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Biological activity and chemical compounds of the hexane extracts from *Chaerophyllum macropodum* in two different habitats

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The chemical constituents of the hexane extracts of *Chaerophyllum macropodum* (Boiss.) from two different localities were obtained in Soxhlet apparatus and analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). The hexane extracts of *C. macropodum* from Givi and Neor were characterized by a higher amount of linoleic acid (23.2 and 29.2%), 6-octadecenoic acid (20.2 and 6.8%), 9,12-octadecadienoic acid (8.8 and 9.9%) and E-β-farnesene (4.6 and 8.7%), respectively. Carotol (16.9%) and (-)-β-acoradiene (5.1%) were the major compounds in the hexane extract of sample from Givi, and germacrene- D (8.8%) and acorenone-B (12.6%) were the major ones in Neor sample. The composition of the extracts was different, although the most abundant components were identical in hexane extract from Neor sample (99.3%). The antimicrobial effects of hexane extracts from *C. macropodum* were studied according to the agar diffusion cup method. The antimicrobial activity of the hexane extracts of those samples were determined against some Gram-positive and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Staphylococcus epidermidis*), as well as three fungi (*Aspergillus niger, Candida albicans* and *Saccharomyces cerevisiae*). The hexane extracts had a moderate effect on some Gram-positive and Gram-negative bacteria.

Key words: *Chaerophyllum macropodum*, umbelliferae, hexane extract, omega-3.

INTRODUCTION

Plant materials widespread use has raised the interest of scientists in basic research of extracts and essential oils. Especially, the anti-microbial and anti-oxidant activities of solvent extracts and essential oils as well as their potential anti-cancer activity have been investigated in recent years (Mimica-Dukic et al., 2004; Shafaghat, 2011a, b; Sylvestre et al., 2005). Organic fatty acids are naturally found in plants and may be formed during processes like fermentation or may be added into food during the manufacturing process. Numerous species of the plants which are rich fatty acids especially in seeds are of great importance as herbs and spices.

The genus *Chaerophyllum* belonging to Umbelliferae family is comprised of about 110 species, 8 are described in the flora of Iran, among which two are endemic: *Chaerophyllum nivale* Hedge et Lamond and *Chaerophyllum khorassanicum* Czern. ex Schischk. (Rechinger, 1987; Mozaffarian, 2007). *Chaerophyllum* species are widely distributed in Europe, Iran, Caucasus and Central Asia, while *C. macropodum* is the only known wild species in Iran and Turkey. During flowering, it is known to be a good source of honey. Few investigations have been made on the composition of *Chaerophyllum* species (Shafaghat, 2009a). These plants are also used in traditional healing practices in most of those countries, fresh stems and leaves are sometimes added to salads, while tea made of dried leaves and roots is used as herbal remedy to soothe cough, allergies

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and sore throat. The essential oil compositions of two *Chaerophyllum* species growing in Azerbaijan, *Chaerophyllum bulbosum* L. and *Chaerophyllum macrospermum* (Willd. ex Spreng.) Fisch, et Mey., were reported (Mamedova, 1994).

However, only a small part of their components was identified. The oil of *C. macropodum* aerial parts from Iran was reported to be predominated by α-pinene (23.0%), β-pinene (17.3%) and fenchyl acetate (13.8%) as the major constituents. The oil was richer in monoterpene than sesquiterpene hydrocarbons (Nematollahi et al., 2005). The essential oil from the roots of *C. macropodum* from Iran was reported to be predominated by myristicin (39.2%), terpinolene (23.1%), *trans*-β-ocimene (21.9%) and γ-terpinene (5.4%) as the main compounds (Shafaghat, 2009b). In the oil of *Chaerophyllum aksekiense* Duran et Duman, endemic in Turkey, 67 components representing 82% of the oil were identified. Paraffin hydrocarbons comprised 21% of the oil of *C. aksekiense* with heptacosane (10.1%) as major constituent (Baser et al., 2000). The oil of *C. macrospermum* (Spreng.) Fisch, et C.A. Mey, growing in Iran, was obtained from aerial parts, and 16 components were identified with high percentage (97.4%) of monoterpenes with (E)-β-ocimene (40.0%) as the main constituent.

In the oil reported here, (E)-β-ocimene (21.9%) was also among the main constituents (Rustaiyan et al., 2002). In the oil of *Chaerophyllum azoricum* Trel., growing in Portugal, 37 components were identified. Terpinolene (44 to 62%) and γ-terpinene (9-31%) were found as major constituents (Pedro Suva et al., 1999). In the oil of *Chaerophyllum bulbosum* ssp. *bulbosum* growing in Greece, sesquiterpenes (20.9%) and alkanes (14.2%) occurred in amounts similar to that encountered in the oil of *C. macropodum*. Apiole (37.1%) was reported as the main constituent of the oil of *C. bulbosum* ssp. *bulbosum* (Kokkalou and Stephanou, 1989). To the best of our knowledge, this is the first report on the analysis of chemical compositions and antimicrobial activity of the hexane extract from *C. macropodum* in tow different habitats from Iran.

MATERIALS AND METHODS

Plants

Plant materials was collected from Givi (sample A) and Neor (sample B) areas, at an altitude of 1450 and 2450 m, respectively in July, 2011 North-west in Iran. A voucher specimen was kept at the Herbarium of Agriculture Research in Ardabil Center (HARAC), Iran.

Isolation of the essential oil

Air dried aerial parts (100 g) of *C. macropodum* of each samples (A and B) were collected from the wild growing plants and subjected to 4 h of hydrodistillation in a clevenger-type apparatus.

Preparation of hexane extracts

Dried and powdered materials were extracted with hexane using a Soxhlet apparatus (70°C, 4 h) to obtain the extracts, aliphatic compounds and the other apolar components. During extraction procedures, hexane (95%) was used. The extracts were concentrated by rotary evaporator under vacuum at 40°C. The extraction yields were presented in the table.

Trans-esterification process

After removing hexane using rotary evaporator, the oily mixtures were derived to their methyl esters by the International Olive Oil Council (IOOC) and International Union of Pure and Applied Chemistry (IUPAC) reports by trans-esterification process (International Olive Oil Council, 2001; Paquat and Hautfenne, 1992). In this process, dried hexane extracts were dissolved in hexane and then extracted with 2 M methanolic KOH at room temperature for 60 s. The upper phases were dried over anhydrous sodium sulfate (Na_2SO_4) and immediately placed into 2 glass tubes and sealed. The samples were stored in the dark at 2°C until analyzing by Gas chromatography/flame ionization detector (GC/FID) and GC/MS systems and other experiments.

GC analysis

GC analysis was performed on a Shimadzu 15 A gas chromatograph equipped with a split/splitless injector (250°C) and a flame ionization detector (250°C). N_2 was used as carrier gas (1 ml/min) and the capillary column used was DB-5 (50 m × 0.2 mm, film thickness 0.32 µm). The column temperature was kept at 60°C for 5 min and then heated to 220°C with a 5°C/min rate and kept constant at 220°C for 5 min. The relative percentages of the characterized components are given in Table 1.

GC/MS analysis

GC/MS analysis was performed using a Hewlett Packard 5973 with an HP-5MS column (30 m × 0.25 mm, film thickness 0.25 µm). The column temperature was kept at 60°C for 5 min and programmed to 220°C at a rate of 5°C/min and kept constant at 220°C for 5 min. The flow rate of helium as carrier gas was 1 ml/min. MS were taken at 70 eV. The fatty acids and terpenoids were identified by comparing their retention times and mass peaks with those given in the literature and with those of authentic samples (Adams, 1995), and by National Institute of Standards and Technology (NIST)-Wiley library data search. Relative percentage amounts were calculated from peak area using a Shimadzu C-R4A chromatopac without the use of correction factors.

Pharmacological screening

The *in vitro* antibacterial and antifungal activities of the essential oils and extracts were evaluated by the disc diffusion method (DDM) using Mueller-Hinton agar for bacteria and Sabouraud Dextrose agar for fungi (Baron and Finegold, 1990). Discs containing 30 µl of the essential oil and hexanic extract were used, and growth inhibition zones were measured after 24 and 48 h of incubation at 37 and 24°C for bacteria and fungi, respectively. Gentamicin and tetracycline for bacteria and nystatin for fungi were used as positive controls. The microorganisms used were: *Bacillus subtilis* ATCC 9372, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 15753, *Staphylococcus aureus* ATCC 25923, *Klebsiella pneumoniae* ATCC 3583, *Pseudomonas aeruginosa*

Table 1. Composition of the hexane extracts of *Chaerophyllum macropodum* from two localities (Givi and Neor).

Compound (related fatty acid)	Rt (min)	Givi%	Neor%
Geraniol	5.0	4.7	-
Neryl acetate	6.5	0.6	-
β-Caryophyllene	6.9	1.3	-
E-β-Farnesene	7.2	4.6	8.7
(-)-β-Acoradiene	7.5	5.1	-
β-Sesquiphellandrene	7.6	1.5	-
(+)-β-Funebrene	7.65	-	4.3
Aromadendrene	7.7	0.4	-
Germacrene- D	7.9	-	8.8
β- Selinene	8.1	-	3.5
Zingiberene	8.2	-	4.4
β-Bisabolene	8.3	0.5	1.7
β- Himachalene	8.4	-	1.4
β-Sesquiphellandrene	8.5	-	2.3
Germacrene B	8.6	-	0.7
Carotol	8.7	16.9	0.7
Acorenone B	9.6	-	12.6
Juniper camphor	9.7	0.5	-
Hexadecanoic acid, methyl ester (Hexadecanoic acid)	11.6	4.5	2.7
9,12-Octadecadienoic acid, methyl ester (linoleic acid)	12.7	8.8	9.9
9,12,15-Octadecatrienoic acid, methyl ester (linolenic acid) or ω- 3	12.8	23.2	29.2
6-Octadecenoic acid, methyl ester (6-Octadecenoic acid)	13.0	20.2	6.8
Octadecanoic acid, methyl ester (Octadecanoic acid)	13.2	0.8	1.6
Bis(2-ethylhexyl) phthalate	16.0	0.4	-
Eicosane	18.1	0.8	-
Total	-	94.8	99.3

Table 2. Class compositions, percentage and yield of the hexane extracts of *Chaerophyllum macropodum* from two localities.

Class composition	*H. EX. (Givi)	H. EX. (Neor)
Oxygenated monoterpenes	5.3	-
Sesquiterpenes hydrocarbons	13.4	35.8
Oxygenated sesquiterpenes	17.4	13.3
Saturated fatty acid (SFA)	6.1	4.3
Unsaturated fatty acid (UFA)	52.6	45.9
UFA/SFA	8.6	10.7
Yield	4.2	4.6

*H. EX. = Hexane extract.

ATCC 27852, *Escherichia coli* ATCC 25922, *Aspergillus niger* ATCC 16404, *Candida albicans* ATCC 5027 and *Saccharomyces cerevisiae* ATCC 9763.

RESULTS AND DISCUSSION

The results obtained in the analyses of the hexane extracts of *C. macropodum* from two localities are listed in Table 1, in which the percentage and retention indices of components are given. The hexane extract composition of *C. macropodum* from Givi and Neor regions were investigated using GC/FID and GC/MS techniques for the first time. Analysis of the compounds of hexane extracts from *C. macropodum* in both samples showed the presence of fatty acids and terpenoid constituents (Table 1). According to the results, the hexane extract yields of the studied *C. macropodum* were found 4.2 and 4.6% on the basis of dry weight of the plant materials in Givi and Neor, respectively (Table 2).

The unsaturated fatty acid contents were higher than saturated ones, and the highest total percentage was detected in Neor sample (Table 1). The percentage and retention time of components are given in Table 1. As it is shown, the total contents of hexane extracts varied from 94.8 (in Givi) to 99.3% (in Neor). The major unsaturated fatty acid in Givi and Neor including linolenic (ω-3) (23.2 and 29.2%), 6-Octadecenoic acid (20.2 and 6.8%) and linoleic acid (ω-6) (8.8 and 9.9%), respectively are shown in the table. The major terpenoid compounds in Givi sample were carotol (16.9%), β-acoradiene (5.1%) and geraniol (4.7%). Three major terpenoids acorenone B (12.6%), germacrene-D (8.8%) and E-β-farnesene (8.7%)

Table 3. Antimicrobial activity of hexane extracts of *"Chaerophyllum macropodum* from Givi and Neor regions.

Tested microorganism	Zone of inhibition (mm)				
	Givi	Neor	Antibiotic		
	*H. EX.	H. EX.	Gentamicin	Nystatin	Tetracycline
B. subtilis	11.9±0.21	11.1±0.21	NT**	NT	22.5±0.4
S. epidermidis	13.6±0.22	12.6±0.11	NT	NT	34.4±0.1
E. faecalis	NA***	NA	NT	NT	9.7±0.3
S. aureus	11.5±0.11	10.6±0.22	NT	NT	21.2±0.2
K. pneumoniae	9.9±0.13	NA	21.4±0.1	NT	NT
P. aeruginosa	NA	NA	12.7±0.2	NT	NT
E. coli	11.3±0.10	10.5±0.15	24.3±0.8	NT	NT
A. niger	NA	6.8±0.10	NT	16.7±0.4	NT
C. albicans	7.8±0.12	7.8±0.32	NT	18.4±0.4	NT
S. cerevisiae	8.8±0.11	7.6±0.22	NT	18.2±0.3	NT

*H. EX. = Hexane extract; ** NT = not tested; ***NA = not active.

were detected in the extract from Neor sample.

As can be seen in Table 1, about 17 components of the extract from Givi and 16 components from Neor sample extract were identified. There were some differences in the fatty acid profiles of the different habitat of this plant. Unsaturated fatty acids (UFAs), essential oil components (EOCs) and some of the saturated fatty acids (SFAs) were observed in both samples of this plant. In fact, both fractions mainly include unsaturated fatty acids (UFAs), with a clear predominance of ω-3 and ω-6. One of the essential fatty acids (EFAs), 9,12,15-octadecatrienoic acid (linolenic acid or ω-3) was a predominant component in Neor sample. Linoleic acid is an omega-6 fatty acid, ranging from 8.8% (in Givi) to 9.9% (in Neor) in which was found little amounts in this work. The ratios of UFAs)/SFAs were 8.6 and 10.7 in extract from Givi and Neor samples, respectively (Table 2). The hexanic extract of this plant in the Givi sample had a higher proportion of UFAs compared to Neor sample (Table 1).

The hexane extracts of *C. macropodum* from Givi and Neor samples were tested against four Gram-positive and three Gram-negative bacteria, as well as three fungi. The results presented in Table 3 show that the hexane extracts from both samples exhibited a different biological activity against all tested fungi and bacteria except for a resistant, *E. faecalis* and *P. aeruginosa*, as well as a fungi, *A. niger.* According to our results, the main constituents of hexane extracts were unsaturated fatty acids and some of the terpenoids compounds. It is clear that there is a significant correlation between the chemical compositions and antimicrobial activity. Thus, it seems that *C. macropodum* from Givi sample may be a good dietary source for extract and effective antimicrobial.

These results showed that the extract constituents from the same plant of the same chemotype vary significantly. This variation is also observed in other species and other localities. Analyzing previously published reports for the examined species and for species that we were inspecting, great variations concerning chemical composition within plant species of the same section as well within plants belonging to same species, collected from different localities, can be observed. Comparison of the results with the literature showed significant differences for the extracts which can be attributed to either climatologically factors or genetic differences of the plants. Both sample extracts from Givi and Neor are rich in terpenoid and fatty acid compounds. The activity of samples may be related to its richness in these constituents.

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REFERENCES

Adams RP (1995). Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Allured Publication Corporation, Carol Stream, IL.

Baron EJ, Finegold SM (1990). Methods for testing antimicrobial effectiveness. In: Stephanie M (ed.), Diagnostic Microbiology. Baltimore, Mosby. pp. 171-194.

Baser KHC, Tabanca N, Ozek T, Demirci B, Duran A, Duman H (2000). Composition of the essential oil of *Chaerophyllum aksekiense* A. Duran et Duman, recently described endemic from Turkey. Flavour Fragr. J. 15:43-44.

International Olive Oil Council (2001). Method of Analysis. International Olive Oil Council COI/T. 20/ Doc. no. 24.

Kokkalou E, Stephanou E (1989). The volatiles of *Chaerophyllum bulbosum* L. spp. *Bulbosum* growing wild in Greece. Pharma. Acta. Helv. 64:133-134.

Mamedova SA (1994). Essential oil of *Chaerophyllum macrospermum*. Chem. Nat. Comp. 30:267-277.

Mimica-Dukic N, Bozin B, Sokovic M, Simin N (2004). Antimicrobial and antioxidant activities of *Melissa officinalis* L. (Lamiaceae) essential oil. J. Agric. Food. Chem. 52:2485-2489.

Mozaffarian V (2007). A Dictionary of Iranian Plant Names. Farhang Moaser Publishers, Tehran, Iran. p 119.
Nematollahi F, Akhgar M, Larijani K, Rustaiyan A (2005). Essential Oils of *Chaerophyllum macropodum* Boiss. and *Chaerophyllum crinitum* Boiss. from Iran. J. Essent. Oil Res. 1:135-138.
Paquat C, Hautfenne A (1992). International Union of Pure and Applied Chemistry. Blackwell Scientific Publications, London.
Pedro Suva LG, DaSilva JA, Barroso JG, Figueiredo AC, Deans SG, Looman A, Scheffer JJC (1999). Composition of the essential oil of *Chaerophyllum azoricum* Trel. An endemic species of the Azores archipelago. Flavour Fragr. J. 14:287-289.
Rechinger KH (1987). *Chaerophyllum*. In: Rechinger KH, Hedge IC (Eds.), Flora Iranica, Umbelliferae. Akademische Druck and Verlagstalt, Graz, Austria. 162:89.
Rustaiyan A, Neekpoor N, Rabani M, Komeilizadeh H, Masoudi S, Monfared A (2002). Composition of the essential oil of *Chaerophyllum macrospermum* (Spreng.) Fisch. and C.A. Mey. from Iran. J. Essent. Oil Res. 14:216-217.
Shafaghat A (2009a). Antibacterial Activity and Composition of Essential Oils from Flower, Leaf and Stem of *Chaerophyllum macropodum* Boiss. from Iran. Nat. Prod. Commun. 4(6):861-864.
Shafaghat A (2009b). Chemical Composition of the Essential Oil from the Roots of *Chaerophyllum macropodum* Boiss. from Iran, J. Essent. Oil Bear. Plants 12(5):615-619.
Shafaghat A (2011a). Antibacterial Activity and GC/MS Analysis of the Essential Oils from Flower, Leaf and Stem of *Origanum vulgare* ssp. *viride* Growing Wild in North-west Iran. Nat. Prod. Commun. 6(9):1351-1352.
Shafaghat A (2011b). Chemical constituents, antimicrobial and antioxidant activity of the hexane extract from root and seed of *Levisticum persicum* Freyn and Bornm. J. Med. Plants Res. 5(20):5127-5131.
Sylvestre M, Legault J, Dufour D, Pichette A (2005). Chemical composition and anticancer activity of leaf essential oil of *Myrica gale* L. Phytomedicine 12:299-304.