*Pure Appl. Chem.*, Vol. 79, No. 12, pp. 2297–2304, 2007.
doi:10.1351/pac200779122297


# Essential fatty acid components and antioxidant activities of eight *Cephalaria* species from southwestern Anatolia*

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*Abstract*: The hexane extracts of eight *Cephalaria* (Dipsacaceae) species, which were collected from southwestern Anatolia, were obtained by Soxhlet apparatus. The fatty acids were derived to methyl esters and determined by gas chromatography/flame ionization detector (GC/FID) and gas chromatography/mass spectrometry (GC/MS) systems. The dominant fatty acid components and maximum percentages were detected as myristic [in *C. joppica* (17.48 %)], palmitic [in *C. cilicica*, *C. elmaliensis*, *C. isaurica*, *C. scoparia* (19.51 %), and *C. gazipashaensis*], linoleic [in *C. joppica* (33.02 %), *C. elmaliensis*, *C. dipsacoides*, and *C. gazipashaensis*], α-linolenic (ALA) [in *C. cilicica*, *C. elmaliensis*, *C. isaurica*, *C. scoparia*, *C. lycica*, and *C. gazipashaensis* (47.95 %)] and oleic [in *C. isaurica* and *C. dipsacoides* (40.66 %)] acids. The antioxidant activity of all hexane extracts was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric thiocyanate (FTC), and thiobarbituric acid (TBA) methods. The results indicate that hexane extracts of *Cephalaria* species possess considerable antioxidant activity. The highest radical scavenging activity was detected in *C. isaurica* ($IC_{50}$ = 741 μg/mL). The most effective species on lipid peroxidation are *C. lycica* and *C. gazipashaensis* in FTC and TBA assays, respectively. This study reveals that *Cephalaria* species are attractive sources of fatty acid components, especially the essential ones, as well as of effective natural antioxidants.

*Keywords*: Dipsacaceae; *Cephalaria* species; fatty acids; antioxidant activity; radical scavenging; lipid peroxidation.

## INTRODUCTION

*Cephalaria* Schrad. is a genus of about 65 species of flowering plants in the family Dipsacaceae and distributed in the Mediterranean region, the Balkan peninsula, and the Middle East [1]. *Cephalaria* species have been used in traditional medicine for many years due to their wide range of biological activities [2,3]. According to the literature, they have antimicrobial [4], antifungal [4], antioxidant [5], and cytotoxic [7,8] activities. The chemical constituents of this genus had generally been reported as triter-

*Invited contribution to a collection of papers for the IUPAC project 2005-042-1-300 "Chemistry for Biology". Other contributions to the project are published in this issue, pp. 2179–2366.

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penoid saponins [9–12], iridoid glycosides [13], flavonoid glycosides [5,6], lignan glycosides [7], hederagenin glycosides [14], and alkaloids [15]. The other two studies on *Cephalaria* species are determination of fatty acid composition of *C. setulifera* and *C. transsylvanica* [16], and the antioxidant activity of *C. pastricensis* [5]. Another member of the Dipsacaceae family, *Dipsacus asper* has been investigated for its antioxidant activity [17].

Here, we aim to determine the fatty acid components of hexane extracts by gas chromatography/flame ionization detector (GC/FID) and gas chromatography/mass spectrometry (GC/MS) techniques and to investigate the antioxidant activities of these extracts by 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric thiocyanate (FTC), and thiobarbituric acid (TBA) methods from eight *Cephalaria* species (*C. joppica*, *C. cilicica*, *C. elmaliensis*, *C. isaurica*, *C. dipsacoides*, *C. scoparia*, *C. lycica*, and *C. gazipashaensis*), six of them are endemic to Turkey, for the first time.

## MATERIALS AND METHODS

### Plant materials

Plant materials were collected from southwestern Anatolia about 5–2000 m height around Antalya, Turkey in June and July 2006 (Table 1). Voucher specimens were botanically established and deposited in the Herbarium Research and Application Center of Akdeniz University with the numbers R. S. Göktürk 5999, 5998, 5991-A, 5992, 5995, 5996, 6000, and 5991 for *C. joppica*, *C. cilicica*, *C. elmaliensis*, *C. isaurica*, *C. dipsacoides*, *C. scoparia*, *C. lycica*, and *C. gazipashaensis*, respectively.

**Table 1** Localities of eight *Cephalaria* species and the yields of hexane extracts.

| No. | Species | Collected area | Height, m | Dry plant, g | Hexane extract, g | Yield, % |
|---|---|---|---|---|---|---|
| 1 | *C. joppica* | Antalya-Manavgat, Side | 5 | 40 | 0.3763 | 0.95 |
| 2 | *C. cilicica** | Antalya-Finike, Elmalı | 500 | 40 | 0.8368 | 2.09 |
| 3 | *C. elmaliensis** | Antalya-Elmalı, Çığlıkara | 1800 | 40 | 0.3978 | 0.99 |
| 4 | *C. isaurica** | Antalya-Gündoğmuş, Söbüçimen | 1100 | 40 | 0.4950 | 1.24 |
| 5 | *C. dipsacoides* | Antalya-Akseki, Çimi | 1400–1500 | 40 | 0.5169 | 1.29 |
| 6 | *C. scoparia** | Antalya-Elmalı | 1200 | 40 | 0.3793 | 0.95 |
| 7 | *C. lycica** | Antalya-Kemer, Tekirova | 1700–2000 | 40 | 0.4150 | 1.04 |
| 8 | *C. gazipashaensis** | Antalya-Gazipaşa, Sugözü | 1200 | 40 | 2.5294 | 6.32 |

*Endemic species.

### Extraction

Dried and powdered plants were extracted with hexane using a Soxhlet apparatus (70 °C, 6 h) to obtain the fatty acids and the other apolar components. During extraction procedures, Merck hexane (95 %, No. 1.04368) was used. The extracts were concentrated by rotary evaporator under vacuum at ~40 °C. The extraction yields were presented in Table 1.

### Methylation of hexane extract

After removing hexane using rotary evaporator, the oily mixtures were derived to their methyl esters by the International Olive Oil Council (IOOC) (2001) and IUPAC (1992) reports by *trans*-esterification process [18,19]. In this process, dried hexane extracts were dissolved in hexane and then extracted with 2 M methanolic KOH at room temperature for 30 s. The upper phases were analyzed by GC/FID and GC/MS systems [20].

### GC/FID and GC/MS analyses

Methyl esters of fatty acids were analyzed using GC-6890 Agilent and MSD (mass selective detector)-5973 Mert Agilent combined system with HP-5 MS apolar column (30 m × 0.25 mm × 0.25 μm). The maximum column temperature, flow of helium, and oven temperature were 350 °C, 0.5 mL/min, and 170–210 °C, respectively. This program was carried out by raising the temperature by 2 °C/min. The fatty acids were identified by comparing their retention times and mass peaks with those of standard methyl ester mixtures and by NIST–Wiley library data search.

All the methyl esters, especially 9,12,15-octadecatrienoic and 9-octadecenoic (*Z*) methyl esters, were also verified by GC-6890 Agilent, with DB-23 polar column (30 m × 0.25 mm × 0.25 μm). The detector and injector temperatures were set at 280 °C, and the flow of hydrogene was 40 mL/min. The other characteristics of the program were adjusted as air flow 450 mL/min, total flow 66 mL/min, oven temperature 50 °C (1 min waiting), rising from 25 °C/min to 175 °C. The program was continued heating the system to 230 °C with 4 °C/min rising (20 min waiting). Inlet temperature, pressure, and split were 250 °C, 15 psi, and 1/50, respectively.

### Antioxidant activity tests

The antioxidant activity of hexane extracts of *Cephalaria* species was assessed by three different methods. Radical scavenging activity was measured by DPPH test [21]. Inhibitory effect on lipid peroxidation was examined by FTC [22] and TBA methods [23]. The main chemicals; ammonium thiocyanate, ferrous chloride, hydrochloric acid, and potassium phosphate salts (Merck), DPPH, linoleic acid (LA), Trolox, butylated hydroxytoluene (BHT), L-ascorbic acid (Sigma), and ethanol (Carlo Erba) were used during the following antioxidant activity tests.

### Free radical scavenging activity

The DPPH assay was carried out according to the modified method of Cheung et al. (2003). Briefly, 0.5 mL of DPPH in ethanol (0.1 mM) was added to 1 mL of hexane extract in different concentrations (0.1–1.6 mg/mL) and kept in the dark for 10 min. The absorbance of the resulting solution was recorded on a spectrometer at 520 nm against a blank of hexane. Vitamin C was used as reference antioxidant. DPPH scavenging activity was expressed as $IC_{50}$ values (μg extract/mL) for comparison. $IC_{50}$ value of each sample defined as the concentration of sample required for the 50 % decrease in absorbance of the blank, was calculated.

### Inhibitory effect on lipid peroxidation

Four milligrams of each extract were dissolved in 4 mL of 99.5 % ethanol and added to lipid peroxidation system, containing 2.51 % LA in 99.5 % ethanol (4.1 mL), 0.02 M phosphate buffer (8.0 mL) (pH 7.0), and distilled water (3.9 mL), in a screw-cap vial (38 mm × 75 mm). Final concentration of the extract was 0.02 % (w/v) in the assay mixture. Hexane was used as negative control. This mixture was incubated in an oven at 40 °C in the dark until the end of the experiment ($8^{th}$ day) and used for FTC

and TBA methods. Each experiment was repeated at least in duplicate, and mean values were used for the evaluation of the results.

### *FTC method*

Hydroperoxides produced by LA oxidation system in the assay mixture were detected as described by Abas et al. (2006). To measure the extent of antioxidant activity, an aliquot (0.1 mL) was taken from the above-mentioned assay mixture and added to 75 % (v/v) ethanol (9.7 mL), followed by 30 % aqueous ammonium thiocyanate (0.1 mL) and 0.02 M $FeCl_2$ (0.1 mL) in 3.5 % HCl were added. After 3 min, absorbance was recorded at 500 nm, as the first measurement. Recordings were repeated at 24 h intervals, until the absorbance of the blank reached its maximum value ($8^{th}$ day). BHT and Trolox were used as reference antioxidants.

### *TBA method*

The same assay mixture used in FTC method was evaluated for TBA test at the final ($8^{th}$) day of the experiment [23]. One milliliter of 20 % aqueous trichloroacetic acid (TCA) and 2 mL of 0.67 % aqueous TBA were added to 2 mL of assay mixture and incubated in a boiling water bath for 10 min. After cooling, the mixture was centrifuged at 1200 *xg* for 5 min. Absorbance of the supernatant was measured at 532 nm and used as an expression of antioxidant activity.

## RESULTS AND DISCUSSION

### Fatty acids profile

The fatty acid composition of *C. joppica*, *C. cilicica*, *C. elmaliensis*, *C. isaurica*, *C. dipsacoides*, *C. scoparia*, *C. lycica*, and *C. gazipashaensis* was investigated using GC/FID and GC/MS techniques for the first time. According to the results, the hexane extract yields of the studied *Cephalaria* species were found between 0.95 and 6.32 % on the basis of dry weight of the plant materials (Table 1). The highest percentage was detected in *C. gazipashaensis.* The total fatty acid contents of hexane extracts varied from 77.94 to 95.23 % (Table 2). The unsaturated fatty acid contents were higher than saturated ones, whereas some of the fatty acids were not observed in all species. In fact, all species mainly include unsaturated fatty acids, with a clear predominance of α-linolenic (ALA), LA, and oleic acids. One of the essential fatty acids (EFAs), ALA was a predominant component in *C. cilicica*, *C. elmaliensis*, *C. isaurica*, *C. scoparia*, *C. lycica*, and *C. gazipashaensis*, whereas the other one, LA, was in *C. joppica* and *C. dipsacoides*. In addition, oleic acid was detected in a high amount in *C. joppica*, *C. isaurica*, and *C. dipsacoides*, as another unsaturated fatty acid. On the other hand, myristic and palmitic acids were mainly found in *C. joppica* and *C. scoparia* as saturated fatty acids, respectively.

**Table 2** Fatty acid compositions of eight *Cephalaria* species, %*.

| Fatty acids | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Dodecanoic acid (lauric acid) | 1.15 | 1.51 | 0.52 | 1.09 | 0.82 | 2.47 | 0.62 | 0.46 |
| Tetradecanoic acid (myristic) | 17.48 | 4.02 | 2.24 | 7.07 | 11.13 | 4.02 | 1.60 | 1.53 |
| Pentadecanoic acid | 0.07 | – | 0.33 | – | 0.20 | 0.68 | 0.43 | 0.15 |
| Hexadeca-7,10,13-trienoic acid | 0.18 | 1.94 | 1.67 | 0.80 | 0.63 | 1.63 | 1.98 | 0.44 |
| Hexadec-9-enoic acid (*Z*) (palmitoleic) | – | – | – | – | 0.15 | – | – | – |
| Hexadec-11-enoic acid (*Z*) | – | – | 0.62 | – | 0.11 | – | 0.47 | – |
| Hexadecanoic acid (palmitic) | 10.42 | 15.11 | 19.42 | 15.18 | 10.92 | 19.51 | 12.28 | 18.40 |

(*continues on next page*)

**Table 2** (*Continued*).

| Fatty acids | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Heptadecanoic acid (margaric) | 0.10 | – | 0.33 | – | 0.24 | 0.21 | 0.32 | 0.23 |
| Octadeca-9,12-dienoic acid (*Z*,*Z*) (linoleic) | 33.02 | 11.37 | 16.09 | 11.37 | 26.07 | 9.73 | 9.14 | 15.75 |
| Octadeca-9,12,15-trienoic acid (α-linolenic) | 7.57 | 43.84 | 36.26 | 26.86 | – | 37.34 | 47.16 | 47.95 |
| Octadec-9-enoic acid (*Z*) (oleic) | 19.10 | 9.36 | 4.94 | 15.43 | 40.66 | 8.75 | 4.49 | 7.36 |
| Octadec-9-enoic acid (*E*) (elaidic) | 0.21 | – | 0.66 | 1.00 | 0.74 | – | 0.56 | 0.67 |
| Octadecanoic acid (stearic) | 1.82 | 2.62 | 2.45 | 3.02 | 2.13 | 3.48 | 2.56 | 1.92 |
| Eicos-11-enoic acid (*Z*) (gondoic) | – | – | – | – | 0.23 | – | 0.42 | 0.22 |
| Eicosanoic acid (arachidic) | 0.34 | 1.91 | 1.52 | 2.54 | 0.85 | 2.32 | 1.77 | 0.78 |
| Heneicosanoic acid | – | – | – | – | – | – | 0.19 | – |
| Docosanoic acid (behenic) | 0.19 | 0.70 | 0.89 | 1.41 | 0.35 | 0.95 | 0.78 | 0.29 |
| **Total fatty acid percentages** | 91.65 | 92.38 | 77.94 | 85.77 | 95.23 | 81.09 | 84.77 | 85.92 |

*In addition to the above data, phytol was detected as a considerable component as 0.68, 6.73, 8.96, 4.81, 2.36, 4.47, 10.44, and 3.41 percentages for the plants **1–8**. Eicosane (for **1–8**), eicos-5-ene (for **4**, **7**), 4,4,7a-trimethyl-5,6,7a tetrahydro-(4*H*)-benzofuran (for **3**, **7**), and 6,10,14-trimethylpentadecan-2-one (for **3–7**) were detected as minor components (~0.1 %).

## Antioxidant activity of extracts

The antioxidant activity of hexane extracts was also reported for the first time. The DPPH scavenging activities expressed as $IC_{50}$ values were presented in Table 3. According to this data, *C. isaurica* was the most efficient free radical scavenger by the lowest $IC_{50}$ value of 741 μg/mL among all the hexane extracts. The activity of the reference antioxidant (vitamin C) was much higher than that of *C. isaurica*. Although *C. isaurica* did not differ considerably in fatty acid composition, it exhibited the best DPPH scavenging activity. This result may arise from the other chemical constituents rather than fatty acids.

**Table 3** DPPH free radical scavenging activity of hexane extracts of *Cephalaria* species and standard antioxidant, vitamin C.

| No. | Samples | $IC_{50}$ value (μg/mL) |
|---|---|---|
| **1** | *C. joppica* | 927 |
| **2** | *C. cilicica* | 800 |
| **3** | *C. elmaliensis* | 928 |
| **4** | *C. isaurica* | 741 |
| **5** | *C. dipsacoides* | 1330 |
| **6** | *C. scoparia* | 1190 |
| **7** | *C. lycica* | 835 |
| **8** | *C. gazipashaensis* | 2880 |
| | Vitamin C | 26 |

The amount of peroxides in the initial stages of lipid peroxidation was measured by the FTC method. Lipid peroxidation was monitored in daily intervals, over a period of eight days. According to the results represented in Fig. 1, all the extracts as well as reference antioxidants (BHT and Trolox) ef-

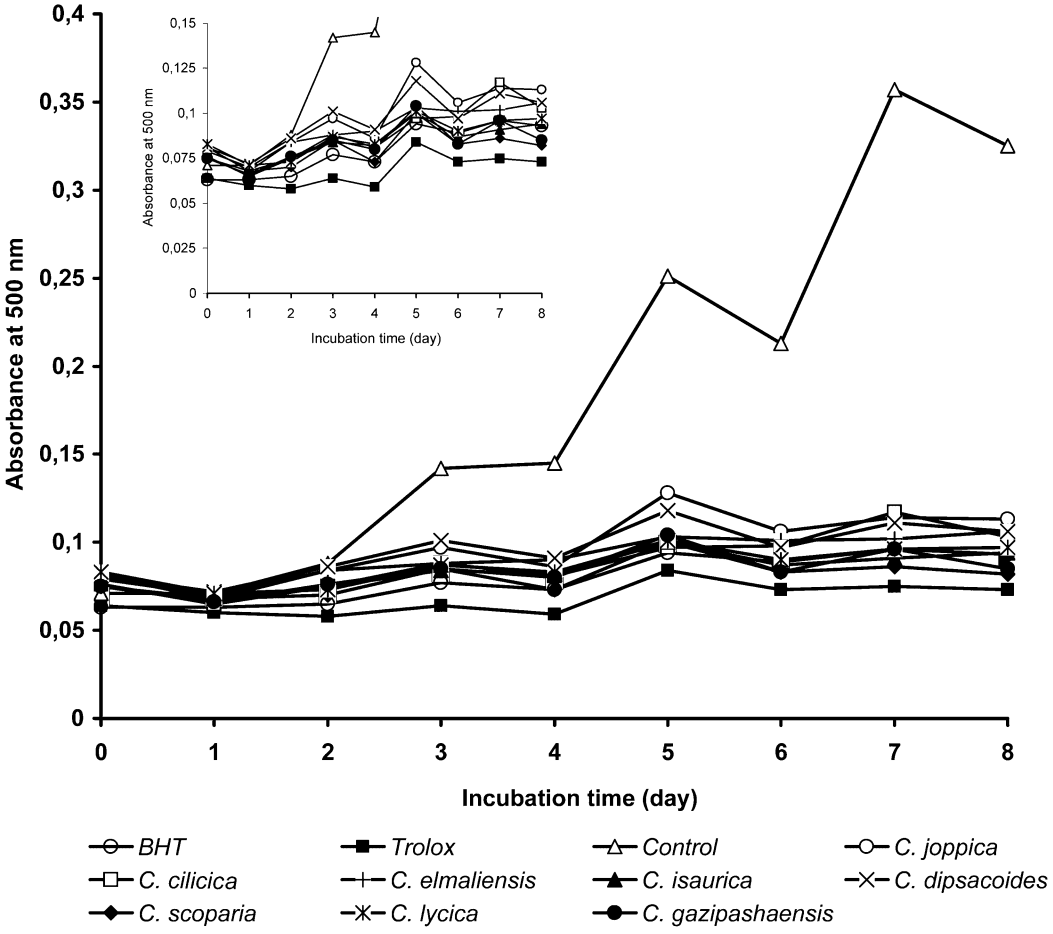


**Fig. 1** Inhibitory effect of *Cephalaria* hexane extracts on the primary oxidation of LA system measured by FTC method. Inset shows the expended plots of the extracts and reference antioxidants.

fectively inhibited the peroxide formation. Especially *C. scoparia* and *C. gazipashaensis* showed significant antioxidant activity even higher than BHT. There was no clear correlation between the unsaturated fatty acid contents and FTC findings.

The amount of malondialdehyde degraded from the peroxides at a later stage of lipid peroxidation ($8^{th}$ day) was measured by the TBA method (Fig. 2). The decrease in absorbance values indicated that all the extracts suppressed the formation of malondialdehyde. The most active species were *C. cilicica*, *C. lycica*, and *C. gazipashaensis*, and their antioxidant activity was similar to that of Trolox. BHT seemed to be the most effective antioxidant under these experimental conditions. It is well known that unsaturated fatty acids that contain more double bonds would be easily oxidized [24]. Here, the results of the TBA assay obtained from the species, including the highest amount of ALA, which contains three double bonds, are inconsistent with this phenomenon.

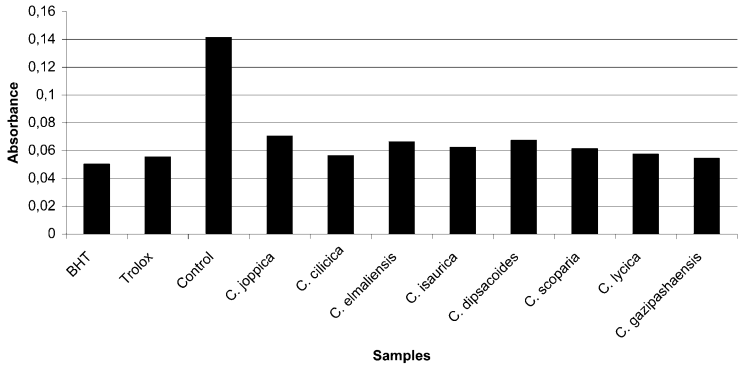


**Fig. 2** Antioxidant activity of hexane extracts measured by TBA method.

## CONCLUSION

It is well known that the EFAs play important roles in preventing many diseases and abnormal differentiation problems. The two EFAs (ALA and LA) cannot be synthesized by human cells and hence have to be obtained from dietary sources. The famous diet model, which is known as the Mediterranean diet, providing oleic acid, LAs, ALAs, antioxidant nutritients, and reduced amounts of saturated fatty acids, resulted in a 70 % reduction in coronary events and 80 % reduction in deaths [25–27].

The lack of EFAs causes several abnormalities and malignant transformations in the human body, such as breast cancer [28], cardiovascular diseases [29], as well as inflammatory and immunological responses [25].

According to our results, the main constituents of all hexane extracts were ALAs and LAs. It is clear that there is a significant correlation between the EFAs and antioxidant activity. Thus, it seems that *Cephalaria* species, especially *C. cilicica*, *C. lycica*, and *C. gazipashaensis* may be a good dietary source for EFAs and/or effective antioxidants.

## ACKNOWLEDGMENTS

The authors acknowledge TUBITAK 106T433 (TBAG-HD/178) for financial support and also Hygene Center of İzmir for running GC/FID and GC/MS analyses.

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