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Antioxidant capacity and fatty acid profile of *Centaurea kotschyi* (Boiss. & Heldr.) Hayek var. *persica* (Boiss.) Wagenitz from Turkey

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RESUMEN

Capacidad antioxidante y perfil de ácidos grasos de *Centaurea kotschyi* (Boiss. & Heldr.) Hayek var. *persica* (Boiss.) Wagenitz de Turquía.

La capacidad antioxidante de extractos metanólicos y composición de ácidos grasos de *C. kotschyi* var. *pérsica* fueron investigados. Seis métodos químicos diferentes fueron realizados para la determinación de la capacidad antioxidante. La composición de ácidos grasos fue analizada por cromatografía de gases. Los valores de IC_{50} de los extractos fueron 37.09 µg/ml (en el ensayo con DPPH). En el sistema β-carotene/ácido linoleico, el extracto mostró un 65.22% de inhibición frente a la oxidación del ácido linoleico. La cantidad total de contenido fenólico y capacidad antioxidante total fueron 36.52 mg equivalentes de ácido gallico (GAE)/g y 74.93 mg equivalentes de ácido ascórbico (AE)/g, respectivamente. El principal ácidos graso encontrado, por análisis de CG, en *C. kotschyi* var. *pérsica* fue el C 18:3 ω3 (ácido α-linolenico). Los resultados presentados aquí indican que *C. kotschyi* var. *pérsica* posee unas fuertes propiedades antioxidantes. Además, las especies pueden ser usadas como aditivos naturales en los alimentos, en cosmética y en industria farmacéutica.

PALABRAS CLAVE: Capacidad antioxidante total - Centaurea kotschyi – *Composición de ácidos grasos – Contenido total de fenoles – Poder reductor – Sistema* β*-caroteno/ácido linoleico*

SUMMARY

Antioxidant capacity and fatty acid profile of *Centaurea kotschyi* (Boiss. & Heldr.) Hayek var. *persica* (Boiss.) Wagenitz from Turkey.

The antioxidant capacity of the methanolic extract and the fatty acid composition of *C. kotschyi* var. *persica* were investigated. Six different chemical methods were used to determine the antioxidant capacity. The fatty acid composition was analyzed using gas chromatography. The IC_{50} value of the extract was determined as 37.09 µg/ml (in the DPPH assay). In the β-carotene/linoleic acid system, the extract exhibited 65.22% inhibition against linoleic acid oxidation. The amount of total phenolic content and total antioxidant capacity were detected as 36.52 mg gallic acid equivalent (GAE)/g and 74.93 mg ascorbic acid equivalent (AE)/g, respectively. The major fatty acid in the composition of *C. kotschyi* var. *persica* was found to be C 18:3 ω3 (α-linolenic acid) by GC analysis. The results presented here indicate that *C. kotschyi* var. *persica* possess strong antioxidant properties. Therefore, the species can be used as a natural additive in food, cosmetic and pharmaceutical industries.

KEY-WORDS: β*-carotene/Linoleic acid system – Centaurea kotschyi – Fatty acid composition – Reducing power – Total antioxidant capacity – Total phenolic content.*

1. INTRODUCTION

Free radical formation is associated with the normal metabolism of aerobic cells. This oxygen consumption inherent in cell growth leads to the generation of a series of free radicals, which may interact with biological systems in a clearly cytotoxic manner (Elmegeed *et al*., 2005). The radicals can cause damage to cellular bio-molecules such as nucleic acids, enzymes, proteins, lipids and carbohydrates (Nilsson *et al*., 2004). Overproduction of free radicals is considered to be the cause of chronic diseases such as cancer, atherosclerosis, ageing, and inflammatory diseases (Braca *et al*., 2002).

All aerobic organisms have antioxidant defenses that protect against free radicals and repair enzymes to remove or repair damaged molecules (Sun *et al*., 1998). If the radical species are not completely scavenged by this endogenous system, a dietary intake of antioxidant compounds becomes important (Espin *et al*., 2000). Although some synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are commonly used in processed foods, it has been reported that these compounds have some side effects (Ito *et al*., 1983). Many plants have been investigated as natural sources of antioxidants that could be safer than synthetic sources (Salazar *et al*., 2008). Therefore, there is a growing interest in natural antioxidants present in medicinal and dietary plants.

Fatty acids are the main components of oils. Especially, essential fatty acids are important in terms of human health. Because these fatty acids are not synthesized by the body itself, their dietary intake through certain plants and various oils is essential (Singer, 1992; Okuyama *et al*., 1996). Clinical and epidemiologic studies have shown that many chronic diseases are related to fatty acid type (WHO, 2003).

The genus *Centaurea* belongs to the Asteraceae family and comprises about 500-600 species. The genus *Centaurea* L. (Asteraceae) is represented in Turkey with 187 taxa, 114 of which are endemic (Wagenitz, 1975). Several *Centaurea* species have been used in folk medicine for various ailments such as antidiarrheic, antipyretic, diuretic, choleretic, antiinflammatory and antibacterial (Arif *et al*., 2004; Kargıoglu *et al*., 2008; Kargıoglu *et al*.,2010). Many *Centaurea* species, such as *C. hadimensis* (Flamini *et al*., 2002), *C. cyanus* and *C. depressa* (Karamenderes *et al*., 2008) were studied upon the determination of essential oil components. Also, the antioxidant properties of the *Centaurea* species such as *C. mucronifera* (Tepe *et al.,* 2006), *C. ensiformis* (Ugur *et al.,* 2009), *C. calolepis* (Karamenderes *et al*., 2007) and *C. solstitialis* (Tekeli *et al*., 2008) were reported using various methods. But no reports have yet been made on the fatty acid composition and antioxidant capacity of *Centaurea kotschyi* var. *persica.* Therefore, the objective of this study was to screen the antioxidant capacity of the methanol extract and the fatty acid composition of *C. kotschyi* var. *persica*.

2. MATERIALS AND METHODS

2.1. Plant material

The aerial parts of *Centaurea kotschyi* (Boiss. et Heldr.) var. *persica* (Boiss.) Wagenitz were collected from Konya in July, 2009. The plant has been identified by Evren Yildiztugay from the Section of Botany, Department of Biology, Faculty of Science, Selcuk University. Voucher specimens have been deposited in KNYA herbarium at the department of Biology, Selcuk University.

2.2. Preparation of methanolic extract

The aerial plant materials were dried at room temperature and powdered to a fine grain using a laboratory mill. A powder plant sample (15 g) was extracted with 100 ml of methanol in a mechanical shaker at room temperature. The extract was filtered and the methanol was evaporated at 40°C using a rotary evaporator. The extract was stored in the dark at -20°C.

2.3. Chemicals

Potassium ferricyanide, ferric chloride, Folin-Ciocalteu's reagent, trichloroacetic acid, methanol, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), ascorbic acid 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 are analytical grade.

2.4. Total Phenolic Content (Folin-Ciocalteu Assay)

The total phenolic content of the extract was determined by the Folin-Ciocalteu method (Slinkard and Singleton, 1977). 0.2 µl of the extract solution (1 mg/ml) mixed with 1 ml Folin-Ciocalteu reagent and 2 ml Na_2CO3 (7.5%). The final volume was brought up to 7 ml with deionized water. The mixture was allowed to stand for 2 h at room temperature. Absorbance was measured at 765 nm with a spectrophotometer (Shimadzu UV-1800). Gallic acid was used to calculate the standard curve and the result was expressed as mg gallic acid equivalent (GAE) per g of extract.

2.5. Total Antioxidant Capacity (Phosphomolybdenum Assay)

The total antioxidant capacity of the extract was determined using the phosphomolybdenum method according to Prieto et al. (1999). 0.3 ml of the methanolic extract (1 mg/ml) was mixed with a 3 ml reagent solution (6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm against a blank. The antioxidant capacity of the extract was expressed as the equivalent of ascorbic acid (mg AE/g).

2.6. Free Radical Scavenging Activity (DPPH, 2,2-diphenyl-1-picrylhydrazyl)

The free radical scavenging activity of the plant extract was determined through slight modifications of the method described by Sarikurkcu *et al*. (2008). 0.5 ml of various concentrations of the extracts in the methanol were added to 3 ml 6.10^{-5} M of a methanol solution of DPPH. The solution was incubated for 30 min in the dark at room temperature. After the incubation, the mixture absorbance was measured at 517 nm. Inhibition activity (I%) 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. The extract concentration providing 50% of the radical scavenging activity (IC_{50}) was calculated from the graph of inhibition percentage against concentration. Synthetic antioxidants (BHA and BHT) were used a standards.

2.7. β-carotene/Linoleic Acid Bleaching Assay

In this assay, the antioxidant capacity of the extract was determined through slight modifications of the procedure described by Sokmen *et al*. (2004). A stock solution of β-carotene-linoleic acid mixture was prepared as follows: 0.5 mg β-carotene was dissolved in 1 ml chloroform, 25 µl linoleic acid and

200 mg Tween 40 were added. The chloroform was completely evaporated and 100 ml distilled water saturated with oxygen were added with vigorous shaking. 2.5 ml of this reaction mixture were dispensed into test tubes and a 350 µl portion of the extract (1 mg/ml) were added. The reaction mixture was incubated at 50°C for 2 h. The same procedure was repeated with a synthetic antioxidant (BHT and BHA) and a blank. After this incubation period, the absorbance of the mixtures was measured at 490 nm and the inhibition ratio was calculated.

2.8. Ferric Ion Reducing Power

The ferric reducing power method was applied with slight modifications of the method of Oyaizu (1986). Different concentrations of the extracts mixed with 2.5 ml 0.2 M phosphate buffer and potassium ferricyanide (1%). The mixture were incubated at 50°C for 20 min. After the incubation, 2.5 ml of 10% trichloroacetic acid were added. 2.5 ml of the reaction mixture were mixed with 2.5 ml distilled water and 0.5 ml of 0.1% ferric chloride. The solution absorbance was measured at 700 nm. The reducing power of the samples increased with the absorbance value. The same procedure was applied with BHA and BHT. The EC_{50} value (the effective concentration at which the absorbance was 0.5) was calculated.

2.9. Cupric Ion Reducing Antioxidant Capacity (CUPRAC Assay)

The cupric ion reducing capacity of the extract and ascorbic acid were determined according to the method of Apak *et al*. (2006). 1 ml each of 10 mM $CuCl_2$, 7.5 mM neocuproine and NH_4Ac buffer (1 M, pH 7.0) solutions were added to a test tube. Then, 0.5 ml of different concentrations of the extract were mixed and the total volume was brought up to 4.1 ml with deionized water. After 30 min incubation at room temperature, the mixture absorbance at 450 nm was recorded against a blank.

2.10. Analysis of fatty acids

The oil extraction of dried and powdered aerial plants (10 g) was carried out at 60°C for 6 h in a Soxhlet extractor using petroleum ether as a solvent. The solvent was evaporated with a rotary evaporator. The oil was esterified to determine its fatty acid composition.

The fatty acids in the total lipids were esterified into methyl esters by saponification with 0.5 N methanolic NaOH and transesterified with 14% BF3 (v/v) in methanol (IUPAC, 1979).

FAMEs (Fatty acid methyl esters) were analyzed on an HP (Hewlett Packard) Agilent 6890N model gas chromatograph (GC), equipped with a flame ionization detector (FID) and fitted to an HP-88 capillary column (100 m length, 0.25 mm i.d. and 0.2 µm thickness). Injector and detector temperatures were 240 and 250°C, respectively. The oven was programmed at 160°C initial temperature and 2 min initial time. Thereafter the temperature was increased up to 185°C at the rate of 4°C/min then increased to 200°C at the rate of 1°C/min and held at 200°C for 46.75 min. The total run time was 70 min. Helium was used as carrier gas (1 ml/min). The identification of fatty acids was carried out by comparing the sample FAME peak relative retention times with those obtained for Alltech and Accu standards. Results were expressed as FID response area in relative percentages.

The antioxidant capacity assays and fatty acid analysis were performed in triplicate. The mean standard deviations, IC_{50} and EC_{50} values were calculated using Microsoft Office Excel. The results are offered as means ± S.D.

3. RESULT AND DISCUSSION

3.1. Antioxidant capacity

The antioxidant capacity of plant extracts cannot be evaluated by only a single method due to the complex nature of phytochemicals (Chu *et al*., 2000). For this reason, six different chemical assays were applied to the determination of antioxidant capacity.

The antioxidative properties of phenolic compounds such as the scavengers of free radicals are very important in the food processing and pharmacology fields (Abdel-Hameed, 2009). The total phenolic content in the extract was determined spectrometrically according to the Folin-Ciocalteu procedure and was calculated as gallic acid equivalents. Results from the colorimetric analysis of total phenol content are presented in Table 1. The total phenolic content of the extract was determined as 36.52 mgGAE/g. Data on the total phenolic contents of the *Centaurea* species are scarce in the literature. The methanolic extract of *Centaurea ammocyanus* has a lower total phenolic content than *C. kotschyi* var. *persica* (Alali *et al*., 2007). The total phenolic contents of *Centaurea ensiformis* were determined as pyrocatechol equivalents and the contents varied from 16.01 to 64.61 µg pyrocatechol in the different extraction solvents (Ugur *et al*., 2009).

The total antioxidant capacity of extract was evaluated by the phosphomolybedum assay. The assay is based on the reduction of Mo(VI) to Mo(V) and is the formation of the green phosphate/Mo(V) complex. Because of its simplicity and cheap reagent, this assay is an alternative to the evaluation of the total antioxidant capacity (Prieto *et al*., 1999). Recently, it has been applied to studying the antioxidant activity of different wild edible plants in the Black Sea Region of Turkey, including *Tussilago farfara* of the Asteraceae family (Özen, 2010). The total antioxidant capacity of *C. kotschyi* var. *persica* was expressed as the ascorbic acid equivalent (AE) and was found to be 74.93 mgAE/g (Table 1).

The free radical scavenging properties and the inhibition effects on the linoleic acid oxidation of *C. kotschyi* var. *persica* are given in Table 2. The

Table 1
Total antioxidant capacity and total phenolic content of *C.kotschyi* var *.persica* and gallic acid

Sample	Total antioxidant capacity (AE mg/g)[a]	Total phenolic content (mg GAE/g)[b]
C. kotschyi var. *persica*	74.93±0.16*	36.52±0.28
Gallic acid	147.20±0.31	–

[a] Total antioxidant capacity expressed as ascorbic acid equivalent (mg AE/g extract). [b] Total phenolic content expressed as gallic acid equivalent (mg GAE/g extract). * Values reported are means ±S.D.

free radical scavenging capacity of the extract was measured by DPPH assay. In the DPPH assay, the plant extracts were able to reduce stable radical DPPH to yellow colored diphenylpricylhydrazine. As can be seen from table 2, The IC_{50} value of *C. kotschyi* var. *persica* was determined to be 37.09 µg/ml. The lower IC_{50} value reflects strong free radical scavenging activity. Therefore, the extract was found to be less effective than synthetic antioxidants (BHA and BHT). The extract of *C. mucronifera* exerted lower free radical scavenging activity than *C. kotschyi* var. *persica* with an IC_{50} value of 67.8 µg/ml (Tepe *et al*., 2006). When the results of our present study were compared to those of a previous study by Shoeb *et al*. (2007), *C. kotschyi* var. *persica* showed better free radical scavenging activity than the *Centaurea* species in this study. Sarker et al. (2005) reported free radical scavenging activity of the seeds of *Centaurea bornmuelleri* (IC_{50}: 0.63 mg/ml), *C. huber-morathii* (IC_{50}: 0.031 mg/ml) and *C. schiskinii* (IC_{50}: 0.15 mg/ml). According to these results, *C. huber-morathii* has greater free radical scavenging activity than *C. kotschyi* var. *percisa*. On the other hand, *C. kotschyi* var. *persica* has higher free radical scavenging properties than *C. bornmuelleri* and *C. schiskinii.*

In the β-carotene/linoleic acid system, the extract of *C. kotschyi* var*. persica* exhibited 65.22% inhibition against linoleic acid oxidation (Table 2). When the results were compared to BHA and BHT, the synthetic antioxidants had higher inhibition capacity than *C. kotschyi* var. *persica.* In the test system, the inhibition rate of oxidation of linoleic acid of *C. mucronifera* was found to be 35.2% which is lower than the inhibition capacity of *C. kotschyi* var. *persica* (Tepe et al., 2006). However, ethyl acetate and the chloroform extract of *C. ensiformis* were superior to those of *C. kotschyi* var. *persica*, with 85.15% and 72.51%, respectively (Ugur *et al*., 2009).

The Ferric ion reducing power of *C. kotschyi* var. *persica* was determined using various concentrations. The EC_{50} (effective concentration at which the absorbance 0.5) value of the extract was found to be 343.20 µg/ml. The lower EC_{50} value demonstrates strong reducing capacity. The EC_{50} value was compared with BHA and BHT and found to be 12.68 and 15.96 µg/ml, respectively (Table 2). As far as our literature survey, there is no study on the reducing power of the members of *Centaurea* in the literature. However, the reducing power of some plants such as *Marribium globosum* subsp. *globosum* (Sarikurkcu *et al*., 2008) and *Calluna vulgaris* (Orhan-Deliorman *et al*., 2009) were reported in the literature. The reducing power of an extract is often used as an indicator of electron-donating ability which is an important mechanism of antioxidant compounds (Dorman *et al*., 2003). The results of the CUPRAC assay are presented in Figure 1 and the extract was found less effective than ascorbic acid. The CUPRAC assay is based on the Cu (II)–Cu (I) reduction by antioxidants in the presence of neocuproine. Due to the fact that it is cost-effective, easy and fast, it is described as an alternative method for antioxidant capacity (Apak *et al*., 2006). Moreover, the assay was correlated with other antioxidant methods (Ozturk *et al*., 2007).

3.2. Fatty acid composition

The fatty acid composition of *C. kotschyi* var. *persica* contains thirty fatty acids. As can be seen from Table 3, C 18:3 ω3 (α-linolenic acid) (32.86%) was identified as the major fatty acid of *C. kotschyi* var. *persica.* Other predominant fatty acids were C 16:0 (palmitic acid) and C 18:2 ω6 (linoleic acid). The fatty acids were found to be 22.37% and 21.82%, respectively. Although there are many reports dealing with the fatty acid composition of the Asteraceae species, there is no data concerning the fatty acid

Table 2
Free radical scavenging capacity, ferric reducing power and inhibition of the linoleic acid of *C. kotschyi* var. *persica* and synthetic antioxidants

Sample	IC_{50} (µg/ml)[a]	Inhibition (%)[b]	EC_{50} (µg/ml)[c]
C. kotschyi var. *persica*	37.09±1.31*	65.22±1.83	343.20±10.60
BHA	1.57±0.19	94.05±1.66	12.68±0.15
BHT	3.56±0.32	96.39±1.61	15.95±0.72

[a] Results of DPPH assay. [b] Results of β-caroten/linoleic acid bleaching assay. [c] Results of ferric ion reducing power. * Values reported are means ±S.D.

Table 3
Fatty acid composition of *C. kotschyi* var. *persica* (%)

Fatty acids	*C. kotschyi* var. *persica*
C 8:0	0.04±0.01*
C 10:0	0.13±0.01
C 11:0	0.39±0.01
C 12:0	1.20±0.01
C 13:0	0.12±0.01
C 14:0	3.39±0.07
C 15:0	1.44±0.08
C 16:0	22.37±0.20
C 17:0	0.54±0.15
C 18:0	2.87±0.02
C 19:0	0.10±0.01
C 20:0	0.23±0.01
C 21:0	0.44±0.01
C 22:0	0.01±0.01
Σ*SFA***	*33.25±0.24*
C 14:1 ω5	0.03±0.01
C 15:1 ω5	0.14±0.02
C 16:1 ω7	0.23±0.01
C 17:1 ω8	0.02±0.01
C 18:1 ω9	4.46±0.05
C 18:1 ω7	0.58±0.01
C 20:1 ω9	0.17±0.01
C 22:1 ω9	0.05±0.01
Σ*MUFA***	*5.66±0.09*
C 18:2 ω6	21.82±0.20
C 18:3 ω3	32.86±0.64
C 18:3 ω6	1.99±0.01
C 20:2 ω6	0.04±0.01
C 20:4 ω6	2.14±0.03
C 20:5 ω3	0.41±0.01
C 22:2 ω6	0.02±0.01
C 22:6 ω3	1.82±0.06
Σ*PUFA***	*61.09±0.34*

* Values reported are means ±S.D.** SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids.

composition of the *Centaurea* species. Linoleic acid was determined as the major fatty acid of the fatty acid composition of some Asteraceae species such as *Chrysanthemum coronarium* (Matthaus *et al*., 2003), *Carthamus oxyacantha* and *Gundelia taurnefartii* (Heidari *et al*., 2000). C 18:1 ω9 (oleic acid) was the highest level fatty acid in MUFAs with

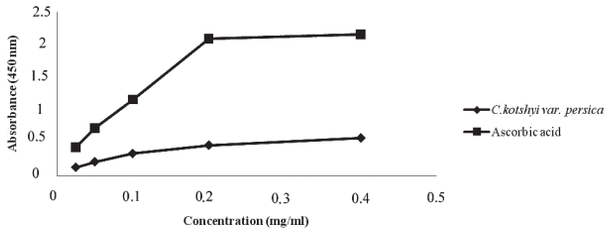

Figure 1
Cupric ion reducing capacity of *C. kotcshyi* var. *persica* and ascorbic acid.

4.46%. Similar results were obtained from the fatty acid composition in the seeds of three safflower species from the Asteraceae family (Sabzalian *et al*., 2008). Our study has also reported that PUFAs were at a higher level than SFAs and MUFAs in the fatty acid composition of *C. kotschyi* var. *persica*. Our results are in agreement with those reported by Dubois (Dubois *et al*., 2007) in that PUFAs were identified as having the highest level in the fatty acid composition of most of the 80 vegetable oils.

4. CONCLUSIÓN

The results of this study revealed that *C. kotschyi* var. *persica* has a considerable amount of phenolic content and significant antioxidant properties. It is believed that *C. kotschyi* var. *persica* can be used as a source of natural antioxidants for the pharmaceutical and food processing fields. We hope that our results will provide a starting point for the investigations to exploit new natural antioxidant substances from other *Centaurea* species. In addition, *C. kotschyi var. persica* could be considered new alternative sources of fatty acids, especially linoleic and α-linolenic acid.

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