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NATURAL DRUGS

FATTY ACIDS ANALYSIS AND ANTIOXIDANT ACTIVITY OF A LIPID EXTRACT OBTAINED FROM *MERCURIALIS ANNUA* L. GROWN WILDLY IN JORDAN

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Abstract: Aerial parts of *Mercurialis annua* L. were used in the present study, and fatty acid content in lipid extract was determined using GC-FID. The major fatty acids identified were α–linolenic acid (20.3%), heptadecanoic acid (12.8%), palmitic acid (11.9%), pentadecanoic acid (11.7%), *cis*-10-pentadecanoic acid (11.2%), linoleic acid (7.7%), tridecanoic Acid (4.6%), stearic (4.4%), *cis*-11,14-eicosadienoic acid (3.8%), beside of minor fatty acids (palmitoleic acid, *cis*-13,16-docosadienoic acid, arachidic acid, behenic acid, *cis*-10-heptadecenoic acid and myristic acid). Antioxidant properties of the extract were determined via DPPH radical scavenging, β-carotene bleaching assay and NO radical scavenging assay. The extract produced significant antioxidant activity *in-vitro*. The data shown here may broaden our knowledge on composition and antioxidant activity of lipid constituents from *Mercurialis annua* L.

Keywords: *Mercurialis annua* L., hexane extract, antioxidant, GC-FID, Fatty acid methyl ester (FAME), DPPH radical scavenging activity, β-carotene bleaching assay, NO radical scavenging activity, FT-IR spectrum

Mercurialis genus belongs to the Acalypho-ideae subfamily, which consists of 457 plant species. This subfamily is within the Euphorbiaceae family which comprises of 300 genera and 7500 species (1, 2). *Mercurialis annua* L. (*M. annua* L.) is flowering plant species known as annual mercury. It is native to the Middle East, Europe, and North Africa. It is an annual herb growing 10-70 cm tall. The aerial parts are commonly used for medicinal purposes as laxative, purgative, emetic, emollient. It is recommended to use the extract of aerial parts for the treatment of warts, sores, ear, and eye problems, as well as gynecological diseases such as menstrual molimina (3-5). A homeopathy remedy is made from the plant which is used in the treatment of rheumatism, dropsy, gall bladder, and liver disorders. *M. annua* L. has been used in folk medicine as a diuretic and anti-syphilitic. These species have been recommended for relief of anxiety in Moroccan folk medicine. Research indicates that the aqueous extract of the aerial parts has an anxiolytic effect (6, 7). Local herbalists in Jordan have used the aqueous extract of aerial parts of *M. annua* L. for the treatment of hematopoietic malignancies.

The pharmacological effects of methanolic extracts of *M. annua* L. leaves were studied in our laboratories and showed potent cytotoxic effect on the colorectal adenocarcinoma cell lines HRT-18 and the breast cancer T47D cell lines (8). The methanolic extract did not produce an appreciable inhibitory effect on the growth of Caco-2, MCF-7, A375-S, and WM 136-1A cell lines. There was also no evidence of stimulation of tumor growth on BJAB Burkett's lymphoma and U266 multiple myeloma cell lines by the extract of this plant. It did not exhibit any antimicrobial activity (9). Different flavonoids have been previously reported from the ethanol extract of the aerial parts of this plant (10). Lorenz et al. reported that the chemotaxonomic differences between the *M. annua* L. and *M. perennis* L. *M. annua* L. solely contains phaselic acid, whereas *M. perennis* L. contains a mixture of mercurialis acid and phaselic acid (4, 5). An exclusive presence of pyridine-3-carbonitrile and nicotinamide were also reported in the aerial parts of *M. annua* L. (5). The fixed oils belong to a class of lipids constituted by saturated and unsaturated fatty acids. This class has emollient properties when incorporated in dermatological formulations and researchers of biological activity

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reveal insecticidal potential and larvicidal for these compounds (11). Lipophilic constituents such as hermidin, hermidin quinone, hermidin dimer, along with benzyl alcohol, 2-phenylethanol, 4-methoxy- and 3,4-imethoxyphenol, *trans*-myrtanol, *cis*-myrtanal and squalene, were reported in *M. perennis* L. root (12). whereas the aerial parts contained phytol derivative, sterols, tocopherols, lutein, carotenes, pheophytins, triglycerides constituted by linolenic acid, oleic acid, stearic and palmitic acid (4-5). The purpose of this study is to investigate the phytochemical constituents present in the fixed oil from the aerial parts of *M. annua* L. that are growing wildly in Jordan and to evaluate the antioxidant activity of the hexane extract of this plant using different methods.

EXPERIMENTAL

Material and methods

Plant material

Plants were collected from different localities in Jordan and identified by Prof. D. Al-Eisawi, plant taxonomist, Department of Biological Sciences, University of Jordan. A herbarium specimen (Pharm-2017-03) was deposited at the Department of Pharmacy, Al-Ahliyya Amman University, Amman, Jordan.

Extraction

The dried powdered aerial parts of the plant (20 g) were extracted with *n*-hexane (150 mL) three times by maceration for 24 hours. The extracted solution was concentrated under vacuum in a rotatory evaporator at 40^{op}C, producing 0.96 g sample.

Infra- red spectroscopy

The infra-red spectrum of the oily liquid separated from hexane extract (as thin film on KBr disc) was recorded on Shimadzu IRPrestige-21 spectrophotometer (Shimadzu Co., Japan).

Fatty acid methyl esters (FAMEs)

FAMEs were synthesized by methylation using sodium methoxide in the presence of methanol at 40^{o}C. In an Iodine flask, 0.1 g of hexane extract of aerial part of *M. annua* L. was transferred and dissolved in 25 mL of methanol with the aid of sonication. Sodium methoxide solution (30% w/v in methanol, 0.1 g) was added to it with constant stirring. The reaction mixture was maintained at 40^{o}C for 45 min. with constant shaking. Twenty-five milliliters of *n*-hexane were added, and the solution was shaken for 20 min. The reaction was terminated using saturated solution of oxalic acid. The precipitated sodium oxalate was removed after centrifuging the mixture at 5000 rpm for 15 min. The supernatant was collected and dried over anhydrous sodium sulfate and was analyzed by GC-FID (13).

Determination of FAME by GC-FID

GC analysis of FAME was performed on a Gas Chromatograph (Model Shimadzu 2010, Shimadzu Co. Japan) equipped with a Teknokroma TRB-WAX-Omega capillary column (60m × 0.25 mm, film thickness 0.25 µm, Spain; Serial No. NF-31995, (14). The injector temperature was maintained at 240^{o}C. Operating conditions were as follows: carrier gas-helium, linear velocity 35 mL/min, injection volume-1 µL and split ratio-1 : 25. Oven temperature was maintained at 70^{o}C for 2 min., then the temperature was raised from 70 to 200^{o}C at a rate of 4^{o}C/min, followed by 15 min hold at 200^{o}C. FAME was identified by comparing their GC retention time with those of 37 pure component FAME mix (Sigma Aldrich Inc., St. Louis, California, USA), methyl ester of C_4-C_{24} saturated and unsaturated fatty acids. The samples were quantitatively determined through the normalization method without using correction factors: the relative peak area for individual constituents was averaged on three different chromatograms of three independent reactions. The fatty acid composition in the oil was expressed as the percentage of the total fatty acids.

Antioxidant activity

DPPH scavenging activity

The extract from *M. annua* L. was analyzed for its free radical scavenging activity using DPPH according to the reported method with slight modification (15). To perform the analysis, the solution of DPPH (0.008 g %) was prepared freshly in normal hexane. Different concentrations of the extract (1.95 to 1000 µg/mL) in hexane were prepared.

The DPPH solution (1 mL) and samples (1 mL) were mixed separately and vortexed for 45 s, and kept in the dark at 25 ± 2^{o}C for around 25 min. The absorbance of the solutions was measured at 517 nm using hexane as blank. DPPH radical scavenging activity was determined and the IC_{50} was calculated.

$$\text{DPPH Scavenging activity} = \left| 1 - \frac{Abs_{control}}{Abs_{sasmple}} \right| \times 100$$

β-Carotene bleaching (BCB) Assay

β-Carotene (5 mg) was dissolved in 50 mL of chloroform. In a separate Erlenmeyer flask linoleic

acid (40 mg) and Tween-40 (400 mg) were taken and an aliquot of β-carotene (3 mL) solution was added. They were mixed and set aside for 2 min. The chloroform was evaporated using N_2 gas. The resultant mixture was dissolved in 100 mL of distilled water. Immediately after preparation, the absorbance of this solution was recorded at 470 and 700 nm. Different solutions of oil (50 µg/mL to 1000 µg/mL) were prepared in methanol (with the aid of 0.05% Tween-40). β-Carotene-linoleic acid emulsion (1 mL) was mixed with different solutions of oil (0.25 mL). All the solutions (control and test) were capped and incubated (at 50°C) for 1 hour. The control sample contains an equivalent amount of methanol (0.05% Tween-40). The absorbance of the solutions (λ_{470} and λ_{700} nm) was determined after 60 min. All determination was carried out in triplicate; the degradation rate (DR) and antioxidant activity were calculated (16).

$$\text{Degradation rate (DR) of } \beta\text{-carotene} = \text{Ln}(A_{initial}/A_{sample})/60$$

$$\text{Antioxidant activity (\%)} = \frac{\text{(degradfation rate of control} - \text{(degradfation rate of samle)}}{\text{degradfation rate of control}} \times 100$$

Nitric oxide radical scavenging activity

The aqueous sodium nitroprusside (SNP) solution reacts with oxygen and generates nitrite ions, these ions can be quantitated by Modified Griess reagent (17). In brief, the reaction mixture contained 10 mM SNP (0.25 mL), phosphate buffered saline (pH 7.4, 0.40 mL) and various concentrations of the test solution (0.10 mL) in test tubes, after incubation at 25°C (150 min.), 0.25 mL of Griess reagent (1×, Sigma-Aldrich, USA) was added. The color generated during diazotization of nitrite ions was measured spectrophotometrically at 546 nm. Quercetin (2.5-175 µg/mL) was used as a standard compound for comparison.

Statistical analysis

Results are expressed as mean ± standard deviation (SD). Graph-Pad Prism 5 (San Diego, CA, USA) for Windows was used for statistical analyses of experimental data.

RESULTS AND DISCUSSION

In the present study, the oil content of the aerial parts of *M. annua* L. was separated by cold extraction with n-hexane as a solvent and determined to be 4.8%. The FT-IR spectrum (as thin film on KBr) reveals characteristic signal at 721 (*cis* –CH=CH– out of plane bending); 837 (=CH_2 out of plane bending); 1170.8 (-CO- stretch), 1201.7 (O-CH_2 stretch), 1331, 1377 (-CH_3 bending), 1456 (–CH_2 bending), 1715,1732 (C=O esters); 2853 and 2924 cm^{-1} (*symmetrical* and *asymmetrical* stretching of-CH_2) and 3001cm^{-1} (*trans* =C–H stretch, merged with *asymmetrical* stretching of-CH_2) and 3445 (bound OH stretching) were observed in IR spectrum (Fig. 1). Presence of a weak signal at 1680 cm^{-1} indicates the presence of non-conjugated fatty acid (18-20).

The fatty acid composition is recorded in Table 1 and structures of various fatty acids identified are

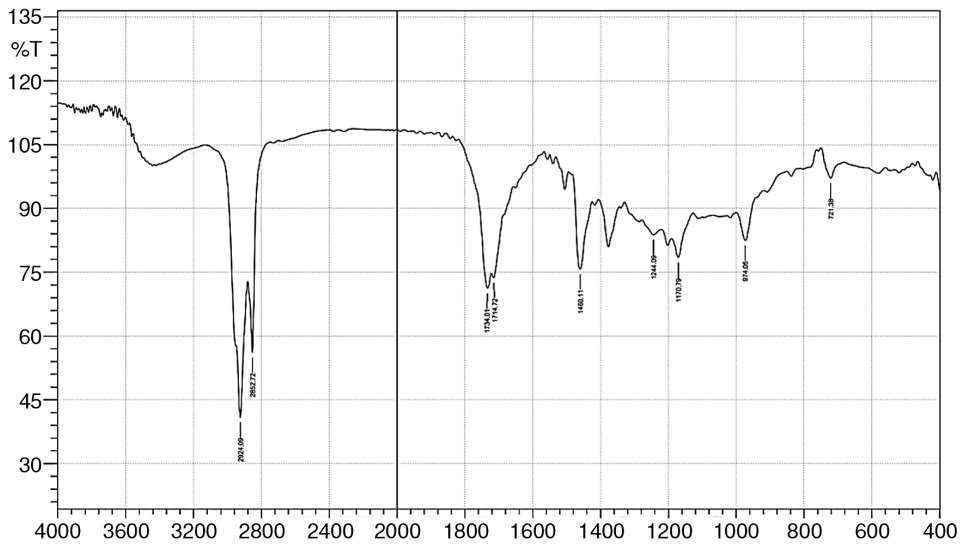

Figure 1. FT-IR spectrum of lipid extract (as a thin film) from aerial parts of *M. annua* L.

depicted in Figure 2. The analysis was carried out using capillary GC-FID, and results are presented as percentage content (Table 1, Fig. 3). Saturated fatty acids (SFA) constitute 50.59% of total fatty acid, which contains heptadecanoic acid (12.78%), palmitic acid (11.90%), pentadecanoic acid (11.75%), tridecanoic acid (4.64%) and stearic acid (4.37%) as the main fatty acids. The monounsaturated fatty acids (MUFA) were *cis*-10-pentadecenoic acid (11.22%), palmitoleic acid (2.81%) and *cis*-10-heptadecenoic acid (1.38%).

The main polyunsaturated fatty acids identified in oil are α-linolenic acid (20.29%), linoleic acid (7.69%), *cis*-11,14-eicosadienoic acid (3.80%) and *cis*-13,16-docosadienoic acid (2.23%). To the best of our knowledge this research, reports for the first time the fatty acid constituents of the aerial parts of *M. annua* L. Mizushina et al. (21) have reported the inhibitory effect of various fatty acids including stearic, linoleic acid, α-linolenic acid, and *Cis*-11,14-eicosadienoic acid on DNA polymerase β, which is responsible for controlling diseases. The α-linolenic acid [C18:3 $\Delta^{9\text{-}12\text{-}15}$ *cis*] has antibacterial activity against *H. pylori*, when present as high proportion in oils or fat (22). Linoleic acid [C18:2 $\Delta^{9\text{-}12}$ *cis*] showed topical anti-inflammatory activity in the TPA-induced mice ear edema assay (23). Among other compounds identified were palmitic and stearic acids, both have an anti-inflammatory effect. The results indicate that the aerial parts of *M. annua* L, have different fatty acid composition than the *M. perennis* L. (4).

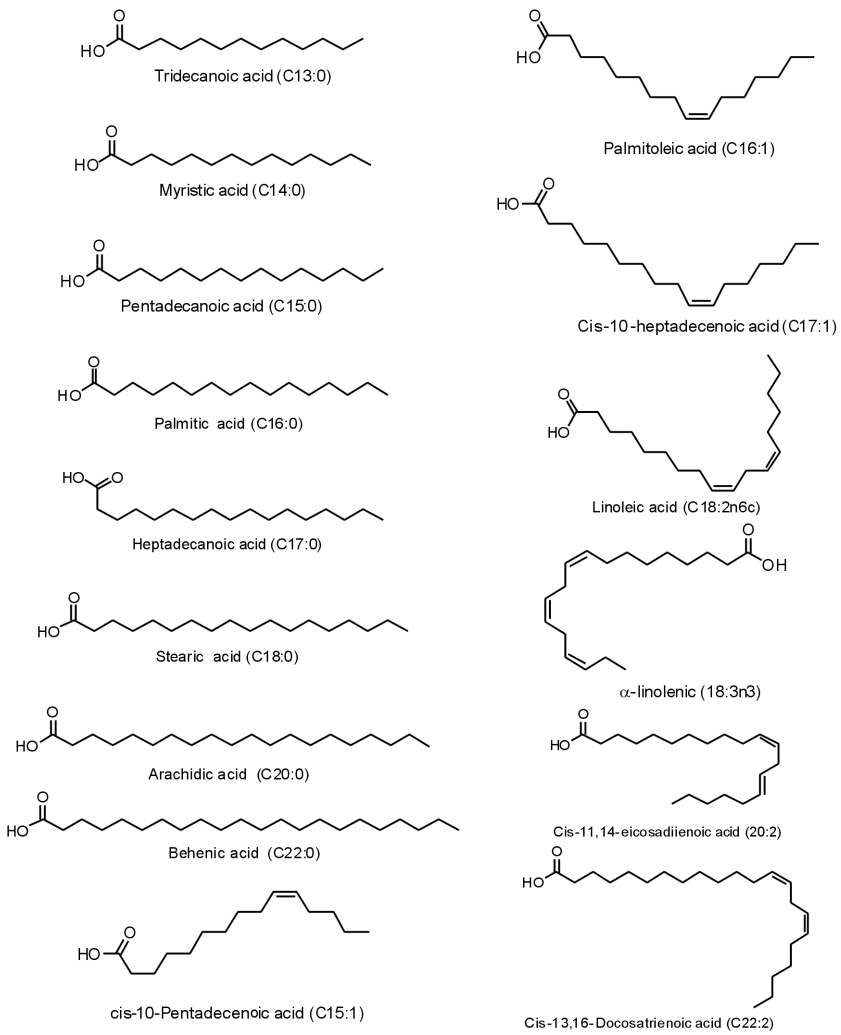

Figure 2. Chemical structures of fatty acids identified as FAME in a sample

Table 1. Fatty acid composition (as a percent of Total Fatty Acids) of lipid extract of *M. annua* L. aerial parts.

Fatty acid[#]	% Content*
Tridecanoic acid [C13:0]	4.64
Myristic acid [C14:0]	1.34
Pentadecanoic acid [C15:0]	11.75
Palmitic acid [C16:0]	11.90
Heptadecanoic acid [C17:0]	12.78
Stearic acid [C18:0]	4.37
Arachidic acid [C20:0]	2.11
Behenic acid [C22:0]	1.70
ΣSFA[a]	50.59
Cis-10-pentadecenoic acid [C15:1 Δ^{10} *cis*]	11.22
Palmitoleic acid (C16:1 Δ^{9} *cis*)	2.81
Cis-10-heptadecenoic acid (C17:1 Δ^{10} *cis*)	1.38
ΣMUFA[b]	15.41
Linoleic acid [C18:2 $\Delta^{9\text{-}12}$ *cis*]	7.68
α-linolenic acid [C18:3 $\Delta^{9\text{-}12\text{-}15}$ *cis*]	20.29
Cis-11,14-eicosadienoic acid [C20:2 $\Delta^{11\text{-}14}$ *cis*]	3.80
Cis-13,16-docosadienoic acid [C22:2 $\Delta^{13\text{-}16}$ *cis*]	2.23
ΣPUFA[c]	34.00

[#] Fatty Acids were analyzed as fatty acid methyl esters (FAME); *Mean of three replicates; [a]SFA = Saturated fatty acids; [b]MUFA= monounsaturated fatty acids; [c]PUFA = Polyunsaturated fatty acids

Table 2. Antioxidant activity of the lipid extract from *M. annua* L. aerial parts.

	IC_{50} (µg/mL)		
Sample	DPPH radical scavenging activity*	β-Carotene bleaching Assay*	NO radical scavenging activity*
Oil	170.5 ± 2.1	265.5 ± 2.8	385.0 ± 4.8
Rutin	-	8.2 ± 0.5	
α-Tocopherol	85.6 ± 1.8	-	-
Quercetin	-	-	51.4 ± 1.8

*Values are given as Mean ± SD (n = 3)

The presence of these constituents in the fixed oil is important phytochemically and therefore its biological activities were evaluated. DPPH scavenging activity (IC_{50}) was calculated (Table 2), the lipid extract at concentrations ranging between 1.95 to 1000 µg/mL showed comparable antioxidant activity. The IC_{50} of oil against DPPH radical was 170.5 ± 2.1 µg/mL, which was comparable to the IC_{50} value of α-tocopherol (85.6 ± 1.8 µg/mL). The antioxidant activity could be attributed due to the presence of unsaturated fatty acids and other compounds. The IC_{50} value in case of β-carotene bleaching assay was 265.5 ± 2.8 µg/mL, compared to 8.2 ± 0.5 µg/mL of rutin. As far as nitric oxide scavenging activity of extract is concerned, the sample produced a weak activity with IC_{50} value of 385.0 ± 4.8 µg/mL, compared to that of quercetin with IC_{50} value of 51.4 ± 1.8 µg/mL. These findings indicate that the extract has significant antioxidant activity which might be responsible for the other biological activities and protecting the oxidation of plant constituents.

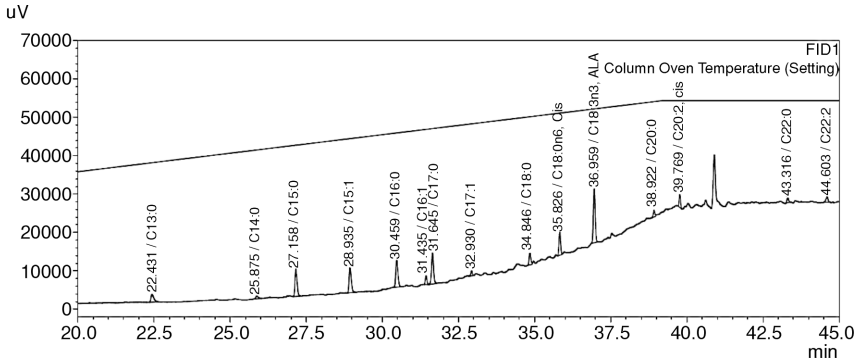

Figure 3. GC-FID analysis of fatty acid methyl esters from a lipid extract of *M. annua* L. aerial parts

CONCLUSION

This study describes the fatty acid composition and quantification of lipid extract of aerial parts of *M. annua* L. growing in Jordan for the first-time using GC-FID. The results indicate the presence of the α-linolenic acid as main polyunsaturated fatty acids along with linoleic acid, and saturated fatty acids like heptadecanoic acid, palmitic, pentadecanoic, tridecanoic and stearic acid. *Cis*-pentadecenoic acid along with small amount of palmitoleic and *cis*-10-heptadecenoic acid were identified as MUFA. The other fatty acids were arachidic acid, behenic acid, myristic acid, and *cis*-13,16-docosadienoic acid. The FT-IR, DPPH scavenging activity, β-carotene bleaching assay and NO radical scavenging activity were recorded also.

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