Saudi Pharmaceutical Journal 27 (2019) 525–531

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

Phytochemical screening and evaluation of the antimicrobial and antioxidant activities of *Ferula caspica* M. Bieb. extracts

Cigdem Kahraman [a,*], Gulacti Topcu [b], Erdal Bedir [c], I. Irem Tatli [d], Melike Ekizoglu [e], Zeliha S. Akdemir [a]

[a] Department of Pharmacognosy, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey
[b] Department of Pharmacognosy, Faculty of Pharmacy, Bezmialem Vakif University, İstanbul, Turkey
[c] Department of Bioengineering, Faculty of Engineering, İzmir Institute of Technology, İzmir, Turkey
[d] Department of Pharmaceutical Botany, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey
[e] Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Hacettepe University, Ankara, Turkey

ARTICLE INFO

Article history:
Received 17 October 2018
Accepted 27 January 2019
Available online 2 February 2019

Keywords:
Ferula caspica
Constituents
NMR
Antimicrobial
Antioxidant

ABSTRACT

Chloroform, ethyl acetate and methanol extracts from the aerial parts of *Ferula caspica* M. Bieb. were tested for their antioxidant capacities by CUPRAC, ABTS, FRAP, Folin–Ciocalteu and aluminum chloride methods and for antimicrobial activities by the broth microdilution method. Chloroform and ethyl acetate extracts showed the highest antioxidant capacity and antimicrobial activity. Three known sesquiterpene derivatives; 1-(2′,4′-dihydroxyphenyl)-3,7,11-trimethyl-3-vinyl-6(E),10-dodecadien-1-one (**1**), 2,3-dihydro-7-hydroxy-2,3-dimethyl-2-[4′,8′-dimethyl-3′,7′-nonadienyl]-furo[3,2,c]coumarin (**2**), 2,3-dihydro-7-hydroxy-2,3-dimethyl-3-[4′,8′-dimethyl-3′,7′-nonadienyl]-furo[3,2,c]coumarin(**3**); phenylpropanoid; laserine/2-epilaserine (**4/5**) and steroid mixtures; stigmasterol and *β*-sitosterol (**6/7**) were isolated from chloroform extract; three known flavonoids; kaempferol-3-*O*-*β*-glucopyranoside (**8**), kaempferol-3-*O*-*α*-rhamnopyranoside (**9**), quercetin-3-*O*-*β*-glucopyranoside (**10**), and one benzoic acid derivative; 2,4-dihydroxybenzoic acid (**11**) were isolated from the ethyl acetate extract. The structures were elucidated by spectroscopic methods.

1. Introduction

The genus *Ferula* L. distributed worldwide (Pimenov and Leonev, 2004) mainly in central and South-west Asia and Mediterranean basin (Yaqoob and Nawchoo, 2016) is the third largest genus of Apiaceae (Pimenov and Leonev, 2004). In Turkey, the genus *Ferula* has 23 species, 13 of which are endemic to Turkish Flora. (Peşmen, 1972; Duman and Sagiroglu, 2005; Sağıroğlu, 2005; Sagiroglu and Duman, 2007, 2010; Pimenov and Kljuykov, 2013; Sağıroğlu and Duman, 2014). *Ferula* species are traditionally used in the treatment of various diseases. Aerial parts of *F. rigidula* DC. subsp. *rigidula*, all parts of *F. orientalis* L., the roots of *F. elaeochytris* Korovin and *F. longipedunculata* Peşmen are used as immunostimulant, aphrodisiac, antidiabetic, anticholesterolemic, emmenagogue, menstrual regulator and for treatment of gastric pain in Anatolia (Altundag and Ozturk, 2011; Güneş and Özhatay, 2011; Demirci and Özhatay, 2012; Güzel et al., 2015; Mükemre et al., 2015). Phytochemical studies on *Ferula* species showed the presence of daucane, germacrane, humulane type sesquiterpenes (Miski and Mabry, 1986; Miski et al., 1987; Miski and Jakupovic, 1990; Ahmed, 1991; Galal et al., 2001; Lhuillier et al., 2005; Alkhatib et al., 2010), sesquiterpene lactone derivatives (Iranshahi et al., 2008; Kurimoto et al., 2012) and sesquiterpene coumarins (Miski et al., 1985; Yang et al., 2006; Abd et al., 2007; Iranshahi et al., 2009; Iranshahi et al., 2010; Dastan et al., 2014; Li et al., 2015a; Li et al., 2015b). Furthermore, furanocoumarin, asetophenone, benzofuran sesquiterpenes (Kojima et al., 1998; Chen et al., 2001; Meng et al., 2013a; Meng et al., 2013b) and flavonoids (Znati et al., 2014) were isolated from these species. In this study, we aimed to investigate the antioxidant and antimicrobial activities of different extracts from *Ferula caspica* M. Bieb. and to isolate polar and apolar secondary metabolites from the active extracts.

* Corresponding author.
E-mail address: cigdemm@hacettepe.edu.tr (C. Kahraman).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jsps.2019.01.016
1319-0164/

2. Material and methods

2.1. General experimental procedures

NMR spectra (600 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) were measured on Agilent spectrometry. HR-ESI-MS spectra were recorded on the Bruker micro Q-TOF/6500 mass spectrometer. Silica gel 60 (0.063–0.200 mm/70–230 mesh, Merck, Germany), Sephadex LH 20 (Merck, Germany) and reversed-phase material (C-18, LiChroprep 25–40 μm, Merck, Germany) were used for column chromatography (CC) and Silica gel 60 F_{254} (Merck, Germany) was used for Thin Layer Chromatography (TLC) and Preparative Thin Layer Chromatography (pTLC). Detection of spots on the plates was done with 1% Vanillin-sulphuric acid or ceric sulfate reagents and UV light (Camag 254 and 366 nm). Mueller-Hinton Broth (MHB, BBL, MD, USA), RPMI-1640 medium (ICN-Flow, Aurora, OH-USA) with L-glutamine and 3-(N-morpholino)propane sulphonic acid (MOPS) (Buffer-ICN-Flow, Aurora, OH-USA) were used for the antimicrobial activity. Reagents and standard compounds used in antioxidant capacity assays were purchased from Sigma-Aldrich (USA).

2.2. Plant material and the extraction procedure

Plant materials were collected from Nallıhan-Davutoğlan Bird Paradise, Ankara province Central Anatolia Region of Turkey in June 2011. The specimens were identified by Prof. Dr. Hayri Duman, Gazi University, Faculty of Science, Department of Botany, Ankara, Turkey. Voucher specimens were deposited at the Herbarium of the Faculty of Pharmacy, Hacettepe University, Ankara (HUEF 11003).

Air dried and powdered aerial parts of *F. caspica* (597 g) were extracted with chloroform (5 L × 4) to give 27 g extract (yield: 4.5%) and methanol (5 L × 4) to give 64 g extract (yield: 10.7%), respectively, using rotary extractor under 40 °C without vacuum. The methanol extract was partitioned between ethyl acetate and water to yield 5 g EtOAc extract (yield: 0.8%) (see Fig. 1).

2.3. Isolation of the compounds 1–11

Chloroform extract (27 g) was fractionated by silica gel column chromatography using *n*-hexane and EtOAc with increasing polarity (95:5–0:100) to give 7 fractions (Frs. A–G). Fr. B (577 mg) was refractionated by silica gel column chromatography (CC) using *n*-hexane and acetone (95:5–90:10) to yield 5 fractions (Frs. B_{1-5}). Then, Fr. B_1 (30 mg) subjected to pTLC (*n*-hexane-acetone, 3:2) to afford the mixture of **4/5** (10 mg). In a sequential manner, Fr. B_4 (150 mg) was chromatographed on silica gel eluting by increasing polarity of acetone in *n*-hexane (90:10–0:100), sephadex LH-20 (cyclohexane-CH_2Cl_2-EtOH, 7:4:1) and pTLC (*n*-Hekzane-EtOAc, 3:2) to purify compound **1** (26 mg). Fr. D (300 mg) was submitted to silica gel CC eluted with *n*-hexane-EtOAc (9:1) and then the mixture of compounds **6/7** was yielded from Fr. D_2 (77.5 mg) with pTLC (toluen-EtOAc-acetonitrile, 40:9:1). Fr. F (1 g) was resubmitted to silica gel CC with the increasing volume of EtOAc in toluene (80:20–0:100). Fr. F_2 (145.5 mg) was subjected to silica gel (toluen-EtOAc, 97:3–92.5:7.5), Sephadex LH-20 CC (cyclohexane-CH_2Cl_2-EtOH, 7:4:1) and pTLC (toluen-EtOAc-acetonitrile, 40:9:1) to obtain compound **3** (10 mg). Compound **2** (6 mg) was purified from Fr. F_4 (147.8 mg) with silica gel CC (toluen-EtOAc, 96:4–91:9) and pTLC (toluen-EtOAc-acetonitrile, 40:9:1).

EtOAc extract (5.0 g) was fractionated by Reverse Phase-Vacuum Liquid Chromatography (RP-VLC) (H_2O-MeOH, 100:0–0:100) to give fractions (Frs. 1–6). Fr. 2 (200 mg) refractionated by RP-VLC (H_2O-MeOH, 100:0–0:100) to yield sequential fractions and then compound **11** (10 mg) was gained from Fr. 2b (40 mg) by pTLC ($CHCl_3$-MeOH-H_2O, 61:32:7). Compound **10** (10 mg) and **8** (7 mg) were purified from Frs. 3 (100 mg) and 5 (100 mg), respectively by applying silica gel CC ($CHCl_3$-MeOH, 90:10–0:100 for Fr. 3 and EtOAc-MeOH-H_2O, 100:5:1 for Fr. 5) and pTLC ($CHCl_3$-MeOH-H_2O, 61:32:7 for Fr. 3 and EtOAc-MeOH-H_2O, 100:13.5:10 for Fr. 5). And finally, Fr. 6 (86.5 mg) was chromatographed on silica gel ($CHCl_3$-MeOH, 98:2–87:13) to give sequential fractions and Fr 6b (27 mg) was applied on Sephadex LH-20 CC (MeOH) to yield compound **9** (13.2).

2.4. Antimicrobial and antioxidant activity assays

The extracts were tested against the bacterial (*Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213) and fungal species (*Candida albicans* ATCC 90028, *C. krusei* ATCC 6258, *C. parapsilosis* ATCC 90018) by using broth microdilution method reported by the Clinical and Laboratory Standards Institute (Wayne, 2008a, 2008b). Gentamycin and fluconazole were used as reference compounds. The test was performed in Mueller-Hinton Broth (MHB, BBL, MD, USA) for bacteria. RPMI-1640 medium (ICN-Flow, Aurora, OH-USA) with L-glutamine, buffered with 3-(N-morpholino) propane sulphonic acid (MOPS) (Buffer-ICN-Flow, Aurora, OH-USA) at pH = 7.4 was used as the test medium for fungi. The microtiter plates were incubated at 35 °C for 18–24 h for bacteria and 48 h for fungi. Minimum inhibitory concentrations (MIC) were defined visually as the lowest concentrations of the extracts that had no turbidity.

Antioxidant capacities of the obtained extracts were evaluated by the following methods:2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) (Re et al., 1999) radical cation scavenging activity, ferric-reducing antioxidant power (FRAP) (Oyaizu, 1986) and cupric ion reducing antioxidant capacity (CUPRAC) (Apak et al., 2004). The quantitative determinations of flavonoids and phenolic compounds of all extracts were conducted aluminum chloride method (Chang et al., 2002) and Folin–Ciocalteu assay (Slinkard and Singleton, 1977), respectively. Briefly, after adding 200 μL of diluted ABTS solution to 20 μL of different concentrations of the test samples in ABTS radical scavenging assay the absorbance were measured at 734 nm. In CUPRAC assay, 50 μL of each of copper(II) chloride, neocuproine, and ammonium acetate solutions were mixed with 25 μL of the test sample and 25 μL of water. After incubating 30 min, absorbance was measured at 450 nm. According to Oyaizu's assay, after incubating at 50 °C for 20 min, the mixture of test sample (20 μL), sodium phosphate buffer (pH 6.6, 0.2 M, 50 μL) and potassium ferricyanide (50 μL, 1%, w/v) were acidified by adding trichloroacetic acid (50 μL, 10% w/v). 50 μL of this solution was mixed with distilled water (50 μL) and iron(III) chloride (10 μL, 0.1% w/v). Absorbance was read at 700 nm after 30 min incubation. The absorbance of the mixture of the test sample (25 μL), ethanol (75 μL, 95%), aluminum chloride (5 μL, 10%), potassium acetate (5 μL, 1 M) and distilled water (140 μL) was measured at 415 nm in aluminum chloride method. Finally, according to Folin-Ciocalteu assay of Slinkard and Singleton, the absorbance of the mixture of 100 μL of diluted Folin reagent, 20 μL of the test sample and 80 μL of sodium carbonate (7.5%) solution, after 2 h incubation, was measured at 765 nm.

2.5. Statistical analysis

Obtained data from antioxidant capacity assays were analyzed using one-way ANOVA followed by Tukey test, p-values < 0.05 were considered statistically significant.

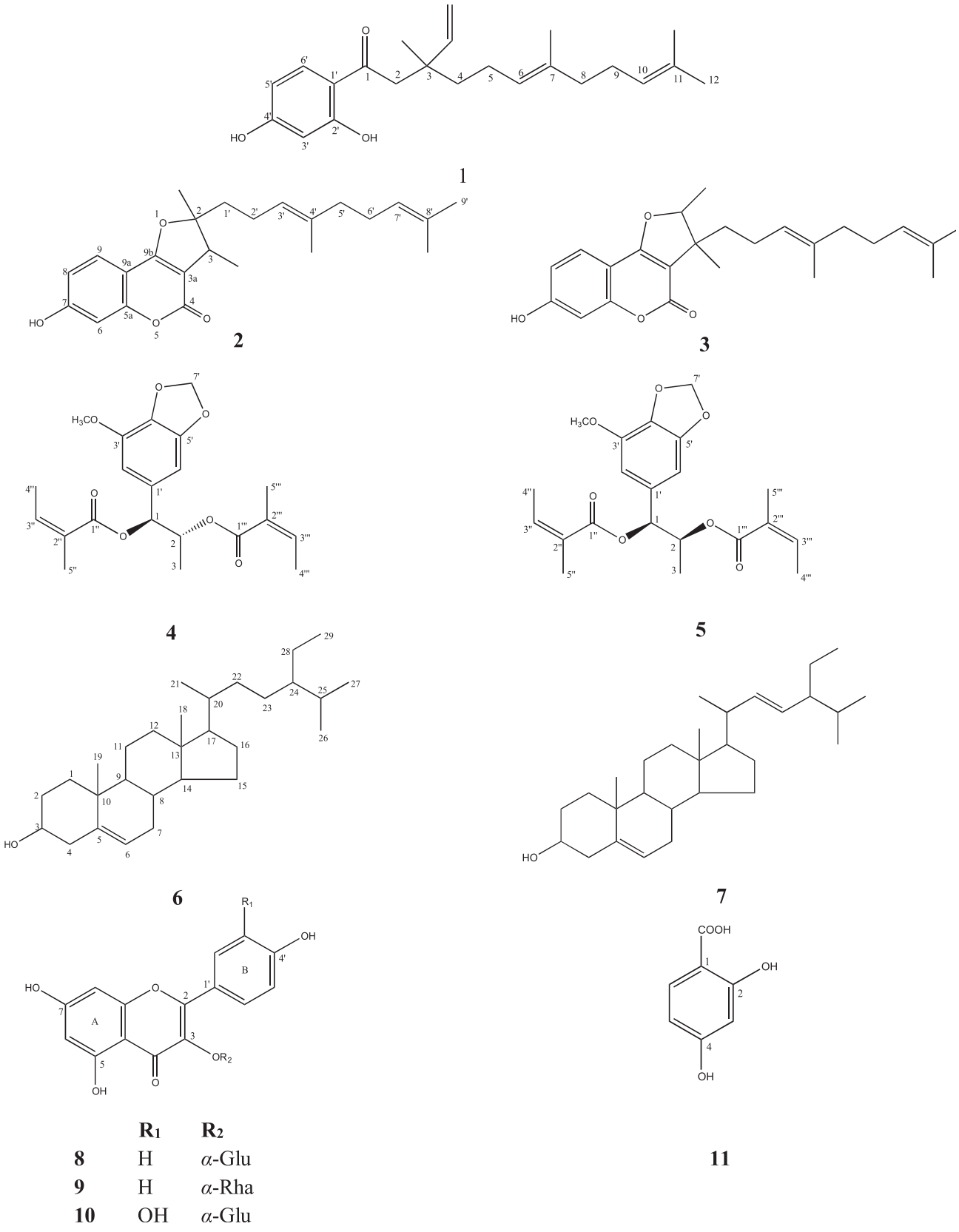

Fig. 1. Structures of compounds 1–11.

3. Results

Isolation studies were performed on chloroform and ethyl acetate extracts showed the highest activity in the assays for antimicrobial and antioxidant activities, respectively.

3.1. Structural elucidation of compounds 1–11

The chloroform extract obtained from the aerial parts of *F. caspica* was fractionated by a serial chromatographic procedures with normal-phase silica gel, Sephadex LH-20 and preparative thin layer chromatography (pTLC) to afford three known sesquiterpene derivatives (**1**–**3**) as well as known phenylpropanoid (**4/5**) and steroid (**6/7**) mixtures; 1-(2′,4′-dihydroxyphenyl)-3,7,11-trimethyl-3-vinyl-6(*E*),10-dodecadien-1-one (**1**) (Kojima et al., 1998), 2,3-dihydro-7-hydroxy-2,3-dimethyl-2-[4′,8′-dimethyl-3′,7′-nonadienyl]-furo[3,2,c]coumarin (**2**) (Isaka et al., 2001), 2,3-dihydro-7-hydroxy-2,3-dimethyl-3-[4′,8′-dimethyl-3′,7′-nonadienyl]-furo[3,2,c]coumarin (**3**) (Kojima et al., 2000), laserine/2-epilaserine mixture (**4/5**) (Barrero et al., 1992), stigmasterol and *β*-sitosterol mixture (**6/7**) (Chaturvedula and Prakash, 2012).

The ethyl acetate fraction of methanolic extract of the dried aerial parts of *F. caspica* was subjected to column chromatography over reversed-phase RP-18 silica gel, normal-phase silica gel, Sephadex LH-20 and pTLC to give three known flavonoids (**8**–**10**) and a benzoic acid derivative (**11**); kaempferol-3-*O*-*β*-glucopyranoside (**8**) (Han et al., 2004), kaempferol-3-*O*-*α*-rhamnopyranoside (**9**) (Correia et al., 2008), quercetine-3-*O*-*β*-glucopyranoside (**10**) (Han et al., 2004) and 2,4-dihydroxybenzoic acid (**11**) (Scott, 1972).

Structures of the compounds were determined by comparison of their spectroscopic data with those reported in the literatures.

3.1.1. Spectral data of compounds 1–11

1-(2′,4′-dihydroxyphenyl)-3,7,11-trimethyl-3-vinyl-6(*E*),10-dodecadien-1-one (1): ^{1}H NMR (600 MHz, $CDCl_3$) of **1**: δ_H 7.64 (1H, d, *J* = 8.2, H-6′), 6.37 (1H, d, *J* = 3, H-5′), 6.36 (1H, s, H-3′), 5.86 (1H, dd, *J* = 17.6/10.5 Hz, Vinyl-CH), 5.09 (1H, †, H-6), 5.08 (1H, †, H-10), 5.02 (1H, d, *J* = 11, Vinyl-CH_{2a}), 4.96 (1H, d, *J* = 17.6 Hz, Vinyl-CH_{2b}), 2.89 (2H, d, *J* = 4.1 Hz, H-2), 2.05 (2H, m, H-9), 1.95 (2H, †, H-5), 1.95 (2H, †, H-8), 1.68 (3H, s, H-12), 1.59 (3H, s, H-11Me), 1.58 (3H, s, H-7Me), 1.53 (2H, m, H-4), 1.17 (3H, s, H-3Me); ^{13}C NMR (150 MHz, $CDCl_3$): δ_C 204.1 (C-1), 165.5 (C-2′), 162.6 (C-4′), 145.7 (Vinyl-CH), 133.2 (C-6′), 135.1 (C-7), 131.3 (C-11), 124.3 (C-10), 124.2 (C-6), 115.1 (C-1′), 112.2 (Vinyl-CH_2), 107.6 (C-5′), 103.5 (C-3′), 47.1 (C-2), 41.1 (C-4), 40.3 (C-3), 39.7 (C-8), 26.7 (C-9), 25.7 (C-12), 23.2 (C-3Me), 22.8 (C-5), 17.7 (C-11Me), 16 (C-7Me).

2,3-dihydro-7-hydroxy-2,3-dimethyl-2-[4′,8′-dimethyl-3′,7′-nonadienyl]-furo[3,2,c]coumarin (2): ^{1}H NMR (600 MHz, $CDCl_3$) of **2**: δ_H 7.53 (1H, d, *J* = 8.6 Hz, H-9), 7.18 (1H, d, *J* = 2.4 Hz, H-6), 6.86 (1H, dd, *J* = 8.6/2.3 Hz, H-8), 5.1 (1H, t, *J* = 7.0 Hz, H-3′), 5.06 (1H, t, *J* = 7.0 Hz, H-7′), 3.29 (1H, q, *J* = 7.0 Hz, H-3), 2.12 (2H, m, H-2′), 2.04 (2H, m, H-6′), 1.95 (2H, m, H-5′), 1.8 (2H, m, H-1′), 1.67 (3H, s, H-9′), 1.58 (3H, s, H-4′Me), 1.58 (3H, s, H-8′Me), 1.46 (3H, s, H-2Me), 1.31 (3H, d, *J* = 7.0 Hz, H-3Me); ^{13}C NMR (150 MHz, $CDCl_3$): δ_C 166.1 (C-9b), 162.5 (C-4), 161.2 (C-7), 156.7 (C-5a), 135.9 (C-4′), 131.4 (C-8′), 124.1 (C-9), 124.1 (C-7′), 123.1 (C-3′), 113.4 (C-8), 105.5 (C-9a), 103.1 (C-3a), 103.1 (C-6), 97.1 (C-2), 41.8 (C-3), 41.7 (C-1′), 39.6 (C-5′), 26.6 (C-6′), 25.6 (C-9′), 22.1 (C-2′), 20.4 (C-2Me), 16 (C-4′Me), 16 (C-8′Me), 14.7 (C-3Me).

2,3-dihydro-7-hydroxy-2,3-dimethyl-3-[4′8′-dimethyl-3′,7′-nonadienyl]-furo[3,2,c]coumarin (3): ^{1}H NMR (600 MHz, $CDCl_3$) of **2**: δ_H 7.54 (1H, dd, *J* = 8.6/1.9 Hz, H-9), 7.21 (1H, d, *J* = 2.2 Hz, H-6), 6.87 (1H, dd, *J* = 8.6/2.2 Hz, H-8), 5.03 (1H, t, *J* = 6.8 Hz, H-3′), 5.03 (1H, t, *J* = 6.8 Hz, H-7′), 4.64 (1H, q, *J* = 6.7 Hz, H-2), 1.99 (2H, †, H-6′), 1.91–2.06 (2H, †, H-2′), 1.88 (2H, †, H-5′), 1.65 (2H, †, H-1′), 1.64 (3H, s, H-9′), 1.56 (3H, s, H-8′Me), 1.54 (3H, d, *J* = 6.8 Hz, H-2Me), 1.48 (3H, s, H-4′Me), 1.45 (3H, s, H-3Me); ^{13}C NMR (150 MHz, $CDCl_3$): δ_C 167.4 (C-9b), 162.4 (C-4), 161.3 (C-7), 156.8 (C-5a), 135.3 (C-4′), 131.3 (C-8′), 124.3 (C-9), 124.2 (C-7′), 124.0 (C-3′), 113.7 (C-8), 105.7 (C-9a), 105.5 (C-3a), 103.2 (C-6), 93.4 (C-2), 46.6 (C-3), 34.8 (C-1′), 39.6 (C-5′), 26.6 (C-6′), 25.7 (C-9′), 23.8 (C-2′), 13.9 (C-2Me), 23.4 (C-3Me), 17.7 (C-8′Me), 16.0 (C-4′Me).

Laserine (4): ^{1}H NMR (600 MHz, $CDCl_3$) of **4**: δ_H 6.58 (1H, d, *J* = 1.5 Hz, H-6′), 6.56 (1H, d, *J* = 0.9 Hz, H-2′), 6.09 (1H, m, H-3″), 6.05* (1H, m, H-3‴), 5.97* (2H, s, H-7′), 5.76 (1H, d, *J* = 7.4 Hz, H-1), 5.35 (1H, dq, *J* = 7.3/6.5 Hz, H-2), 3.87 (3H, s, OMe), 1.97 (3H, dq, *J* = 7.3/1.5 Hz, H-4″), 1.93* (3H, dq, *J* = 7.3/1.5 Hz, H-4‴), 1.88 (3H, p, *J* = 1.5 Hz, H-5″), 1.85* (3H, p, *J* = 1.5 Hz, H-5‴), 1.16 (3H, d, *J* = 6.5 Hz, H-3); ^{13}C NMR (150 MHz, $CDCl_3$): δ_C 167.1* (C-1‴), 166.6** (C-1″), 148.9 (C-5′), 143.5* (C-3′), 139.0 (C-3″), 138.2* (C-3‴), 135.4 (C-4′), 131.7* (C-1′), 127.8* (C-2‴), 127 (C-2″), 107.2 (C-2′), 101.6 (C-6′), 101.6 (C-7′), 77.1 (C-1), 71.2 (C-2), 56.6 (OMe), 20.6 (C-5″), 20.6* (C-5‴), 16.8 (C-3), 15.8 (C-4″), 15.7 (C-4‴).

2-Epilaserine (5): ^{1}H NMR (600 MHz, $CDCl_3$) of **5**: δ_H 6.58 (1H, d, *J* = 1.5 Hz, H-6′), 6.56 (1H, d, *J* = 0.9 Hz, H-2′), 6.13 (1H, m, H-3″), 6.06* (1H, m, H-3‴), 5.96* (2H, s, H-7′), 5.91 (1H, d, *J* = 4 Hz, H-1), 5.28 (1H, dq, *J* = 6.5/4.5 Hz, H-2), 3.87 (3H, s, OMe), 2.01 (3H, dq, *J* = 7.3/1.5 Hz, H-4″), 1.95* (3H, dq, *J* = 7.5/1.5 Hz, H-4‴), 1.96 (3H, p, *J* = 1.5 Hz, H-5″), 1.84* (3H, p, *J* = 1.5 Hz, H-5‴), 1.26 (3H, d, *J* = 6.5 Hz, H-3); ^{13}C NMR (150 MHz, $CDCl_3$): δ_C 167.2* (C-1‴), 166.5* (C-1″), 149 (C-5′), 143.4** (C-3′), 139.3 (C-3″), 138.3* (C-3‴), 135 (C-4′), 131.6* (C-1′), 127.7* (C-2‴), 127 (C-2″), 106.9 (C-2′), 101.2 (C-6′), 101.6 (C-7′), 75.9 (C-1), 71.6 (C-2), 56.6 (OMe), 20.6 (C-5″), 20.5* (C-5‴), 15.8 (C-4″), 15.7 (C-4‴), 15.1 (C-3).

***β*-sitosterol (6):** ^{1}H NMR (600 MHz, $CDCl_3$) of **6:** δ_H 5.35 (1H, †, H-6), 3.51 (1H, m, H-3), 1.01 (3H, s, H-18), 0.92 (3H, d, *J* = 6.5 Hz, H-21), 0.84 (3H, t, *J* = 7.1 Hz, H-29), 0.83 (3H, d, *J* = 7 Hz, H-27), 0.81 (3H, d, *J* = 7 Hz, H-26), 0.68 (3H, s, H-19).

Stigmasterol (7) ^{1}H NMR (600 MHz, $CDCl_3$) of **7:** δ_H 5.35 (1H, †, H-6), 5.15 (2H, dd, *J* = 15.2/8.8 Hz, H-23), 5.02 (2H, dd (*J* = 15.3/8.8 Hz, H-22), 3.51 (1H, m, H-3).

Kaempferol 3-O-*β*-D-glucopyranoside (8): ^{1}H NMR (600 MHz, CD_3OD) of **8:** δ_H 8.03 (2H, dd, *J* = 6.9/1.9 Hz, H-2′, H-6′), 6.86 (2H, dd, *J* = 6.9/1.9 Hz, H-3′, H-5′), 6.24 (1H, d, *J* = 2 Hz, H-8), 6.08 (1H, d, *J* = 2 Hz, H-6), 5.12 (1H, d, *J* = 7.5 Hz, H-1″), 3.38–3.45 (2H, †, H-2″, H-3″), 3.30 (H, †, H-4″), 3.18 (H, m, H-5″), 3.67 (1H, dd, *J* = 11.9/2.4 Hz, H-6a″), 3.53 (1H, dd, *J* = 11.9/5.4 Hz, H-6b″); ^{13}C NMR (150 MHz, CD_3OD): δ_C 178.8 (C-4), 163 (C-7), 161.7 (C-5), 161.7 (C-4′), 158.9 (C-9), 158.3 (C-2), 135.3 (C-3), 132.2 (C-2′/C-6′), 122.9 (C-1′), 116.2 (C-3′/C-5′), 104.1 (C-10), 104.6 (C-1″), 101.2 (C-6), 96.2 (C-8), 78.4 (C-3″), 78.1 (C-5″), 75.8 (C-2″), 71.3 (C-4″), 61.6 (C-6″).

Kaempferol-3-*O*-*α*-rhamnopyranoside (9): ^{1}H NMR (600 MHz, CD_3OD) of **9:** δ_H 7.76 (2H, d, *J* = 8.8 Hz, H-2′, H-6′), 6.93 (2H, d, *J* = 8.8 Hz; H-3′, H-5′), 6.36 (1H, d, *J* = 1.7 Hz, H-8), 6.19 (1H, d, *J* = 1.8 Hz, H-6), 5.38 (1H, d, *J* = 1.5 Hz, H-1″), 4.22 (1H, m, H-2″), 3.32–3.34 (2H, †, H-4″, H-5″), 0.92 (3H, d, *J* = 5,9 Hz, H-6″); ^{13}C (150 MHz, CD_3OD): δ_C 178.2 (C-4), 164.7 (C-7), 161,7 (C-5), 160.1 (C-4′), 157.8 (C-9), 157.1 (C-2), 134.8 (C-3), 130.5 (C-2′, C-6′), 121.2 (C-1′), 115.2 (C-3′, C-5′), 104.4 (C-10), 102.1 (C-1″), 98.5 (C-6), 93.4 (C-8), 71.8(C-4″), 70.7 (C-3″), 70.5 (C-2″), 70.4 (C-5″), 16.2 (CH_3).

Quercetin-3-*O*-*β*-glucopyranoside (10): ^{1}H NMR (600 MHz, CD_3OD) of **10:** δ_H 7.9 (1H, d, *J* = 2.1 Hz, H-2′), 7.45 (1H, dd, *J* = 8.5/2.1 Hz, H-6′), 6.82 (1H, d, *J* = 8.5 Hz, H-5′), 6.39 (1H, d, *J* = 2.1 Hz, H-8), 6.16 (1H, d, *J* = 2.0 Hz, H-6), 5.13 (1H, d, *J* = 7.3 Hz, H-1″), 3.63 (1H, dd, *J* = 11.8/2.5 Hz, H-6a″), 3.47 (1H, dd, *J* = 11.8/5.4 Hz, H-6b″), 3.32–3.38 (2H, †, H-2″, H-3″), 3.26 (1H,

Table 1
Antimicrobial activities of *F. caspica* and *F. halophila* extracts (MIC, μg/mL).

	Bacteria				Fungi		
	S. aureus ATCC 29,213	*E. faecalis* ATCC29212	*P. aeruginosa* ATCC27853	*E. coli* ATCC 25,922	*C. parapsilosis* ATCC 90,018	*C. krusei* ATCC6258	*C. albicans* ATCC 90,028
FC/AP/$CHCl_3$	**32**	**64**	512	512	128	256	256
FC/AP/MeOH	1024	512	512	512	256	128	256
FC/AP/EtOAc	512	512	1024	1024	256	256	128
Gentamicin	**0.12**	**8**	**1**	**0.5**	–	–	–
Fluconazole	–	–	–	–	**0.5**	**16**	**0.5**

FC: *Ferula caspica*; AP: Aerial parts; $CHCl_3$: Chloroform extract; MeOH: Methanolic extract; EtOAc: Ethyl acetate extract.
Bold value indicates FC/AP/$CHCl_3$ showed the highest antibacterial activity against *S. aureus* and *E. faecalis*

Table 2
Antioxidant capacities of *F. caspica* and *F. halophila* extracts.

Extracts	CUPRAC (mg GA/g extract)	ABTS (mg Trolox/g extract)	FRAP (mg Quercetin/g extract)	Total phenol (mg GA/g extract)	Total flavonoid (mg Quercetin/g extract)
FC/AP/$CHCl_3$	42.64 ± 5.78	88.57 ± 1.24	12.94 ± 0.55	46.51 ± 2.03	8.17 ± 0.34
FC/AP/MeOH	53.75 ± 4.48	78.66 ± 3.07	58.65 ± 0.99	59.16 ± 3.42	12.14 ± 1.67
FC/AP/EtOAc	**177.23** ± 1.17	**268.28** ± 1.84	**207.38** ± 7.42	**214.04** ± 3.21	**97.66** ± 1.89

FC: *Ferula caspica*; AP: Aerial parts; $CHCl_3$: Chloroform extract; MeOH: Methanolic extract; EtOAc: Ethyl acetate extract; GA: Gallic acid; values statistically different at $p < 0.05$.
Bold value indicates FC/AP/EtOAc was the most active extract in all antioxidant capacity test assays

m, H-4″), 3.22 (1H, m, H-5″); ^{13}C NMR (150 MHz, CD_3OD) δ_C 179.2 (C-4), 165.4 (C-7), 162.9 (C-5), 158.9 (C-2), 158.0 (C-9), 149.3 (C-4′), 145.3 (C-3′), 135.7 (C-3), 124.1 (C-1′), 122.7 (C-6′), 118.1 (C-2′), 115.9 (C-5′), 105.4 (C-10), 105.1 (C-1″), 99.8 (C-6), 94.7 (C-8), 78.1 (C-3″), 77.8 (C-5″), 75.5 (C-2″), 71.0 (C-4″), 62.8 (C-6″).

2,4-dihydroxy benzoic acid (11): ^{1}H NMR (600 MHz, CD_3OD) of **11:** δ_H 7.65 (1H, d, J = 8.5 Hz, H-6), 6.21 (1H, dd, J = 8.5/2.4 Hz, H-5), 6.18 (1H, d, J = 2.3 Hz, H-3); ^{13}C NMR (150 MHz, CD_3OD): δ_C 164.3 (C-2 or C-4), 162.9 (C-2 or C-4), 133.0 (C-6), 112.4 (C-1), 108.9 (C-5), 102.9 (C-3).

[†]Unclear due to overlapping.

[*]Signals of laserine and 2-epilaserine are interchangeable due to the similarity of the signals.

3.2. Antimicrobial activities and antioxidant capacities of the extracts

The chloroform extract of the aerial parts from *F. caspica* showed the highest antibacterial activity against gram-positive bacteria (*Enterococcus faecalis* and *Staphylococcus aureus*) with the MIC value of 32 and 64 μg/mL (Table 1), respectively.

The ethyl acetate extract from the aerial parts of *F. caspica* exhibited the highest antioxidant activity in all test assays with the value of 177.23 ± 1.17 mg gallic acid equivalent/g extract in CUPRAC assay and 268.28 ± 1.84 mg Trolox equivalent/g extract in ABTS radical cation scavenging assay as well as 207.38 ± 7.42 mg quercetin equivalent/g extract in FRAP assay (Table 2). In addition, the same extract was found to have the highest total flavonoid and total phenolic content; 97.66 ± 1.89 mg quercetin equivalent/g extract and 214.04 ± 3.21 gallic acid equivalent/g extract (Table 2), respectively.

4. Discussion

In the literature, the antioxidant capacity of *F. caspica* has not been reported, previously, and there aren't any recent studies on this species. To evaluate the antioxidant capacities of the extracts, ABTS radical cation scavenging activity, CUPRAC, and FRAP assays were tested. These methods are based on an electron transfer reaction resulted in a color change, the degree of which is related to the antioxidant concentration (Huang et al., 2005). $ABTS^{+}$, Cu(II) and Fe (III) are reduced by acquiring an electron from antioxidant in corresponding assays (Re et al., 1999; Apak et al., 2004; Berker et al., 2007). According to the literature, phenolics/flavonoids are well known with their antioxidant activities varying by structural changes (Rice-Evans et al., 1997). Dehghan et al. (2007) determined that the total phenolic contents of hexane, diethyl ether, ethyl acetate, and methanol extracts of the aerial parts and roots of *F. szovitsiana* were correlated with DPPH radical scavenging activities and FRAPs. Thereby, the high antioxidant capacity of the ethyl acetate extract of *F. caspica* is accordant with the high phenolic/flavonoid content and the literature findings (Rice-Evans et al. 1997; Dehghan et al., 2007).

The antimicrobial activity of *F. caspica* has not been reported so far. Tamemoto et al. (2001) determined the significant antimicrobial activities of sesquiterpenes isolated from *F. kuhistanica* against methicillin-sensitive and methicillin-resistant *S. aureus*. Among the petroleum ether, hexane, hot/cold water and ethanol extracts of *F. asafoetida*, the hexane extract showed the highest antimicrobial activity and apolar extracts were found to be more active than the polar extracts (Bhatnager et al., 2015). The essential oil from the seeds of *F. tunetana* inhibited the Gram (+)/Gram (−) bacteria and phenylpropanoid-rich essential oil of the underground parts of the *F. heuffelii* exhibited antimicrobial activity against Gram (+) bacteria and fungi (Pavlović et al., 2012; Znati et al., 2017). The previous reports on *F. ferulioides*, Liu et al. (2013, 2015) tested the antimicrobial activities of isolated acetophenone and furocoumarin sesquiterpenes, including compounds **1**–**3**. Compounds **1**–**3** showed antimicrobial activity against tetracycline-resistant *S. aureus* strain with the MIC values of 2 μg/mL, 2 μg/mL and 4 μg/mL, respectively. Therefore, the highest activity of the chloroform extract can be attributed to isolated compounds **1**–**5**.

5. Conclusion

This is the first report of antioxidant capacity and antimicrobial activity of *F. caspica* M Bieb. In this research, eleven secondary metabolites were isolated from chloroform (**1**–**7**) and ethyl acetate extracts (**8**–**11**). The compounds **1**, **2**, **3**, **4**, **5**, **6**, **7** and **11** have been previously isolated from several *Ferula* species, while three flavonoid derivatives (**8**–**10**) were reported for the first time in *Ferula*

species obtained from the ethyl acetate extract, which was the most active extract for antioxidant activity.

Acknowledgement

The authors thank Dr. Barış Özüdoğru, Hacettepe University, Faculty of Science, Department of Botany, Ankara, Turkey for supporting the collecting plant material and Prof. Dr. Hayri Duman, Gazi University, Faculty of Science, Department of Botany, Ankara, Turkey, for authentification of the plant specimen.

Funding

This study was supported by Hacettepe University Scientific Research Projects Coordination Unit. Project Number: 014 D03 301 002-552).

References

Abd, E.-R.M.H., Wu, Y.-C., Chang, F.-R., 2007. Sesquiterpene coumarins from *Ferula foetida*. J. Chin. Chem. Soc. 54 (1), 235–238. https://doi.org/10.1002/jccs.200700035.

Ahmed, A.A., 1991. Daucanes and other constituents from *Ferula sinica*. Phytochemistry 30 (4), 1207–1210. https://doi.org/10.1016/s0031-9422(00)95203-8.

Alkhatib, R., Hennebelle, T., Joha, S., Roumy, V., Guezel, Y., Biabiany, M., Idziorek, T., Preudhomme, C., Quesnel, B., Sahpaz, S., Bailleul, F., 2010. Humulane and germacrane sesquiterpenes from *Ferula lycia*. J. Nat. Prod. 73 (4), 780–783. https://doi.org/10.1021/np900827a.

Altundag, E., Ozturk, M., 2011. Ethnomedicinal studies on the plant resources of east Anatolia, Turkey. Proc. Soc. Behav. Sci. 19, 756–777. https://doi.org/10.1016/j.sbspro.2011.05.195.

Apak, R., Guclu, K., Ozyurek, M., Karademir, S.E., 2004. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. J. Agric. Food Chem. 52 (26), 7970–7981. https://doi.org/10.1021/jf048741x.

Barrero, A.F., Herrador, M.M., Arteaga, P., 1992. Sesquiterpenes and phenylpropanoids from *Seseli vayredanum*. Phytochemistry 31 (1), 203–207. https://doi.org/10.1016/0031-9422(91)83036-k.

Berker, K.I., Güçlü, K., Tor, İ., Apak, R., 2007. Comparative evaluation of Fe (III) reducing power-based antioxidant capacity assays in the presence of phenanthroline, batho-phenanthroline, tripyridyltriazine (FRAP), and ferricyanide reagents. Talanta 72 (3), 1157–1165. https://doi.org/10.1016/j.talanta.2007.01.019.

Bhatnager, R., Rani, R., Dang, A.S., 2015. Antibacterial activity of *Ferula asafoetida*: a comparison of red and white type. J. App. Biol. Biotech. 3 (2), 18–21. https://doi.org/10.7324/jabb.2015.3204.

Chang, C.C., Yang, M.H., Wen, H.M., Chern, J.C., 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal. 10 (3), 178–182.

Chaturvedula, V.S.P., Prakash, I., 2012. Isolation of stigmasterol and β-sitosterol from the dichloromethane extract of *Rubus suavissimus*. Int. Curr. Pharm. J. 1 (9), 239–242.

Chen, B., Takaishi, Y., Kawazoe, K., Tamemoto, K., Honda, G., Ito, M., Takeda, Y., Kodzhimatov, O.K., Ashurmetov, O., 2001. Farnesyl hydroxybenzoic acid derivatives from *Ferula kuhistanica*. Chem. Pharm. Bull. 49 (6), 707–710. https://doi.org/10.3329/icpj.v1i9.11613.

Correia, S.J., David, J.M., Silva, E.P., David, J.P., Lopes, L.M.X., Guedes, M.L.S., 2008. Flavonóides, norisoprenóides e outros terpenos das folhas de *Tapirira guianensis*. Flavonoids, norisoprenoids and other terpenes from leaves of *Tapirira guianensis*. Quím. Nova. 31, 2056–2059. https://doi.org/10.1590/s0100-40422008000800027 (Portuguese).

Dastan, D., Salehi, P., Gohari, A.R., Ebrahimi, S.N., Aliahmadi, A., Hamburger, M., 2014. Bioactive sesquiterpene coumarins from *Ferula pseudalliacea*. Planta Med. 80 (13), 1118–1123. https://doi.org/10.1055/s-0034-1382996.

Dehghan, G., Shafiee, A., Ghahremani, M.H., Ardestani, S.K., Abdollahi, M., 2007. Antioxidant potential of various extracts from *Ferula szovitsiana*. in relation to their phenolic content. Pharm. Biol. 691–699. https://doi.org/10.1080/13880200701575098.

Demirci, S., Özhatay, N., 2012. An ethnobotanical study in Kahramanmaraş (Turkey); wild plants used for medicinal purpose in Andırın, Kahramanmaraş. Turk. J. Pharm. Sci. 9 (1), 75–92.

Duman, H., Sagiroglu, M., 2005. A new species of *Ferula* (Apiaceae) from South Anatolia, Turkey. Bot. J. Linn. Soc. 147 (3), 357–361. https://doi.org/10.1111/j.1095-8339.2005.00360.x.

Galal, A.M., Abourashed, E.A., Ross, S.A., ElSohly, M.A., Al-Said, M.S., El-Feraly, F.S., 2001. Daucane sesquiterpenes from *Ferula hermonis*. J. Nat. Prod. 64 (3), 399–400. https://doi.org/10.1021/np000526x.

Güneş, F., Özhatay, N., 2011. An ethnobotanical study from Kars (Eastern) Turkey. Biol. Divers. Conserv. 4 (1), 30–41.

Güzel, Y., Güzelşemme, M., Miski, M., 2015. Ethnobotany of medicinal plants used in Antakya: a multicultural district in Hatay Province of Turkey. J. Ethnopharmacol. 174, 118–152. https://doi.org/10.1016/j.jep.2015.07.042.

Han, J.-T., Bang, M.-H., Chun, O.-K., Kim, D.-O., Lee, C.-Y., Baek, N.-I., 2004. Flavonol glycosides from the aerial parts of *Aceriphyllum rossii* and their antioxidant activities. Arch. Pharm. Res. 27 (4), 390–395. https://doi.org/10.1007/bf02980079.

Huang, D., Ou, B., Prior, B., 2005. The chemistry behind antioxidant capacity assays. J. Agric. Food Chem. 53 (6), 1841–1856. https://doi.org/10.1021/jf030723c.

Iranshahi, M., Hosseini, S.T., Shahverdi, A.R., Molazade, K., Khan, S.S., Ahmad, V.U., 2008. Diversolides A-G, guaianolides from the roots of *Ferula diversivittata*. Phytochemistry 69 (15), 2753–2757. https://doi.org/10.1016/j.phytochem.2008.08.009.

Iranshahi, M., Kalategi, F., Sahebkar, A., Sardashti, A., Schneider, B., 2010. New sesquiterpene coumarins from the roots of *Ferula flabelliloba*. Pharm. Biol. 48 (2), 217–220. https://doi.org/10.3109/13880200903019226.

Iranshahi, M., Rezaee, R., Sahebkar, A., Bassarello, C., Piacente, S., Pizza, C., 2009. Sesquiterpene coumarins from the fruits of *Ferula badrakema*. Pharm. Biol. 47 (4), 344–347. https://doi.org/10.1080/13880200902752884.

Isaka, K., Nagatsu, A., Ondognii, P., Zevgeegiin, O., Gombosurengyin, P., Davgiin, K., Kojima, K., Ogihara, Y., 2001. Sesquiterpenoid derivatives from *Ferula ferulaeoides* [correction of *ferulioides*]. V. Chem. Pharm. Bull. 49 (9), 1072–1076. https://doi.org/10.1248/cpb.49.1072.

Kojima, K., Isaka, K., Purev, O., Jargalsaikhan, G., Suran, D., Mizukami, H., Ogihara, Y., 1998. Sesquiterpenoid derivatives from *Ferula ferulioides*. Chem. Pharm. Bull. 46 (11), 1781–1784. https://doi.org/10.1248/cpb.46.1781.

Kojima, K., Isaka, K., Ondognii, P., Zevgeegiin, O., Gombosurengyin, P., Davgiin, K., Mizukami, H., Ogihara, Y., 2000. Sesquiterpenoid derivatives from *Ferula ferulioides*. IV. Chem. Pharm. Bull. 48 (3), 353–356. https://doi.org/10.1248/cpb.48.353.

Kurimoto, S., Suzuki, K., Okasaka, M., Kashiwada, Y., Kodzhimatov, O.K., Takaishi, Y., 2012. New sesquiterpene lactone glucosides from the roots of *Ferula varia*. Phytochem. Lett. 5 (4), 729–733. https://doi.org/10.1016/j.phytol.2012.08.001.

Lhuillier, A., Fabre, N., Cheble, E., Oueida, F., Maurel, S., Valentin, A., Fouraste, I., Moulis, C., 2005. Daucane Sesquiterpenes from *Ferula hermonis*. J. Nat. Prod. 68 (3), 468–471. https://doi.org/10.1021/np049652h.

Li, G., Li, X., Cao, L., Zhang, L., Shen, L., Zhu, J., Wang, J., Si, J., 2015a. Sesquiterpene coumarins from seeds of *Ferula sinkiangensis*. Fitoterapia. 103, 222–226. https://doi.org/10.1016/j.fitote.2015.03.022.

Li, G., Wang, J., Li, X., Cao, L., Lv, N., Chen, G., Zhu, J., Si, J., 2015b. Two new sesquiterpene coumarins from the seeds of *Ferula sinkiangensis*. Phytochem. Lett. 13, 123–126. https://doi.org/10.1016/j.phytol.2015.06.002.

Liu, T., Wang, S., Xu, L., Fu, W., Gibbons, S., Mu, Q., 2015. Sesquiterpenoids with Anti-MDR *Staphylococcus aureus* activities from *Ferula ferulioides*. Chem. Biodivers. 12 (4), 599–614. https://doi.org/10.1002/cbdv.201400150.

Liu, T., Osman, K., Kaatz, G.W., Gibbons, S., Mu, Q., 2013. Antibacterial sesquiterpenoid derivatives from *Ferula ferulaeoides*. Planta Med. 79 (8), 701–706. https://doi.org/10.1055/s-0032-1328461.

Meng, H., Li, G., Huang, J., Zhang, K., Wang, H., Wang, J., 2013a. Sesquiterpene coumarin and sesquiterpene chromone derivatives from *Ferula ferulaeoides* (Steud.) Korov. Fitoterapia. 86, 70–77. https://doi.org/10.1016/j.fitote.2013.02.002.

Meng, H., Li, G., Huang, J., Zhang, K., Wei, X., Ma, Y., Zhang, C., Wang, J., 2013b. Sesquiterpenoid derivatives from *Ferula ferulaeoides* (Steud.) Korov. Phytochemistry 86, 151–158. https://doi.org/10.1016/j.phytochem.2012.10.013.

Miski, M., Jakupovic, J., 1990. Daucane esters from *Ferula rigidula*. Phytochemistry 29 (1), 173–178. https://doi.org/10.1016/0031-9422(90)89032-5.

Miski, M., Mabry, T.J., 1986. New daucane esters from *Ferula tingitana*. J. Nat. Prod. 49 (4), 657–660. https://doi.org/10.1021/np50046a016.

Miski, M., Mabry, T.J., Saya, O., 1987. New daucane and germacrane esters from *Ferula orientalis* var. *orientalis*. J. Nat. Prod. 50 (5), 829–934. https://doi.org/10.1021/np50053a009.

Miski, M., Ulubelen, A., Lee, E., Mabry, T.J., 1985. Sesquiterpene-coumarin ethers of *Ferula tingitana*. J. Nat. Prod. 48 (2), 326–327. https://doi.org/10.1021/np50038a024.

Mükemre, M., Behçet, L., Çakilcioğlu, U., 2015. Ethnobotanical study on medicinal plants in villages of Çatak (Van-Turkey). J. Ethnopharmacol. 166, 361–374. https://doi.org/10.1016/j.jep.2015.03.040.

Oyaizu, M., 1986. Studies on products of browning reactions-antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44, 307–315. https://doi.org/10.5264/eiyogakuzashi.44.307.

Pavlović, I., Petrović, S., Radenković, M., Milenković, M., Couladis, M., Branković, S., Drobac, M., Niketić, M., 2012. Composition, antimicrobial, antiradical and spasmolytic activity of *Ferula heuffelii* Griseb. ex Heuffel (Apiaceae) essential oil. Food Chem. 130 (2), 310–315. https://doi.org/10.1016/j.foodchem.2011.07.043.

Peşmen, H., 1972. Ferula L. In: Davis, P.H. (Ed.), Flora of Turkey and the East Aegean Islands. University Press, Edinburgh, pp. 440–453.

Pimenov, M.G., Kljuykov, E.V., 2013. *Ferula divaricata* (Umbelliferae), a new species from Central Anatolia, Turkey. Phytotaxa 99 (1), 35–39 https://doi.org/10.11646/phytotaxa.99.1.2.

Pimenov, M.G., Leonev, M.V., 2004. The Asian Umbelliferae biodiversity database (ASIUM) with particular reference to South-West Asian taxa. Turk. J. Botany. 28, 139–145.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26 (9–10), 1231–1237. https://doi.org/10.1016/s0891-5849(98)00315-3.

Rice-Evans, C.A., Miller, N.J., Paganga, G., 1997. Antioxidant properties of phenolic compounds. Trends Plant Sci. 2 (4), 152–159. https://doi.org/10.1016/S1360-1385(97)01018-2.

Sağıroğlu, M., 2005. Türkiye *Ferula* L. (Umbelliferae) cinsi'nin revizyonu [Revision of Turkish *Ferula* L. (Umbelliferae)] [dissertation]. Gazi Üniversitesi, Ankara.

Sagiroglu, M., Duman, H., 2007. *Ferula mervynii* (Apiaceae), a distinct new species from North-East Anatolia, Turkey. Bot. J. Linn. Soc. 153 (3), 357–362. https://doi.org/10.1111/j.1095-8339.2007.00615.x.

Sagiroglu, M., Duman, H., 2010. Ferula *brevipedicellata* and *F. duranii* (Apiaceae), two new species from Anatolia, Turkey. Ann. Bot. Fenn. 47 (4), 293–300. https://doi.org/10.5735/085.047.0405.

Sağıroğlu, M., Duman, H., 2014. Are *Ferula tenuissima* and *F. amanicola* distinct species or not? Biol. Divers. Conserv. 7 (3), 74–77.

Scott, K.N., 1972. Carbon-13 nuclear magnetic resonance of biologically important aromatic acids. I. Chemical shifts of benzoic acid and derivatives. J. Am. Chem. Soc. 94 (24), 8564–8568. https://doi.org/10.1021/ja00779a045.

Slinkard, K., Singleton, V.L., 1977. Total phenol analysis: automation and comparison with manual methods. Am. J. Enol. Vitic. 28 (1), 49–55.

Tamemoto, K., Takaishi, Y., Chen, B., Kawazoe, K., Shibata, H., Higuti, T., Honda, G., Ito, M., Takeda, Y., Kodzhimatov, O.K., Ashurmetov, O., 2001. Sesquiterpenoids from the fruits of *Ferula kuhistanica* and antibacterial activity of the constituents of *F. kuhistanica*. Phytochemistry 58 (5), 763–767. https://doi.org/10.1016/S00319422(01)00307-7.

Yaqoob, U., Nawchoo, I.A., 2016. Distribution and taxonomy of *Ferula* L.: a review. Res. Rev. J. Bot. 5 (3), 15–23.

Wayne, P., 2008a. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically: Approved Standard. Clinical and Laboratory Standards Institute.

Wayne, P., 2008b. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts: Approved Standard. Clinical and Laboratory Standards Institute.

Yang, J.-R., An, Z., Li, Z.-H., Jing, S., Qin, H.-L., 2006. Sesquiterpene coumarins from the roots of *Ferula sinkiangensis* and *Ferula teterrima*. Chem. Pharm. Bull. 54 (11), 1595–1598. https://doi.org/10.1248/cpb.54.1595.

Znati, M., Ben Jannet, H., Cazaux, S., Souchard, J.P., Skhiri, F.H., Bouajila, J., 2014. Antioxidant, 5-lipoxygenase inhibitory and cytotoxic activities of compounds isolated from the *Ferula lutea* flowers. Molecules 19 (10), 16959–16975. https://doi.org/10.3390/molecules191016959.

Znati, M., Filali, I., Jabrane, A., Casanova, J., Bouajila, J., Ben Jannet, H., 2017. Chemical composition and in vitro evaluation of antimicrobial, antioxidant and antigerminative properties of the seed oil from the Tunisian endemic Ferula *tunetana* Pomel ex Batt. https://doi.org/10.1002/cbdv.201600116.