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Fatty Acid Composition and Antioxidant Potential of Ten *Cephalaria* Species

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Abstract: This paper focused on the assessment of fatty acid composition and antioxidant properties of ten *Cephalaria* (*C. aytachii, C. taurica, C. tuteliana*, *C. procera*, *C. speciosa*, *C. tchihatchewii, C. hirsuta*, *C. elazigensis* var. *elazigensis, C. anatolica* and *C. aristata*) species. The principal fatty acids in all species were oleic acid (10.28-31.65%), linoleic acid (17.81–37.67%) and palmitic acid (10.54–23.81%). Linolenic acid was also the most abundant fatty acid component in *C. tuteliana* (24.42%) and in *C. speciosa* (36.65%). *In vitro* antioxidant capacity of the hexane extracts of ten *Cephalaria* species was investigated by CUPRAC and DPPH methods. Total phenolic content of hexane extracts was also examined. The results showed that all species of *Cephalaria* have antioxidant properties with the highest trolox equivalent antioxidant capacity (1.005 ± 0.13 mmol trolox equivalent per gram extact) in *C. aristata* and the highest radical scavenging activity (IC_{50} value 3.768 ± 0.67 mg/mL) in *C. tchihatchewii*. It was found that reducing power of *C. aristata* and radical scavenging potential of *C. tchihatchewii* were mainly due to highest phenolic contents of these species (2.907 ± 0.146 and 3.037 ± 0.156 mg gallic acid equivalent per gram extract, respectively). These findings suggest that the *Cephalaria* species might be used as a potential source of unsaturated fatty acids as well as phenolic constituents possessing antioxidant activity in food, cosmetics and pharmaceutical industries.

Keywords: Dipsacaceae; *Cephalaria*; fatty acid; GC-FID; antioxidant activity; total phenolic content; chemotaxonomy. © 2015 ACG Publications. All rights reserved.

1. Introduction

It is known that fatty acids, especially essential ones (EFAs), are important for human being not only as essential nutrients but also may favorably modulate for many diseases. They regulate body functions such as heart rate, blood pressure, blood clotting, fertility and conception. They also play an important role in immune function by arranging the immune system inflammation against harmful waste products. They construct and repair the cell membranes [1-2]. The lack of EFAs causes several abnormalities and malignant transformations in human body. Mammalian cells can not convert α-

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linoleic acid (LA) to α-linolenic acid (ALA) because they lack the converting enzyme, omega-3 desaturase [3]. Thus, they should be obtained through dietary intake. Dietary intake of EFAs and EFA balance are important for good health and development. The ratio of the main EFAs, omega-6 to omega-3 is very important for preventing and treatment of coronary artery disease, hypertension, diabetes, arthritis, osteoporosis, other inflammatory and autoimmune disorders and cancer. According to Richard and coworkers, it was proposed that omega-3 series of fatty acids could be able to act as antioxidants in vascular endothelial cells, thus reduce the risk of atherosclerosis and cardiovascular disease by diminishing inflammation. Polyunsaturated fatty acid micelles have been shown to scavenge superoxide anion in an unsaturation-dependent manner [4]. The ideal intake of LA to ALA ratio is recommended as 1:1 to 4:1 for traditional nutrition. The minimum healthy intake per adult for both LA and ALA via diet is 1.5 g/day. [1-2, 5-7]. *Cephalaria* Schrad. ex Roem. & Schult. (Dipsacaceae) is a large genus with 94 species which are spread out in the Mediterranean Region, Balkan Peninsula, the Middle East and South Africa. Forthy *Cephalaria* species, 24 of them are endemic, and widely distributed in Turkey [8–9]. Several biological properties have been attributed to *Cephalaria* species, such as antibacterial, antifungal, antioxidant and cytotoxic activities [10–13]. The roots of *C. gigantean* (Ledeb.) Bobrov, is well known in traditional medicine remedies because of its sedative and anti-inflammatory effects [14]. *Cephalaria* species are characterized by an extremely rich phytochemical diversity. Iridoid, triterpene, flavonoid glycosides and alkaloids have been reported to be important chemical constituents of these species [10-13, 15-16]. There was little documentation on the fatty acid composition of *Cephalaria* species except our recent projects [17-18]. In addition, there is a specific report about the fatty acid composition of *C. syriaca* Schrad. which is used as bread additive material in Turkey [19].

The objective of this work was to investigate the fatty acid profile of ten *Cephalaria* species (*C. aytachii* Gokturk & Sumbul*, C. taurica* Szabó*, C. tuteliana* Kus & Gokturk, *C. procera* Fisch. & Avé-Lall*, C. speciosa* Boiss. & Kotschy, *C. tchihatchewii* Boiss.*, C. hirsuta* Stapf, *C. elazigensis* Gokturk & Sumbul var. *elazigensis, C. anatolica* Shkhiyan and *C. aristata* K.Koch) growing in Turkey, by GC-FID method as well as to evaluate the antioxidant potentials of *n*-hexane extracts of these species, for the first time. Antioxidant capacity of these extracts was examined by CUPRAC (**cup**ric ion **r**educing **a**ntioxidant **c**apacity), while free radical scavenging activity was evaluated by DPPH (1,1-**d**i**p**henyl-2-**p**icryl**h**ydrazyl) test. Total phenolic content of the extracts was measured by spectrophotometric method using Folin-Ciocalteu's reagent.

2. Materials and Methods

2.1. Plant Material

Plant materials were collected in July-September 2012 from northwest, central and southeastern Anatolia. Voucher specimens were deposited at the Herbarium Research and Application Center of Akdeniz University, Antalya, Turkey (Table 1).

2.2 Extraction

Dried and powdered aerial parts of the plants (50 g each) were extracted with HPLC grade *n*-hexane (Merck No. 1.04391) (600 mL) using a Soxhlet apparatus at 70^0C for 6 h, to obtain the fatty acids and other apolar components of the plant materials. The solvents were removed under vacuum at ~40^0C and the residue was kept at -20^0C for GC-FID analysis and antioxidant activity assays.

2.3. Methylation of hexane extracts

In order to analyze the oily mixture of *Cephalaria* species, first they were derived to their methyl esters. These derivatizations were carried out using International Olive Oil Council and the IUPAC reports according to *trans*-esterification process [20-21]. In this process, the *n*-hexane extracts were dried with anhydrous sodium sulphate attentively. After dissolving the extracts in HPLC grade *n*-

hexane, they were treated with 2M methanolic KOH at room temperature for 30 s. The upper phases of the reaction mixtures were analyzed by GC-FID system [22].

Table 1. Localities of ten *Cephalaria* species (each 50 g) and the yields of *n*-hexane extracts

No	Species	Collected Area	Height, m	Oil Yield, %
1	*C. aytachii** Gokturk & Sumbul (RSG 7483)	Eskisehir: Sivrihisar	942	0.9
2	*C. taurica** Szabó (RSG 7484)	Adana: Pozanti, Kamisli	980	1.2
3	*C. tuteliana** Kus & Gokturk (RSG 7526)	Istanbul: Bahcesehir, Kirac	90	1.0
4	*C. procera* Fisch. & Avé-Lall (RSG 7672)	Sivas: Zara, Zara Imranli	1665	1.6
5	*C. speciosa** Boiss. & Kotschy (RSG 7673)	Erzincan	1600	1.1
6	*C. tchihatchewii* Boiss. (RSG 7678)	Mus: Varto, Seferek Gate	1910	1.3
7	*C. hirsuta* Stapf (RSG 7676)	Erzurum: Erzurum-Cat	2100	0.9
8	*C. elazigensis* Gokturk & Sumbul var. *elazigensis** (RSG 7679)	Elazig: Maden	900	1.7
9	*C. anatolica** Shkhiyan (RSG 7675)	Erzurum: Tortum	1050	1.0
10	*C. aristata* K. Koch (RSG 7480)	Kayseri: Pinarbasi	1435	0.9

*Endemic species

2.4. Gas chromatography analysis

Methyl esters of fatty acids were analyzed using GC-6890 Agilent system with a Supelco SP TM-2560 polarizable column (100 m x 0.25 mm x 0.20 µm) and a flame ionization detector (FID). In the GC programme; flow of helium, split ratio and detector temp. were 0.5 mL/min., 1/100 and 260 ^{0}C, respectively. The column temperature was adjusted to 140 ^{0}C and then increased to 240 ^{0}C by 4 ^{0}C per minute. Identification of each component was exhibited by comparing their retention times with those of standard fatty acid methyl esters mixture (Sigma L 2626 and Supelco^{TM} 37 Catalog No: 47885-U) and by Nist-Wiley (Wiley 7.n- 2005) library data search. All measurements were carried out in triplicate.

2.5. Antioxidant activity tests

The antioxidant activities of *Cephalaria* extracts were evaluated by two different test methods: CUPRAC [23] and DPPH [24]. All tests were carried out in triplicate and data was expressed by means ± S.D.

CUPRAC method, also known as trolox equivalent antioxidant capacity (TEAC) assay exhibits the total antioxidant activity of the samples [23]. The molar absorptivity of Trolox (ε_{TR}) was 1.74×10^4 $Lmol^{-1}cm^{-1}$ in our assay system. Absorbance of each sample was divided by the ε_{TR}, and TEAC value was calculated as mmol trolox equivalent (TE) per gram dry weight of extract (DWE).

The DPPH free radical scavenging activity was estimated according to the method of Cheung and co-workers [24]. Briefly, 0.2 mM DPPH in methanol (DPPH reagent) was added to each extract in a volume ratio of 4:1. The mixture was left under subdued light for 10 min. and the absorbance at 520 nm was measured. Percentage inhibition of DPPH was calculated using the following formula:

$\%Inhibition = [(A_{control}-A_{sample})/A_{control}]x100$

$A_{control}$ is the absorbance of DPPH reagent + hexane (or methanol for blank) (4:1, v:v),

A_{sample} is the absorbance of DPPH reagent + extract (or ascorbic acid as reference antioxidant).

The results were given as IC_{50} values, which is defined as the concentration of sample decreasing the absorbance of DPPH reagent by 50%.

2.6. Determination of Total Phenolic Content

Total phenolic content was measured by using Folin-Ciocalteu's reagent as described as earlier [25] with some modifications. Briefly, 500 µL of each extract was mixed with Folin-Ciocalteu's reagent and then 1.5 mL of Na_2CO_3 (200 g/L) was added to this mixture and finally 2.75 mL of dH_2O was added and then samples were centrifuged at 1250 *g* for 5 min. The absorbance of supernatants were measured at 725 nm. The total phenolic content was expressed as mg gallic acid equivalents (GAE)/g extract. All measurements were carried out in triplicate and data was expressed by means ± S.D.

3. Results and Discussion

The fatty acid composition of the *n*-hexane extracts of *C. aytachii, C. taurica, C. tuteliana*, *C. procera, C. speciosa*, *C. tchihatchewii, C. hirsuta*, *C. elazigensis* var. *elazigensis, C. anatolica* and *C. aristata* species was investigated using GC-FID technique for the first time. Mean values and standard deviations of the fatty acid compositions in *n*-hexane extracts are shown in Table 2. According to the results, the oil yields of the studied *Cephalaria* species were detected between 0.9% and 1.7% on the basis of dry weight of the plant materials (Table 1). The fatty acids profile is composed by 22 fatty acids ranging their carbon numbers from C_{12} to C_{22}.

Linoleic acid was detected as the most abundant one for all samples varying between 17.81% to 37.67% except *C. tuteliana, C. speciosa* and *C. tchihatchewii.* Linolenic acid was the major fatty acid for *C. speciosa* (36.65%) and *C. tuteliana* (24.42%). Oleic acid which was observed between 10.28% and 31.65% was the second order of importance. Palmitic acid was detected in all *n*-hexane extracts and was the main component among the saturated fatty acids (SFAs). The highest percentage of palmitic acid was detected for *C. tchihatchewii* (23.70%) and *C. aytachii* (23.81%).

The results of this study indicated that *n*-hexane extracts of these ten *Cephalaria* species became rich sources of polyunsaturated fatty acids (PUFAs) ranging from 40.37% to 66.13% and monounsaturated fatty acids (MUFAs), ranging from 11.20% to 31.82%. American Heart Association and National Academy of Sciences/-Institute of Medicine have made dietary recommendations recently that focus not only on the quantity but also on the types of fatty acids in the diet, and generally recommended substituting MUFAs and PUFAs [26]. Our study showed that among PUFAs, linoleic and linolenic acids were the most prevalent ones in all *Cephalaria* species. LA (ω-6) and ALA (ω-3) are essential fatty acids, because mammals like -humans cannot synthesize and must obtain them in their diet. Thus, they should be obtained through dietary intake. These fatty acids play an important role in human growth and development and are positively associated with health and the prevention and treatment of heart disease, arthritis, inflammatory and autoimmune diseases and cancer [27-30]. The balance of EFAs (ω-6/ω-3) is also important for good health and development. The optimal ratio of LA (ω-6) to ALA (ω-3) between 1:1 and 4:1 is recommended as traditional nutrition and a lower ratio is more desirable in reducing the risk of many of the chronic diseases of high prevalence in the developing countries [31]. The analyzed *n*-hexane extracts contain omega-6 to omega-3 fatty acid in the ratios of 0.5 for *C. speciosa*, nearly 1 for *C. aytachii, C. tuteliana, C. tchihatchewii, C. hirsuta* and *C. anatolica*, between 3 and 10 for *C. taurica, C. procera, C. elazigensis var. elazigensis* and *C. aristata*.

Antioxidant potency of *n*-hexane extracts of *Cephalaria* species, containing fatty acids and other apolar components was investigated for the first time in this study (Table 3). The results showed that especially hexane extracts of *C. aristata* (**10**), *C. speciosa* (**5**), *C. tchihatchewii* (**6**), *C. elazigensis* var. *elazigensis* (**8**), *C. hirsuta* (**7**) , *C. anatolica* (**9**) and *C. procera* (**4**) were capable to compete with those of *C. davisiana*, *C. elazigensis* var. *purpurea*, *C. stellipilis* and *C. paphlagonica* reported earlier [17], and with some well-known antioxidant flavonoids such as naringenin (0.22 mmol TE per gram dry weight of extract) and *p*-coumaric acid (0.55 mmol TE per gram dry weight of extract) [32].

Table 2. Fatty acid composition of ten *Cephalaria* species[a-c]

[a]**1**: *C. aytachii,* **2**: *C. taurica,* **3**: *C. tuteliana*, **4**: *C. procera,* **5**: *C. speciosa*, **6**: *C. tchihatchewii,* **7**: *C. hirsuta*,

R_T	Name	Fatty Acids	Fatty Acid Percentages (%)									
			1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
16.25	12:0	Lauric acid	1.18 ±0.01	2.15 ±0.02	1.39 ±0.04	0.74 ±0.02	0.91 ±0.01	1.62 ±0.02	1.37 ±0.02	0.86 ±0.03	0.78 ±0.01	0.44 ±0.01
17.80	13:0	Tridecanoic acid	nd	0.15 ±0.01	0.08 ±0.01	0.03 ±0.00	nd	0.10 ±0.00	0.05 ±0.00	0.04 ±0.00	nd	0.02 ±0.00
20.85	14:0	Myristic acid	4.21 ±0.02	8.37 ±0.08	7.27 ±0.12	9.55 ±0.19	3.40 ±0.00	4.62 ±0.03	7.71 ±0.02	12.79 ±0.24	2.54 ±0.16	9.53 ±0.05
20.96	15:0	Pentadecanoic acid M.E.	0.55 ±0.02	0.58 ±0.03	0.42 ±0.02	0.19 ±0.01	0.53 ±0.02	0.59 ±0.01	0.42 ±0.01	0.15 ±0.00	0.25 ±0.00	0.16 ±0.01
22.59	16:0	Palmitic acid	23.81 ±0.03	21.32 ±0.07	15.36 ±0.13	11.77 ±0.06	17.66 ±0.24	23.70 ±0.03	14.34 ±0.04	10.54 ±0.06	14.57 ±0.61	11.97 ±0.01
23.81	16:1	Palmitoleic acid	0.29 ±0.00	0.25 ±0.02	0.29 ±0.01	0.27 ±0.01	0.63 ±0.03	0.42 ±0.03	0.56 ±0.11	0.13 ±0.00	0.30 ±0.03	0.17 ±0.01
24.33	17:0	Heptadecanoic acid	0.71 ±0.01	1.06 ±0.02	0.55 ±0.03	0.19 ±0.01	0.64 ±0.04	0.91 ±0.03	0.40 ±0.04	0.23 ±0.00	0.50 ±0.03	0.22 ±0.00
25.72	18:0	Stearic acid	4.52 ±0.03	3.83 ±0.07	3.13 ±0.08	2.67 ±0.03	3.55 ±0.07	4.61 ±0.01	2.70 ±0.02	2.35 ±0.03	3.35 ±0.16	3.18 ±0.01
26.58	18:1 n-9	*trans*-Oleic acid	nd	nd	nd	nd	0.45 ±0.39	nd	nd	nd	nd	nd
26.75	18:1 n-9	Octadeca-9-enoic acid (*Z*) M.E. (Oleic)	17.47 ±0.06	18.10 ±0.12	15.64 ±0.06	27.42 ±0.20	10.28 ±0.05	15.42 ±0.25	20.89 ±0.07	26.86 ±0.12	10.90 ±0.17	31.65 ±0.04
28.24	18:2 n-6	Octadeca- 9,12- dienoic acid (Z,Z) M.E. (Linoleic)	25.24 ±0.02	28.99 ±0.08	21.40 ±0.06	34.02 ±0.08	17.81 ±0.08	22.24 ±0.06	26.13 ±0.04	37.67 ±0.15	29.23 ±0.38	37.14 ±0.04
28.72	20:0	Arachidic acid	3.52 ±0.11	2.50 ±0.09	3.26 ±0.17	1.21 ±0.02	3.48 ±0.02	3.32 ±0.04	2.05 ±0.02	0.37 ±0.03	0.70 ±1.20	0.91 ±0.03
29.64	20:1	Gondoic acid	nd	nd	nd	0.39 ±0.02	nd	nd	nd	nd	nd	nd
29.91	18:3 n-3	Linolenic acid	15.58 ±0.10	8.39 ±0.30	24.42 ±0.10	7.84 ±0.10	36.65 ±0.07	19.60 ±0.21	21.95 ±0.06	6.29 ±0.13	18.97 ±0.17	3.38 ±0.03
30.16	21:0	Heneicosanoic acid	nd	nd	nd	nd	nd	nd	0.78 ±0.04	nd	nd	nd
31.56	22:0	Behenic acid	2.93 ±0.03	1.34 ±0.06	nd	0.59 ±0.02	2.19 ±0.03	2.04 ±0.07	nd	nd	nd	nd
32.07	20:3 n-6	*cis*-8,11,14-eicosatrienoic acid	nd	nd	nd	nd	nd	nd	nd	nd	17.93 ±0.26	nd
32.72	23:3 n-3	*cis*-11,14,17-eicosatrienoic acid	nd	nd	nd	0.27 ±0.03	nd	nd	nd	nd	nd	nd
34.02	22:2	*cis*-13,16 -Docasadienoic acid	nd	1.33 ±0.06	nd	nd	nd	nd	nd	nd	nd	nd
34.33	24:0	Lignoceric acid	nd	nd	3.23 ±0.07	nd	nd	nd	nd	nd	nd	nd
34.87	20:5	*cis*-5,8,11,14,17-Eicosapentaenoic acid	nd	nd	2.45 ±0.07	nd	1.56 ±0.07	nd	nd	nd	nd	0.44 ±0.01
39.38	22:6	*cis*-4,7,10,13,16,19-Docasahexaenoic acid	nd	1.66 ±0.07	1.13 ±0.03	2.87 ±0.04	0.25 ±0.01	0.80 ±0.02	0.66 ±0.02	1.72 ±0.05	nd	0.78 ±0.00
		Total SFA	**41.43**	**41.30**	**34.69**	**26.94**	**31.96**	**41.51**	**29.82**	**27.33**	**22.69**	**26.43**
		Total MUFA	**17.76**	**18.35**	**15.93**	**28.08**	**11.36**	**15.84**	**21.45**	**26.99**	**11.20**	**31.82**
		Total PUFA	**40.82**	**40.37**	**49.40**	**45.00**	**56.27**	**42.64**	**48.74**	**45.68**	**66.13**	**41.74**
		ω-6/ω-3 Ratio	**1.62**	**3.46**	**0.88**	**4.34**	**0.49**	**1.14**	**1.19**	**5.99**	**1.54**	**10.99**
		Total Fatty Acid	**100.01**	**100.02**	**100.02**	**100.02**	**99.59**	**99.99**	**100.01**	**100.00**	**100.02**	**99.99**

8: *C. elazigensis* var. *elazigensis,* **9**: *C. anatolica,* **10**: *C. aristata*
[b]nd: not detected; SFA: Saturated Fatty Acid; MUFA: MonoUnsaturated Fatty Acid; PUFA: PolyUnsaturated Fatty Acid
[c]Results are expressed as mean ± S.D. (n=3)

Interestingly, different varieties of *C. elazigensis* (var. *elazigensis* and var. *purpurea*) exhibited different TEAC values (trolox equivalent antioxidant capacity) (0.571 and 0.252 mmol ± 0.13 TE/g extract, respectively). This twofold difference probably resulted from different constituents of these two species collected from different locations and altitudes. *C. elazigensis* var. *elazigensis* was collected from eastern part of Turkey (Elazig-Maden, 900 m) in this study whereas *C. elazigensis* var. *purpurea* from Anatolian part of Turkey (Kirikkale-Kirsehir, 1255 m) earlier [17]. In other words,

it is important that different locations, climatic conditions, harvest time and seasons affect the secondary metabolites composition of plant species. So, in this study fatty acid composition and the optimal ratio of LA (ω-6) to ALA (ω-3) of the hexane extracts was found to be 1.6, 3.5, 0.9, 4.3, 1.1, 1.2 and 1.5 for *C. aytachii*, *C. taurica*, *C. tuteliana*, *C. procera*, *C. tchihatchewii*, *C. hirsuta* and *C. anatolica*, which could be used as a chemotaxonomical marker for *Cephalaria* species, respectively.

Table 3. Antioxidant activity[a] and total phenolic content of ten *Cephalaria* species.

No	Species	CUPRAC value (mmol TE/g extract)	IC_{50} value of DPPH scavenging activity (mg/mL)	Total phenolic content (mg GAE/g extract)
1	*C. aytachii*	0.150 ± 0.02	15.125 ± 7.31	0.057 ± 0.009
2	*C. taurica*	0.230 ± 0.12	14.177 ± 8.65	0.154 ± 0.007
3	*C. tuteliana*	0.072 ± 0.07	10.555 ± 1.38	0.724 ± 0.117
4	*C. procera*	0.485 ± 0.17	6.938 ± 2.56	1.561 ± 0.042
5	*C. speciosa*	0.596 ± 0.09	6.169 ± 3.13	2.658 ± 0.100
6	*C. tchihatchewii*	0.596 ± 0.11	3.768 ± 0.67	3.037 ± 0.156
7	*C. hirsuta*	0.537 ± 0.03	5.131 ± 1.04	1.192 ± 0.038
8	*C. elazigensis* var. *elazigensis*	0.571 ± 0.04	5.279 ± 0.46	1.365 ± 0.058
9	*C. anatolica*	0.506 ± 0.02	5.196 ± 0.92	1.490 ± 0.161
10	*C. aristata*	1.005 ± 0.13	12.569 ± 3.97	2.907 ± 0.146
	Ascorbic acid [b]	-	6.24 ± 0.03 [b]	-

[a] Sample concentration of all tested hexane extracts was 1 mg/mL. Data represent the mean ±S.D. (n=3)
[b] Ascorbic acid (reference) concentration was 6.24 µg/mL.

The highest TEAC value (1.005 mmol ± 0.13 TE/g extract) was detected for *C. aristata*. Reducing power of the extracts was in following order: **10>5=6>8>7>9>4>2>1>3**.

DPPH test revealed that hexane extracts of *C. tchihatchewii* (**6**), *C. hirsuta* (**7**), *C. anatolica* (**9**), *C. elazigensis* var. *elazigensis* (**8**) and *C. speciosa* (**5**) possessed significant radical scavenging activity, with the IC_{50} values of 3.768±0.67, 5.131±1.04, 5.196±0.92, 5.279±0.46 and 6.169±3.13 mg/mL, respectively (Table 3), with the order of **6>7>9>8>5>4>3>10>2>1**. Although, the *n*-hexane extract of *C. aristata* showed the highest activity in CUPRAC method, this extract was less active in DPPH method as a free radical scavenger due to different active constituents acting through different mechanisms. Wang et al. [33] reported that fatty acids of *Camellia sinensis* L. (tea plant) seed oil extract has DPPH radical scavenging activity with the IC_{50} value of 35.8 mg/mL. Radical scavenging activity in *n*-hexane extracts of these ten *Cephalaria* species was found to be higher than that of *Camellia sinensis* L. which play an important role as a health-promoting role in human diets.

Total phenolic contents of the *n*-hexane extracts were in the order of extract numbers; **6>10>5>4>9>8>7>3>2>1** (Table 3). Thus, *C. tchihatchewii* (**6**), *C.aristata* (**10**) and *C. speciosa* (**5**) were found to contain the highest (at least two fold) phenolic content. As unsaturated fatty acid percentage of *C. tchihatchewii* (**6**) was not significantly higher than the ones of other species, this result gave rise to thought that radical scavenging capacity of *C. tchihatchewii* arised from phenolic constituents rather than fatty acids.

On the other hand, *n*-hexane extract of *C. aristata* (**6**) containing high level of phenolic substances, was effective as trolox in CUPRAC assay. Thus, reducing power of this extract seems to be related to phenolic content as well as to the highest total MUFA percentage (31.82 %) and omega 6/3 ratio (10.99).

As the DPPH reaction depends on the hydrogen donating ability of antioxidant [34], DPPH scavenging potential of *Cephalaria* species may be attributed to constituents, which could donate electrons to DPPH. It is well known that phenolic compounds play a key role as antioxidants due to the presence of hydroxyl substituents and their aromatic structure, which enables them to scavenge free radicals [35]. However, it is not clear which phenolic(s) are responsible in *Cephalaria* hexane extracts.

The mechanism of CUPRAC method has been fully described by Apak and coworkers [36]. In this reaction, the reactive -OH groups of phenolic antioxidants are oxidized to the corresponding quinones and Cu(II)-bis(neocuproine) is reduced to the a chelate, Cu(I)-bis(neocuproine) showing maximum absorption at 450 nm. Hence, correlation between CUPRAC values and phenolic contents of **6**, **10** and **5** is consistent with this phenomenon.

In conclusion, distinct activities of *n*-hexane extracts of the *Cephalaria* species depend on their different compositions and the different percentages of the constituent fatty acids. Furthermore, it may be suggested that these factors are directly related to or may even be prominent contributors of antioxidative properties. Further studies are required to exhibit molecular mechanisms underlying antioxidant activity.

In the light of the findings of this study, the *Cephalaria* species might be regarded as an alternative source of unsaturated fatty acids and new natural antioxidants, which could be used in the food, cosmetics and pharmaceutical industries.

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