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APOLAR CONSTITUENTS OF SOME BIOLOGICALLY ACTIVE *Dianthus* SPECIES FROM WESTERN ANATOLIA

Inci Durucasu,[1] Kiymet Mutlu,[1] Levent Sik,[2] Ihsan Yasa,[3] Nazli Arda,[4] and Suheyla Kirmizigul[5*]

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The apolar constituents of four Dianthus *(Caryophyllaceae) species were determined by GC-MS. Palmitic, linoleic, and oleic acids were detected as dominant components in all species.* D. elegans *d'Urv. var.* elegans *had the highest antioxidant activity. All four species also showed considerable antimicrobial activity against* S. epidermidis *and* C. albicans.

Key words: Caryophyllaceae, *Dianthus*, fatty acids, antioxidant activity, antimicrobial activity.

Dianthus L. (Caryophyllaceae) is a genus of about 300 species in the world, distributed in the Mediterranean Region and in the Middle East [1]. In Turkey, 67 species are present, especially in Western Anatolia. Some *Dianthus* species are used in traditional medicine in addition to agricultural purposes [2–4]. According to the literature, they have antimicrobial [2], antihepatotoxic [4], analgesic [4], diuretic [5], and dermatologic [3] importance. The chemical constituents of this genus have generally been reported as triterpenoid saponins [4, 6], hydropyranes [7], and cyclic peptides [5]. Some GC-MS and LC-MS studies have determined that this genus consists of different types of terpenes [8], essential fatty acids [9, 10], and volatile compounds [11].

In this study, we have determined the fatty acid components of the hexane extracts by GC-MS and investigated the antioxidant (by the DPPH method) and antimicrobial (by microdilution assay) activities of four *Dianthus* species, three of which are endemic to Turkey, for the first time.

In this study, the apolar constituents of four *Dianthus* species were investigated using GC-MS for the first time. According to our results, the hexane extract yields were between 2.62% and 4.96% based on dry weight of the plant materials (Table 1). The highest percentage was detected in *D. erinaceus* var. *erinaceus* (4.96%). The total fatty acid components of hexane extracts varied from 95.23% to 98.48% (Table 2). The remainder of these percentages consisted mainly of phytol, eicosanol, and eicosane. 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 contained unsaturated fatty acids, with a clear predominance of linoleic (LA) and oleic acids. One of the essential fatty acids (EFAs), LA, was a major component in all species, especially in *D. elegans* var. *elegans* with a component value of 51.98%. The other important essential fatty acid was present in the ratio 20:0, which was detected only in *D. erinaceus* var. *erinaceus* at 2.07%. Oleic acid was detected at high levels in all species as another unsaturated fatty acid. The highest oleic acid content was found in *D. lydus* at 48.54%. Palmitoleic acid was found only in *D. lydus* at 4.11%. In addition to these findings, myristic, palmitic, and stearic acids were found in all plants at more than 14.0% as saturated fatty acids.

1) Celal Bayar University, Faculty of Science and Arts, Department of Chemistry, 45140, Muradiye, Manisa, Turkey; 2) Celal Bayar University, Faculty of Science and Arts, Department of Biology, 45140, Muradiye, Manisa, Turkey; 3) Ege University, Faculty of Science, Department of Biology, 35100, Bornova, Izmir, Turkey; 4) Istanbul University, Faculty of Science, Department of Molecular Biology and Genetics, 34118, Vezneciler, Istanbul, Turkey; 5) Ege University, Faculty of Science, Department of Chemistry, 35100, Bornova, Izmir, Turkey, fax: +90 232 3888264, e-mail: suheyla.kirmizigul@ege.edu.tr.
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TABLE 1. Localities of Four *Dianthus* Species and the Yields of Their Hexane Extracts

Species	Height, m	Yield, %
Dianthus elegans var. *elegans**	950	2.62
Dianthus erinaceus var. *erinaceus**	1500	4.96
Dianthus lydus	800	2.77
Dianthus zonatus var. *zonatus**	1000	2.70

*Endemic.

TABLE 2. Fatty Acid Compositions of Four *Dianthus* Species, %*

Fatty acid	*D. elegans* var. *elegans*	*D. erinaceus* var. *erinaceus*	*D. lydus*	*D. zonatus* var. *zonatus*
12:0	–	1.36	1.35	1.82
14:0	1.81	5.46	3.04	4.87
15:0	1.05	0.80	0.44	–
16:1	–	–	4.11	–
16:0	14.09	24.89	29.49	27.25
18:2	51.98	17.80	8.34	20.77
18:1	22.44	32.19	48.54	36.17
18:0	1.63	4.15	2.00	4.10
20:0	–	2.07	–	–
22:0	5.48	8.58	0.49	0.63
Total	98.48	95.23	97.80	95.61

*In addition to the above data, the other apolar components were as folows: phytol was detected as a considerable component comprising 1.79% and 3.49% in *D. lydus* and *D. zonatus* var. *zonatus*, respectively. Eicosanol (1.01%) was found only in *D. elegans* var. *elegans.* Eicosane was detected as a minor component (~0.5%) in all plants except *D. erinaceus* Boiss. var. *erinaceus* (~2.70%). Note the high levels of total fatty acids in all four *Dianthus* species.

TABLE 3. DPPH Free Radical Scavenging Activity of Hexane Extracts of *Dianthus* Species

Sample	DPPH inhibition, %	IC_{50} value, mg/mL
D. elegans var. *elegans*	31.00	1.93
D. erinaceus var. *erinaceus*	3.50	17.14
D. lydus	18.20	3.29
D. zonatus var. *zonatus*	5.00	12.00
Vitamin C	59.00	0.169

TABLE 4. Antimicrobial Activities of Hexane Extracts of Four *Dianthus* Species

Microorganism	*D. elegans*	*D. erinaceus*	*D. lydus*	*D. zonatus*	Gentamycine, μg/mL
Staphylococcus aureus	–	–	–	–	1.25
Staphylococcus epidermidis	0.625	0.312	1.25	0.312	1.25
*Candida albicans**	–	1.25	–	0.312	–
Escherichia coli	–	20.00	20.00	20.00	1.25
Bacillus cereus	–	–	–	–	1.25
Pseudomonas aeruginosa	20.00	–	20.00	–	2.50

*Clotrimazole – 0.78 μg/mL.

The antioxidant activity of hexane extracts was also reported for the first time. The DPPH scavenging activities expressed as IC_{50} values presented in Table 3 indicated that *D. elegans* var. *elegans* was the most efficient free radical scavenger with the lowest IC_{50} value of 1.93 mg/mL among all hexane extracts. The activity of the reference antioxidant (vitamin C) was higher than that of *D. elegans* var. *elegans.* Although *D. elegans* var. *elegans* did not differ considerably from the other species in fatty acid composition, it exhibited the best DPPH scavenging activity. This result may be due to the total unsaturated fatty acids (~75%), namely the linoleic and oleic acid contents of the materials. It is well known that unsaturated fatty acids can be easily oxidized as they contain double bonds. Thus, the highest activity observed in those species that contain the highest amount of linoleic and oleic acids is consistent with this phenomenon.

The antimicrobial activity results expressed as mg/mL by MIC experiment in Table 4 demonstrated that *D. elegans* d′Urv. var. *elegans*, *D. erinaceus* Boiss. var. *erinaceus*, and *D. zonatus* Fenzl. var. *zonatus* showed considerable antimicrobial activity against *S. epidermidis* ATCC 12228, in addition to the activity of *D. erinaceus* Boiss. var. *erinaceus* against *C. albicans* ATCC 10239 by microdilution assay. *C. albicans* was moderately affected by *D. zonatus* var. *zonatus*. *B. cereus* and *S. aureus* were considered resistant to all extracts since no inhibition of growth was observed. It has previously been reported that the ethanol extract [12] and dimethylsulfoxide–methanol 1:1 extract [2] from *D. caryophyllum* possesed antimicrobial effect against *S. epidermidis*, *P. aeruginosa*, *E. coli*, and *C. albicans*, which were similar to our findings.

It is well known that the essential fatty acids play important roles in preventing many diseases and abnormal differentiation problems. Some fatty acids cannot be synthesized by human cells and hence have to be obtained from dietary sources. The famous diet model known as the Mediterranean diet, providing oleic, linoleic, and α-linolenic acids, has been established as offering many health benefits. This type of diet was shown to reduce coronary events and deaths due to its high antioxidant nutrient levels [13, 14]. The lack of EFAs is linked with several abnormalities and malignant transformations such as breast cancer [15], cardiovascular diseases [16], as well as inflammatory and immunological responses [13]. Furthermore, a significant correlation has been established between the essential fatty acids and antioxidant and antimicrobial activity data [2, 12]. From this aspect, our results showed a clear similarity between the constituent levels of all hexane extracts and the antioxidant and antimicrobial activities. Thus, the four *Dianthus* species, especially *D. elegans* var. *elegans* and *D. lydus*, are rich sources of unsaturated fatty acids with antioxidant and antimicrobial properties in addition to their chemotaxonomic significance.

EXPERIMENTAL

Plant Materials. Plant materials were collected at varying altitude levels between 800 m and 1500 m on the Spil Mountain located in Western Anatolia in the city of Manisa, Turkey in June and July 2006.

Extraction. Dried and powdered plants (60 g each) were extracted with hexane using a Soxhlet apparatus (70°C, 6 h). The extracts were concentrated by a rotary evaporator under vacuum at ~40°C. The extraction yields are presented in Table 1.

Methylation of Hexane Extract. After removal of the hexane, the oily mixtures were converted to their methyl esters by IOOC and analyzed by GC-MS system [17].

GC-MS Analysis. Methyl esters of fatty acids were analyzed using a GC (Gas Chromatography)-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). Maximum column temperature, flow of helium, oven temperature, and pressure were 350°C, 1 mL/min, 170–210°C, and 14.3 psi, respectively. This program was carried out with a temperature rise of 2°C/min and an injection volume of 2 μL. 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.

Antioxidant Activity Test. Free radical scavenging activity was measured by the DPPH test. The main chemicals 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma), L-ascorbic acid (Sigma), and ethanol (Carlo Erba) were used during the test. The DPPH assay was carried out according to the modified method of Cheung et al. [18]. Briefly, 0.5 mL of DPPH in ethanol (0.1 mM) was added to 1 mL of hexane extract in different concentrations (0.1 to 1.2 mg/mL) and the mixture 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 (0.2 mg/mL) was used as reference antioxidant. DPPH scavenging activity was expressed as IC_{50} values (mg/mL) for comparison. IC_{50} value of each sample defined as the concentration of sample required for a 50% decrease in absorbance of the blank was calculated. Each experiment was repeated at least in duplicate, and mean values were used for the evaluation of the results.

Antimicrobial Activity Test. Antibacterial and anticandidal activities of all extracts were evaluated using minimum inhibitory concentration (MIC) measurements. The MIC values were determined for the bacterial strains and the unicellular fungus *C. albicans* by a microdilution assay [19].

In vitro antimicrobial tests were carried out against *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 6633, and *Pseudomonas aeruginosa* ATCC 27853 bacteria strains and *Candida albicans* ATCC 10239 unicellular fungus, which were obtained from the Microbiology Department Culture Collection of Ege University, Faculty of Science, Izmir, Turkey. The bacteria strains and *C. albicans* were inoculated on Mueller-Hinton Broth (Difco) and incubated for 24 h at 37±0.1°C. Inoculates of the strains were prepared from 24 h broth cultures, and the suspensions were adjusted to 0.5 McFarland standard turbidity. A series of test tubes containing from 0.156 to 40 mg/mL extracts was examined. The 96-well plates were prepared by dispensing into each well 160 µL of broth and 20 µL of the inoculums (1×10^5 CFU/mL). A 20 µL sample from stock solutions of the extracts initially prepared at a concentration of 40 mg/mL was added into the first well. Then, 20 µL from their serial dilutions was transferred into consecutive wells. The last well contained 195 µL of Mueller-Hinton Broth without extract, and 5 µL of the inoculums on each strip was used as a negative control. The plate was covered with a sterile plate sealer. Gentamycin (Sigma) for bacterial strains and clotrimazole (Sigma) for *C. albicans* ATCC 10239 were used as reference antibiotics. The microtiter plates were incubated at 37°C for 24 h. After incubation, the MIC values were measured by adding 50 µL of a 0.5% TTC (triphenyl tetrazolium chloride, Fluca) aqueous solution. The MIC is defined as the lowest concentration of extracts that inhibited visible growth as indicated by the TTC reduction. In the presence of bacterial growth by reduction reactions, TTC changed the color of microorganisms from colorless to red. This provided clearly defined and easily readable endpoints [20]. All of the assays were performed in duplicate.

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REFERENCES

1. P. H. Davis, *Flora of Turkey,* Edinburg University Press, Vol. **2**, 1967, 121 pp.
2. G. H. S. Bonjar, *Fitoterapia,* **75**, 231 (2004).
3. S. Mansouri, *Pharm. Biol.,* **37**, 375 (1999).
4. H. Hikino, T. Ohsawa, Y. Kiso, and Y. Oshima, *Planta Med.,* **50**, 353 (1984).
5. P. W. Hsieh, F. R. Chang, C. C. Wu, K. Y. Wu, C. M. Li, S. L. Chen, and Y. C. Wu, *J. Nat. Prod.,* **67**, 1522 (2004).
6. K. Koike, H. Li, H. Muraoka, S. Fukui, M. Inoue, and T. Ohmato, *Tetrahedron*, **50**, 12811 (1994).
7. V. Plouvier, M. T. Martin, and J. P. Brouard, *Phytochemistry,* **25**, 546 (1986).
8. A. Jurgens, T. Witt, and G. Gottsberger, *Biochem. Syst. Ecol*., **31**, 345 (2003).
9. H. Tunon, W. Thorsell, A. Miviker, and I. Malander, *Fitoterapia*, **77**, 257 (2006).
10. J. L. Guil-Guerrero, J. C. Lopez-Martinez, R. Navarro-Juarez, F. Garcia-Maroto, and P. Campa-Madrid, *J. Am. Oil Chem. Soc.*, **81**, 659 (2004).
11. F. Schade, R. L. Legge, and J. E. Thomson, *Phytochemistry*, **56**, 703 (2001).
12. O. Erturk, *Biologia*, **61**, 275 (2006).
13. J. L. Quiles, J. R. Huertas, J. J. Ochoa, M. Battino, J. Mataix, and M. Manas, *Nutrition*, **19**, 363 (2003).
14. S. M. Innis, *Am. J. Clin. Nutr.*, **71**, 38 (2000).
15. A. R. Eynard, *Nutrition*, **19**, 386 (2003).
16. J. X. Kang and A. Leaf, *Am. J. Clin. Nutr.*, **71**, 202 (2000).
17. F. David, P. Sandra, and P. L. Wylie, *Agilent Technol.*, 2003.
18. L. M. Cheung, P. C. K. Cheung, and V. E. C. Ooi, *Food Chem.*, **81**, 249 (2003).
19. R. M. Atlas, L. C. Parks, and A. E. Brown, *Laboratory Manual of Experimental Microbiology*, Mosby-Year Book, Inc., St. Louis, Missouri, 1995, 341 pp.
20. J. Uno, M. L. Shigematsu, and T. Arai, *Antimicrob. Agents Chemother.*, **21**, 912 (1982).