Antioxidative Coumarins from the Roots of *Ferulago subvelutina*

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From the ethyl acetate extract of the roots of *Ferulago subvelutina* (Apiaceae) six coumarins, osthol (**1**), oxypeucedanin (**2**), xanthotoxin (**3**), isoimperatorin (**4**), oxypeucedanin hydrate (**5**), meranzine hydrate (**6**) and one sterol, β -sitosterol linoleate (**7**), were isolated by silica gel column chromatography and identified by spectroscopic methods. Antioxidant activities of the isolated compounds were tested by the DPPH radical scavenging assay. Compounds exhibited moderate antioxidant activities. DPPH scavenging abilities of compounds were lower than that of synthetic antioxidant *tert*-butyl hydroxytoluene (BHT) ($IC_{50} = 27 \mu\text{g/mL}$) in the order BHT > oxypeucedanin hydrate > meranzin hydrate > oxypeucedanin > isoimperatorin > xanthotoxin > osthole.

Key Words: *Ferulago subvelutina*, Apiaceae, Coumarin, Osthol, Oxypeucedanin, Xanthotoxin, Isoimperatorin, Oxypeucedanin hydrate, Meranzine hydrate.

INTRODUCTION

The genus *Ferulago* (Apiaceae) consists of about 40 species. Five species of which are exclusively growing in the north-east and central parts of Iran including *F. subvelutina*, *F. carduchorum*, *F. contracta*, *F. phialocarpa* and *F. trifida*. Other species are growing abundantly in central Asia¹. There are some evidences that *Ferulago*, *Ferula* and *Prangos* species have been used in folk medicine for their sedative, tonic, digestive, aphrodisiac properties and in the treatment of intestinal worms and hemorrhoid in different regions of Turkey. In addition, some of *Ferulago* species have been used in folk medicines against ulcers, snakebite, as well as headache and spleen disorders²⁻⁴. *F. subvelutina* Rech. F., a perennial herbaceous plant, grows widely in the north-east and central parts of Iran¹.

A literature review shows that there are a few studies on phytochemical analysis of *F. subvelutina*. The essential oil of the aerial parts of *F. subvelutina* has been reported to contain 36 volatile compounds. Limonene (27.5 %), α -phellandren (23.1 %) and α -pinene (13.3 %) were major components. The essential oil showed antimicrobial activity against three species of *Salmonella*, *Shigella flexneri* and *E. coli*⁵. In this study, we report the isolation and identification of the main constituents of the ethyl acetate extract of the roots of *F. subvelutina* and antioxidant activities of the isolated compounds were tested by the DPPH radical scavenging assay which has not been previously reported.

EXPERIMENTAL

¹H and ¹³C NMR spectra were recorded on a Bruker Avance 500 DRX spectrometer® with tetramethylsilane as an internal standard and chemical shifts are given in δ (ppm). Multiple-pulse experiments (HSQC, HMBC and H-H COSY) were performed using the standard Bruker® programs. Silicagel 60 F₂₅₄ and Silicagel 60 RP-18 F₂₅₄S pre-coated plates (Merck®) were used for TLC. The spots were detected by spraying with anisaldehyde-H₂SO₄ reagent followed by heating.

The roots of *Ferulago subvelutina* Rech. F. were collected in June 2009 from north-east of Iran, Khorasan province, Esfarayen, Rouein, 1650 m height. The plant was identified by Mr. Y. Ajani. A voucher specimen (No. 246 ACECR) has been deposited at the Herbarium of Complex of Academic Center for Educational and Cultural Research, Tehran, Iran.

Isolation process: The milled roots of *F. subvelutina* (2500 g) was extracted in ethyl acetate by percolation method at room temperature three times, each for 48 h. Obtained extract was concentrated by rotary-evaporator, dried by freeze drier and preserved in refrigerator. The ethyl acetate extract (97 g) was subjected to silicagel column chromatography (CC). The column was eluted by different gradients of hexane-chloroform, chloroform, chloroform-ethyl acetate, respectively, to yield 13 fractions (A-M). The compound **1** was isolated from the fraction B and crystallized as a pure compound. The

fraction D (4.8 g) was submitted to silica gel CC with chloroform: ethyl-acetate (9:1) and ethyl-acetate to give seven fractions (D₁-D₇). The fraction D₅ was crystallized as compound **2**. The fraction D₄ was subjected to sephadex LH₂₀ CC with methanol: chloroform (7:3) to obtain four fractions (D₄₁-D₄₄). The fraction D₄₃ was precipitated as needle crystals (compound **3**, 72 mg). The fraction D₂ was loaded on sephadex LH₂₀ CC and eluted by methanol:chloroform (8:2) to result five fractions (D₂₁-D₂₅). The fraction D₂₃ was pure (compound **4**, 19 mg). The fraction H was submitted to silica gel CC with ethyl acetate and ethyl acetate:methanol (9:1), respectively, to obtain eight fractions. The fraction H₅ (637 mg) was compound **5**. The fraction H₇ was subjected to silica gel CC connected to MPLC apparatus resulted in isolation of five fractions, of which fraction H₇₃ was pure (compound **6**). The fraction A subjected to silica gel CC with hexane: chloroform (7:3), chloroform and chloroform:ethyl acetate (1:1), respectively. Among the eight obtained fractions, the fraction A₂ was pure (compound **7**, 162 mg).

Free radical scavenging activity: Free radical scavenging activity was evaluated by measuring the scavenging activity of the compounds in the solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). One milliliter of DPPH solution (500 µM) in methanol was thoroughly mixed with an equal volume of the test solution at various concentrations and kept in the dark for 0.5 h. The absorbance of the solutions, including a blank (without sample) and a positive control (BHT, *tert*-butylated hydroxytoluene), was read at 517 nm after 1 h incubation without light at room temperature on a Shimadzu UV-2100 spectrophotometer. Each sample assay was carried out in triplicate and the data presented as a mean of the three values. A decrease in absorbance of DPPH solution indicates

the increase in DPPH radical scavenging activity. The values were calculated as a percentage using the following formula:

$$\text{DPPH \% radical scavenging} = \left[\frac{(\text{Absorbance of blank} - \text{Absorbance of sample})}{\text{Absorbance of blank}} \right] \times 100$$

Antioxidant activities of the compounds were tested by the DPPH radical scavenging assay. The effect of antioxidant on DPPH radical scavenging was thought to be due to their radical scavenging activity. When a solution of DPPH is mixed with that of a substance, this gives rise to the reduced form diphenylpicrylhydrazyl (non radical) with the loss of this violet colour⁶.

RESULTS AND DISCUSSION

From the ethyl acetate extract of the roots of *F. subvelutina*, six coumarins, osthol (**1**)⁷, oxypeucedanin (**2**)⁸, xanthotoxin (**3**)⁹, isoimperatorin (**4**)⁹, oxypeucedanin hydrate (**5**)¹⁰, meranzine hydrate (**6**)¹¹ and one sterol, β -sitosteryl linoleate (**7**)¹², were isolated by silica gel column chromatography and identified by comparison of their spectral data (¹H NMR, ¹³C NMR) with those reported in the literature. The NMR data of the compounds **1-6** have been summarized in Tables 1-3. The structures of the isolated compounds are shown in the Fig. 1.

β -Sitosteryl linoleate (7): ¹H NMR (500 MHz, CDCl₃), δ H: 5.36 (5H, m, H-6, H-9', H-10', H-12', H-13'), 4.63 (1H, m, H-3), 1.03 (3H, s, Me-19), 0.93 (3H, d, *J* = 6.5 Hz, Me-21), 0.88 (3H, m, Me-18'), 0.84 (6H, m, Me-26 and Me-29), 0.82 (3H, d, *J* = 6.6 Hz, Me-27), 0.69 (3H, s, Me-18). ¹³C NMR (125 MHz, CDCl₃), δ C: 37.0 (C-1), 27.8 (C-2), 73.7 (C-3), 38.3 (C-4), 139.7 (C-5), 122.6 (C-6), 31.8 (C-7), 31.9

TABLE-1
¹H NMR DATA OF THE COMPOUNDS **1**, **3** AND **6** (δ VALUES: ppm, CDCl₃)

Carbon	1	3	6
3	6.18 (d, <i>J</i> = 9.4 Hz, 1H)	6.37 (d, <i>J</i> = 9.5 Hz, 1H)	6.23 (d, <i>J</i> = 9.5 Hz, 1H)
4	7.57 (d, <i>J</i> = 9.4 Hz, 1H)	7.77 (d, <i>J</i> = 9.5 Hz, 1H)	7.63 (d, <i>J</i> = 9.5 Hz, 1H)
5	7.25 (d, <i>J</i> = 8.5 Hz, 1H)	7.35 (s, 1H)	7.34 (d, <i>J</i> = 8.5 Hz, 1H)
6	6.80 (d, <i>J</i> = 8.5 Hz, 1H)	—	6.87 (d, <i>J</i> = 8.5 Hz, 1H)
1'	3.50 (d, <i>J</i> = 7 Hz, 2H)	—	2.99 (dd, <i>J</i> = 14, 10 Hz, 1H)
2'	5.20 (t, <i>J</i> = 7 Hz, 1H)	7.69 (d, <i>J</i> = 2.2 Hz, 1H)	3.08 (dd, <i>J</i> = 14, 2.5 Hz, 1H)
3'	—	6.82 (d, <i>J</i> = 2.2 Hz, 1H)	3.62 (dd, <i>J</i> = 10, 2.5 Hz, 1H)
4'	1.64 (s, 3H)	—	—
5'	1.81 (s, 3H)	—	1.32 (s, 3H)
OCH ₃	3.89 (s, 3H)	4.28 (s, 3H)	1.33 (s, 3H)
			3.92 (s, 3H)

TABLE-2
¹H NMR DATA OF THE COMPOUNDS **2**, **4** AND **5** (δ VALUES: ppm, CDCl₃)

Carbon	2	4	5
3	6.32 (d, <i>J</i> = 9.7 Hz, 1H)	6.26 (d, <i>J</i> = 9.7 Hz, 1H)	6.22 (d, <i>J</i> = 9.5 Hz, 1H)
4	8.20 (d, <i>J</i> = 9.7 Hz, 1H)	8.15 (d, <i>J</i> = 9.7 Hz, 1H)	8.14 (d, <i>J</i> = 9.5 Hz, 1H)
8	7.20 (s, 1H)	7.14 (s, 1H)	7.08 (s, 1H)
2'	7.61 (s, 1H)	7.58 (d, <i>J</i> = 2.3 Hz, 1H)	7.58 (d, <i>J</i> = 2.5 Hz, 1H)
3'	6.95 (s, 1H)	6.95 (d, <i>J</i> = 2.3 Hz, 1H)	6.97 (d, <i>J</i> = 2.5 Hz, 1H)
1''	4.44 (dd, <i>J</i> = 10.3, 6.4 Hz, 1H)	4.91 (d, <i>J</i> = 6.8 Hz, 2H)	4.43 (dd, <i>J</i> = 9.5, 7.5 Hz, 1H)
	4.59 (dd, <i>J</i> = 10.3, 6.6 Hz, 1H)		4.54 (dd, <i>J</i> = 9.5, 3 Hz, 1H)
2''	3.23 (m, 1H)	5.53 (t, <i>J</i> = 6.8 Hz, 1H)	3.90 (dd, <i>J</i> = 7.5, 3 Hz, 1H)
4''	1.33 (s, 3H)	1.69 (s, 3H)	1.35 (s, 3H)
5''	1.41 (s, 3H)	1.79 (s, 3H)	1.30 (s, 3H)

TABLE-3
¹³C NMR CHEMICAL SHIFTS OF THE COUMARINS
 ISOLATED FROM *F. subvelutina* (δ_C: ppm, CDCl₃)

Carbon	1	2	3	4	5	6
2	161.2	161.0	160.5	161.3	161.3	161.3
3	112.7	113.2	114.7	112.5	112.8	113.0
4	143.7	139.0	144.3	139.6	139.2	144.0
5	126.1	148.4	112.9	148.9	148.5	127.0
6	107.2	114.2	126.1	114.2	114.1	107.4
7	160.1	158.3	147.6	158.1	158.0	160.5
8	117.7	94.9	132.8	94.2	94.6	115.7
4a	112.8	107.5	116.5	107.3	107.1	113.1
8a	152.7	152.6	143.0	152.6	152.4	153.4
1'	21.8	—	—	—	—	26.1
2'	121.0	145.3	146.6	144.9	145.2	78.3
3'	132.4	104.5	106.7	105.0	104.8	73.1
4'	25.7	—	—	—	—	24.0
5'	17.8	—	—	—	—	25.6
1''	—	72.3	—	69.7	71.7	—
2''	—	61.1	—	119.1	74.4	—
3''	—	58.3	—	139.8	76.5	—
4''	—	19.0	—	18.2	26.6	—
5''	—	24.6	—	25.8	25.1	—
OCH ₃	55.9	—	61.3	—	—	56.2

Fig. 1. Structures of the isolated compounds from *F. subvelutina*

(C-8), 50.0 (C-9), 36.6 (C-10), 21.0 (C-11), 39.7 (C-12), 42.3 (C-13), 56.7 (C-14), 24.3 (C-15), 28.2 (C-16), 56.0 (C-17), 11.8 (C-18), 19.3 (C-19), 36.1 (C-20), 18.8 (C-21), 33.9 (C-22), 26.1 (C-23), 45.8 (C-24), 29.1 (C-25), 19.0 (C-26), 19.8 (C-27), 23.0 (C-28), 12.0 (C-29), 173.3 (C-1'), 34.7 (C-2'), 25.0 (C-3'), 29.1 (C-4'), 29.3 (C-5'), 29.6 (C-6'), 29.6 (C-7'), 27.2 (C-8'), 130.1 (C-9'), 127.9 (C-10'), 25.6 (C-11'), 128.0 (C-12'), 130.2 (C-13'), 27.2 (C-14'), 29.3 (C-15'), 31.5 (C-16'), 22.5 (C-17'), 14.1 (C-18').

The literature review shows that the coumarins **1**, **2**, **4** and **5** have been previously reported from various *Ferulago* species. A summary of the widespread coumarins within *Ferulago* species is shown in Table-4. Two other isolated coumarins **3** and **6** have not been reported from this genus until now, so that this is the first report for presence of xanthotoxin (**3**) and meranzin hydrate (**6**) in *Ferulago*.

TABLE-4
 DISTRIBUTION OF THE ISOLATED
 COUMARINS WITHIN *FERULAGO* SPECIES

Compound	Plant	Source	Ref.
Osthol	<i>F. brachyloba</i>	Roots	13
	<i>F. campestris</i>	Roots	14
	<i>F. capillaries</i>	Roots	13
	<i>F. turcomanica</i>	Roots	15
Oxypeucedanin	<i>F. bernardii</i>	Aerial parts	16
	<i>F. brachyloba</i>	Roots	13
	<i>F. capillaries</i>	Aerial parts and roots	13
	<i>F. granatensis</i>	Roots	17
	<i>F. meoides</i>	Umbels	18
	<i>F. platycarpa</i>	Roots	19
	<i>F. sylvatica</i>	Roots	20
	<i>F. turcomanica</i>	Roots	15
Isoimperatorin	<i>F. capillaries</i>	Aerial parts and roots	13
	<i>F. grandatensis</i>	Umbels	17
	<i>F. meoides</i>	Roots	18
	<i>F. sylvatica</i>	Roots	20
	<i>F. turcomanica</i>	Roots	15
Oxypeucedanin hydrate	<i>F. brachyloba</i>	Roots	13
	<i>F. capillaries</i>	Roots	13
	<i>F. meoides</i>	Umbels	18
	<i>F. sylvatica</i>	Roots	20
	<i>F. turcomanica</i>	Roots	15

Free radical scavenging properties of the compounds are presented in Table-5. Lower IC₅₀ value indicates higher antioxidant activity. All the tested compounds exhibited moderate antioxidant activities. DPPH scavenging abilities of compounds were lower than that of synthetic antioxidant BHT (IC₅₀ = 27 µg/mL). In this study, DPPH radical scavenging activity of the test samples was in the order BHT > oxypeucedanin hydrate > meranzin hydrate > oxypeucedanin > isoimperaturin > xanthotoxin > osthole. As it is shown in Table-5, peucedanin hydrate and meranzin hydrate presented higher antioxidant activity than the other isolated coumarins.

TABLE-5
ANTIOXIDATIVE ACTIVITIES OF THE ISOLATED
COMPOUNDS AGAINST DPPH (IC₅₀)

Compound	DPPH IC ₅₀ (µg/mL)
Oxypeucedanin hydrate	160
Meranzin hydrate	180
Osthole	210
Oxypeucedanin	217
Isoimperaturin	245
Xanthotoxin	270
BHT	27

Conclusion

The result of this study revealed that *F. subvelutina* contains various coumarins as the main components of the roots, which presented diverse antioxidative activity. Phytochemical investigation on the arial parts of this species is suggested for future studies.

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