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Essential Oil Analysis and Isolation of Coumarins and Flavonol Glycosides of *Ferulago angulata* (Schltdl.) Boiss. Fruits

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Running title: Phytochemical Constitutes of *Ferulago angulata* Fruits

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Abstract

Background: *Ferulago angulata* (Schltdl.) Boiss. is a herbaceous perennial plant distributed in Iran, Turkey and Iraq. The aromatic aerial parts of this plant are commonly used as antiseptic, sedative, wound healing, analgesic and food additive.

Methods: Column chromatography on silica gel (normal phase and RP-18) and Sephadex LH-20, along with recrystallization method were applied to isolation of the phytochemicals extracted from *F. angulata* fruits. The structures of the isolated compounds were characterized by ^{1}H-NMR and ^{13}C-NMR spectral analysis. Chemical composition of the fruits essential oil obtained by hydrodistillation (HD) and steam distillation (SD) methods were also analyzed using GC-MS technique.

Results: Six coumarin derivatives; suberosin (**1**), isoimperatorin (**2**), imperatorin (**3**), bergapten (**4**), tamarin (**5**) and suberenol (**6**), a monoterpene glycoside; verbenone-5-O-β-D-glucopyranoside (**7**), together with five flavonol-3-O-glycosides; isorhamnetin-3-O-rutinoside (narcissin) (**8**), kaempferol-3-O-rutinoside (nicotiflorin) (**9**), quercetin-3-O-rutinoside (rutin) (**10**), isorhamnetin-3-O-β-D-glucuronide (**11**), isorhamnetin-3-O-β-D-glucopyranoside (**12**) were isolated from *F. angulata* fruits. Essential oil extraction using HD and SD methods afforded colorless oils in 4.1 and 1.8% (v/w) yields, respectively. A total of 28 compounds were identified in essential oils, of which (*Z*)-β-ocimene (HD; 48.97%, SD; 50.02%), α-pinene (HD; 21.32%, SD; 23.06%) and *allo*-ocimene (HD; 6.98%, SD; 5.61%) were the main compounds.

Conclusion: This study introduces *F. angulata* fruits as a new source of coumarin derivatives and flavonoid glycosides. The presence of these compounds with known biological properties provides more medicinal potentials for the fruits of *F. angulata*. The present study also reports hydrodistillation, as an efficient method for extraction of essential oil from these aromatic fruits.

Keywords: *Ferulago angulata* (Schltdl.) Boiss, Coumarin derivatives, flavonoid glycosides, essential oil, GC-MS

Introduction

Ferulago angulata (schltdl.) Boiss. is one of nine species from the genus *Ferulago* W.D. Koch. represented in flora of Iran.[1] In Iran and Turkey, the aromatic aerial parts of this plant (Local names; Chavil, Chavir and Chevir) are added by indigenous peoples to local dairy and oils as flavour and preservative.[2,3] Ethnobotanical studies have also reported some traditional uses such as antiseptic, sedative, wound healing, as well as for the alleviation of renal pains for the aerial parts of *F. angulata*.[2,4,5] Previous studies have shown antioxidant, antimicrobial, anxiolytic, antidepressant, antiamnesic, antitumor and hepatoprotective effects of *F. angulata* aerial parts.[6-12] In 2006, Sajjadi *et al*. reported the isolation of two prenylated coumarins, suberosin and suberosin epoxide, from aerial parts of this plant.[13] In their further studies, suberosin epoxide has been demonstrated to possess considerable antiprotozoal activity against *Leishmania major* and *Plasmodium berghei*.[13,14] Xanthotoxin, isoimperatorin, oxypeucedanin, oxypeucedanin hydrate, prantschimgin, quercetin, rutin, along with stigmasterol, β-sitosterol and β-sitosterol linoleate are the other compounds reported from the aerial parts of *F. angulata* during previous phytochemical studies.[15-17] There are also some reports in the literature on essential oil constituents of *F. angulata*.[7,8,18,19] Nevertheless, data on phytochemical constituents and biological activity of the fruits of this species are still scare. Therefore, the present study was designed to isolate and identify the phytochemical constituents of the dichloromethane and hydroalcoholic extracts of *F. angulata* fruits, as well as to analyse its essential oil composition extracted by hydrodistillation and steam-distillation methods.

Materials and methods

Plant material

The fruits of *Ferulago angulata* (Schltdl.) Boiss. were purchased from Pakan-Bazr Co., Isfahan, Iran (Plant source: Fereydunshahr region, Isfahan, Iran.; Collection date: July 2017). The identity of the fruits was authenticated by botanist Dr. Yousef Ajani and the code of PMP-2672 was assigned for it at the herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Extraction

Extraction procedure from the dried and comminuted fruits of *F. angulata* (1.5 kg) was done using maceration method with dichloromethane and methanol-water (70:30), successively (4×5 L, each). The obtained extracts were concentrated using a rotary evaporator at 45 °C under the reduced pressure and then dried in vacuum oven (Extraction yields; 14.6 and 12.0% (w/w), for dichloromethane and hydroalcoholic extracts, respectively).

Isolation and purification of the compounds

A portion of the dichloromethane extract (50 g) was chromatographed on a normal phase silica gel column (Mesh 230-400, Merck) using a gradient solvent mixture of *n*-Hexane-EtOAc (90:10 to 50:50) to get nineteen fractions (D1-19). Fraction D3 was recrystallized from boiling ethanol to give compound **1** (45 mg). Compound **2** (9.9 mg) was obtained from fraction D9 through silica gel column chromatography with *n*-Hexane-EtOAc (90:10). Recrystallization of fractions D12 and D15 by boiling ethanol resulted in the purification of compounds **3** (42.8 mg) and **4** (25.3 mg), respectively. Column chromatography of fraction D17 on silica gel using $CHCl_3$-EtOAc (90:10) led to the isolation of compounds **5** (18.8 mg) and **6** (7.4 mg).

Hydroalcoholic extract (284 g) was suspended in water and fractionated by enough volume of *n*-butanol. A portion of *n*-butanol fraction (10 g) was divided to seventeen subfractions (B1-17) via sephadex LH-20 (GE Healthcare) column chromatography with methanol as mobile phase. Subfraction B1 yielded a yellow amorphous solid which was identified as compound **7** (32 mg). Reversed phase chromatography of subfraction I on a RP18 (Mesh 230-400, Fluka) column with methanol-water (2:8 to 5:5) afforded compound **8** (22 mg). Subfraction J was eluted on a sephadex LH-20 column using methanol-water (8:2) to get compound **9** (7 mg). Chromatography of subfraction K on a silica gel column ($CHCl_3$-MeOH, 9:1 to 7:3) led to eleven subfractions K1-11. Compound **10** (23) was obtained from subfraction K9 by chromatography on sephadex LH-20 column using methanol-water (8:2). Normal phase column

chromatography of subfraction N on a silica gel column with $CHCl_3$-MeOH (8:2) resulted in the isolation of compounds **11** (14 mg) and **12** (8 mg).

Monitoring of the column chromatography was carried out by thin layer chromatography (Pre-coated silica gel GF_{254} plates, Merck) and fractions giving similar spots under UV (254 and 366 nm) were combined. The structures of the compounds were elucidated by ^{1}H-NMR and ^{13}C-NMR (Bruker Avance 400 DRX, 400 MHz for ^{1}H and 100 MHz for ^{13}C) spectral analysis, as well as by comparing with the data published in the literature.

Essential oils extraction

The comminuted fruits (100 g) were subjected to hydrodistillation and steam-distillation for 3 hours, individually, using a Clevenger apparatus. The obtained oils were dried over anhydrous sodium sulfate and keep at 4 °C until analyses.

GC-MS analysis

The obtained essential oils were analyzed on a Agilent 6890 gas chromatograph equipped with a BPX5 fused silica column (30 m × 0.25 mm id, 0.25μm film thickness), coupled with Agilent 5973N mass detector (Ionization energy: 70 eV) under the following conditions; 5 min after injection, oven temperature was increased from 50°C to 240 °C at a rate of 3 °C min^{-1} and then reached to 300 °C at rate of 15 °C min^{-1} and hold 3 min in this temperature. Injector temperature: 250 °C, detector temperature: 220 °C, injection volume: 1.0 μl, split ratio: 1:35, carrier gas: helium (99.999%, Flow rate: 0.5 ml min^{-1}). The retention indices (RIs) were calculated for all identified compounds using a homologous series of *n*-alkanes injected under the same conditions described to samples. Identification of the compounds was carried out based on computer matching, as well as by comparison of RRIs and mass fragmentation patterns with those published for standard compounds.[20]

Results and Discussion

Phytochemical analysis of the fruits of *F. angulata* using chromatography on normal and reversed phase (RP-18) silica gel and Sephadex LH-20 columns resulted in the isolation of six compounds (**1**-**6**) from

dichloromethane extract and six compounds (**7**-**12**) from hydroalcoholic extract. The structures of the isolated compounds were characterized as two 5/8-prenyloxy linear furanocoumarins; isoimperatorin (**2**) and imperatorin (**3**), three 6-C-prenyl coumarins; suberosin (**1**), tamarin (**5**) and suberenol (**6**), a simple linear furanocoumarins; bergapten (**4**), a monoterpene glycoside; verbenone-5-O-β-D-glucopyranoside (**7**), together with five flavonol-3-O-glycosides; isorhamnetin-3-O-rutinoside (narcissin) (**8**), kaempferol-3-O-rutinoside (nicotiflorin) (**9**), quercetin-3-O-rutinoside (rutin) (**10**), isorhamnetin-3-O-β-D-glucuronide (**11**) and isorhamnetin-3-O-β-D-glucopyranoside (**12**) (Figure **1**) using ^{1}H-NMR and ^{13}C-NMR spectral analysis, as well as by comparison with literature data.[20-29]

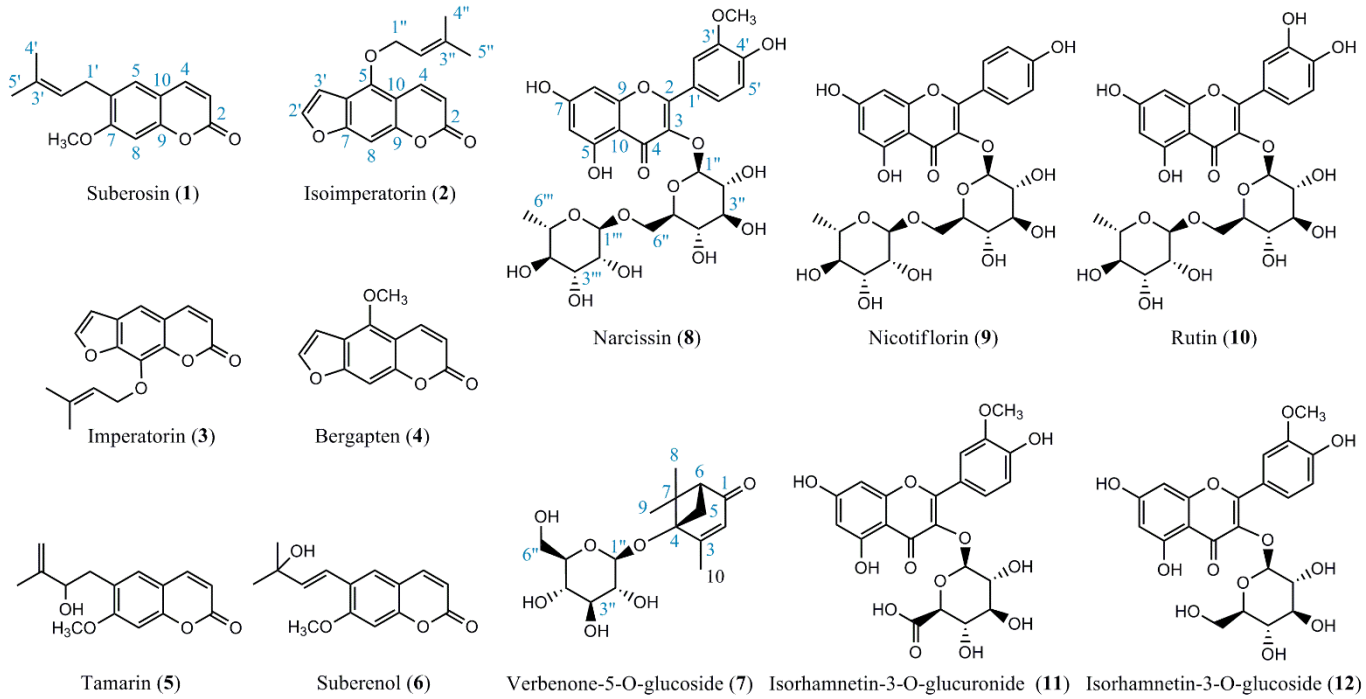

Figure 1. Structures of the isolated compounds from *F. angulata* fruits

Spectroscopic data of the isolated compounds

Compound **1**; *Suberosin* ($C_{15}H_{16}O_3$); White needle crystals; R_f = 0.70 (*n*-hexane-EtOAc, 8:2); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 7.6 (1H, *d*, *J*= 9.4 Hz, H-4), 7.1 (1H, *s*, H-5), 6.7 (1H, *s*, H-8), 6.2 (1H, *d*, *J*= 9.4 Hz, H-3), 5.2 (1H, *t sep*, *J*= 7.3, 1.2 Hz, H-2'), 3.8 (3H, *s*, OCH3), 3.3 (2H, *t*, *J*= 7.4, 1.2 Hz, H-1'), 1.77 (3H, *s*, H-4'), 1.70 (3H, *s*, H-5'). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ_C 161.6 (C-2), 160.6 (C-7), 154.5 (C-9),

143.7 (C-4), 133.7 (C-3'), 127.5 (C-6), 127.4 (C-5), 121.3 (C-2'), 112.7 (C-3), 111.7 (C-10), 98.5 (C-8), 55.9 (OCH_3), 27.79 (C-1'), 25.85 (C-4'), 17.78 (C-5').[21]

Compound **2**; *Isoimperatorin* ($C_{16}H_{14}O_4$); White needle crystals; R_f = 0.70 (*n*-hexane-EtOAc, 8:2); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 8.1 (1H, *d*, *J*= 9.7 Hz, H-4), 7.6 (1H, *d*, *J*= 2.3 Hz, H-2'), 7.1 (1H, *s*, H-8), 6.9 (1H, *d*, *J*= 2.3 Hz, H-3'), 6.2 (1H, *d*, *J*= 9.7 Hz, H-3), 5.5 (1H, *t*, *J*= 7 Hz, H-2''), 4.9 (2H, *d*, *J*= 7 Hz, H-1''), 1.8 (3H, *s*, H-4''), 1.7 (3H, *s*, H-5''). ^{13}C-NMR (100 MHz, $CDCl_3$): δ 160.1 (C-2), 157.5 (C-7), 152.0 (C-9), 148.6 (C-5), 146.0 (C-2'), 139.5 (C-4), 138.9 (C-3''), 119.6 (C-2''), 113.7 (C-6), 112.3 (C-3), 106.6 (C-10), 105.5 (C-3'), 93.5 (C-8), 69.2 (C-1''), 25.4 (C-5''), 18.0 (C-4'').[22]

Compound **3**; *Imperatorin* ($C_{16}H_{14}O_4$); White cube crystals; R_f = 0.50 (*n*-hexane-EtOAc, 8:2); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 7.77 (1H, *d*, *J*= 9.6 Hz, H-4), 7.70 (1H, *d*, *J*= 2.2 Hz, H-2'), 7.3 (1H, *s*, H-5), 6.8 (1H, *d*, *J*= 2.2 Hz, H-3'), 6.3 (1H, *d*, *J*= 9.6 Hz, H-3), 5.6 (1H, *t*, *J*= 7.2 Hz, H-2''), 5 (2H, *d*, *J*= 7.2 Hz, H-1''), 1.74 (3H, *s*, H-4''), 1.72 (3H, *s*, H-5''). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ_C 160.6 (C-2), 148.6 (C-7), 146.6 (C-2'), 144.4 (C-4), 143.8 (C-9), 139.8 (C-3''), 131.6 (C-8), 125.9 (C-6), 119.8 (C-2''), 116.5 (C-10), 114.6 (C-5), 113.2 (C-3), 106.8 (C-3'), 70.1 (C-1''), 25.8 (C-5''), 18.1 (C-4'').[23]

Compound **4**; Bergapten ($C_{12}H_8O_4$); White amorphous powder; R_f = 0.45 (*n*-hexane-EtOAc, 8:2); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 8.1 (1H, *d*, *J*= 9.8 Hz, H-4), 7.59 (1H, *d*, *J*= 2.3 Hz, H-2'), 7.1 (1H, *s*, H-8), 7.02 (1H, *d*, *J*= 2.3 Hz, H-3'), 6.2 (1H, *d*, *J*= 9.8 Hz, H-3), 4.27 (3H, *s*, OCH3). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ_C 161.3 (C-2), 158.4 (C-7), 152.7 (C-9), 149.6 (C-5), 144.8 (C-2'), 139.3 (C-4), 112.6 (C-6), 112.5 (C-3), 106.3 (C-10), 105.1 (C-3'), 93.8 (C-8), 60.1 (OCH_3).[23]

Compound **5**; *Tamarin* ($C_{15}H_{16}O_4$); White amorphous powder; R_f = 0.50 ($CHCl_3$-EtOAc, 9:1); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 7.6 (1H, *d*, *J*= 9.4 Hz, H4), 7.2 (1H, *s*, H5), 6.7 (1H, *s*, H8), 6.2 (1H, *d*, *J*= 9.4 Hz,

H3), 4.93 (1H, *s*, H4'a), 4.84 (1H, *s*, H4'b), 4.3 (1H, *dd*, *J*= 8.6, 4.0 Hz, H2'), 3.9 (3H, *s*, OCH3), 3.0 (1H, *dd*, *J*= 13.8, 4.0 Hz, H1'a), 2.7 (1H, *dd*, *J*= 13.8, 8.6 Hz, H1'b), 1.8 (3H, *s*, H5'-Me). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ_C 161.3 (C-2), 160.8 (C-7), 154.8 (C-9), 147.1 (C-3'), 143.5 (C-4), 129.7 (C-5), 124.4 (C-6), 113.0 (C-10), 112.0 (C-3), 110.8 (C-4'), 98.8 (C-8), 74.9 (C-2'), 55.9 (OCH_3), 36.2 (C-1'), 18.1 (C-5').[24]

Compound **6**; *Suberenol* ($C_{15}H_{16}O_4$); White amorphous powder; R_f = 0.45 ($CHCl_3$-EtOAc, 9:1); ^{1}H-NMR ($CDCl_3$, 400 MHz): δ_H 7.6 (1H, *d*, *J*= 9.5 Hz, H4), 7.4 (1H, *s*, H5), 6.8 (1H, *d*, *J*= 16.2 Hz, H1'), 6.7 (1H, *s*, H8), 6.3 (1H, *d*, *J*= 16.2 Hz, H2'), 6.2 (1H, *d*, *J*= 9.5 Hz, H3), 3.9 (3H, *s*, H7-OCH3), 1.4 (6H, *s*, H4' and 5'); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 160.4 (C-2), 159.3 (C-7), 154.5 (C-9), 144.4 (C-4), 140.5 (C-2'), 125.3 (C-1'), 123.2 (C-6), 118.0 (C-5), 112.8 (C-3), 112.1 (C-10), 99.2 (C-8), 69.5 (C-3'), 56.3 (OCH_3), 30.1 (C-4',5').[25]

Compound **7**; *Verbenone-5-O-β-D-glucopyranoside* ($C_{16}H_{24}O_7$); Yellow amorphous solid; R_f = 0.45 (EtOAc-Formic acid-Acetic acid-water, 26:1:1:2); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ_H 5.6 (1H, *br s*, H-2), 4.4 (1H, *d*, *J*= 7.7 Hz, H-1'), 3.0-3.7 (7-H, *overlapped signals*, H5b & H2'-H6'), 2.4 (1H, *dd*, *J*= 6.8, 2.4 Hz , H-6), 2.3 (1H, *d*, 9.2 Hz, H-5a), 2.0 (3H, *br s*, H-10), 1.4 (3H, *s*, H-8), 0.9 (3H, *s*, H-9). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ_C 201.7 (C-1), 173.9 (C-3), 120.6 (C-2), 99.2 (C-1'), 83.0 (C-4), 77.3 (3'), 77.2 (C-5'), 74.0 (C-2'), 70.6 (C-4'), 61.5 (C-7), 59.4 (C-6'), 51.4 (C-6), 43.4 (C-5), 22.7 (C-8), 20.6 (C-9), 20.1 (C-10).[26]

Compound **8**; *Isorhamnetin-3-O-rutinoside* (*Narcissin*) ($C_{28}H_{32}O_{16}$); Yellow amorphous solid; R_f = 0.3 (EtOAc-Formic acid-Acetic acid-water, 26:1:1:2); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ_H 7.8 (1H, *d*, *J*= 1.5 Hz , H-2'), 7.5 (1H, *dd*, *J*= 8.4, 1.5 Hz, H-6'), 6.9 (1H, *d*, *J*= 8.4 Hz, H-5'), 6.4 (1H, *d*, *J*= 2 Hz, H-8), 6.2 (1H, *d*, *J*= 2 Hz, H-6), 5.4 (1H, *d*, *J*= 7.1 Hz, H-1''), 4.4 (1H, *br s*, H1'''), 3.8 (3H, *s*, OCH_3), 3.0-3.8 (9H, *overlapped signals*, H2''-H6'' and H2'''-H5'''), 1.0 (3H, *d*, *J*= 6.1 Hz, H6'''). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ_C 177.7 (C-4), 166.6 (C-7), 161.6 (C-5), 157.0 (C-9), 156.9 (C-2), 149.9 (C-4'), 147.3 (C-3'), 133.4 (C-3), 122.7 (C-6'), 121.5 (C-1'), 115.7 (C-5'), 113.7 (C-2'), 104.3 (C-10), 101.7 (C-1''), 101.4 (C-

1'''), 99.3 (C-6), 94.3 (C-8), 76.8 (C-5''), 76.4 (C-3''), 74.7 (C-2''), 72.2 (C-4'''), 71.0 (C-3'''), 70.8 (C-2'''), 70.5 (C-4''), 68.8 (C-5'''), 67.3 (C-6''), 56.1 (OCH_3), 18.2 (C-6''').[27]

Compound **9**; *Kaempferol-3-O-rutinoside* (*Nicotiflorin*) ($C_{27}H_{30}O_{15}$); Yellow amorphous solid; R_f = 0.27 (EtOAc-Formic acid-Acetic acid-water, 26:1:1:2); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ_H 8.0 (2H, *d*, *J*= 8.8 Hz, H-2' and H-6'), 6.9 (2H, *d*, *J*= 8.8 Hz, H-3' and H-5'), 6.4 (1H, *d*, *J*= 1.8 Hz, H-8), 6.2 (1H, *d*, *J*= 1.8 Hz, H-6), 5.3 (1H, *d*, *J*= 7.4 Hz, H-1''), 4.4 (1H, *br s*, H-1'''), 3.1-3.7 (11H, *overlapped signals*, H2''-H6'' and H2'''-H5'''), 1.0 (3H, *d*, *J*= 6.2 Hz, H-6'''). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ_C 176.4 (C-4), 164.1 (C-7), 160.4 (C-5), 159.1 (C-4'), 156.2 (C-9), 156.0 (C-2), 132.5 (C-3), 130.2 (C-2', C-6'), 120.1 (C-1'), 114.3 (C-3', C-5'), 103.1 (C-10), 100.8 (C-1''), 100.1 (C-1'''), 98.2 (C-6), 93.1 (C-8), 75.7 (C-5''), 75.1 (C-3''), 73.6 (C-2''), 71.1 (C-4'''), 70.0 (C-3'''), 69.7 (C-2'''), 69.3 (C-4''), 67.6 (C-5'''), 66.3 (C-6''), 17.2 (C-6''').[28]

Compound **10**; *Quercetin-3-O-rutinoside* (*Rutin*) ($C_{27}H_{30}O_{16}$); Yellow amorphous solid; R_f = 0.25 (EtOAc-Formic acid-Acetic acid-water, 26:1:1:2); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ_H 7.6 (1H, *br d*, *J*= 8.1 Hz, H-6'), 7.5 (1H, *br s*, H-2'), 6.8 (1H, *d*, *J*= 8.1 Hz, H-5'), 6.4 (1H, *br s*, H-8), 6.2 (1H, *br s* ,H-6), 5.3 (1H, *d*, *J*= 6.8 Hz, H-1''), 4.4 (1H, *br s*, H-1'''), 3.0-3.8 (11H, *overlapped signals*, H2''-H6'' and H2'''-H5'''), 1.0 (3H, *d*, *J*= 6.1 Hz, H-6'''). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ_C 178.0 (C-4), 164.8 (C-7), 166.6 (C-5), 157.0 (C-9), 156.9 (C-2), 148.9 (C-4'), 45.2 (C-3'), 133.7 (C-3), 122.0 (C-6'), 121.6 (1'), 116.7 (C-5'), 115.7 (C-2'), 104.3 (C-10), 101.6 (C-1'''), 101.2 (C-1''), 99.2 (C-6), 94.1 (C-8), 76.9 (C-3''), 76.3 (C-5''), 74.5 (C-2''), 72.3 (C-4'''), 71.0 (C-3'''), 70.8 (C-2'''), 70.4 (C-4''), 68.7 (C-5'''), 67.4 (C-6''), 18.2 (C-6''').[27]

Compound **11**; *Isorhamnetin-3-O-β-D-glucuronide* ($C_{22}H_{20}O_{13}$); Yellow amorphous solid; R_f = 0.80 (EtOAc-Formic acid-Acetic acid-water, 26:1:1:2); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ_H 7.9 (1H, *d*, *J*= 1.6 Hz, H-2'), 7.4 (1H, *dd*, *J*= 6.7, 1.6 Hz, H-6'), 6.9 (1H, *d*, *J*= 6.7 Hz, H-5'), 6.2 (1H, *br s*, H-8), 6.0 (1H, *br*

s, H-6), 5.5 (1H, *d*, *J*= 5.9 Hz, H-1"), 3.8 (3H, *s*, OCH_3), 3.1-3.7 (4H, *overlapped signals*, H2"-H5"). ^{13}C-NMR (DMSO-d_6, 100 MHz): δ_C 176.3 (C-4), 170.1 (C-6"), 165.2 (C-7), 162.1 (C-5), 157.2 (C-9), 155.7 (C-2), 149.6 (C-4'), 147.3 (C-3'), 133.2 (C-3), 122.3 (C-6'), 121.7 (1'), 115.5 (C-5'), 113.8 (C-2'), 102.7 (C-10), 101.4 (C-1"), 100.2 (C-6), 94.8 (C-8), 77.7 (C-3"), 76.7 (C-5"), 74.7 (C-2"), 70.1 (C-4"), 56.1 (OCH_3).[29]

Compound **12**; *Isorhamnetin-3-O-β-D-glucopyranoside* ($C_{22}H_{22}O_{12}$); Yellow amorphous solid; R_f = 0.70 (EtOAc-Formic acid-Acetic acid-water, 26:1:1:2); ^{1}H-NMR (DMSO-d_6, 400 MHz): δ_H 7.9 (1H, *d*, *J*= 1.8 Hz, H-2'), 7.9 (1H, *dd*, *J*= 8.4, 1.5 Hz, H-6'), 6.9 (1H, *d*, *J*= 8.4 Hz, H-5'), 6.4 (1H, *d*, *J*= 1.5 Hz, H-8), 6.2 (1H, *d*, *J*= 1.5 Hz, H-6), 5.6 (1H, *d*, *J*= 7.2 Hz, H-1"), 3.8 (3H, *s*, OCH_3), 3.1-3.6 (6H, *overlapped signals*, H2"-H6"). ^{13}C-NMR (DMSO-*d*6, 100 MHz): δ_C 177. 8 (C-4), 164.6 (C-7), 161.4 (C-5), 156.9 (C-9),156.7 (C-2), 149.6 (C-3′), 147.3 (C-4′), 133.5 (C-3), 122.5 (C-6′) , 121.6 (C-1′), 115.6 (C-5′), 113.9 (C-2′), 104.4 (C-10), 101.2 (C-1″), 99.1 (C-6), 94. 2 (C-8), 77.8 (C-5″), 76.6 (C-3″), 74.6 (C-2″), 70.1 (C-4″) , 60.9 (C-6″), 56.1 (OCH_3).[30]

This study reports the isolation of mentioned compounds (**1**-**12**) from the fruits of *F. angulata* for the first time. Moreover, this is the first report on isolation of tamarin (**5**), verbenone-5-O-β-D-glucopyranoside (**7**), isorhamnetin-3-O-rutinoside (**8**), kaempferol-3-O-rutinoside (**9**), isorhamnetin-3-O-β-D-glucuronide (**11**) and isorhamnetin-3-O-β-D-glucopyranoside (**12**) from the genus *Ferulago* W. Koch..

Among the isolated compounds, suberosin (**1**), isoimperatorin (**2**), and rutin (**10**) have previously been isolated from the aerial parts of *F. angulata*.[13,15,17] The present study reveals some differences between coumarin derivatives present in the fruits (suberosin (**1**), isoimperatorin (**2**), imperatorin (**3**), bergapten (**4**), tamarin (**5**) and suberenol (**6**)) and those previously reported from the aerial parts of *F. angulata* (Xanthotoxin, isoimperatorin, oxypeucedanin, oxypeucedanin hydrate, prantschimgin, suberosin and suberosin epoxide),[13,15,16] which may be raised from difference in their biosynthetic pathways. In 2018,

Tavakoli *et al*. reported the isolation of thirteen coumarin derivatives from the roots and fruits of *Ferulago trifida*, among them suberosin showed higher preferential toxicity against MDA-MB-23 cells (IC_{50}: 0.21 mM, Selectivity index: 5.0), in comparison with tamoxifen (IC_{50}: 0.012 mM, Selectivity index: 2.45) and strong antibacterial activity against *S. epidermidis* (Inhibition zone; 19 mm, MIC; 250 µg/ml).[25] In the mentioned study, suberenol was also found as a potent antioxidant in ferric reducing antioxidant power (FRAP) test (251.2 ± 6.2 mmol FSE/100 g).[25] In another study, phytochemical analysis of *Ferulago bracteata* roots using a bioassay guided approach resulted in the isolation of suberosin, bergapten, imperatorin, and other nine compounds, of which suberosin showed the potent α-glucosidase inhibitory activity (IC_{50}: 0.89 mg/ml) in comparison with reference standard acarbose (IC_{50} 4.95 mg/ml).[31] Anti-inflammatory potential of suberosin, a major prenylated coumarin isolated from *F. angulata* fruits, has been demonstrated by its inhibitory effects of proliferation of human peripheral blood mononuclear cells (HPBC) proliferation through reduction of intracellular Ca^{2+} concentration and inhibition of ERK, NF-AT, and NF-κB activation.[32] Imperatorin, another major phytochemical present in *F. angulata* fruits, has been documented in literature for its various biological effects such as antihypertensive,[33] cardioprotective,[34] hepatoprotective,[35] anticancer,[36] anticonvulsant,[37] antidepressant,[38] and protective effect against memory impairment.[39] Recently, Xian *et al*. reported the inhibitory role of imperatorin on inflammatory cytokines production and inflammatory cells infiltration in OVA-induced airway remodeling model.[40] They showed the involvement of Nrf2/HO-1 signaling pathway in anti-inflammatory effects of imperatorin.[40]

Verbenone-5-O-β-D-glucopyranoside (**7**) is a rare monoterpene glycoside, previously reported from the aerial parts of *Prangos tschimganica* and *Echinophora cinerea* (Apiaceae).[26, 41]

Five flavonol-3-O-glycosides, namely, narcissin (**8**), nicotiflorin (**9**), rutin (**10**), isorhamnetin-3-O-β-D-glucuronide (**11**) and isorhamnetin-3-O-β-D-glucopyranoside (**12**) were isolated from the fruits of *F. angulata* in the present study. There are few reports in literature on flavonoids present in the genus *Ferulago*. In an experiment, Doganca *et al*. reported two flavonoid derivatives, isorhamnetin 3-

galactoside and 6-hydroxyapigenin 6-methyl ether, from chloroform extract of the aerial parts of *Ferulago aucheri*.[42] Moreover, the presence of flavonoid aglycons; quercetin, isorhamnetin and luteolin have been detected in some *Ferulago* species from Turkey.[43] The potential role of flavonoids with free radical scavenging activity in prevention of oxidative stress related diseases such as diabetes, cancers, neurodegenerative, cardiovascular and inflammatory diseases is well-known.[44] Therefore, the presence of flavonoids with the mentioned health beneficial effects in the fruits of *F. angulata* provides more medicinal potentials for this medicinal plant.

In this work, chemical constituents of the essential oils obtained from the fruits of *F. angulata* using hydrodistillation (HD) and steam-distillation (SD) methods were also investigated. The yield of essential oil extraction in HD (4.1 % (v/w)) was found higher than SD (1.8 % (v/w)). GC-MS and GC-FID analysis of the oils obtained by HD and SD methods led to the identification of the 24 and 23 compounds, representing 97.28 and 97.17% of the oils, respectively (Table 1). Among the identified compounds, (*Z*)-β-ocimene (HD; 48.97%, SD; 50.02%), α-pinene (HD; 21.32%, SD; 23.06%) and *allo*-ocimene (HD; 6.98%, SD; 5.61%) were the main compounds (Table 1). In both analyzed oils, monoterpene hydrocarbons were the main group of constituents (HD; 92.78%, SD; 94.88%). The results showed ethyl 2-methlbutyrate, ethyl butyrate, propyl 2-methylbutyrate and α-cubebene present in SD oil were not identified in the oil obtained by HD method. In contrary, HD method was more efficient to extract some oxygenated monoterpenes such as α-phellandren-8-ol, *p*-cymen-8-ol and levoverbenone (Table 1). In 2018, Moghaddam *et al*. reported (*Z*)-β-ocimene (19.93%), α-pinene (15.50%), *p*-cymene (7.67%), sabinene (7.49%) and β-phellandrene (5.50%) as main compounds of the hydro-distillated oil of *F. angulata* fruits (8). In another study, (*Z*)-β-ocimene and α-pinene were characterized with relative percentages of 64.8-76.1% and 7.3-15.4%, respectively, in essential oils of *F. angulata* fruits collected from two different regions of western Iran.[19] Limonene (38.1 and 34.9%), along with α-pinene (18.2 and 13.9%) and β-phellandrene (7.3 and 6.6%) have also been reported as main compounds of the essential oil of *F. angulata* fruits extracted by the hydrodistillation and microwave-assisted hydrodistillation

methods.[45] Beside intrinsic factors, mentioned varieties may be related to differences in environmental and geographical conditions.

Essential oil of the fruits of *F. trifida*, a species with close morphological similarities to *F. angulata*, has been reported to have very strong antibacterial activity against *Salmonella paratyphi* A and *Shigella dysenteriae* (IZ: 25 mm, MIC: 125 μg ml^{-1}), as well as potent acetylcholine-esterase (AChE) inhibitory effects (78.7% inhibition at the final concentration 500 μg ml^{-1}), containing (*E*)-β-ocimene (19.93%), α-pinene (15.50%) and bornyl acetate (7.67%) as main compounds.[46] In other study conducted by Baser *et al*., a high variety was observed in composition of micro-distilled essential oils obtained from twelve *Ferulago* species growing in Turkey.[47] (*Z*)-β-ocimene, the main compound identified in our study, was only found at rather high amounts in essential oil of *Ferulago humilis* fruits with relative percentage of 31.9 %.[47]

Conclusion

The results of this study on isolation of suberosin (**1**), isoimperatorin (**2**), imperatorin (**3**), bergapten (**4**), tamarin (**5**) suberenol (**6**), verbenone-5-O-β-D-glucopyranoside (**7**) narcissin (**8**), nicotiflorin (**9**), rutin (**10**), isorhamnetin-3-O-β-D-glucuronide (**11**), isorhamnetin-3-O-β-D-glucopyranoside (**12**) from *F. angulata* fruits introduce it as a new source of coumarin derivatives (**1-7**) and flavonoid glycosides (**8-12**). Biological properties reported in literature for the isolated compounds provide more medicinal potentials for the fruits of *F. angulata* and suggest it as an appropriate candidate for further biological studies. Furthermore, reports on antioxidant and antimicrobial activity of some compounds isolated from *F. angulata* fruits represent rationales for its uses as a natural flavour and preservative. This study also reports hydrodistillation, as a more efficient method rather than steam-distillation for the extraction of essential oil from these aromatic fruits.

Table 1. Chemical constituents of the essential oils of *F. angulata* fruits obtained by hydrodistillation (HD) and steam-distillation (SD) methods.

No.	Compounds[a]	Method		RI[b]
		HD (%)	SD (%)	
1	Ethyl 2-methylbutyrate	-	0.06	866
2	Ethyl valerate	-	0.07	870
1	α-Thujene	0.07	0.10	936
2	α-Pinene	21.32	23.06	945
5	Propyl 2-methylbutyrate	-	0.11	957
3	Camphene	1.50	1.81	962
4	Verbenene	0.32	0.56	966
5	Sabinene	0.37	0.36	985
6	β-Pinene	0.98	1.04	990
7	β-Myrcene	1.68	1.62	1002
8	Isobutyl 2-methylbutyrate	0.13	-	1015
9	α-Phellandrene	2.40	2.33	1020
10	*o*-Cymene	1.10	1.27	1040
11	Limonene	1.49	1.63	1043
12	β-Phellandrene	1.29	1.31	1046
13	(*Z*)-β-Ocimene	48.97	50.02	1053
14	(*E*)-β-Ocimene	2.85	2.71	1060
15	γ-Terpinene	0.22	0.23	1073
16	α-Terpinolene	1.15	1.13	1099
17	*allo*-Ocimene	6.98	5.61	1144
18	*neo-allo*-Ocimene	0.10	0.10	1152
19	(*E*)-Verbenol	0.64	0.11	1166
20	α-Phellandren-8-ol	0.18	-	1171
21	*p*-Cymen-8-ol	0.20	-	1211
22	Levoverbenone	0.08	-	1231
23	(*E*)-Bornyl acetate	2.45	1.58	1302
24	Carvacrol	0.26	0.05	1325
24	α-Cubebene	-	0.04	1391
25	Germacrene D	0.26	0.13	1501
26	bicyclogermacrene	0.08	0.05	1516
27	δ-Cadinene	0.14	0.09	1538
28	Germacrene B	0.08	-	1582
	Monoterpene hydrocarbons	92.78	94.88	
	Oxygenated monoterpenes	3.81	1.74	
	Sesquiterpene hydrocarbons	0.56	0.31	
	Oxygenated sesquiterpenes	0	0	
	Other	0.13	0.24	
	Total identified	97.28	97.17	

Note: A dash (–) indicate the absence of compound in the sample; [a]Compounds listed in order of elution from HP-5MS column; [b]Retention indices to C8–C24 *n*-alkanes on HP-5MS column.

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References

1. Mozaffarian V. Flora of Iran, No.54: Umbelliferae. Tehran: Publication of Research Institute of Forests and Rangelands; 2007.
2. Amiri MS, Joharchi MR. Ethnobotanical knowledge of Apiaceae family in Iran: A review. Avicenna J Phytomed. 2016;6(6):621-35. doi:10.22038/AJP.2016.6696
3. Kaval İ, Behçet L, Çakilcioğlu U. Survey of wild food plants for human consumption in Geçitli (Hakkari, Turkey). Indian J Tradit Knowle. 2015;14(2):183-90. doi:10.1016/j.jep.2014.05.014
4. Farida SHM, Ghorbani A, Ajani Y, Sadr M, Mozaffarian V. Ethnobotanical applications and their correspondence with phylogeny in Apiaceae-Apioideae. Res J Pharmacogn. 2018;5(3):79-97. doi:10.22127/RJP.2018.64880
5. Jahantab E. Ethnobotanical study of medicinal plants of Boyer Ahmad and Dena regions in Kohgiluyeh and Boyer Ahmad province, Iran. Adv Herb Med. 2018;4(4):12-22. doi:10.12927/whp.2016.24519
6. Azarbani F, Saki Z, Zareei A, Mohammadi A. Phenolic contents, antibacterial and antioxidant activities of flower, leaf and stem extracts of *Ferulago angulata* (schlecht) boiss. Int J Pharm Pharm Sci. 2014;6(10):123-5.
7. Ghasemi Pirbalouti A, Izadi A, Malek Poor F, Hamedi B. Chemical composition, antioxidant and antibacterial activities of essential oils from *Ferulago angulata*. Pharm Biol. 2016;54(11):2515-20. doi:10.3109/13880209.2016.1162816
8. Moghaddam M, Mehdizadeh L, Mirzaei Najafgholi H, Ghasemi Pirbalouti A. Chemical composition, antibacterial and antifungal activities of seed essential oil of *Ferulago angulata*. Int J Food Prop. 2018;21(1):158-70. doi:10.1080/10942912.2018.1437626
9. Bagci E, Aydin E, Mihasan M, Maniu C, Hritcu L. Anxiolytic and antidepressant-like effects of *Ferulago angulata* essential oil in the scopolamine rat model of Alzheimer's disease. Flavour Frag J. 2016;31(1):70-80. doi:10.1002/ffj.3289
10. Hritcu L, Bagci E, Aydin E, Mihasan M. Antiamnesic and antioxidants effects of *Ferulago angulata* essential oil against scopolamine-induced memory impairment in laboratory rats. Neurochem Res. 2015;40(9):1799-809. doi:10.1007/s11064-015-1662-6

11. Heidari S, Akrami H, Gharaei R, Jalili A, Mahdiuni H, Golezar E. Anti-tumor activity of *Ferulago angulata* Boiss. extract in gastric cancer cell line via induction of apoptosis. Iran J Pharm Res. 2014;13(4):1335-45. doi:10.22037/IJPR.2014.1592
12. Kiziltas H, Ekin S, Bayramoglu M, Akbas E, Oto G, Yildirim S, et al. Antioxidant properties of *Ferulago angulata* and its hepatoprotective effect against N-nitrosodimethylamine-induced oxidative stress in rats. Pharm Biol. 2017;55(1):888-97. doi:10.1080/13880209.2016.1270974
13. Sajjadi SE, Eskandarian AA, Shokoohinia Y, Yousefi H-A, Mansourian M, Asgarian-Nasab H, et al. Antileishmanial activity of prenylated coumarins isolated from *Ferulago angulata* and *Prangos asperula*. Res Pharm Sci. 2016;11(4):324-31. doi:10.4103/1735-5362.189314
14. Sajjadi SE, Pestechian N, Kazemi M, Mohaghegh MA, Hosseini-Safa A. Evaluation of the antimalarial effect of *Ferulago angulata* (Schlecht.) Boiss. extract and suberosin epoxide against plasmodium berghei in comparison with chloroquine using in-vivo test. Iran J Pharm Res. 2016;15(3):515-21. doi:10.9734/bmrj/2016/29262
15. Ameen BAH. Phytochemical study and cytotoxic activity of *Ferulago angulata* (Schlecht) Boiss, from Kurdistan-region of Iraq. Int J Innov Res Adv Eng. 2014;1(9):1-5.
16. Razavi SM, Ravansalar A, Mirinejad S. The investigation on phytochemicals from *Ferulago angulata* (Schlecht) Boiss, indigenous to central parts of Iran. Nat Prod Res. 2015;29(21):2037-40. doi:10.1080/14786419.2015.1017725
17. Khanahmadi M, Shahrezaei F, Alizadeh A. Isolation and structural elucidation of two flavonoids from *Ferulago angulata* (Schlecht) Boiss. Asian J Res Chem. 2011;4(11):1667-70.
18. Rustaiyan A, Sedaghat S, Larijani K, Khossravi M, Masoudi S. Composition of the essential oil of *Ferulago angulata* (Schlecht.) Boiss. from Iran. J Essent Oil Res. 2002;14(6):447-8. doi:10.1080/10412905.2002.9699917
19. Ghasempour H, Shirinpour E, Heidari H. The constituents of essential oils of *Ferulago angulata* (Schlecht.) Boiss at two different habitats, Nevakoh and Shahoo, Zagross Mountain, western Iran. Iran J Sci Tech. 2007;31(3):309-12. doi:10.3923/pjbs.2007.814.817
20. Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. Carol Stream: Allured Publishing Corporation; 2007.
21. Karakaya S, Şimşek D, Özbek H, Güvenalp Z, Altanlar N, Kazaz C, et al. Antimicrobial activities of extracts and isolated coumarins from the roots of four *Ferulago* species growing in Turkey. Iran J Pharm Res. 2019;18(3):1516-29. doi:10.22037/IJPR.2019.1100718
22. Delnavazi MR, Soleimani M, Hadjiakhoondi A, Yass N. Isolation of phenolic derivatives and essential oil analysis of *Prangos ferulacea* (L.) Lindl. aerial parts. Iran J Pharm Res. 2017;16(Suppl):207-15. doi:10.22037/IJPR.2017.1992
23. Muller M, Byres M, Jaspars M, Kumarasamy Y, Middleton M, Nahar L, et al. 2D NMR spectroscopic analyses of archangelicin from the seeds of *Angelica archangelica*. Acta Pharmaceut. 2004;54(4):277-85.

24. Burke BA, Parkins H. Coumarins from *Amyris balsamifera*. Phytochemistry. 1979;18(6):1073-5. doi:10.1016/s0031-9422(00)91488-2
25. Tavakoli S, Delnavazi MR, Hadjiaghaee R, Jafari-Nodooshan S, Khalighi-Sigaroodi F, Akhbari M, et al. Bioactive coumarins from the roots and fruits of *Ferulago trifida* Boiss., an endemic species to Iran. Nat Prod Res. 2018;32(22):2724-8. doi:10.1080/14786419.2017.1375915
26. Shikishima Y, Takaishi Y, Honda G, Ito M, Takeda Y, Kodzhimatov OK, et al. Terpenoids and γ-pyrone derivatives from *Prangos tschimganica*. Phytochemistry. 2001;57(1):135-41. doi:10.1016/s0031-9422(00)00407-6
27. Güvenalp Z, Özbek H, Ünsalar T, Kazaz C, Demirezer LÖ. Iridoid, flavonoid, and phenylethanoid glycosides from *Wiedemannia orientalis*. Turk J Chem. 2006;30(3):391-400.
28. Lee JM, Lee KH, Yoon YH, Cho EJ, Lee S. Identification of triterpenoids and flavonoids from the seeds of tartary buckwheat. Nat Prod Sci. 2013;19(2):137-44.
29. Im SH, Wang Z, Lim SS, Lee OH, Kang IJ. Bioactivity-guided isolation and identification of anti-adipogenic compounds from *Sanguisorba officinalis*. Pharm Biol. 2017;55(1):2057-64. doi:10.1080/13880209.2017.1357736
30. Aliotta G, Della Greca M, Monaco P, Pinto G, Pollio A, Previtera L. In vitro algal growth inhibition by phytotoxins of *Typha latifolia* L. J Chem Ecol. 1990;16(9):2637-46. doi:10.1007/bf00988075
31. Karakaya S, Gözcü S, Güvenalp Z, Özbek H, Yuca H, Dursunoğlu B, et al. The α-amylase and α-glucosidase inhibitory activities of the dichloromethane extracts and constituents of *Ferulago bracteata* roots. Pharm Biol. 2018;56(1):18-24. doi:10.1080/13880209.2017.1414857
32. Chen YC, Tsai WJ, Wu MH, Lin LC, Kuo YC. Suberosin inhibits proliferation of human peripheral blood mononuclear cells through the modulation of the transcription factors NF-AT and NF-κB. Brit J Pharmacol. 2007;150(3):298-312. doi:10.1038/sj.bjp.0706987
33. Zhou N, Wang T, Song J, He H, He J, He L. Antihypertensive and vascular remodelling effects of the imperatorin derivative OW 1 in renovascular hypertension rats. Clin Exp Pharmacol P. 2014;41(8):571-8. doi:10.1111/1440-1681.12248
34. Zhang Y, Cao Y, Duan H, Wang H, He L. Imperatorin prevents cardiac hypertrophy and the transition to heart failure via NO-dependent mechanisms in mice. Fitoterapia. 2012;83(1):60-6.
35. Okamoto T, Yoshida S, Kobayashi T, Okabe S. Inhibition of concanavalin A-induced mice hepatitis by coumarin derivatives. Jap J Pharmacol. 2001;85(1):95-7. doi:10.1254/jjp.85.95
36. Luo K-w, Sun J-g, Chan JY-W, Yang L, Wu S-h, Fung K-P, et al. Anticancer effects of imperatorin isolated from *Angelica dahurica*: induction of apoptosis in HepG2 cells through both death-receptor-and mitochondria-mediated pathways. Chemotherapy. 2011;57(6):449-59. doi:10.1159/000331641
37. Luszczki JJ, Wojda E, Andres-Mach M, Cisowski W, Glensk M, Glowniak K, et al. Anticonvulsant and acute neurotoxic effects of imperatorin, osthole and valproate in the maximal electroshock seizure and chimney tests in mice: a comparative study. Epilepsy Res. 2009;85(2-3):293-9. doi:10.1016/j.eplepsyres.2009.03.027

38. Cao Y, Liu J, Wang Q, Liu M, Cheng Y, Zhang X, et al. Antidepressive-like effect of imperatorin from *Angelica dahurica* in prenatally stressed offspring rats through 5-hydroxytryptamine system. Neuroreport. 2017;28(8):426-33. doi:10.1097/wnr.0000000000000778
39. Budzynska B, Boguszewska-Czubara A, Kruk-Slomka M, Skalicka-Wozniak K, Michalak A, Musik I, et al. Effects of imperatorin on scopolamine-induced cognitive impairment and oxidative stress in mice. Psychopharmacology. 2015;232(5):931-42. doi:10.1007/s00213-014-3728-6
40. Xian Z, Choi YH, Zheng M, Jiang J, Zhao Y, Wang C, et al. Imperatorin alleviates ROS-mediated airway remodeling by targeting the Nrf2/HO-1 signaling pathway. Biosci Biotech Biochem. 2020:1-13. doi:10.1080/09168451.2019.1710107
41. Shokoohinia Y, Khajouei S, Ahmadi F, Ghiasvand N, Hosseinzadeh L. Protective effect of bioactive compounds from *Echinophora cinerea* against cisplatin-induced oxidative stress and apoptosis in the PC12 cell line. Iran J Basic Med Sci. 2017;20:438-45. doi:10.7324/japs.2019.90403
42. Doganca S, Ulubelen A, Tuzlaci E. 1-Acetylhydroquinone 4-galactoside from *Ferulago aucheri*. Phytochemistry. 1991;30(8):2803-5. doi:10.1016/0031-9422(91)85152-p
43. Akalin E, Pimenov MG. *Ferulago trojana* (Umbelliferae), a new species from western Turkey. Bot J Linn Soc. 2004;146(4):499-504. doi:10.1111/j.1095-8339.2004.00308.x
44. Shahidi F, Ambigaipalan P. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. J funct Food. 2015;18:820-97. doi:10.1016/j.jff.2015.06.018
45. Mollaei S, Sedighi F, Habibi B, Hazrati S, Asgharian P. Extraction of essential oils of *Ferulago angulata* with microwave-assisted hydrodistillation. Ind Crops Prod. 2019;137:43–51. doi: 10.1016/j.indcrop.2019.05.015
46. Tavakoli S, Yassa N, Delnavazi MR, Akhbari M, Hadjiakhoondi A, Hajimehdipoor H, et al. Chemical composition and biological activities of the essential oils from different parts of *Ferulago trifida* Boiss. J Essent Oil Res. 2017;29(5):407-19. doi:10.1080/10412905.2017.1313178
47. Baser KHC, Demirci B, Özek T, Akalin E, Özhatay N. Micro-distilled volatile compounds from Ferulago species growing in western Turkey. Pharm Biol. 2002;40(6):466-71. doi:10.1076/phbi.40.6.466.8439