Phytochemical analysis of *Ferulogo Bernardii* Tomk & M.Pimen

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ABSTRACT

From the hexane extract of the aerial parts of *Ferulago Bernardii* (Apiaceae) four coumarins, namely prantschimgin **1**, oxypeucedanin **2**, psoralen **3** and umbelliferone **4**; β-sitosterol **5**; and nonacosane **6** were isolated by Column Chromatography (CC), Preparative Thin Layer Chromatography (PTLC) and crystallization. The structures were elucidated by melting point, UV, IR, MS, ^{1}H and ^{13}C-NMR spectra. The presence of compounds **1**, **2**, **3** and **5** in some others *Ferulago* species could be used as chemotaxonomic marker in genus *Ferulago*. This is the first report on phytochemical analysis of *Ferulago Bernardii* Tomk. & *M. Pimen*.

Keywords: *Ferulago Bernardii*, Apiaceae, Prantschimgin, Oxypeucedanin, Psoralen, Umbelliferone, β-Sitosterol, Nonacosane

INTRODUCTION

Ferulago W. D. Koch, is a perennial genus of the Apiaceae family which is distinguished by the presence of persistent bracts and bracteoles (1). The genus *Ferulago* is represented by 40 species in the world. Eight species exist in Iran of which three are endemic. *Ferulago Bernardii* Tomk. & M. Pimen. is a glabrous plant distributed in southeast Turkey and west of Iran (1,2).

Ferulago, *Ferula* and *Prangos* species have been used in folk medicine in different regions of Turkey for their sedative, tonic, digestive, aphrodisiac properties and for treatment of intestinal worms and hemorrhoids (3,4). Moreover the plants of the genus *Ferulago* have been used since antiquity in folk medicine against ulcers, snake bite and for treatment of headache and diseases of the spleen (5).

The chemical compositions of some *Ferulago* species have previously been reported. Coumarins have been found in *F. meoides*, *F. turcomanica*, *F. granatensis*, *F. aucheri*, *F. asparagifolia*, *F. nodosa*, *F. capillaris* and *F. brachyloba* (6-13). Isolation of flavonoids from *F. aucheri* and *F. asparagifolia* (10,11), *β*-sitosterol from *F. granatensis* (9), aromatic compounds from *F. F. aucheri* (10), monoterpenes from *F. nodosa* (12), phenylpropanoid and sesquiterpene aryl esters from *F. antiochia* have also been reported (14).

A number of *Ferulago* species have previously been investigated for their essential oil compositions and antimicrobial activities (3,5,15-27). In addition the chemical composition and antimicrobial activity of the essential oil of *Ferulago Bernardii* Tomk. & M. Pimen have been reported (28). In this report the isolation of four coumarins, prantschimgin (1), oxypeucedanin (2), psoralen (3) and umbelliferone (4); β-sitosterol (5); and nonacosane (6) from the aerial parts of *Ferulago Bernardii* of Iran (Figure 1) is described.

MATERIALS AND METHODS

General

Melting points were recorded by a Reichert-jung apparatus and are uncorrected. EIMS spectra were recorded on a Finnigan MAT TSQ-70. ^{1}H and ^{13}C-NMR spectra were measured on a Varian FT-400 unity plus spectrometer with tetramethylsilane as an internal standard. Chemical shifts are given in δ. The FT-IR spectra were recorded on a Nicolet 550 instrument. The UV spectra were obtained using a Shimadzu 160A

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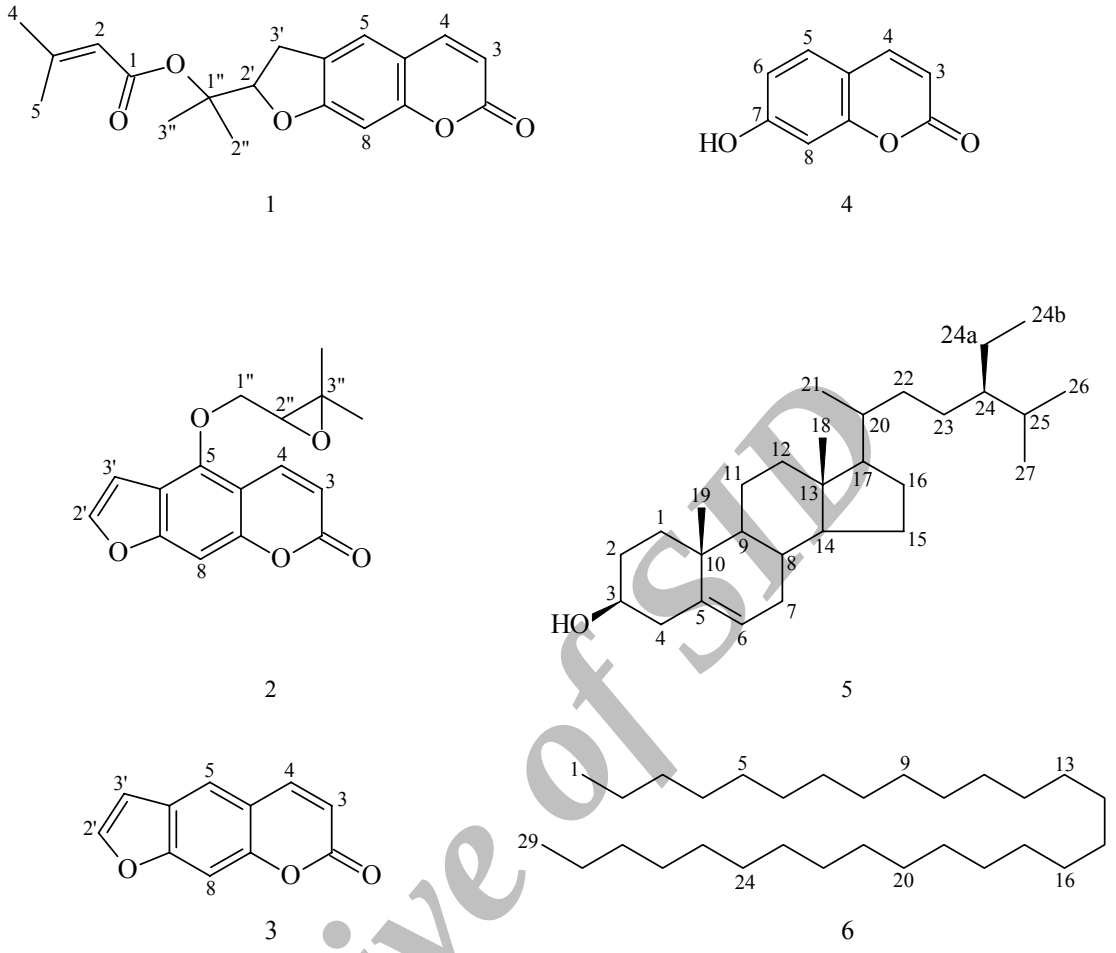


Figure 1. Structures of compounds **1-6** isolated from *Ferulago Bernardii* (Apiaceae)

spectrophotometer. Silica gel for column chromatography (Mesh 70 – 230) was purchased from Merck Company. Preparative TLC was conducted on Merck $60F_{254}$ silica gel plates (absorbent thickness: 0.75 mm). Silica gel $60F_{254}$ precoated plates (Merck) were used for TLC. Spots were detected under UV (254 and 365 nm) and by spraying KOH 5-10% in EtOH as reagent.

Plant Material and Isolation Procedure

The aerial parts of *F. Bernardii* in fruiting stage were collected in September 2001 from west of Iran, the height of Ariz (in the road of Sanandaj to Marivan), with an altitude ca. 2140 m. The plant was identified by the Department of Botany of the Research Institute of Forests and Rangelands (TARI), Tehran. A voucher specimen No. 71608 has been deposited at the Herbarium of TARI.

The air-dried and finely grounded aerial parts (500 g) were extracted with n-hexane at room temperature. The extract was concentrated in vacuo (16 g) and a part of it was subjected to preparative thin layer chromatography (PTLC) using petroleum ether-EtOAc (60:40) as solvent system. Several bands were separated from PTLC under UV light (254 and 365 nm). Bands 3 and 4 contained two main compounds showing a violet fluorescence. These bands were subjected to PTLC for further purification using petroleum ether-EtOAc (70:30) as solvent system. After separation of compounds from silica gel, they were further purified by crystallization from MeOH to yield compound **1** (2 mg) and **3** (1.2 mg). The hexane extract (2.8 g) was chromatographed on a silica gel column (3.5×60 cm) eluted with a petroleum ether-EtOAc-MeOH gradient. The volume of each fraction was 20 ml. Fractions 121-132 (0.8 g) were subjected to second silica gel column (2×30 cm) eluted with a petroleum ether-EtOAc-MeOH gradient. Fractions 10-14 of the second CC were combined and further purified by crystallization from EtOH and water to yield compound **2** (1.8 mg). Fractions 134-150 (0.9 g) of the first CC were subjected to third silica gel

column (2×30 cm) eluted with a petroleum ether-EtOAc-MeOH gradient. Fractions 15-21 of the third CC were combined and further purified by crystallization from EtOH and water to yield compound **4** (2.2 mg). Fractions 79-85 of the first CC were combined and further applied to PTLC over silica gel, using toluene-ether (1:1) saturated with 10% acetic acid as solvent system. Three bands were separated from silica gel. Band **1** was dissolved in EtOAc from which after a few hours compound **5** (2.6 mg) as white needles crystallized. Some of the hexane extract (1 g) was dissolved in $CHCl_3$ and subjected to PTLC over silica gel, using petroleum ether-EtOAc (50:50) as solvent system. Band **6** (R_f 0.74) was separated from silica gel, by using EtOAc as solvent. Compound **6** (20 mg) was isolated and further purified by crystallization from $CHCl_3$.

Compound **1** (Prantschimgin): White needle crystal (2 mg); Mp 137-139˚C (lit. (29) Mp: 138-140˚C); R_f 0.47 toluene-ether (1:1 saturated with 10% acetic acid); UV λ_{max} nm 244 and 335 nm; FTIR ($CHCl_3$) ν_{max} cm^{-1}: 2919, 2851, 1727 (lactone C=O), 1627, 1570, 1263, 1229, 1123; EIMS (70 eV) m/z (%): 328 $[M]^+$ (40), 228 [M–OSen]+ (87), 213 (100), 187 (32), 159 (5), 131 (15), 83 (85), 55 (50); formula: $C_{19}H_{20}O_5$; 1H-NMR, see Table 1; ^{13}C-NMR, see Table 2. The spectral data of the compound **1** were in agreement with the reported data for prantschimgin (13,29,30).

Compound **2** (Oxypeucedanin): Yellow powder (1.8 mg); Mp 138-140˚C (lit. (31) Mp: 139-140˚C); R_f 0.43 toluene-ether (1:1 saturated with 10% acetic acid); UV λ_{max} nm 251 and 306 nm; FTIR ($CHCl_3$) ν_{max} cm-1: 1726 (lactone C=O), 1603-1451 (aromatic C=C), 1257, 1209, 1082, 821, 750; EIMS (70 eV) m/z (%): 286 (100), 202 (42), 173 (20), 145 (25), 85 (35), 59 (60); formula: $C1_6H_{14}O_5$; ^{1}H-NMR, see Table 1; ^{13}C-NMR, see Table 2. The spectral data of the compound **2** were in agreement with the reported data for oxypeucedanin (13,31,32).

Compound **3** (Psoralen): White powder (1.2 mg); Mp 161-163˚C (lit. (33) Mp: 163-164˚C); R_f 0.42 (toluene-ether (1:1, saturated with 10% acetic acid)); UV λ_{max} nm 249, 290 and 331 nm; FTIR ($CHCl_3$) ν_{max} cm^{-1}: 1715 (lactone C=O), 1126 (cyclic ether C-O), 1014, 756; EIMS (70 eV) m/z (%): 186 (100), 158 (80), 130 (18), 102 (35), 51 (15); formula: $C_{11}H_6O_3$; ^{1}H-NMR, see Table 1; ^{13}C-NMR, see Table 2. The spectral data of the compound 3 were in agreement with the reported data for psoralen (13,33-37).

Compound **4** (Umbelliferone): White needle crystals (2.2 mg); Mp 229-231˚C (lit. (38) Mp: 230-232˚C); R_f 0.28 toluene-ether (1:1 saturated with 10% acetic acid; UV λ_{max} nm 206 and 325 nm; FTIR (KBr) ν_{max} cm^{-1}: 3159, 1709, 1681 (C=O), 1568 (phenolic C–O), 1322 (phenolic C–O), 1235 (C=O), 1134, 835; EIMS (70 eV) m/z (%): 162 (100), 134 (81), 51 (21); formula: C9H6O3; ^{1}H-NMR, see Table 1; ^{13}C-NMR, see Table 2. The spectral data of the compound **4** were in agreement with the reported data for umbelliferone (13,38-41).

Compound **5** (β-Sitosterol): White needle crystal (2.6 mg); Mp 135-136˚C (lit. (29) Mp: 136-137˚C); R_f 0.25 (EtOH: $CHCl_3$ (98:2)); UV λmax 247 nm; FTIR ($CHCl_3$) ν_{max} cm^{-1}: 3438 (O-H), 2934 (C-H), 2865, 1462, 1047 (C-O), 757; EIMS (70 eV) m/z (%): 414 (75), 396 (20), 381 (10), 329 (10), 255 (7), 213 (35), 199 (10), 69 (21), 57 (45), 43 (100); formula: $C_{29}H_{50}O$; ^{1}H and ^{13}C-NMR, see Table 3. The spectral data of the compound 5 were in agreement with the reported data for β-sitosterol (29,42-44).

Compound **6** (Nonacosane): White amorphous powder (20 mg); Mp 62-64˚C (lit. (29) Mp: 64˚C); FTIR (KBr) ν_{max} cm^{-1}: 2918, 2849, 1467, 1378 (methyl), 724 (long chain); EIMS (70 eV) m/z (%): 408 (5), 351 (3), 295 (5), 239 (5), 211 (7), 141 (15), 113 (20), 99 (30), 85 (70), 57 (100); formula: C29H60; ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm: 1.25 (s, methylene groups), 0.88 (*t, J*=6 Hz, methyl groups). The spectral data of the compound 6 were in agreement with the reported data for nonacosane (29,45).

RESULTS AND DISCUSSION

From the hexane extract of the aerial parts of *Ferulago Bernardii* (Apiaceae) four coumarins, prantschimgin **1**, oxypeucedanin **2**, psoralen **3** and umbelliferone **4**; one steroid, β-sitosterol **5**; and a normal alkane, nonacosane **6** were isolated by CC, PTLC and crystallization. The structure of these compounds were elucidated by melting point, UV, IR, MS, ^{1}H and ^{13}C-NMR spectra and by comparison of their physical data with those reported in literature.

Compound **1**: The mass spectrum of the compound **1** had [M]+ at m/z 328 suggesting the molecular formula $C_{19}H_{20}O_5$. In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum, H-3 and H-4 were observed at δ 6.22 (1H, *d*, *J*=9.8 Hz) and 7.61 (1H, *d*, *J*=9.8 Hz) as AB-type signals, respectively. The presence of two singlets proton

Table 1. [1]H-NMR spectral data for compounds **1**, **2**, **3**[a] and 4[b].

H	1 [*mult.*, *J* (Hz)]	2 [*mult.*, *J* (Hz)]	3 [*mult.*, *J* (Hz)]	4 [*mult.*, *J* (Hz)]
3	6.22 *d* (9.8)	6.32 *d* (9.6)	6.39 *d* (9.6)	6.19 *d* (9.4)
4	7.61 *d* (9.8)	8.21 *d* (9.6)	7.81 *d* (9.6)	7.93 *d* (9.4)
5	7.22 *s*	-	7.69 *s*	7.52 *d* (8.4)
6	-	-	-	6.78 *dd* (0.2, 8.4)
8	6.75 *s*	7.19 *s*	7.48 *m*	6.70 *d* (0.16)
2'	5.14 *dd* (8.0, 8.8)	7.62 *d* (2)	7.70 *d* (2.2)	-
3'	3.23 *m*	6.96 *d* (2)	6.84 *dd* (1.2, 2.2)	-
1"	-	4.62 *dd* (10.8,4.2) 4.44 *dd* (10.8,6.8)	-	-
2"	1.60 *s*	3.24 *dd* (4.2, 6.8)	-	-
3"	1.54 *s*	-	-	-
Me-3"	-	1.42 *s* 1.34 *s*	-	-
1(OSen)	-	-	-	-
2(OSen)	5.56 *s*	-	-	-
3(OSen)	-	-	-	-
4(OSen)	2.10 *s*	-	-	-
5(OSen)	1.86 *s*	-	-	-

[a] Chemical shifts were determined at 400 ([1]H) MHz in CDCl3 with TMS as internal standard.
[b] Chemical shifts were determined at 400 ([1]H) MHz in DMSO-d6.

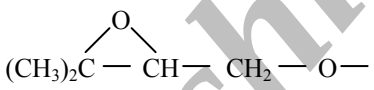
Table 2. [13]C-NMR spectral data for compounds **1**, **2**, **3**[a] and **4**[b].

C	**1**	**2**	**3**	**4**
2	161.5	161.0	161.0	161.3
3	112.6	113.1	114.6	111.3
4	143.7	139.0	144.1	144.5
4a	112.2	107.3	115.4	111.4
5	123.2	148.3	119.8	129.7
6	124.5	114.1	124.8	113.1
7	163.4	158.0	156.4	160.4
8	97.9	94.8	99.8	102.1
8a	155.7	152.5	152.0	155.5
2'	88.8	145.3	146.9	-
3'	29.6	104.5	106.4	-
1"	81.3	61.1	-	-
2"	22.3	72.2	-	-
3"	21.2	58.3	-	-
Me-3"	-	24.6 19.0	-	-
1(OSen)	165.8	-	-	-
2(OSen)	116.9	-	-	-
3(OSen)	156.7	-	-	-
4(OSen)	27.4	-	-	-
5(OSen)	20.1	-	-	-

[a] Chemical shifts were determined at 100 ([13]C) MHz in CDCl3 with TMS as internal standard.
[b] Chemical shifts were determined at 100 ([13]C) MHz in DMSO-d6.

at δ 6.75 (1H, *s*, H-8) and 7.22 (1H, *s*, H-5), H-2' at δ 5.14 (1H, *dd*, *J*=8.0, 8.8 Hz), H-3' at δ 3.23 (2H, *m*) and the deshielding of H-4, suggest the structure of a linear dihydrofuranocoumarin (13). A substituent at C-2' was identified by ^{1}H-NMR according to the following signals: two singlets at δ 1.60 (3H, *s*, H-2") and 1.54 (3H, *s*, H-3") (methyl groups) and the remaining signals in agreement with the presence of a senecioyl group; three singlets at δ 5.56 (1H, *s*, H-2), 2.10 (3H, *s*, H-4) and 1.86 (3H, *s*, H-5) (13,30). The ^{13}C-NMR spectral data (Table 2) showed nineteen signals. The peaks at δ 165.8 (C-1(OSen)) and 161.5 (C-2) ppm showed a characteristic senecioyloxy and pyrone carbonyl group, respectively. The infrared spectrum of compound **1** displayed two significant absorptions at 1727 and 1627 cm^{-1} due to the carbonyl and olefinic functional groups, respectively (30). On the basis of these data and the reported data for prantschimgin (13,29,30), the compound **1** was assigned as prantschimgin.

Compound **2**: The mass spectrum of the compound **2** had $[M]^+$ at m/z 286.1 suggesting the molecular formula $C_{16}H_{14}O_5$ (32). The ^{1}H-NMR spectrum shows two AB system at δ 6.32, δ 8.21 (1H each, *d*, *J*=9.6 Hz, H-3, H-4, respectively) and at δ 6.96, δ 7.62 (1H each, *d*, *J*=2 Hz, H-3', H-2', respectively) which are characteristic of the furanocoumarin skeleton. The presence of a singlet proton at δ 7.19 (1H, *s*, H-8) and the deshielding of H-4, suggest a linear furanocoumarin structure with a substituent at C-5 for compound **2**. This substituent was identified by ^{1}H-NMR spectroscopy as



group according to these signals: two singlets at δ 1.42 and δ 1.34 (methyl group) and an ABX system with signals at δ 4.62 (1H, *dd*, *J*=10.8, 4.2 Hz, H-1"), δ 4.44 (1H, *dd*, *J*=10.8, 6.8 Hz, H-1") and δ 3.24 (1H, *dd*, *J*=4.2, 6.8 Hz, H-2") (13). The ^{13}C-NMR spectral data (Table 2) showed sixteen signals. The peak at δ 161.0 (C-2) showed a characteristic pyrone carbonyl group. The signals at δ 158.0 (C-7), 152.5 (C-8a), 148.3 (C-5) and 145.3 (C-2') were related to , =C-O whereas the signals at δ 72.2 (C-2"), 61.1 (C-1") and 58.3 (C-3") were related to –C–O (13). The infrared spectrum of compound **2** displayed absorption at 1726 cm^{-1} due to the pyrone carbonyl group. On the basis of these and the reported data for oxypeucedanin (13,31,32), the compound **2** was assigned as oxypeucedanin.

Compound **3**: The mass spectrum of the compound **3** had $[M]^+$ at m/z 186 suggesting the molecular formula $C_{11}H_6O_3$ (34,35). The C-4, C-5 and C-8 protons in coumarin are markedly more deshielded than that on C-3 as a result of the linear fusion of a furan moiety in psoralen (36). The ^{1}H-NMR spectrum shows two AB system at δ 6.39, δ 7.81 (1H each, *d*, *J*=9.6 Hz, H-3, H-4, respectively) and at δ 6.84 (1H, *dd*, *J*=1.2, 2.2 Hz, H-3'), δ 7.70 (1H, *d*, *J*=2.2 Hz, H-2') which are characteristic of the furanocoumarin skeleton. The presence of a multiplet proton at δ 7.48 (1H, *m*, H-8) and the deshielding of H-4, suggest a linear furanocoumarin structure for compound 3. H-5 was observed at δ 7.69 (1H, *s*) (13). The ^{13}C-NMR spectral data (Table 2) showed eleven signals. The peak at δ 161.0 ppm shows a characteristic pyrone carbonyl group and δ 156.4 ,152.0 and 146.9 ppm are related to =C-O (34). The infrared spectrum of compound 3 displayed absorption at 1715 cm^{-1} due to the pyrone carbonyl group (37). On the basis of these and the reported data for psoralen (13,33-37), the compound **3** was assigned as psoralen.

Compound **4**: The mass spectrum of the compound **4** had $[M]^+$ at m/z 162 suggesting the molecular formula $C_9H_6O_3$. In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum, H-3 and H-4 were observed at δ 6.19 (*d*, *J*=9.4 Hz, 1H) and 7.93 (*d*, *J*=9.4 Hz, 1H) as AB-type signals, respectively. The ^{1}H-NMR spectrum showed an ABX system with signals at δ 7.52 (1H, *d*, *J*=8.4 Hz, H-5), δ 6.78 (1H, *dd*, *J*=0.2, 8.4 Hz, H-6) and δ 6.70 (1H, *d*, *J*=0.16 Hz, H-8) (13). The ^{13}C-NMR spectral data (Table 2) showed eleven signals. The peak at δ 161.3 ppm shows a characteristic pyrone carbonyl group and δ 160.4 (C-7) and 155.5 (C-8a) are related to =C-O (39,40). The infrared spectrum of compound **4** exhibits two strong bands at 1681 and 1235 cm^{-1}, which may be assigned to ν_{as} (C=O) and ν_s (C=O), respectively. The low carbonyl frequency (1681 cm^{-1}) is presumably due to intermolecular hydrogen bonding of the 7-hydroxyl hydrogen either with O(1) or with O(2). The ν_{as} (C–O) (phenolic) and ν_s (C–O) (phenolic) have been assigned at 1568 and 1322 cm^{-1} as very strong bands in umbelliferone, respectively (39). On the basis of these and the reported data for umbelliferone (13,38-41), the compound **4** was assigned as umbelliferone.

Compound **5**: The mass spectrum of the compound **5** had $[M]^+$ at m/z 414.3 suggesting the molecular formula $C_{29}H_{50}O$. The ^{1}H-NMR spectrum of the compound **5** displayed a multiplet at δ 3.53 for the H-3. This is a deshielded proton as a result of OH group. The ^{1}H-NMR spectrum displayed olefinic proton (H-6) at δ 5.36 as a distorted doublet. The same spectrum showed signals for Me-18 at δ 0.68 (*s*), Me-19 at δ 1.01 (*s*), Me-21 at δ 0.92 (*d*, *J*=6 Hz), Me-26 and 27 at

Table 3. 1H-NMR & 13C-NMR spectral data for compound **5**[a].

C/H atoms	δ C	δ H [*mult.*, *J* (Hz)]	C/H atoms	δ C	δ H [*mult.*, *J* (Hz)]
1	37.2	-	16	28.2	-
2	31.6	-	17	56.0	-
3	71.8	3.53 (*m*, 1H)	18	11.8	0.68 (*s*, 3H)
4	42.3	-	19	19.8	1.01 (*s*, 3H)
5	140.7	-	20	36.1	-
6	121.7	5.36 (*d*, 1H)	21	18.8	0.92 (*d*, *J*=6 Hz, 3H)
7	31.9	-	22	33.9	-
8	31.9	-	23	26.0	-
9	50.1	-	24	45.8	-
10	36.5	-	24a	23.0	-
11	21.1	-	24b	12.0	0.84 (*brs*, 3H)
12	39.7	-	25	29.1	-
13	42.3	-	26	19.0	0.82 (*d*, *J*=6.8 Hz, 3H)
14	56.7	-	27	19.4	0.82 (*d*, *J*=6.8 Hz, 3H)
15	24.3	-			

[a] Chemical shifts were determined at 400 (^{1}H) and 100 (^{13}C) MHz in CDCl3 with TMS as internal standard.

Table 4. Reports in the literature about compounds in the *Ferulago* species

Compound	Plant	Occurrence
Oxypeucedanin	F. Bernardii	Aerial parts
	F. brachyloba (13)	Roots
	F. capillaris (13)	Aerial parts
	F. capillaris (13)	Roots
	F. granatensis (9)	Umbels
	F. meoides (6)	Roots
	F. turcomanica (8)	Bark
	F. turcomanica (7)	Roots
Prantschimgin	*F. asparagifolia* (11)	Aerial parts
	F. aucheri (10)	Aerial parts
	F. Bernardii	Aerial parts
	F. brachyloba (13)	Roots
	F. capillaris (13)	Aerial parts
	F. capillaris (13)	Roots
	F. granatensis (9)	Umbels
	F. meoides (6)	Roots
Psoralen	F. Bernardii	Aerial parts
	F. turcomanica (8)	Bark
β-Sitosterol	F. Bernardii	Aerial parts
	F. granatensis (9)	Umbels

δ 0.82 (*d*, *J*=6.8 Hz) and Me-24b at δ 0.84 (*brs*) (42). The ^{13}C-NMR spectral data (Table 2) showed twenty-nine signals. The peaks at δ 140.7 (C-5) and 121.7 (C-6) are related to olefinic carbons. The signal at δ 71.8 (C-3) ppm may be related to C-O (43,44). The infrared spectrum of compound **5** displayed an absorption at 3438 cm^{-1} due to the hydroxyl function (42). On the basis of these and the reported data for β-sitosterol (29,42-44), the compound **5** was assigned as β-sitosterol.

Compound **6**: The mass spectrum of the compound **6** had $[M]^+$ at m/z 408 suggesting the molecular formula $C_{29}H_{60}$. The absence of an ion at m/z 393 $[M\text{-}Me]^+$ indicated the compound 6 not to be branched having a methyl group as substituent (45). The ^{1}H-NMR spectrum of the compound **6** displayed a triplet at δ 0.88 (*J*=6 Hz) for the two terminal methyl groups and a singlet at δ 1.25 for the methylene groups (45). The infrared spectrum of compound **6** displayed two absorptions at 1378 (methyl) and 724 cm^{-1} (long chain). On the basis of these and the reported data for nonacosane (29,45), the compound **6** was assigned as nonacosane.

CONCLUSION

This is the first report on the phytochemical analysis of *Ferulago Bernardii* Tomk. & M. Pimen. From the hexane extract of the aerial parts of *Ferulago Bernardii* four coumarins, prantschimgin, oxypeucedanin, psoralen and umbelliferone; one steroid, β-sitosterol; and a normal alkane, nonacosane were isolated by CC, PTLC and crystallization were characterized by melting point, and spectral data.

Four of these compounds have been found in some others *Ferulago* species (see Table 4). The presence of these compounds (oxypeucedanin, prantschimgin, psoralen and β-sitosterol) could be used as chemotaxonomic marker in genus *Ferulago*.

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REFERENCES

1. Rechinger KH, Hedge IC, Lamond JM. Flora Iranica. Graz: Akademische Druck- und Verlagsanstalt; 1987, No. 162, p. 428-430.
2. Mozaffarian V. A Dictionary of Iranian Plant Names. Tehran: Farhang Moaser; 1996. p. 230.
3. Baser KHC, Demirci B, Demirci F, Hashimoto T, Asakawa Y, Noma Y. Ferulagone: A new monoterpene ester from *Ferulago thirkeana* essential oil. Planta Med 2002; 68: 564-567.
4. Ozturk B, Gur S, Coskun M, Kosan M, Erdurak C, Hafez G, Ozgunes O, Cetinkaya M. Relaxant effect of *Ferulago syriaca* root extract on human corpus cavernosum. Eur Urol Suppl 2004; 3: 62.
5. Demetzos C, Perdetzoglou D, Gazouli M, Tan K, Economakis C. Chemical analysis and antimicrobial studies on three species of *Ferulago* from Greece. Planta Med 2000; 66: 560-563.
6. Ognyanov I, Bocheva D. Natural coumarins. II: Coumarins in *Ferulago meoides*. Planta Med 1969; 17: 65-70.
7. Andrianova VB, Sklyar YuE, Pimenov MG. Coumarins of *Ferulago turcomanica* roots. Khim Prir Soedin 1975; 11: 514 (Russ); CA 1976; 84: 27990u.
8. Serkerov SV, Kagramanov AA, Abbasov RM. Coumarins of Ferulago turcomanica. Khim Prir Soedin 1976; (1): 94 (Russ); CA 1976; 85: 59559x.
9. De Pascual TJ, Jimenez B, Corrales B, Grande M. Coumarins from Ferulago granatensis group: isovaleryl marmesin. An Quim 1979; 75: 175-176 (Span); CA 1979; 91: 39263s.
10. Doganca S, Ulubelen A, Tuzlaci E. 1-Acetylhydroquinone 4-galactoside from Ferulago aucheri. Phytochemistry 1991; 30: 2803-2805.
11. Doganca S, Tuzlaci E, Ulubelen A. Constituents of *Ferulago asperigifolia*. Fitoterapia 1992; 63: 552.
12. Ruberto G, Cannizzo S, Amico V, Bizzini M, Piattelli M. Chemical constituents of *Ferulago nodosa*. J Nat Prod 1994; 57: 1731-1733.
13. Jimenez B, Grande MC, Anaya J, Torres P, Grande M. Coumarins from *Ferulago capillaris* and *F. brachyloba*. Phytochemistry 2000; 53: 1025-1031.
14. Miski M, Moubasher HA, Mabry TJ. Sesquiterpene aryl esters from *Ferulago antiochia*. Phytochemistry 1990; 29: 881-886.
15. Baser KHC, Koyuncu M, Vural M. Composition of the essential oil of *Ferulago trachycarpa* (Fenzl) Boiss. J Essent Oil Res 1998; 10: 665-666.
16. Baser KHC, Demirci B, Duman H. Composition of the essential oil of *Ferulago asparagifolia* Boiss. from Turkey. J Essent Oil Res 2001; 13: 134-135.
17. Baser KHC, Demirci B, Ozek T, Akalin E, Ozhatay N. Micro-distilled volatile compounds from *Ferulago* species growing in western Turkey. Pharm Biol 2002; 40: 466-471.
18. Baser KHC. Aromatic biodiversity among the flowering plant taxa of Turkey. Pure Appl Chem 2002; 74: 527-545.

19. Ruberto G, Biondi D, Renda A. The composition of the volatile oil of *Ferulago* nodosa obtained by steam distillation and supercritical carbon dioxide extraction. Phytochem Anal 1999; 10: 241-246.
20. Rustaiyan A, Yari M, Masoudi Sh, Aghjani Z. Chemical constituents of the essential oil of *Ferulago contracta* Boiss. et Hausskn., a species endemic to Iran. J Essent Oil Res 1999; 11: 609-610.
21. Rustaiyan A, Sedaghat S, Larijani K, Khossravi M, Masoudi Sh. Composition of the essential oil of *Ferulago angulata* (Schlecht.) Boiss. from Iran. J Essent Oil Res 2002; 14: 447-448.
22. Demirci F, Iscan G, Guven K, Kirimer N, Demirci B, Baser KHC. Antimicrobial activities of *Ferulago* essential oils. Z Naturforsch, C: J Biosci 2000; 55: 886-889.
23. Hadjiakhoondi A, Aghel N, Etemadi R. Chemical and biological study of essential oil of *Ferulago macrocarpa* (Fenzi) Boiss. Hamdard Med 2002; 45: 35-38.
24. Rezazadeh S, Yazdani D, Shahnazi S. Chemical composition of essential oil of *Ferulago angulata* Boiss. in florescence from west of Iran. J of Medicinal Plants 2003; 2: 49-52.
25. Sefidkon F, Omidbaigi R. Chemical composition of the essential oil of *Ferulago angulata* from Iran. Journal of Essential Oil Bearing-Plants 2004; 7: 60-63.
26. Masoudi S, Rustaiyan A, Ameri N. Volatile Oils of *Ferulago phialocarpa* Rech. f. et H. Reidl. and Leutea elbursensis Mozaffarian from Iran. J Essent Oil Res 2004; 16: 143-144.
27. Erdurak CS, Coskun M, Demirci B, Baser KHC. Composition of the essential oil of fruits and roots of *Ferulago isaurica* Pesmen and *F. syriaca* Boiss. (Umbelliferae) from Turkey. Flavour Fragr J (In press). Published online in Wiley InterScience (www.interscience.wiley. com). DOI: 10.1002/ffj.1540.
28. Khalighi-Sigaroodi F, Hadjiakhoondi A, Shahverdi AR, Mozaffarian V, Shafiee A. Chemical composition and antimicrobial activity of the essential oil of *Ferulago Bernardii* Tomk. & M. Pimen. Daru 2005; 13: 100-104.
29. Djerassi C, et al. Dictionary of natural products. London: Chapman and Hall; 1994.
30. Nemoto T, Ohshima T, Shibasaki M. Enantioselective total syntheses of (+)-decursin and related natural compounds using catalytic asymmetric epoxidation of an enone. Tetrahedron 2003; 59: 6889-6897.
31. Woo WS, Lee CK, Shin KH. Isolation of drug metabolism modifiers from roots of *Angelica koreana*. Planta Med 1982; 45: 234-236.
32. Chaudhary SK, Ceska O, Tetu C, Warrington PJ, Ashwood-Smith MJ, Poulton GA. Oxypeucedanin, a major furocoumarin in parsley, *Petroselinum* crispum. Planta Med 1986; 45: 462-464.
33. O'Neil. The merck index: an encyclopedia of chemicals, drugs and biologicals, 30th ed. Whitehouse Station, NJ: Merck & CO., INC.; 2001.
34. Wang X, Wang Y, Yuan J, Sun Q, Liu J, Zheng C. An efficient new method for extraction, separation and purification of psoralen and isopsoralen from Fructus Psoraleae by supercritical fluid extraction and high-speed counter-current chromatography. J Chromat A 2004; 1055: 135-140.
35. Jiangning G, Xinchu W, Hou W, Qinghua L, Kaishun B. Antioxidants from a Chinese medicinal herb – *Psoralea corylifolia* L. Food Chem 2005; 91: 287-292.
36. Abu-Mustafa EA, Fayez MBE. Natural coumarins.VI. Nuclear magnetic resonance spectra of some coumarin and coumarilic acid derivatives. Can J Chem 1967; 45: 325-327.
37. Valenciennes E, Smadja J, Conan JY. Screening for biological activity and chemical composition of *Euodia borbonica* var. *borbonica* (Rutaceae), a medicinal plant in Reunion Island. J Ethnopharmacol 1999; 64: 283-288.
38. Das Gupta AK, Chatterje RM, Das KR. Coumarins and related compounds. Part IV. Aluminium chloride-catalyzed reaction of phenols with methyl acrylate: a new approach to synthesis of hydroxycoumarins. J Chem Soc 1969; C: 29-33.
39. Nath M, Jairath R, Eng G, Song X, Kumar A. Triorganotin (IV) derivatives of umbelliferone (7-hydroxycoumarin) and their adducts with 1,10-phenanthroline: synthesis, structural and biological studies. J Organomet Chem 2005; 690: 134–144.
40. Cussans NJ, Huckerby TN. Carbon-13 NMR spectroscopy of heterocyclic compounds-IV: a 20 MHz study of chemical shifts and carbon-proton coupling constants in a series of hydroxy, methoxy and glucosyl coumarins. Tetrahedron 1975; 31: 2719-2726.
41. Abu-Eittah RH, El-Tawil BAH. The electronic absorption spectra of some coumarins: a molecular orbital treatment. Can J Chem 1985; 63: 1173-1179.
42. Shaiq Ali M, Saleem M, Ahmad W, Parvez M, Yamdagni R. A chlorinated monoterpene ketone, acylated β-sitosterol glycosides and a flavanone glycoside from *Mentha longifolia* (Lamiaceae). Phytochemistry 2002; 59: 889-895.
43. Goad LJ, Akihisa T. Analysis of sterols. London: Blackie Academic and Prophessional; 1997.p.375.
44. Rauter AP, Filipe MM, Prata C, Noronha JP, Sampayo MAM, Justino J, Bermejo J. A new dihydroxysterol from the marine phytoplankton *Diacronema* sp. Fitoterapia 2005; 76: 433-8.
45. Shukla YN, Thakur RS. Aliphatic constituents from *Dubosia myoporoides*. Phytochemistry 1984; 23: 799-801.