*. These species contained considerable amounts of PUFA based on the concentrations of the two major fatty acids, linoleic acid (C18:2) and oleic acid (C18:1). Strong correlations and relationships were determined between antioxidant capacity and PUFA and TPC or TF concentrations. The data obtained from the present study also revealed a regional difference in the contents of chemical components. The results of the current study suggested that the *Centaurea* phenolics and oil could be utilized as a potential source of natural antioxidants and unsaturated fatty acids for nutritional and pharmacological applications as ingredients in the formulation of functional foods. However, these results should be considered preliminary. Further studies are needed to verify these findings, and several other taxa with wider-ranging populations from different geographical locations, as well as different genera in the sub-family Carduoideae, should be included and also needed to isolate and characterize active phenolic compounds that are responsible for the antioxidant capacities.

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