SHORT REPORT

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Fatty Acid Profile and Biological Data of Four Endemic *Cephalaria* Species Grown in Turkey

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Abstract: The fatty acid compositions of the *n*-hexane extracts of the aerial parts of four Turkish *Cephalaria* species (*C. paphlagonica, C. stellipilis, C. davisiana* and *C. elazigensis* var. *purpurea*) were analyzed by GC-MS for the first time. The oil yields of these species were determined as ranging from 0.07% to 0.36 %. Seventeen fatty acids as methyl esters were identified. All extracts were found to contain significant quantities of palmitic, linoleic (LA), stearic and *alpha*-linolenic acid (ALA). ALA was the most abundant fatty acid in all species (29.00%, 30.51%, 32.49% and 34.87% for *C. stellipilis, C. elazigensis, C. davisiana* and *C. paphlagonica,* respectively). Other dominant fatty acid was palmitic acid, which ranged from 19.10% to 28.23% for all species. LA was detected in a considerable amount of 19.44 % for *C. paphlagonica*. The *n*-hexane extracts of the plants were also checked for their antimicrobial and antioxidant activities.

Keywords: *Cephalari;* Dipsacaceae; fatty acid; α-linolenic acid; linoleic acid; palmitic acid; GC-MS; antimicrobial activity; antioxidant activity.

1. Plant Source

Cephalaria Schrad. (Dipsacaceae) is a genus with 94 species worldwide, which are spread out in the Mediterranean Region, Balkan Peninsula and the Middle East. Forty *Cephalaria* species, 24 of them are endemic, are widely distributed in Turkey [1-3].

Plant materials were collected in July 2007 from central and southeastern Anatolia. Voucher specimens were deposited at the Herbarium Research and Application Center of Akdeniz University, Antalya, Turkey (Table 1).

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Table 1. Characteristics of four *Cephalaria* species and yields of *n*-hexane extracts

Species	Collected Area	Altitude, m	*n*-Hexane extracts yield, %
C. paphlagonica* Bobrov. (R. S. Göktürk 6100)	Cankiri- Kastamonu Ilgaz,	1200-1300	0.19
C. stellipilis Boiss. (R. S. Göktürk 6078)	Kahramanmaras- Goksun, Degirmendere	1428	0.17
C. davisiana* R. S. Göktürk & Sümbül (R. S. Göktürk 6083)	Kahramanmaras- Goksun, Binboga Mountain	1850	0.36
C. elazigensis* var. *purpurea* R. S. Göktürk & Sümbül (R. S. Göktürk 6089)	Kirikkale-Kirsehir	1255	0.07

*Endemic species

2. Previous Studies

According to literature data, this genus contain mainly iridoid, triterpene and flavonoid glycosides, and alkaloids [4-9]. All these natural products have used for different areas from medicine to agriculture because of their biological activities [8, 10]. In addition, there is a specific report about the fatty acid composition of *C. syriaca*, which is used as bread additive material in Turkey [11]. There are no data on fatty acid composition as well as antimicrobial and antioxidant activities of *Cephalaria* species, except the one derived from our recent research [12]. It is known that fatty acids, especially the essential fatty acids (EFAs), are important for human being. They may regulate the body functions such as heart rate, blood pressure, blood clotting and fertility. They also arrange the immune system inflammation against harmful waste products. The balance of EFA is important for good health and normal development of human [13, 14].

3. Present Study

Dried and powdered aerial parts of the plants (40 g each) were extracted with HPLC grade *n*-hexane (Merck No. 1.04391) (600 mL) using a Soxhlet apparatus (70 °C, 6 h) to obtain the fatty acids and other apolar components. The solvents were evaporated to dryness under vacuum at ~40 °C and the residue was kept at -20 °C before using for GC-MS analysis.

In order to analyze the oily mixtures of *Cephalaria* species, firstly methyl esters were obtained by *trans*-esterification process [15]. In this process, the *n*-hexane extracts were dried with anhydrous sodium sulphate attentively. After dissolving in HPLC grade *n*-hexane, they treated with 2 M methanolic KOH at room temperature for 30 s. The upper phases of the reaction mixtures (1 µL) were analyzed by GC-MS system [16].

Methyl esters of fatty acids were analyzed using Agilent 6890 Series gas chromatography coupled with Agilent MSD-5973 Mert mass selective detector and fitted with a HP-5MS (5 % Phenyl methyl siloxane) column (30 m x 0.25 mm x 0.25 µm). The GC programme was followed for maximum column temperature, flow of helium and oven temperature as, 325 ˚C, 0.5 mL/min and 170-210 ˚C, respectively. This programme was carried out increasing the temperature at 2 °C for 1 min, split 1/50. The EI-MS data was provided using 70 eV ionization energy ranging from 30 to 500 a.m.u. Identification of each component was exhibited by comparing their retention times and mass peaks with those of standard fatty acid methyl esters mixture (Sigma L 2626 and Supelco^{TM} 37 Catalog No: 47885-U) and Wiley 7th library data search.

Antimicrobial and antioxidant activities of *n*-hexane extracts were determined using convensional methods [17-19]. The minimum inhibitory concentration (MIC) and cupric (II) reducing antioxidant capacity (CUPRAC) values were presented for antimicrobial and antioxidant activities, respectively (*see* supporting information for detailed procedures).

The fatty acid composition of the *n*-hexane extracts of aerial parts of *C. paphlagonica*, *C. stellipilis, C. davisiana* and *C. elazigensis* var. *purpurea* species was investigated using GC-MS technique for the first time. According to the results, the oil yields of the studied *Cephalaria* species were 0.07-0.36% (of dry plant weight) (Table 1). In total, 17 different fatty acid components were identified ranging their carbon numbers from C12 to C26. The identified fatty acid percentages of the *n*-hexane extracts were varied from 97.66% (*C. paphlagonica*) to 89.82% (*C. davisiana*) (Table 2).

The high amounts of EFAs were detected in all species with a clear predominance of *α*-linolenic acid, which was the most abundant fatty acid with contents of 34.87%, 32.49% 30.51% and 29.00 % for *C. stellipilis, C. elazigensis, C. davisiana* and *C. paphlagonica,* respectively. Other dominant fatty acid was palmitic acid, ranging from 19.10% (*C. davisiana*) to 28.23% (*C. elazigensis*). All extracts were contained significant amounts of linoleic acid (LA) and stearic acid with small amount of arachidic, behenic, myristic, lignoceric acid (Table 2). Interestingly, arachidic acid (6.19%) and lignoceric acid (5.07%) contents were considerably high in *C. davisiana* and LA content was significantly high in *C. paphlagonica* (19.44%). It was found the high concentration of phytol known as cancer preventive agent [20] especially in *C. davisiana* (10.18 %).

The *n*-hexane extracts of *Cephalaria* species exhibited as a very low anti-bacterial activity against *S. aureus*, *E. coli* and *K. pneumonia* with the MIC values of 500 to 1625 µg/mL and no activity against other bacteria tested, when compared with standard antibiotic, gentamycine. *C. stellipilis*, containing the highest amount of ALA was the most effective species on *S. aureus*. This result is consistent with the finding that ALA is the most effective antimicrobial fatty acid among the 15 different fatty acids, tested against *S. aureus* [21]. The most susceptible strain to the *n*-hexane extracts was *E. coli*. Similar results have been obtained by Uma et al (2010) [22] for the hexane extract of sea urchin (*Temnopleurus alexandri*). The CUPRAC values of the *n*-hexane extracts from *C. davisiana, C. elazigensis, C. stellipilis,* and *C. paphlagonica* were 0.334, 0.252, 0.136 and 0. 120 mmol TR/g dry extract, respectively. Most of the reports indicated that ALA is the most abundant fatty acid in active extracts. Phytol is also a major constituent with antioxidant activity [23]. Thus, it seems that antioxidant activity of these species resulted from synergistic effect of ALA and phytol. This effect was also noticed in our previous study exhibiting significant inhibitory activity on lipid peroxidation containing > 40% ALA [12]. To our knowledge, this is the first report of the antimicrobial and antioxidant activities of *n*-hexane extracts of four *Cephalaria* species.

It is known that the prevention of many diseases is possible to have healthy and balance diet, especially the balance of EFAs. The ideal intake ratio of LA to ALA between 1:1 and 4:1 is recommended as traditional nutrition. In the Western countries this value was ~1:1 because of the types and amounts of the artificial production of some oil reach plants like canola, corn and sunflowers. It is clear to know that the products of these plants have high amount of LA. Increased dietary intake of LA may decrease the ALA amounts through some oxidation reactions. So, it is essential to increase the ALA and decrease the LA intake, in order to balance the ALA and LA ratio in the background diet [13,14].

In this study, the high concentrations of ALA were determined four *Cephalaria* species especially in *C. stellipilis,* which was contained the highest percentage of ALA (34.87 %). The *C. davisiana*, which was the most effective cupric (II) reducer, was contained the great levels of phytol and arachidic acid, as well as heptadecanoic, heneicosanoic, tricosanoic and tetracosanic acids along with ALA. In conclusion, *Cephalaria* taxa were viewed to be potential additives in human diet, because of their fatty acid composition, especially the essential ones. In this manner, all species were shown the similar fatty acid profile and *C. davisiana* is the most remarkable species for antioxidant capacity.

Table 2. Fatty acid composition and phytol content of four *Cephalaria* species, %[a]

Symbols	Names	M^+	Quant Masses	*Cephalaria* species[b]			
				CP	CS	CD	CE
12:0	Dodecanoic acid ME	214	74	0.98	0.89	0.74	0.98
14:0	Tetradecanoic acid ME	242	74	3.41	2.45	2.33	3.28
15:1	*cis*-10 Pentadecenoic acid ME	254	74	0.46	0.48	0.43	0.90
15:0	Pentadecanoic acid ME	256	74	0.39	0.44	0.55	0.50
15:3	2-Pentadecanone 6,10,14-tri ME	268	58	0.53	1.19	1.36	1.30
16:0	Hexadecanoic acid ME	270	74	21.46	23.01	19.10	28.23
17:0	Heptadecanoic acid ME	284	74	0.62	0.59	0.71	0.65
18:2ω6	Octadeca- 9,12- dienoic acid ME	294	67	19.44	6.71	8.65	9.01
18:3ω3	Octadeca-9,12,15-trienoic acid ME	292	79	29.00	34.87	30.51	32.49
18:1ω9	Octadeca-9-enoic acid ME	296	55	0.85	0.64	0.59	0.50
18:0	Octadecanoic acid ME	298	74	5.30	5.51	5.22	5.60
20:0	Eicosanoic acid ME	326	74	3.96	4.61	6.19	3.09
21:0	Heneicosanoic acid ME	340	74	0.73	0.72	1.07	0.54
22:0	Docosanoic acid ME	354	74	3.04	4.32	3.99	4.21
23:0	Tricosanoic acid ME	368	74	0.89	0.66	1.39	0.41
24:0	Tetracosanic acid ME	382	74	3.82	2.85	5.07	2.72
26:0	Hexacosanoic acid ME	410	74	2.77	1.94	1.92	2.26
	Total saturated fatty acid %			**47.90**	**49.18**	**49.64**	**53.77**
	Total unsaturated fatty acid %			**49.75**	**42.70**	**40.18**	**42.90**
	Total fatty acid %			**97.65**	**91.88**	**89.82**	**96.67**
-	Phytol	296	71	2.35	8.13	10.18	3.35

[a]Percentages of total fatty acids. [b]**CP**: *C.paphlagonica,* **CS**: *C. stellipilis,* **CD**: *C. davisiana* and **CE**: *C. elazigensis*

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