

Supporting information:

***Rec. Nat. Prod.* 6:2 (2011) 151-155**

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

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Activity tests of the fatty acid mixtures

Antimicrobial activity test

The antimicrobial tests were run by the following standard organisms, which were obtained from the Microbiology Department Culture Collection of Ege University, Faculty of Science, Izmir, Turkey. The antibacterial activities of the *n*-hexane extracts of four *Cephalaria* species were tested against four gram negative (*Escherichia coli* ATCC 23999, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* CCM 5445 and *Klebsiella pneumoniae* CCM 2318) and four gram positive (*Staphylococcus aureus* ATCC 6538/P, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212 and *Bacillus cereus* ATCC 7064) bacteria strains.

The minimum inhibitory concentration of the samples was determined using the method described by Atlas et. al. and NCCLS [21-22]. Firstly, the bacteria strains were inoculated on Mueller-Hinton broth (Difco) and incubated for 24 h at 37 ± 0.1 °C. Before inoculation of the test organisms, the bacteria strains were adjusted to 0.5 McFarland standards and diluted 1:100 (v/v) in Mueller-Hinton broth (MHB). Dilution series of the *n*-hexane extracts were prepared in test tubes and then transferred to the broth in 96-well microtiter plates. Final concentrations in the medium were ranged from 2000 to 50 µg/mL. The plates [sterile, polypropylene, 250 µL, standard 96 well (8 rows x 12 columns) U shape bottom, transparent microplates] were prepared by dispensing into each well 80 µL of two fold MHB and 20 µL of the inoculums (1×10^8 CFU/ mL). A 100 µL of the stock solutions of *n*-hexane extracts initially prepared at the concentration of 2000 µg/mL was added into the first wells. Then, 100 µL from their serial dilutions was transferred into consecutive wells. The last well containing 195 µL of nutrient broth without extract and 5 µL of the inoculums on each strip was used as negative control. All the plates were covered with a sterile plate sealer and incubated at 37 °C for 24 h. The MIC was defined as the lowest concentration of the extract inhibiting the growth of microorganisms, observed as a clear background (no visible growth) against blank (containing equal volume of *n*-hexane instead of extract). Samples from clear wells were sub-cultured by plotting onto Mueller Hinton agar. Gentamycine (Sigma) (128 to 0.25 µg/mL in each well) was the positive control.

Antioxidant activity test

The cupric (II) reducing antioxidant capacity was determined using the CUPRAC method described by Apak et. al. [23]. This method is based on the redox chemistry of copper (II). The assay mixture consisting of 1.0 mL CuCl_2 (1×10^{-2} M), 1.0 mL freshly prepared neocuproine alcoholic solution (7.5×10^{-3} M, in 96% EtOH), 1.0 mL ammonium acetate buffer at pH 7.0 (1 M), 0.1 mL sample solution and 1.0 mL distilled water was incubated at room temperature for 30 min, and subsequently the absorbance at 450 nm was measured in a multiwell spectrophotometer (BioLab µQuant), against a reagent blank of *n*-hexane. Trolox (Fluka 56510) solution (1×10^{-3} M, in 96% EtOH) was used as standard antioxidant. The dried extracts were dissolved in an initial volume of *n*-hexane, and diluted so as to give a final absorbance (at 450 nm) around 0.2 in the CUPRAC measurements, for highest photometric precision. CUPRAC values indicating cupric (II) reducing antioxidant capacity of the samples were calculated as mmol trolox (TR) equivalent/g dry extract, using the equation:

$$\text{Capacity (mmol TR/g)} = (A_{450}/\epsilon_{\text{TR}}) \times (V_f/V_s) \times r(V_{\text{cup}}/m)$$

A_{450} is final absorbance at 450 nm; ϵ_{TR} is molar absorptivity of trolox in this system ($\epsilon_{\text{TR}} = 1.74 \times 10^4$ L/mol/cm); V_f is final volume of assay mixture (4.1 mL); V_s is sample volume (0.1 mL); r is dilution factor; V_{cup} is initial volume of the extract and m is amount of dried extract as g.