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Antimicrobial Antioxidant Daucane Sesquiterpenes from *Ferula hermonis* Boiss

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Seventeen daucane sesquiterpenoid esters, including a new one (4), were isolated from the root of *Ferula hermonis* Boiss. The structures of the isolated compounds were elucidated on the basis of spectroscopic evidence and correlated with known compounds. The relative stereochemistry of the new compound was determined using 2D NOESY and the most stable and the lowest energy conformation was determined using molecular modelling. The antimicrobial activity was evaluated by determination of MIC using the broth microdilution method against six bacterial strains and one fungal strain (*Pseudomonas aeruginosa* PAO1, *Escherichia coli*, *Bacillus subtilis* ATCC6633, *Mycobacterium bovis* BCG Pasteur, *Mycobacterium tuberculosis* H37Rv, *Staphylococcus aureus* ATCC6538 and *Candida albicans* SC5314). There was a significant indication that compounds 15, 16, 17 demonstrated potent activity against Gram +ve (*S. aureus*, *B. subtilis*), as well as *Mycobacterium* strains *M. bovis* BCG and *M. tuberculosis* H37Rv. None of the isolated compounds exhibited a significant antifungal activity. In the antioxidant study using the DPPH assay method, the highest radical scavenging activity was observed for compounds 15, 16, 17. **Copyright © 2011 John Wiley & Sons, Ltd.**

Keywords: Ferula hermonis; daucane sesquiterpene esters; antimicrobial activity; radical scavenging activity.

Supporting information may be found in the online version of this article (Supplementary Material)

INTRODUCTION

The genus *Ferula* (Apiaceae) comprises about 130 species distributed throughout the Mediterranean area and Central Asia (Frensh, 1971). The chemistry of this genus has been studied by many research groups. The widespread sesquiterpene compounds in this genus are characteristic daucanes, humulanes, himachalanes, germacranes, eudesmanes and guainanes (Gonzalez and Barrera, 1995). Several species of the genus *Ferula* have been used in traditional medicine for a variety of therapeutic purposes such as tranquilizers, and for the treatment of digestive disorders, rheumatism, headache, arthritis, dizziness, toothache, etc. (Gonzalez and Barrera, 1995). *Ferula hermonis* Boiss., commonly known as ‘Shilsh-el-zallouh’ or ‘Hashishat-al-kattira’, is a small shrub that grows abundantly on the Hermon Mountain between Syria and Lebanon (Said *et al.*, 2002; Lev and Amar, 2002). This plant has long been used in the Middle East as an aphrodisiac, and for the treatment of frigidity and impotence (El-Taher *et al.*, 2001; Hadidi *et al.*, 2003). Previous phytochemical investigation on *F. hermoni*, root and seed, revealed the presence of various sesquiterpenes, mainly of the daucane type (Abourashed *et al.*, 2001; Galal, 2000; Galal *et al.*, 2001; Diab *et al.*, 2001a; Lhuillier *et al.*, 2005; Nazari and Iranshahi, 2010). Extensive research was done to study the hormonal activity of the isolated sesquiterpenes from *F. hermonis* and recently herbal products containing *F. hermonis* extract have been sold in the dietary supplement market claiming a sexual function enhancement (Abourashed *et al.*, 2001; Appendino *et al.*, 2002, 2004; Greige-Gerges *et al.*, 2008; Khleifat *et al.*, 2001; Homady *et al.*, 2002; Ikeda *et al.*, 2002; Zanoli *et al.*, 2003, 2005a, 2005b, 2009; Zavatti *et al.*, 2006, 2009). Different activities were also reported including antiinflammatory (Geroushi *et al.*, 2011), cytotoxic (Auzi *et al.*, 2008; Elouzi *et al.*, 2008) and other activities (Abdallah *et al.*, 2005; Severini *et al.*, 2006; Macho *et al.*, 2004; Zamaraeva *et al.*, 1997; Palumbo *et al.*, 2009; Ferretti *et al.*, 2010; Hanafi *et al.*, 2010). Concerning the antimicrobial activity, the literature survey revealed the antimicrobial activity of the essential oil of some *Ferula* sp. such as *F. narthex* (Kar and Jain, 1971), *F. gummosa* (Eftekhar *et al.*, 2004) and *F. lycia* (Kose *et al.*, 2010) as well as the gum-resin of *F. gumosa* (Vaziri, 1975) and isolated constituents of *F. kuhistanica* fruit (Tamemoto *et al.*, 2001), *F. persica* root (Shahverdi *et al.*, 2005), *F. communis* rhizomes (Al-Yahya *et al.*, 1998) but until now, little work has been done on the the antimicrobial (Hilan *et al.*, 2007; Abourashed *et al.*, 2011) and antioxidant (Dehghan *et al.*, 2007; Kose *et al.*, 2010) activities of *F. hermonis* constituents.

This study reports the isolation and structural elucidation of 17 daucane esters from the roots of *F. hermonis* (Fig. 1), one of which (**4**) is a compound. The structure of **4** was established by spectroscopic analyses, especially 2D-NMR techniques (^{1}H-^{1}H COSY, ^{1}H-^{13}C

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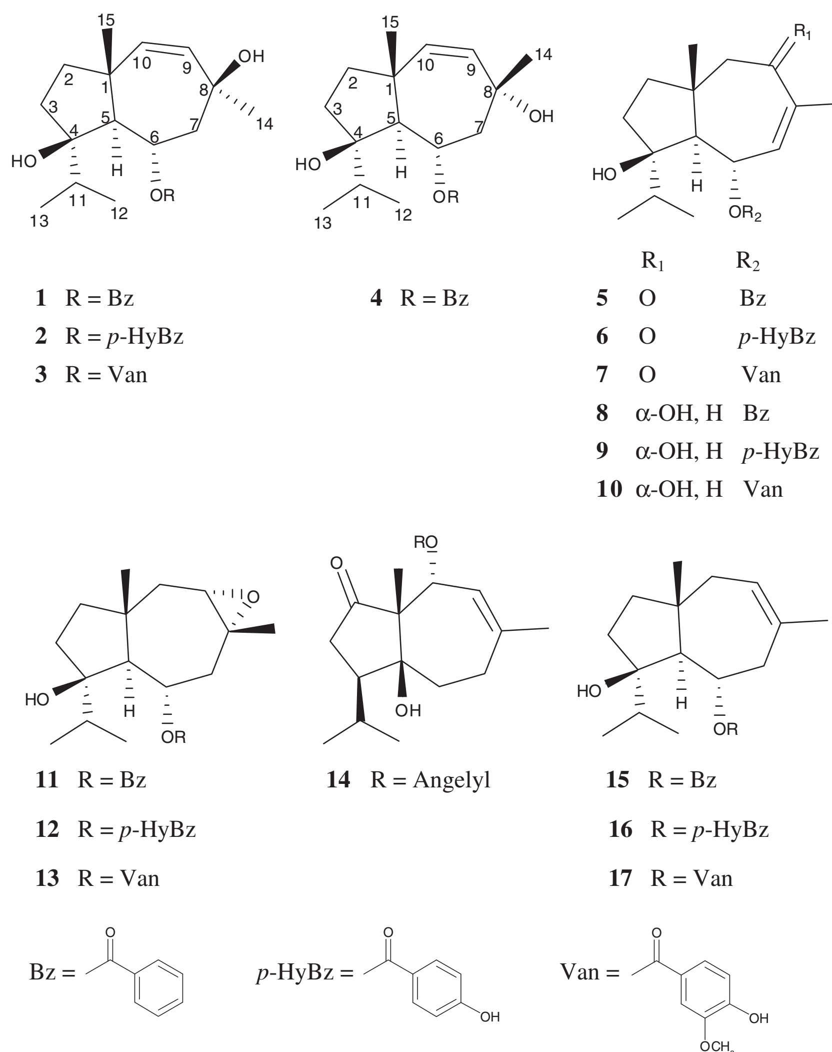

Figure 1. Chemical structures of compounds **1–17**.

HSQC, ^{1}H-^{13} HMBC, NOESY) as well as using accurate mass measurement. The most stable and the lowest energy conformation was determined using molecular modelling. The isolated compounds were tested on a panel of pathogenic microbial strains for antimicrobial activities, following the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) broth microdilution method in 96-well microplates. The radical scavenging-activity of the isolated compounds was also evaluated using the DPPH assay method.

MATERIALS AND METHODS

General experimental procedures. The IR spectra were measured on an Ati Mattson Genesis Series FTIR machine. The 1D and 2D-NMR experiments were recorded on Varian Unity INOVA 400 MHz NMR systems. Low resolution ESI-MS data were obtained using a Perseptive Biosystems Mariner LC-MS, and high-resolution ESI-MS data were obtained on a Finnigan MAT 900 XLT system. The HPLC separations were carried out using a Phenomenex reversed-phase column (Jupiter 4 μm Proteo 90 Å, 250 × 10 mm, 4 μm) and an Agilent 1200 series gradient pump monitored using a DAD G1315B variable-wavelength UV detector. Column chromatography (CC) was performed using a silica gel (Kieselgel 60 Å, 40–63 μM mesh size, Fluorochem, UK), size exclusion chromatography (Sephadex LH-20, 25–100 mm mesh size, Sigma, Germany) and using TLC pre-coated silica-gel 60 F254 (0.25 mm, Alugram® SIL G/UV$_{254}$, Macherey-Nagel, Germany) and RP-18 F254S plates (0.25 mm, Merck, Germany). All flash chromatography was performed on Biotage cartridge flash columns (Si 40 M and C18 HS 40 M) using a Biotage Flash system, Charlottesville, USA.

Plant materials. The roots of *Ferula hermonis* Boiss. (2.1 kg) were obtained from Damascus, Syria in July 2008. The plant was kindly authenticated by Professor G. El-Naggar, Professor of Taxonomy, Faculty of Science, Assiut University, Assiut, Egypt. A voucher specimen (No 2008FH) was deposited at the Pharmacognosy Department, Assiut University, Assiut, Egypt.

Extraction and isolation. The air-dried powdered root of *Ferula hermonis* (500 g) was extracted exhaustively in a soxhlet apparatus with ethyl alcohol. The ethanol extract was concentrated *in vacuo* to give a residue, which was partitioned between chloroform and water. The chloroform layer was evaporated under reduced pressure to dryness to give an oily yellow mass (55 g, 11%). Part (20 g) of the chloroform fraction was

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chromatographed on a silica gel column (700 g) and eluted with a gradient mixture of *n*-hexane–EtOAc. Fractions (200 mL each) were collected and monitored on TLC using benzene–EtOAc mixtures as solvent systems and H_2SO_4 (10% in MeOH) as a spraying reagent. Similar fractions were mixed and concentrated to yield four groups.

Group 1 (fractions 1–10, *n*-hexane–EtOAc, 9:1) was further re-chromatographed on a silica gel column using 5% to 15% EtOAc in *n*-hexane gradiently as the eluent. Four compounds were obtained: compound **15** (200 mg, yellow oil), compound **5** (70 mg, colourless oil), compound **14** (45 mg, colourless oil) and **11** (47 mg, amorphous powder).

Group 2 (fractions 11–25, *n*-hexane–EtOAc, 4:1) was further re-chromatographed on silica gel column using gradient elution of 10% to 30% EtOAc in *n*-hexane followed by 5% to 15% MeOH in $CHCl_3$ as eluent to give three sub-fractions. The sub-fractions were subjected to gel filtration chromatography using a Sephadex LH-20 column with $CHCl_3$–MeOH (3:2) followed by HPLC (silica gel, Hex–EtOAc, 3:2) to give compounds **16** (660 mg, yellow oil), **17** (90 mg, amorphous powder), **6** (63 mg, colourless needles), **7** (12 mg, amorphous powder), **8** (23 mg, oily substances) and **4** (19 mg, amorphous powder).

Group 3 (fractions 26–30, *n*-hexane–EtOAc, 7:3) was subjected to gel filtration chromatography using a Sephadex LH-20 column with $CHCl_3$–MeOH (3:2) followed by reversed-phase HPLC (Jupiter 4 μm Proteo 90 Å, 250 × 10 mm, 4 μm) using a gradient of 0–100% CH_3CN–H_2O over 40 min to give compound **1** (35 mg, colourless oil), **9** (14 mg, amorphous substance) and **10** (5 mg, amorphous substance).

Group 4 (fractions 31–40, *n*-hexane–EtOAc, 3:2) was subjected to gel filtration chromatography using a Sephadex LH-20 column with $CHCl_3$–MeOH (3:2) followed by reversed-phase HPLC (Jupiter 4 μm Proteo 90 A°, 250 × 10 mm, 4 μm) using a gradient of 0–100% CH_3CN–H_2O over 40 min to give compound **12** (29 mg, amorphous substance), **13** (6.2 mg, amorphous substance), **2** (13 mg, amorphous substance) and **3** (7.3 mg, amorphous substance).

Compound 4. Amorphous powder (19 mg). $[\alpha]_D^{25}$ -27 (*c* 0.2, MeOH); IR (KBr) ν_{max} 3463, 3190, 2982, 1685, 1610, 1520, 1455, 1366, 1255, 1117, 1085 cm^{-1}; ESIMS *m*/*z* 381.2 $[M + Na]^+$; HRESIMS *m*/*z* 381.20407 ($[M + Na]^+$, calcd for $C_{22}H_{30}NaO_4$, 381.20418, Δ = –0.29 ppm); ^1H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR data in Table 1.

Compound 1. Colourless oil (35 mg). $[\alpha]_D^{25}$ +4.7 (*c* 0.5, MeOH); IR (KBr) ν_{max} 3520, 3194, 2977, 1681, 1614, 1523, 1452, 1369, 1247, 1110, 1077 cm^{-1}; ESIMS *m*/*z* 381.2 $[M + Na]^+$; HRESIMS *m*/*z* 381.20416 ($[M + Na]^+$, calcd for $C_{22}H_{30}NaO_4$, 381.20418, Δ = –0.05 ppm), ^1H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR data in Table 1.

Evaluation of antimicrobial activity. The fungal strain used in the antifungal activity assay was *Candida albicans* SC5314 as a representative fungus for candidiasis. Bacterial strains used were *Pseudomonas aeruginosa* PAO1 (PA), *Escherichia coli* (EC), *Bacillus subtilis* ATCC6633 (BS), *Staphylococcus aureus* ATCC6538 (SA), *Mycobacterium bovis* BCG Pasteur 1173P2 and *Mycobacterium tuberculosis* H37Rv (MTB). Strains were preserved in glycerol stocks at −80 °C, and sub-cultured at 37 °C before use.

Chemicals for antimicrobial assay. Vancomycin hydrochloride (Van), tetracycline hydrochloride (Tet), chloramphenicol (Chl), ciprofloxacin (Cip), ketoconazole (Kez) and isoniazid (INH) were purchased from Sigma-Aldrich Co., used as positive controls in the susceptibility testing assay. Chemical agents were dissolved in DMSO with a concentration of 1 mg/mL, stored at –20 °C until usage. Compounds were adjusted to the proper concentration before use.

Assay for anti-SA, anti-PA, anti-*B. subtilis* and anti-*E. coli* activity. All the screening assays were performed according to the Antimicrobial Susceptibility Testing Standards outlined by the Clinical and Laboratory Standards Institute (CLSI) (2006).

The SA, PA, *B. subtilis* and *E. coli* strains were recovered from glycerol stocks, respectively, by culturing on a LB agar plate overnight aerobically at 37 °C, single colonies were picked and adjusted to approximately 1×10^4 CFU/mL with Mueller-Hinton broth as bacterial suspensions. Aliquots (80 μL) of the bacterial suspension were added to each well of the 96-well microplates (F-bottom, Greiner Bio-One Ltd), followed by the addition of 2 μL compound solutions (1 mg/mL) in each test well, which gave 25 μg/mL as the final test concentration of each compound. Serial dilutions of control drugs were added to the left column on each plate as positive controls (positive control drugs were settled as Van for SA assay, Tet for BS assay, Cip for PA assay, and Chl for EC assay). Two μL of DMSO was added to each well of the right column (column 12), serving as a negative control. After 16 h incubation at 37 °C, bacterial growth in each well was inspected by the measurement of OD_{600} in a multi-label plate reader (Perkin Elmer En Vision). Active hits were defined as compounds that exhibited a growth inhibitory effect at a concentration of 25 μg/mL in the primary screening assay.

For MIC determination, overnight cultures of the bacteria strains were diluted with fresh M-H broth, and standardized to 1×10^4 CFU/mL as the bacteria suspension. Two μL of 2-fold serial dilution of each compound (concentration ranging from 1 mg/mL to7.8 μg/mL) was added to each row on a 96-well plate containing 78 μL of bacteria suspension in each well. Positive and negative controls were included in each plate as described in the primary screening assay. Plates were incubated at 37 °C for 16 h and checked for bacteria growth. The MIC here is defined as the lowest concentration of compound that resulted in inhibition of visible bacterial growth (no turbidity) compared with the positive control antibiotics.

Assay for anti-BCG and MTB (H37Rv) in logarithmic phase of growth. The in *vitro* activity of compounds against *M. bovis* BCG Pasteur and *M. tuberculosis* H37Rv was determined in a 96-well plate format as described previously (Wang *et al.*, 2010), modified from that reported by Collins *et al.* (1998). The BCG and MTB strains used here were transformed with green fluorescent protein (GFP) constitutive expression

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Table 1. ^{1}H (400 MHz) and ^{13}C-NMR (100 MHz) data of 1 and 4 in $CDCl_3$

	1		4	
Position	δ^1H/ppm, mult., J/Hz	$\delta^{13}C$/ppm, mult.	δ^1H/ppm, mult., J/Hz	$\delta^{13}C$/ppm, mult.
1	–	46.2, C	–	46.1, C
2	**a**. 1.41-1.33 (1H,m) **b**. 1.67-1.59 (1H,m)	41.4, CH_2	**a**. 1.47-1.39 (1H,m) **b**. 1.67-1.59 (1H,m)	41.4, CH_2
3	**a**. 1.63-1.54 (1H,m) **b**. 1.97-1.89 (1H,m)	31.2, CH_2	**a**. 1.63-1.54 (1H,m) **b**. 2.00-1.90 (1H,m)	31.2, CH_2
4	–	85.6, C	–	85.9, C
5	2.47 (1H,d, 10.8)	52.3, CH	2.90 (1H,d, 10.6)	52.7, CH
6	5.83 (1H, dddd, 1.95, 5.5, 10.8, 12.8)	73.0, CH	5.86 (1H, q, 5.5)	72.5, CH
7	**a**. 2.49 (1H, dd, 5.5, 15.5) **b**. 2.10 (1H, dt, 1.7, 15.5)	46.0, CH_2	**a**. 2.43 (1H, dd, 5.5, 16.2) **b**. 2.12 (1H, m)	44.6, CH_2
8	–	71.1, C	–	71.6, C
9	5.46 (1H, dd, 2.0, 11.6)	134.8, CH	5.46 (1H, d, 11.4)	132.4, CH
10	5.56 (1H, d, 11.6)	138.2, CH	5.65 (1H, d, 11.4)	141.6, CH
11	1.58-1.51 (1H, m)	36.8, CH	1.64-1.56 (1H, m)	36.9, CH
12	0.84 (3H, d, 6.6)	18.6, CH_3	0.88 (3H, d, 6.7)	18.7, CH_3
13	0.82 (3H, d, 6.6)	17.6, CH_3	0.83 (3H, d, 6.7)	17.6, CH_3
14	1.60 (3H, s)	29.2, CH_3	1.33 (3H, s)	34.2, CH_3
15	1.26 (3H,s)	21.5, CH_3	1.24 (3H,s)	20.5, CH_3
1′	–	130.5, C	–	130.5, C
2′	8.00 (1H, d, 8.0)	129.9, CH	8.02 (1H, d, 8.0)	129.9, CH
3′	7.44 (1H, t, 8.0)	128.8, CH	7.44 (1H, t, 8.0)	128.8, CH
4′	7.57 (1H, t, 8.0)	133.5, CH	7.60 (1H, t, 8.0)	133.5, CH
5′	7.44 (1H, t, 8.0)	128.8, CH	7.44 (1H, t, 8.0)	128.8, CH
6′	8.00 (1H, d, 8.0)	129.9, CH	8.02 (1H, d, 8.0)	129.9, CH
7′	–	167.1, C	–	167.1, C

plasmid pSC301 (Cowley and Av-Gay, 2001), thus the fluorescence could be a direct readout reflecting bacterial viability and growth (Changsen *et al*., 2003). Briefly, BCG and MTB H37Rv were grown at 37 °C to mid log phase in Middlebrook 7H9 broth supplemented with 10% OADC enrichment (Becton Dickinson) 0.05% Tween-80 and 0.2% glycerol, which was then adjusted to OD_{600} nm = 0.025 with culture medium as the bacterial suspension. For primary screening, aliquots (80 μL) of the bacterial suspension were added to each well of the clear flat-bottom 96-well microplates, followed by the addition of 2 μL compound solutions (1 mg/mL). Subsequent serial dilutions of isoniazid were conducted in the left column, serving as the positive control, while the right column containing 2 μL of DMSO served as the drug free control. The plates were sealed with breathable film and incubated at 37 °C for 3 days for *M. bovis* BCG or 10 days for *M. tuberculosis*. The GFP fluorescence was measured with a Perkin-Elmer Envision 2103 multi-label reader using the bottom read mode, with excitation at 485 nm and emission at 535 nm. Hits were defined as compounds that could inhibit more than 90% of bacterial growth compared with the drug free control group.

For MIC determination of the hits compounds, bacterial suspensions were prepared and dispensed as described above, and compounds were serially diluted two-fold from 25 to 0.19 μg/mL in each column. Isoniazid as a positive control (800 ng/mL to 6.25 ng/mL) and DMSO as a blank control were also included in each plate. After incubation at 37 °C for an appropriate time, the bacterial growth condition was inspected by GFP fluorescence measurement. Here, MIC is defined as the minimum concentration of drug that inhibited more than 90% of bacteria growth reflected by fluorescence value.

Antifungal and synergistic antifungal screening. *Candida albicans* SC5314 was used as a test strain for the antifungal and synergistic antifungal bioassay (Zhang *et al*., 2007). Experiments were carried out in 96-well microplates (F-bottom, Greiner Bio-One Ltd), using a broth microdilution protocol modified from the CLSI M-27A methods. Overnight cultures were chosen to prepare the culture suspension with medium RPMI 1640 at a concentration of 1×10^4 CFU/mL. Two μL of the test compounds was added to the test wells in 96 well-plates followed by the addition of 80 μL of the culture suspension. The test plates were incubated at 35 °C for 16 h. The antifungal positive control was ketoconazole, and antifungal minimal inhibition concentrations (MICs) were determined by measuring and comparing the optical diversities of the blank control and tested wells. For the synergistic antifungal assay, ketoconazole was supplemented into the culture suspension with a concentration of one quarter the MIC according to the antifungal result, and the remaining procedures were conducted as for the antifungal assay. All the experiments were tested in triplicate.

DPPH radical-scavenging assay. Firstly, radical scavenging activity of sesquiterpenes against stable DPPH· was performed with a rapid TLC screening method using 0.2% DPPH in methanol. Then 30 min after spraying, the active compounds appeared as yellow spots against purple background (Braca *et al*., 2002; Yrjönen *et al*., 2003).

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In a second experiment, the spectrophotometric assay was carried out according to the method of Yen and Chen (1995). Briefly, 2.0 mL of a wide range of concentrations (2.5–120 μM) of test sample (in methanol) was added to 2.0 mL of 100 μM of methanol solution of DPPH. The mixture was vortexed for 1 min and then left to stand at room temperature for 30 min in the dark. When DPPH· reacts with an antioxidant compound, which can donate hydrogen, it is reduced and the changes in colour (from deep-violet to light-yellow) were measured at 517 nm on a UV/visible light spectrophotometer (Perkin-Elmer Instruments, Lambda 25 (UV/VIS) spectrometer). Absorption of the blank sample containing the same amount of methanol and DPPH solution was prepared and measured daily. The experiment was carried out in triplicate, using ascorbic acid as a positive control standard. The percentage reduction of the DPPH, Q, referred to 'inhibition or quenching' was calculated by the following formula (Shin-ya *et al.*, 1993; Abdel-Mageed *et al.*, 2010):

$$Q(\%\ \text{Inhibition}) = \frac{A_B - A_A}{A_B} \times 100$$

where A_B is the absorption of the blank sample ($t = 0$ min) and A_A is the absorption of the tested extract solution ($t = 30$ min).

RESULTS AND DISCUSSION

A combination of flash or medium-pressure liquid chromatography (MPLC), gel filtration and HPLC of the chloroform fraction obtained from the roots of *F. hermonis* afforded 17 daucane sesquiterpenes (**1–17**), one of which is a new sesquiterpene ester. The structures of all compounds were elucidated by IR, MS and extensive NMR analysis.

Sixteen known daucane sesquiterpenes (**1–3**, **5–17**) (Fig. 1) were identified as 4ß,8ß-dihydroxy-6α-benzoyl-dauc-9-ene (feruhermonin B) (**1**) (Auzi *et al.*, 2008); 4ß,8ß-dihydroxy-6α-*p*-hydroxybenzoyl-dauc-9-ene (**2**) (Garg and Agarwal, 1987); 4ß,8ß-dihydroxy-6α-(4-hydroxy-3-methoxybenzoyl)-dauc-9-ene (**3**) (Auzi *et al.*, 2008); 4ß-hydroxy-6α-benzoyl-7-daucen-9-one (feruhermonin A) (**5**) (Auzi *et al.*, 2008); 4ß-hydroxy-6α-*p*-hydroxybenzoyl-7-daucen-9-one (lancerdiol *p*-hydroxybenzoate) (**6**) (Fraga *et al.*,1985); 4ß-hydroxy-6α-(4-hydroxy-3-methoxybenzoyl)-7-daucen-9-one (lancerdiol vanillate) (**7**) (Miski, 1987); 4ß,9α-dihydroxy-6α-benzoyl-dauc-7-ene (lancerotriol benzoate) (**8**) (Auzi *et al.*, 2008); 4ß,9α-dihydroxy-6α-*p*-hydroxybenzoyl-dauc-7-ene (lancerotriol *p*-hydroxybenzoate) (**9**) (Fraga *et al.*, 1985); 4ß,9α-dihydroxy-6α-(4-hydroxy-3-methoxybenzoyl)-dauc-7-ene (lancerotriol vanillate) (**10**) (Miski and Jakupovic, 1990); 2,3-epoxy-jaeschkeanadiol benzoate (**11**) (Diab *et al.*, 2001b); 2,3-epoxy-jaeschkeanadiol *p*-hydroxybenzoate (jaeschkeanin) (**12**) (Diab *et al.*, 2001b); 2,3-epoxy-jaeschkeanadiol vanillate (**13**) (Chen *et al.*, 2000); vaginatin (**14**) (Miski and Mabry, 1986; Yang *et al.*, 2008); jaeschkeanadiol benzoate (teferidin) (**15**) (Diab *et al.*, 2001b); jaeschkeanadiol *p*-hydroxybenzoate (ferutinin) (**16**) (Diab *et al.*, 2001b); jaeschkeanadiol vanillate (teferin) (**17**) (Diab *et al.*, 2001b). All the physical and spectral data (Supplementary data, Tables 1S-3S) of these compounds were in agreement with the respective published data.

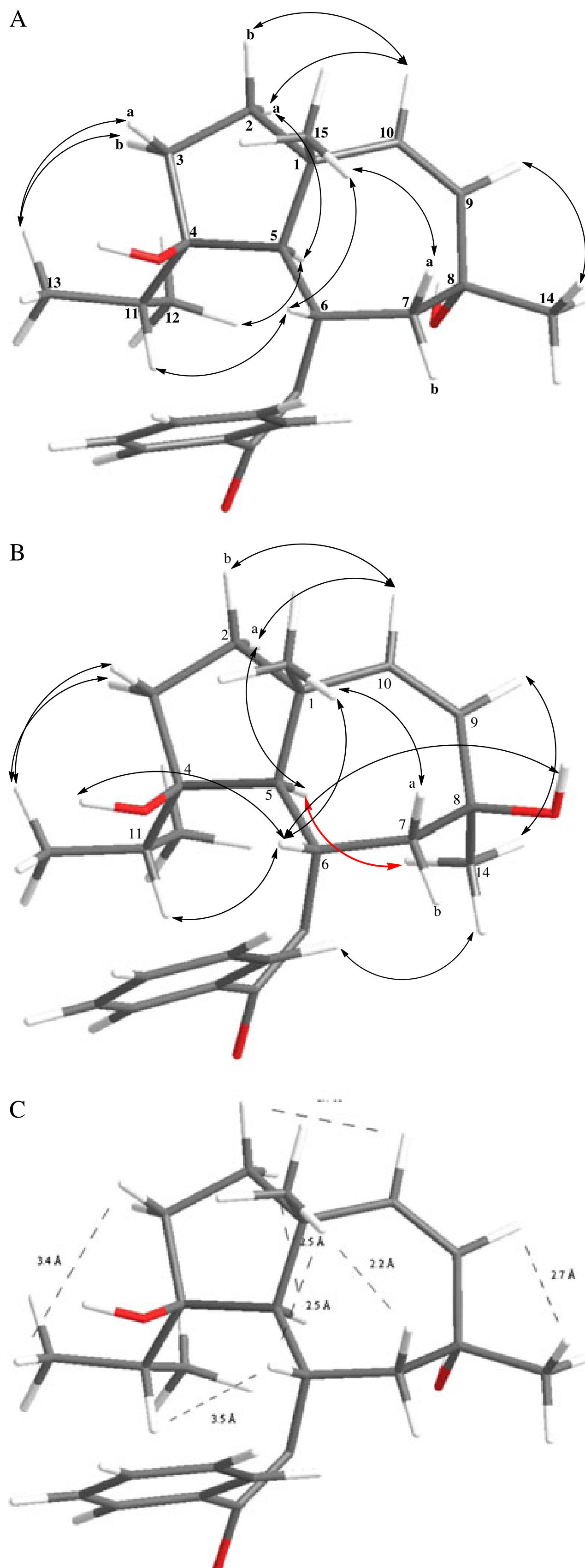

Figure 2. (A) Key NOESY correlations for compound **4**. (B) Key NOESY correlations of compound **1**. (C) Global energy minimum for compound **4**. (Figures were generated using Chem 3D Ultra 10.0®).

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Compound **4** was obtained as a colourless amorphous powder. ESIMS showed pseudomolecular ion peaks at m/z 381.2 $[M+Na]^+$, consistent with a molecular weight of 358 amu. By accurate mass measurements, the molecular formula was established as $C_{22}H_{30}O_4$, thus implying eight degrees of unsaturation. The IR spectrum showed strong absorption bands attributed to the hydroxyl and ester groups at 3463 and 1685 cm^{-1}.

The 1D and 2D NMR spectra in $CDCl_3$ (Table 1) of **4** revealed the daucane nucleus and were very similar to those of **1** (Auzi *et al.*, 2008) (Supplementary data, Figures 1S-14S). The ^{13}C NMR spectrum of **4** exhibited a downfield shift of the methyl group CH_3-14 (δ 34.2), whereas the corresponding signal appeared at (δ 29.2) in **1**, with an upfield shift of CH_2-7 (δ 44.6) in **4** instead of (δ 46.0) in **1** and CH-9 (δ 132.4) to (δ 134.8) respectively. The ^1H-NMR showed a slight upfield shift of CH_3-14 (δ 1.33 in **4**, instead of δ 1.60 in **1**) and a downfield shift of H-5 (δ 2.90 in **4**, instead of δ 2.47 in **1**) which confirmed the relative stereochemistry change of CH_3-14 at C-8 to be ß with 6α-OH in **4** rather than α with 6ß-OH in **1** (Ahmed, 1990; Chen *et al.*, 2000). The 2D NOESY experiment (Fig. 2A) confirmed the stereochemistry change at C-8 through the absence of any mutual NOE correlation between CH_3-14 and H-5 which was observed in the case of α position in **1** (Fig. 2C) to confirm its ß position in **4**. The relative stereochemistry at centres C-1, C-4, C-5 and C-6 was also established based on the results of a 2D NOESY experiment (Fig. 2A) and by comparison with previously reported compounds. So, compound **4** was assigned to (9*Z*, 1*R**, 4*R**, 5*S**, 6*S**, 8*R**) carotane.

The global minimum energy conformation of compound **4** was calculated using a Chem3D Ultra 10.0® (CambridgeSoft, Cambridge, MA, USA) (10000 steps, global minimum shown in Fig. 2C). The molecular mechanics calculation was consistent with an outcome of NOESY that showed protons H-6 and H_3-15 are oriented *syn* with regards to H-7a based on the mutual diagnostic NOE cross-peaks between each other. In a similar manner, NOE correlations were observed between H-5, H_3-12 and H-2a that indicates that they are oriented *syn* based on the mutual NOE correlations. Also, NOE correlations were observed between H_3-14 and H-9; H-10 and H-2a/b as well as H_3-13 and H-3a/b.

On the basis of the similarity in terms of the remaining coupling constants, ^{13}C-NMR chemical shifts at all stereocentres, the comparable results of the 2D NOESY experiment and molecular modelling (Fig. 2B) as described above, it was evident that **4** is an isomer of **1**. As a result, **4** was identified as a new natural product for which we propose the name feruhermonin D.

The antifungal and antibacterial activity for the isolated compounds was evaluated by minimum inhibitory concentration (MIC) determined by microdilution

Table 3. DPPH radical scavenging activity of isolated sesquiterpenes and ascorbic acid.

Compound Number	DPPH radical scavenging activity (IC_{50}/μM)
1	179.2
2	134.6
3	127.1
4	183.5
5	73.2
6	66.7
7	61.8
8	95.5
9	84.0
10	77.6
11	37.4
12	31.7
13	26.2
14	97.8
15	17.3
16	13.2
17	11.5
Ascorbic acid	12.5

Table 2. MICs of isolated sesquiterpenes against different bacteria and fungi.

Compound Number	MRSA MICs	*Bacillus subtilis* MICs	*E. coli* MICs	*M. tuberculosis* MICs	BCG MICs	*Candida albicans* MICs
1	> 50	> 50	> 50	ND	50	> 50
2	> 50	> 50	> 50	ND	50	> 50
3	> 50	> 50	> 50	ND	> 50	> 50
4	> 50	> 50	> 50	ND	> 50	> 50
5	> 50	> 50	> 50	ND	25	> 50
6	> 50	> 50	> 50	ND	50	> 50
7	> 50	> 50	> 50	ND	> 50	> 50
8	> 50	> 50	> 50	ND	50	> 50
9	> 50	> 50	> 50	ND	> 50	> 50
10	> 50	> 50	> 50	ND	> 50	> 50
11	> 50	> 50	> 50	ND	50	> 50
12	> 50	> 50	> 50	ND	50	> 50
13	> 50	> 50	> 50	ND	50	> 50
14	> 50	> 50	> 50	ND	50	> 50
15	0.78	< 0.39	> 50	0.69	3.125	> 50
16	< 0.39	< 0.39	> 50	2	1.56	> 50
17	1.56	1.56	> 50	8	6.25	> 50

ND, not determined.

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method. The MICs of the isolated compounds to fungi (*Candida albicans* SC5314), Gram-positive bacteria (SA) and Gram-negative bacteria (*E. coli*, PA) were determined and compared with those for commercially available antibiotics (Table 2). No activity was observed against *Candida albicans*, *Escherichia coli* and PA. Compounds **15** (teferidin), **16** (ferutinin) and **17** (teferin) exhibited strong activity against MRSA (MICs 0.78, < 0.39 and 1.56), *Bacillus subtilis* (MICs < 0.39, < 0.39 and 1.56), *Mycobacterium bovis* BCG (MICs 3.12, 1.56 and 6.25) and *Mycobacterium tuberculosis* (MICs 0.69, 2.0 and 8.0). From the MIC values, it was obvious that teferidin and its derivatives are considered a promising potential candidate for further evaluation of their activity against pathogenic bacteria such as MRSA, *Bacillus* sp. and *Mycobacterium* sp.

Compounds (**15**, **16**, **17**) displayed the strongest radical scavenging activity (IC_{50s} 17.3, 13.2 and 11.5 μM), respectively, whilst the weakest radical scavenging activity was observed with compounds (**1**, **2**, **3**, **4**). Obviously improvement of the radical scavenging activity accompanied by the increase in the oxygenation pattern of the acyl moiety of jaeschkeanadiol nucleus (Table 3).

In conclusion, 17 daucane sesquiterpenoid esters (**1–17**) were isolated from the root of *Ferula hermonis* and one of them was a new compound. Compounds (**15**, **16**, **17**) demonstrated potent activity against Gram +ve (*S. aureus*, *B. subtilis*), as well as *Mycobacterium* strains *M. bovis* BCG and *M. tuberculosis* H37Rv. None of the isolated compounds exhibited a significant antifungal activity. In the antioxidant study, compounds (**15**, **16**, **17**) have the highest radical scavenging activity.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

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