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Assessment of the antioxidant potential and fatty acid composition of four *Centaurea* L. taxa from Turkey

Abdurrahman Aktumsek [a,*], Gokhan Zengin [a], Gokalp Ozmen Guler [c], Yavuz Selim Cakmak [a], Ahmet Duran [b]

[a] Department of Biology, Science Faculty, Selcuk University, Konya, Turkey
[b] Department of Biological Education, Ahmet Kelesoglu Education Faculty, Konya University, Konya, Turkey
[c] Department of Biotechnology and Molecular Biology, Faculty of Science and Arts, Aksaray

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ABSTRACT

This paper focused on the assessment of antioxidant property and fatty acid composition of four *Centaurea* species. The antioxidant activity of its methanol extract was evaluated by several *in vitro* experiments including phosphomolybedum assay, DPPH assay, β-carotene/linoleic acid, ferric and cupric reducing power. Total phenolic and flavonoid contents were also evaluated. The methanol extract of *Centaurea pulcherrima* var. *pulcherrima* showed the superior free radical scavenging activity, linoleic acid inhibition capacity, reducing power and also had the highest total phenolic content. A significant relationship between antioxidant capacity and total phenolic components was found. The oils of *Centaurea* taxa were also analysed for fatty acid concentration by gas chromatography. The principal fatty acids in the species were palmitic acid (23.38–30.49%) and linoleic acid (20.19–29.93%). These findings suggest that the *Centaurea* species could be used as a potential source of new natural antioxidants and unsaturated fatty acids in food industry, cosmetics and pharmaceutical preparations.

1. Introduction

Reactive oxygen species (ROS) are a class of highly reactive molecules derived from oxygen and generated by metabolic process in humans and external factors such as pollution, radiation or some dietary habits (e.g. smoking and poor diet). Free radicals, when in excess or in the absence of endogenous antioxidant defences, can cause oxidative damage. Oxidative damage or oxidative stress associated with many chronic and degenerative diseases such as cancer, heart disease and Alzhemier's disease (Oseni, Rat, Bogdan, Warnet, & Touitou, 2000). Antioxidants play a major role in the prevention of these diseases because of their ability to scavenge or repair the damage caused by the radicals (Alonso, Guillen, Barroso, Puertas, & Garcia, 2002). Epidemiologic studies have indicated an inverse relationship between dietary intake of antioxidant-rich foods and mortality from these degenerative processes (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993; Pietta, 2000; Sun, 1990). Therefore, antioxidants should be considered be important nutraceuticals for preventing oxidative damage.

Synthetic antioxidants such as butylated hydroxyanisol (BHA), butylated hydroxytoulene (BHT), propyl gallate (PG) and tertiary butyl hydroquinone (TBHQ) are used in foods to prevent lipid oxidation. However, some studies suggest synthetic antioxidants are harmful to human health (Valentao et al., 2002). Consequently, there is increasing interest in finding naturally occurring alternatives from plants for use in food and medicines.

The genus *Centaurea* (Asteraceae) comprises more than 600 species worldwide, but particular Mediterranean region and in West Asia (Garcia-Jacas, Susanna, Mozaffarian, & Ilarslan, 2000). The genus is represented by more than 180 species in Turkey alone (Davis, 1988) including 120 native species. Therefore, Turkey is the main gene center for *Centaurea*. Many *Centaurea* species are used traditionally for medicinal purposes, including *Centaurea pulchella*, *Centaurea drabifolia*, *Centaurea triumfetti*, *Centaurea depressa* and *Centaurea virgata* (Honda et al., 1996; Kargıoglu et al., 2008; Sezik et al., 2001). Basal leaves of *C. triumfetti* and *Centaurea urvillei* subsp. *stepposa* are used as foodstuff and eaten while fresh (Özüdoğru, Akaydın, Erik, & Yesilada, 2011). Moreover, fresh stem of *Centaurea iberica* is eaten after peeling of the outer part (Sarper, Akaydın, Şimşek, & Yeşilada, 2009). Several *Centaurea* species have been investigated for essential oil composition, antioxidant capacity and secondary metabolites (Karamenderes, Konyalioglu, Khan, & Khan, 2007; Tepe, Sokmen, Akpulat, Yumrutas, & Sokmen, 2006).

To our knowledge, there are no reports about antioxidant properties and fatty acid composition in *Centaurea* species. The objectives of the this study were to (i) determine total phenolic and flavonoid content (ii) screen antioxidant properties used different *in vitro* assays (iii) observe possible correlations between total phenolic components and antioxidant activities (iv) identify fatty acid

* Corresponding author. Tel.: +90 332 2231866; fax: +90 332 2410106.
E-mail address: aktumsek@selcuk.edu.tr (A. Aktumsek).

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compositions of the plants by using GC (v) evaluate as a potential natural antioxidant for the food and pharmaceutical industry.

2. Methods

2.1. Plant materials

Centaurea species were collected from different localities in Turkey during vegetation season of 2010. The plants were identified by botanist Professor. Dr. Ahmet Duran. The voucher specimens were deposited in Selcuk University Science Faculty Herbarium, Konya. The complete list of the plant materials with taxonomical information, location and voucher number are given below:

(1) *Centaurea pseudoscabiosa* Boiss et Buhse subsp. *araratica* (Azn.) Wagenitz: Erzincan, Tercan-Erzurum road, 11 km, 1576 m, AD 9131.
(2) *Centaurea pulcherrima* Willd var. *pulcherrima* Willd.: Kars, Arpacay, Kardestepe-Dag koyu road, 2245 m, AD 9195.
(3) *Centaurea salicifolia* Bieb. ex Willd. subsp. *abbreviata* C. Koch: Artvin, Savsat, Sahara national park 1700 m, AD 9197.
(4) *Centaurea babylonica* (L) L.: Antalya, Gazipasa-Saydas road, 250 m, AD 9211.

2.2. Preparation of the extracts

The aerial plant materials were dried at the room temperature. The dried plant materials were ground to a fine powder using a laboratory mill. Fifteen grams of powdered plant were mixed with 250 mL methanol and extracted in a Soxhlet apparatus for 6–8 h. The extracts concentrated under vacuum at 40 °C by using a rotary evaporator. Extracts were stored at +4 °C in dark until use.

2.3. Chemicals

Potassium ferricyanide, ferric chloride, Folin–Ciocalteau's reagent, trichloroacetic acid, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and methanol were purchased from Merck (Darmstadt, Germany). 2,2-diphenyl-1-picrylhydrazyl (DPPH), β-carotene, linoleic acid and Tween 40 were purchased from Sigma Chemical Co. (Sigma–Aldrich GmbH, Sternheim, Germany). All other chemicals and solvents were analytical grade.

2.4. Assay for total phenolics

Total phenolic constituents of the methanolic extracts were determined by employing the methods given in the literature (Slinkard & Singleton, 1977) involving Folin–Ciocalteau reagent and gallic acid as standard. Absorbance was measured at 760 nm (Shimadzu-UV1800). The total phenolic content was determined as gallic acid equivalents (mg GAE g^{-1} extract).

2.5. Assay for total flavonoids

The total flavonoid content in extracts was determined spectrophotometrically according to Arvouet-Grand, Vennat, Pourrat, and Legret (1994). Briefly, 1 mL of 2% aluminium trichloride ($AlCl_3$) methanolic solution was mixed with the same volume of extract solution (at 2 mg mL^{-1} concentration). The absorbance values of the reaction mixtures were determined at 415 nm after 10 min duration against a blank. Rutin was used as the standard and the total flavonoids content of the extracts was expressed as milligram rutin equivalents per gram of extract (mg RE g^{-1} extract).

2.6. Evaluation of total antioxidant capacity by phosphomolybdate assay

The total antioxidant capacities of extracts were evaluated by phosphomolybdenum method according to Prieto, Pineda, and Aguilar (1999). The antioxidant capacity of extracts was expressed as equivalents of ascorbic acid (mg AE g^{-1} extract).

2.7. Scavenging activity on DPPH

The hydrogen atoms or electrons donation ability of the corresponding extracts and some pure compounds were measured based on bleaching of the purple-coloured methanol solution of DPPH. The effect of methanolic extracts on DPPH radical was estimated according to Kirby and Scmidt (1997). 1 mL of various concentrations of the methanolic extract was added to a 4 mL of a 0.004% methanol solution of DPPH. The mixture was shaken vigorously and allowed to stand for 30 min; the absorbance of the resulting solution was measured at 517 nm with a spectrophotometer. Inhibition activity was calculated in following way:

$$I\,(\%) = (A_0 - A_1)/A_0 \times 100$$

where A_0 is the absorbance of the control, A_1 is the absorbance of the extract/standard. 50% of free radical inhibition (IC_{50}) of extract was calculated. The lower the IC_{50} value indicates high antioxidant capacity.

2.8. β-Carotene–linoleic acid bleaching assay

In this assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Dapkevicius, Venskutonis, van Beek, & Linssen, 1998). A stock solution of β-carotene–linoleic acid mixture was prepared as following: 0.5 mg β-carotene was dissolved in 1 mL of chloroform (HPLC grade). 25 μL linoleic acid and 200 mg Tween 40 was added. Chloroform was completely evaporated using a vacuum evaporator. Then 100 mL of oxygenated distilled water was added with vigorous shaking; 2.5 mL of this reaction mixture was dispersed to test tubes and 0.35 mL of the methanolic extract (2 mg mL^{-1}) were added and the emulsion system was incubated for up to 2 h at 50 °C. The same procedure was repeated with the positive control BHT, BHA and a blank. After this incubation period, absorbance of the mixtures was measured at 490 nm. Measurement of absorbance was continued until the colour of β-carotene disappeared. The bleaching rate (R) of β-carotene was calculated according to Eq. (1).

$$R = \ln(a/b)/t \quad (1)$$

where ln = natural log, a = absorbance at time 0, b = absorbance at time t (120 min). The antioxidant activity (AA) was calculated in terms of percent inhibition relative to the control using Eq. (2).

$$AA = [(R_{Control} - R_{Sample})/R_{Control}] \times 100 \quad (2)$$

Antioxidative activities of the extracts were compared with those of BHT and BHA at 2.0 mg/mL.

2.9. Reducing power activity (Iron (III) to iron (II) reduction)

The ferric reducing power method applied with slight modifications of the method of Oyaizu (1986). The EC_{50} value (the effective concentration at which the absorbance was 0.5) was calculated for extracts and BHT.

2.10. Cupric ion reducing antioxidant capacity (CUPRAC assay)

The cupric ion reducing capacity of extracts was determined according to the method of Apak, Guclu, Ozyurek, Karademir, and Ercag (2006). Ascorbic acid was used as a standard antioxidant. The results of the assay were evaluated by using EC_{50} values.

2.11. Extraction of oils

The oil extraction of dried and powdered aerial plants (10 g) was carried out at boiling point (34 °C) for 6 h using a Soxhlet extractor and diethyl ether as a solvent. The solvent was evaporated by rotary evaporator. The oil was esterified to determine fatty acid composition.

2.12. Fatty acids methyl esters (FAMEs) preparation

The fatty acids in the oil were esterified into methyl esters by saponification with 0.5 mol L^{-1} methanolic NaOH and transesterified with 14% BF_3 (v/v) in methanol (IUPAC, 1979).

2.13. Gas chromatographic analysis

FAMEs were analysed on a HP (Hewlett Packard) Agilent 6890 N model gas chromatograph (GC), equipped with a flame ionisation detector (FID) and fitted to a HP-88 capillary column (100 m, 0.25 mm i.d. and 0.2 μm). Injector and detector temperatures were set at 250 and 280 °C, respectively. The oven was programmed at 60 °C initial temperature and 1 min initial time. Thereafter the temperature was increased up to 190 °C rate of 20 °C/min^{-1} held for 60 min then increased at 1 °C/min to 220 °C and held for 10 min at 220 °C. Total run time was 107.5 min. Helium was used as carrier gas (1 mL min^{-1}).

Identification of fatty acids was carried out by comparing sample FAME peak relative retention times with those obtained for Alltech and Accu standards. Results were expressed as FID response area in relative percentages. Each reported result is given as the average value of three GC analyses. The results are offered as means ± S.D. Atherogenic index (AI) and thrombogenicity index (TI) were calculated according to Ulbricht and Southgate (1991). AI = [12:0 + (4 × 14:0) + 16:0]/[(ω6 + ω3) PUFA + 18:1 + other MUFA]; TI = [14:0 + 16:0 + 18:0]/[0.5 × 18:1 + 0.5 × other MUFA + 0.5 × ω6 PUFA + 3 × ω3 PUFA + (ω3 PUFA/ω6 PUFA)]. The cluster analysis of data on fatty acid profiles of the studied samples were performed with Ward's method using Statistica 8.0.

3. Results and discussion

3.1. Total phenolic, flavonoid and antioxidant capacity

Phenolic compounds contribute to multiple biological effects, including antioxidant activity. Therefore, assessment of phenolic content in plant extracts is very important issue. The total phenolic content of each *Centaurea* species was determined according to the Folin–Ciocalteu method and expressed as mg GAE g^{-1} of extract. A wide variation was observed in the phenolic contents found in the *Centaurea* species. As shown in Table 1, the highest levels of phenolics were found in *C. pulcherrima* var. *pulcherrima* (348.56 mg GAE g^{-1} extract). In contrast, the content of *C. pseudoscabiosa* subsp. *araratica* (146.06 mg GAE g^{-1} extract) was the lowest. The total phenolic content of studied species can be ranked: *C. pulcherrima* var. *pulcherrima* > *C. salicifolia* subsp. *abbreviata* > *C. babylonica* > *C. pseudoscabiosa* subsp. *araratica*. The amount of phenolics was comparable with the results described in the literature for other *Centaurea* species. For example, the content was detected as 175.404 and 10.9 mg GAE g^{-1} for *Centaurea cheirolopha* and *Centaurea ammocyanus*, respectively (Aktumsek, Zengin, Guler, Cakmak, & Duran, 2011; Alali et al., 2007). Total flavonoid content of studied *Centaurea* species were also evaluated by the aliminium–chloride method and presented as rutin equivalents (RE) in Table 1. Total extractable flavonoid contents in the extracts varied from 13.12 mg RE g^{-1} in *C. babylonica* to 182.56 mg RE g^{-1} in *C. pulcherrima* var. *pulcherrima*.

Phosphomolybdenum assay was performed in order to evaluate total antioxidant capacity of studied *Centaurea* species. Among the *Centaurea* species studied, total antioxidant capacity showed a similar trend to total phenolics (Table 1). Thus, the strong reduction activity (from Mo(VI) to Mo(V)) might be attributed the presence of phenolic compounds. The result was consistent with previous reports because some authors have shown that high total phenol content increases the total antioxidant activity (Kumaran & Karunakaran, 2007; Sagdic et al., 2011).

3.2. Free radical scavenging activity

DPPH assay is widely used to test the free radicals scavenging activities of plant extracts. This activity was evaluated by the determination of the IC_{50} values (the amount of extract required to scavenge 50% of DPPH radicals) for each extracts (Table 2). A lower IC_{50} value indicated a greater antioxidant activity. *C. pulcherrima* var. *pulcherrima*, which has the highest level of phenolic compound, displayed the strongest DPPH radical quenching ability with an IC_{50} = 187.42 μg mL^{-1}. Values for two *Centaurea* extracts were the greater than 400 μg mL^{-1}. When compared with *Centaurea*, the synthetic antioxidant (BHT) has very high free radical scavenging activity. In the present work, IC_{50} values reported were lower than previous studies on some *Centaurea* species for example *Centaurea rigida* and *Centaurea amanicola* (Aktumsek et al., 2011). Because *C. rigida* and *C. amanicola* had low phenolic contents, these species exhibited lower free radical scavenging activity in comparison with *C. pulcherrima* var. *pulcherrima*.

3.3. Ferric and cupric reducing power

Reducing power is one of antioxidant mechanism and is a powerful assay to indicate of this activity in a plant extract (Jayapraka-

Table 1
Extraction yield, total antioxidant capacity, total phenolic and flavonoid content of four *Centaurea* species.

Species	Yield (%)	TPC[a]	TFC[b]	TAC[c]
C. pseudoscabiosa subsp. *araratica*	16.79	146.06 ± 18.46*	54.39 ± 2.19	120.81 ± 2.00
C. pulcherrima var. *pulcherrima*	18.18	348.56 ± 11.00	182.56 ± 2.13	179.33 ± 1.45
C. salicifolia subsp. *abbreviata*	22.09	309.39 ± 20.82	149.19 ± 1.18	161.18 ± 3.93
C. babylonica	13.96	274.94 ± 5.11	13.12 ± 1.01	125.33 ± 3.76

[a] Total phenolic content (TPC) expressed as gallic acid equivalent (mg GAE g^{-1} extract).
[b] Total flavonoid content (TFC) expressed as rutin equivalent (mg RE g^{-1} extract).
[c] Total antioxidant capacity (TAC) expressed as ascorbic acid equivalent (mg AE g^{-1} extract).
* Values expressed are means ± S.D.

Table 2
Antioxidant activities of four *Centaurea* species and standard antioxidants expressed as IC_{50} and EC_{50} values, based on DPPH and reducing power assays, respectively.

Centaurea species	DPPH /IC_{50} (μg/mL)	Ferric reducing power/EC_{50} (μg/mL)	CUPRAC/EC_{50} (μg/mL)
Centaurea pseudoscabiosa subsp. *araratica*	670.59 ± 19.43*	736.86 ± 8.49	713.52 ± 43.52
Centaurea pulcherrima var. *pulcherrima*	187.42 ± 3.11	200.95 ± 4.99	202.58 ± 20.22
Centaurea salicifolia subsp. *abbreviata*	284.46 ± 3.07	276.11 ± 2.98	247.41 ± 1.54
Centaurea babylonica	464.23 ± 9.36	440.57 ± 24.14	488.22 ± 22.70
BHT	43.74 ± 3.04	24.35 ± 7.85	–
Ascorbic acid	–	–	13.73 ± 0.48

* Values expressed are means ± S.D.

sha, Singh, & Sakariah, 2001). For these reason, ferric and cupric reducing power assay were performed to evaluate antioxidant capacity of studied *Centaurea* species. The reducing power activities exhibited by studied extracts were concentration-dependent. *C. pulcherrima* var. *pulcherrima* demonstrated a strong ferric reducing power comparable to that of other species. The absorbances were found to be 0.57, 1.95, 1.45 and 0.91 at 800 μg/mL for *C. pseudoscabiosa* subsp. *araratica*, *C. pulcherrima* var. *pulcherrima*, *C. salicifolia* subsp. *abbreviata* and *C. babylonica*, respectively. As to CUPRAC assay, the order of the tested *Centaurea* species in the cupric reducing capacity assay was similar in the ferric reducing power. Thus, a very good correlation was observed between antioxidant capacities determined by ferric and cupric reducing power. Our results were in accordance with other investigators, who have also reported that ferric reducing power could be strongly correlated with cupric reducing power (Celep, Aydın, & Yesilada, 2012; Ozturk, Aydogmus-Ozturk, Duru, & Topcu, 2007). Both ferric and cupric reducing activity of studied extracts was also evaluated using EC_{50} values (Table 2). Based on EC_{50} values, the same trends were observed in *Centaurea* species. Previous studies examined the reducing capacity of some *Centaurea* species. For instance, EC_{50} values in ferric and cupric reducing power tests were 205.929 and 154.534 μg/mL for *C. cheirolopha*, which exhibited the strongest antioxidant activity in our previous study (Aktumsek et al., 2011). From the results, it can be concluded that *C. cheirolopha* has strongest reducing power activity compared to *Centaurea* species studied in the present work.

3.4. β-Carotene/linoleic acid assay

β-Carotene/linoleic acid test system is a useful method to evaluate lipid peroxidation inhibitory activity of plant extracts. Fig. 1 shows the antioxidant capacity of the extracts as measured by bleaching of β-carotene. In this assay, *C. pulcherrima* var. *pulcherrima* had the best antioxidant activity, but it was not good as BHA and BHT. At the same concentration (2 mg mL^{-1}), the values for BHA (93.370%) and BHT (94.324%) were 1.27-fold greater than that for *C. pulcherrima* var. *pulcherrima* (73.875%). The antioxidant activity of extracts and standard antioxidant can be ranked as BHT > BHA > *C. pulcherrima* var. *pulcherrima* > *C. salicifolia* subsp. *abbreviata* > *C. babylonica* > *C. pseudoscabiosa* subsp. *araratica* . The same trend was observed for studied *Centaurea* species in DPPH assay. β-Carotene/linoleic acid and DPPH assays are reported as complementary tests in the literature. In the same test system, inhibition values of linoleic oxidation were found to be 63.60% in *C. pulchella* (Zengin, Cakmak, Guler, & Aktumsek, 2010), 85.15% in ethyl acetate extract of *Centaurea ensiformis* (Ugur et al., 2009) and 35.2% in *Centaurea mucronifera* (Tepe et al., 2006).

3.5. Correlations between total phenolic /flavonoid content and antioxidant properties

Table 3 shows that the antioxidant activity of studied *Centaurea* extracts correlated well with their total phenolic and flavonoid content. The contents of phenolic and flavonoid exhibited good correlation ($p < 0.05$) with total antioxidant capacity ($R^2 = 0.84$–0.96). Reducing power activities presented a significant correlation with the level of phenolic contents. The results also show a good linear correlation free radical scavenging activity ($R^2 = 0.88$), in relation to the amount of phenolic compounds. Similar correlation was reported by Dudonne, Vitrac, Coutıerre, Woillez, and Merillon (2009) who found $R^2 = 0.939$ as the correlation factor between free radical scavenging activity and total phenolics in some plant extracts. In the present study, the correlation coefficient between the inhibition capacity by β-carotene/linoleic acid test system for flavonoids were lower than for total polyphenols ($R^2 = 0.76$–0.97).

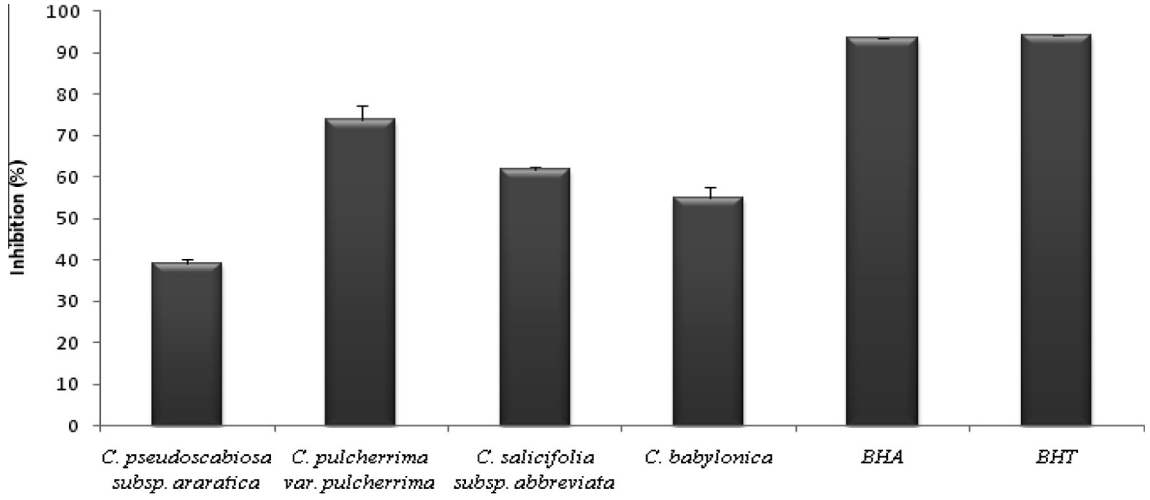

Fig. 1. Inhibition capacities of four *Centaurea* species and synthetic antioxidants in β-carotene/linoleic acid test system.

Table 3
Correlation between antioxidant assays and each of total phenolic and flavonoid contents of four *Centaurea* species.

Assays	Total phenolic content	Total flavonoid content
Total antioxidant capacity	0.84	0.96
Inhibition capacity	0.97	0.76
Ferric ion reducing power	0.91	0.88
Free radical scavenging activity	0.88	0.91
CUPRAC assay	0.89	0.93

These results are in agreement with previous reports, stating that the phenolic compounds contribute significantly to the antioxidant activity in different medicinal plants (Duan, Wu, & Jiang, 2007).

3.6. Fatty acid composition

Table 4 shows data on oil content and fatty acid composition of studied *Centaurea* species. The oil content in the species varied from 2.50% to 3.16%, the highest value being observed in *C. salicifolia* subsp. *abbreviata* while *C. pseudoscabiosa* subsp. *araratica* showed the lowest value. The oil contents of *Centaurea* species obtained in this study were higher than that reported by Aktumsek et al. (2011). Many authors reported that differences were observed in oil content of plants depending on several factors including temperature, soil content and altitude (Hassan, Kaleem, & Ahmad, 2011; Marguard & Schuster, 1980; Sakla & Ghali, 1988). Because of these factors, oil content of *Centaurea* species may be change to a relatively small extent. Thirty individual fatty acids were identified in the *Centaurea* oils. The major fatty acids have 16 or 18 carbon atoms with a variable degree of unsaturation. Palmitic acid was the major fatty acid, contributing 29.37 to 30.49 % of the total fatty acids in *C. salicifolia* subsp. *abbreviata* and *C. babylo-*

Table 4
Fatty acid composition of four *Centaurea* species (%).

Fatty acids	*C. pseudoscabiosa* subsp. *araratica*	*C. pulcherrima* var. *pulcherrima*	*C. salicifolia* subsp. *abbreviata*	*C. babylonica*
C 8:0	0.01 ± 0.01*	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
C 10:0	0.05 ± 0.01	0.80 ± 0.01	0.03 ± 0.01	0.05 ± 0.01
C 11:0	0.07 ± 0.01	0.09 ± 0.05	0.16 ± 0.03	0.16 ± 0.01
C 12:0	1.70 ± 0.04	1.01 ± 0.01	2.37 ± 0.01	1.81 ± 0.06
C 13:0	0.18 ± 0.01	0.04 ± 0.01	0.07 ± 0.05	0.08 ± 0.02
C 14:0	0.88 ± 0.01	0.93 ± 0.09	0.85 ± 0.01	0.76 ± 0.18
C 15:0	0.24 ± 0.01	0.49 ± 0.02	0.63 ± 0.02	0.54 ± 0.01
C 16:0	25.79 ± 0.13	23.38 ± 0.13	29.37 ± 0.45	30.49 ± 0.13
C 17:0	0.59 ± 0.01	1.01 ± 0.01	0.64 ± 0.06	0.67 ± 0.23
C 18:0	4.16 ± 0.03	5.48 ± 0.01	4.58 ± 0.11	8.03 ± 0.04
C 19:0	0.20 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.03 ± 0.01
C 20:0	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.12 ± 0.04
C 21:0	0.26 ± 0.10	0.14 ± 0.01	0.33 ± 0.08	0.19 ± 0.01
C 22:0	0.04 ± 0.02	0.09 ± 0.04	0.03 ± 0.01	0.16 ± 0.06
ΣSFA[a]	34.19 ± 0.16	33.57 ± 0.05	39.15 ± 0.35	43.10 ± 0.27
C 14:1 ω5	0.04 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.02 ± 0.01
C 15:1 ω5	0.71 ± 0.01	1.24 ± 0.01	1.67 ± 0.07	1.16 ± 0.28
C 16:1 ω7	0.39 ± 0.05	0.32 ± 0.01	0.40 ± 0.06	0.36 ± 0.02
C 17:1 ω8	1.00 ± 0.13	0.45 ± 0.01	0.50 ± 0.01	0.60 ± 0.01
C 18:1 ω9	17.37 ± 0.30	12.92 ± 0.01	6.90 ± 0.23	9.69 ± 0.03
C 18:1 ω7	1.04 ± 0.21	0.54 ± 0.09	0.23 ± 0.19	0.24 ± 0.01
C 20:1 ω9	0.28 ± 0.10	0.28 ± 0.01	0.30 ± 0.01	0.27 ± 0.01
C 22:1 ω9	0.04 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01
ΣMUFA[a]	20.87 ± 0.03	15.83 ± 0.70	10.10 ± 0.40	12.41 ± 0.25
C 18:2 ω6	29.81 ± 0.26	29.93 ± 0.33	26.94 ± 0.66	20.19 ± 0.01
C 18:3 ω3	9.96 ± 0.10	13.65 ± 0.04	17.27 ± 0.42	10.75 ± 0.48
C 18:3 ω6	2.92 ± 0.06	3.88 ± 0.18	4.38 ± 0.06	10.22 ± 0.06
C 20:2 ω6	0.42 ± 0.06	0.04 ± 0.03	0.01 ± 0.01	0.03 ± 0.01
C 20:4 ω6	1.52 ± 0.47	1.45 ± 0.03	1.03 ± 0.54	2.45 ± 0.51
C 20:5 ω3	0.15 ± 0.01	0.72 ± 0.10	0.48 ± 0.06	0.58 ± 0.04
C 22:2 ω6	0.06 ± 0.04	0.06 ± 0.02	0.12 ± 0.01	0.11 ± 0.01
C 22:6 ω3	0.09 ± 0.01	0.87 ± 0.16	0.52 ± 0.11	0.16 ± 0.02
ΣPUFA[a]	44.94 ± 0.17	50.60 ± 0.65	50.75 ± 0.16	44.49 ± 0.03
ΣUFA[a]	65.81 ± 0.14	66.43 ± 0.05	60.85 ± 0.75	56.90 ± 0.28
ΣEFA	39.77 ± 0.36	43.58 ± 0.51	44.21 ± 0.48	30.94 ± 0.06
Σω3	10.20 ± 0.08	15.24 ± 0.24	18.27 ± 0.31	11.49 ± 0.07
Σω6	34.73 ± 0.25	35.36 ± 0.41	32.48 ± 0.47	33.00 ± 0.04
ω3/ω6	0.29 ± 0.01	0.43 ± 0.01	0.56 ± 0.02	0.35 ± 0.01
ω6/ω3	3.40 ± 0.05	2.32 ± 0.01	1.78 ± 0.09	2.87 ± 0.02
AI[b]	0.47 ± 0.01	0.42 ± 0.01	0.58 ± 0.02	0.62 ± 0.01
TI[b]	0.53 ± 0.01	0.42 ± 0.01	0.46 ± 0.01	0.68 ± 0.01
Oil content	2.50	2.85	3.16	2.62

[a] SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids, UFA: Unsaturated fatty acids, EFA: Essential fatty acids.
[b] AI: Atherogenic index, TI: Thrombogenicity index.
* Values expressed are means ± S.D.

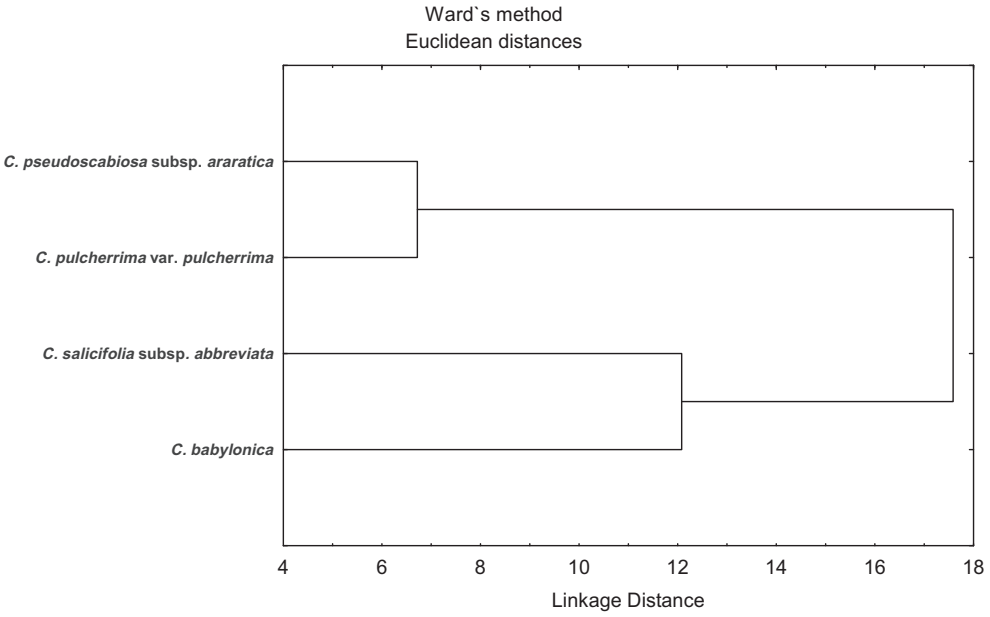

Fig. 2. Dendrogram of *Centaurea* species using Ward's method based on fatty acid compositions.

nica, respectively while *C. pseudoscabiosa* subsp. *araratica* and *C. pulcherrima* var. *pulcherrima* oils were rich linoleic acid (29.81% and 29.93%). Similar results were observed by Yildirim, Sunar, Agar, Bozari, and Aksakal (2009) who reported that palmitic acid was the predominant fatty acid in some *Centaurea* oils.

The same profile (C18:2 > C16:0) was also described for some *Centaurea* (*C. cheirolopha*, *C. iberica* and *C. balsamita*) from Turkey (Aktumsek et al., 2011; Tekeli, Sezgin, Aktumsek, Guler, & Sanda, 2010). Linoleic and linolenic acid content of the studied oils account for up to 30% in the total fatty acids. With regard to linolenic acid, *C. salicifolia* subsp. *abbreviata* had the highest (17.27%) amount while *C. pseudoscabiosa* subsp. *araratica* showed the lowest (9.96%) value. The amounts of linoleic and linolenic acid in the *Centaurea* oils are noteworthy because these fatty acids are not synthesised by human body. Therefore, they must be obtained through the diet. Studied *Centaurea* species, especially *C. salicifolia* subsp. *abbreviata* (44.21%) and *C. pulcherrima* var. *pulcherrima* (43.58%) oils, can be considered as a source of essential fatty acids. Other major polyunsaturated fatty acids were 18:3 ω6 and 20:4 ω6. Among the minor fatty acids there were ω6 PUFAs such as 20:2 ω6 and 22:2 ω6 as well as PUFAs of the ω3 family, 20:5 ω3 and C 22:6 ω3. Oleic acid was the most abundant MUFA in all the samples. The oleic acid level reached 17.37% in the *C. pseudoscabiosa* subsp. *araratica* oil. PUFAs comprised the largest percent composition of all fatty acid groups (saturated, monounsaturated, polyunsaturated) in all samples and their contents ranged from 44.49% in *C. babylonica* to 50.75% in *C. salicifolia* subsp. *abbreviata*.

Some parameters, currently used to assess nutritional quality of oils, ω6/ω3 ratios, and AI and TI values, which are measures of atherogenic and trombogenic characteristics of an oil. Lower of ω6/ω3 ratios and AI and TI values favour good health. Therefore, *C. pulcherrima* var. *pulcherrima* and *C. salicifolia* subsp. *abbreviata* oils had an important nutritional quality. Interestingly, the species had stronger antioxidant properties compared to other *Centaurea* species. Also, in our previous studies, were observed similar results (Aktumsek et al., 2011; Zengin et al., 2010). Therefore, the situation can be recommended a hypothesis, i.e. that plants accumulate higher amounts of antioxidant compound in order to protect polyunsaturated fatty acids.

The dendrogram obtained from Cluster analysis classified the *Centaurea* species in two groups (Fig. 2). *C. pseudoscabiosa* subsp. *araratica* and *C. pulcherrima* var. *pulcherrima* clearly separated from the remaining species in the dendrogram (Group 1). These species were characterised with high level linoleic acid. Among *Centaurea* species, the greatest similarity was observed in this group. Other group (Group 2) had higher contents of palmitic acid and lowest contents of linoleic acid than first group. *C. babylonica* aligned distantly with this group because its the highest percentage of palmitic and low content of linoleic acid.

4. Conclusion

The results obtained in the present study clearly demonstrate that *Centaurea* species, especially *C. pulcherrima* var. *pulcherrima,* exhibited the strong antioxidant activity in test *in vitro*. Our study showed a significant correlation between the phenolic components and antioxidant properties. The amounts of polyunsaturated fatty acids were higher than mono-and saturated fatty acids in all examined *Centaurea* oils. Based on the above-mentioned results, we can conclude that these species have good potential to be used as ingredient (as a source of natural antioxidant and polyunsaturated fatty acids) for the formulation of functional foods. However, further studies are needed to isolate and characterise the active compounds that are responsible for the antioxidant activities.

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