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ISOLATION AND IDENTIFICATION OF FURANOCOUMARINS AND A PHENYLPROPANOID FROM THE ACETONE EXTRACT AND IDENTIFICATION OF VOLATILE CONSTITUENTS FROM THE ESSENTIAL OIL OF *Peucedanum pastinacifolium*

Seyed Ebrahim Sajjadi,[1*] Yalda Shokoohinia,[2] and Saeed Hemmati[3]

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Peucedanum pastinacifolium Boiss. & Hausskn. has been used by local inhabitants in Central and Western Iran as an antihyperlipidemic vegetable, which has been verified *in vivo* [1, 2]. The ethanolic extract of the aerial parts of *P. pastinacifolium* can lower total serum cholesterol, low-density lipoprotein cholesterol, triglyceride levels, and atherogenic indices in hypercholesterolemic rats after eight days [1]. In another study, one-month treatment of streptozotocin-induced diabetic rats with a hydroalcoholic extract of *P. pastinacifolium* aerial parts was shown to ameliorate their blood lipid profile [2].

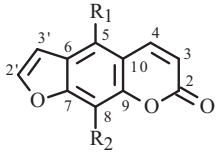
In our continuing investigation of *Apiaceaous* plants, it was decided to analyze *P. pastinacifolium* aerial parts. We identified three linear furanocoumarins, bergapten, isopimpinellin, and methoxsalen, along with a phenylpropanoid named elemicin from the acetone extract. The essential oil of the aerial parts of the plant was also analyzed using GC/MS. Literature survey revealed that the aerial parts of *P. pastinacifolium* have not been chemically studied to date; therefore, the present paper is the first phytochemical investigation on this plant.

By using several chromatographic separations, a number of furanocoumarins **1–3** and a phenylpropanoid **4** have been isolated.

Compounds **1–3** show characteristic peaks of furanocoumarins in ^{1}H NMR analysis. Detailed data are presented in Table 1. EI-MS revealed a molecular ion at *m/z* 216 for compounds **1** and **3**, and at *m/z* 246 for compound **2**, representing a further methoxy for compound **2** compared to the other two. This is confirmed as well by the peak around δ 4 (OCH_3) for these compounds, which is two singlets for compound **2**. The other doublets between δ 6.5 to 8 are assigned to hydrogens attached to unsaturated carbons of the furanocoumarin general skeleton. Compounds **1** and **3** are isomers characterized as bergapten and methoxsalen (xanthotoxin), respectively, by comparison of the ^{1}H NMR data of these compounds with those reported in the literature [3]. Compound **2** with a further OCH_3 was identified as isopimpinellin [3, 4].

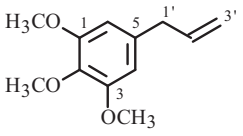
Compound **4** was a pale yellow liquid characterized as elemicin from the EI-MS spectrum, which revealed the molecular ion peak at *m/z* 208, and from ^{1}H NMR data (Table 1) that included one multiplet, one triplet, one doublet, and two singlets, which were in consistent with what has been reported for elemicin [5].

Bergapten (**1**) and isopimpinellin (**2**) have previously shown antifeedant [6] and antimicrobial activity [7]. Bergapten has shown antimutagenic effects [8] as well. They have been reported before from *Apiaceaous* plants [9].



1 - 3

1: $R_1 = OCH_3$, $R_2 = H$
2: $R_1 = R_2 = OCH_3$
3: $R_1 = H$, $R_2 = OCH_3$



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1) Department of Pharmacognosy, School of Pharmacy, Isfahan University of Medical Sciences, Isfahan, Iran, e-mail: sajjadi@pharm.mui.ac.ir; 2) Department of Pharmacognosy and Biotechnology, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran; 3) Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran. Published in *Khimiya Prirodnykh Soedinenii*, No. 4, July–August, 2012, pp. 598–600. Original article submitted May 23, 2011.

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TABLE 1. ^{1}H NMR Data of Isolated Compounds **1–4** (500 MHz, $CDCl_3$, δ)

Atom	**1**	**2**	**3**	**4**
3	6.300 d	6.320 d	6.405 d	–
4	8.185 d	8.153 d	7.795 d	6.435 s
5	–	–	7.384 s	–
6	–	–	–	6.435 s
8	7.167 s	–	–	–
1-OMe	–	–	–	3.869 s
2-OMe	–	–	–	3.849 s
3-OMe	–	–	–	3.869 s
5-OMe	4.302 s	4.204 s	–	–
8-OMe	–	4.200 s	4.335 s	–
1′	–	–	–	3.356 d
2′	7.625	7.032 d	7.725 d	5.981 m
3′	7.052 d	7.159 d	6.853 d	5.135 d
				5.107 d

TABLE 2. Percentage Composition of the Essential Oil of *Peucedanum pastinacifoium*

Compound	RI*	%	Compound	RI*	%
Hexanal	800	0.1	*trans*-Carveol	1216	0.2
trans-2-Hexenal	853	0.2	Citronellol	1227	0.2
Tricyclene	926	Tr.	Thymol methyl ether	1233	Tr.
α-Thujene	930	0.4	Pulegone	1237	0.1
α-Pinene	938	4.9	Bornyl acetate	1283	0.9
Camphene	952	0.5	*trans*-Pinocarvyl acetate	1296	Tr.
Sabinene	976	0.6	Methyl geraniate	1321	0.2
β-Pinene	980	2.7	*trans*-Carvyl acetate	1335	0.2
Myrcene	991	1.3	Eugenol	1353	Tr.
α-Phellandrene	1005	1.1	α-Copaene	1371	0.1
δ-3-Carene	1012	2.9	*trans*-Jasmone	1394	0.1
α-Terpinene	1018	0.3	Methyl eugenol	1404	6.1
p-Cymene	1027	0.9	β-Caryophyllene	1413	0.2
Limonene	1030	11.6	2,5-Dimethoxy-*p*-cymene	1422	2.1
cis-β-Ocimene	1041	2.4	*trans*-α-Bergamotene	1432	0.2
Phenylacetaldehyde	1046	0.1	*ar*-Curcumene	1479	0.4
trans-β-Ocimene	1052	5.9	Zingiberene	1491	0.9
γ-Terpinene	1061	0.3	β-Bisabolene	1504	1.5
Terpinolene	1088	1.6	Myristicin	1522	8.2
Linalool	1098	0.3	Elemicin	1569	31.1
trans-Verbenol	1146	Tr.	2,3,4,5-Tetramethoxyallylbenzene	1596	0.3
Umbellulone	1172	0.1	Dillapiole	1626	5.9
Terpinen-4-ol	1176	0.3	*trans*-Isoelemicin	1648	Tr.
p-Cymen-8-ol	1183	0.2	Acorenone	1682	0.5
α-Terpineole	1188	Tr.	Total		98.1

*RI: retention index experimental. Tr.: trace (≤ 0.05%).

Elemicin has been reported from many plants [10–12], where it is cited only as a part of the essential oil and not as an isolated pure compound, and antimicrobial [11] and antifungal effect [5] are the most prominent effects of the compound. The plant has shown antilipidemic action [1, 2] with good effect on glucose level [13], which is popular in folk medicine as well. Although some coumarins have previously shown anti-lipidemic effects [14–16], there is no information on the probable effect of the currently isolated coumarins on blood lipids. It is proposed to investigate the antilipidemic effects of these compounds.

The air-dried aerial parts of *P. pastinacifolium* yielded 0.2% of essential oil. Forty-nine components, comprising 98.1% of the total oil, were identified in the aerial parts of *P. pastinacifolium.* The list of compounds identified in the oil sample is presented in Table 2. As was clarified, the main components were elemicin (31.1%), limonene (11.6%), myristicin (8.2%), methyl eugenol (6.1%), dillapiole (5.9%) , *trans*-β-ocimene (5.9%), and α-pinene (4.9%).

The oil of aerial parts of *P. pastinacifolium* consisted of 16 monoterpene hydrocarbons (37.4%), 16 oxygenated monoterpenes (4.9%), six sesquiterpene hydrocarbons (3.3%), and one oxygenated sesquiterpene (0.5%). Six phenylpropanoid compounds were also detected in the volatile oil of *P. pastinacifolium* (51.3%). As is seen in Table 2, other nonterpenoic compounds comprised 0.7% of the oil.

However, the latest report on the essential oil of *Zeravschania pastinacifolia,* the synonym of *Peucedanum pastinacifolium*, gathered from central Iran, showed a very different composition of the essential oil [17]. While Yassa et al. reported the main component of the plant as β-bisabolene, we have found elemicin as the major constituent, which is confirmed by isolation of the compound from acetone extract and NMR analysis. Furthermore, Yassa et al. mentioned that sesquiterpene hydrocarbons were the dominant fraction (43.8%), followed by phenylpropanoids (31%) and only 2.3% monoterpenes. The current collected sample from Isfahan Province showed the major fraction to be phenylpropanoids (51%), followed by monoterpene hydrocarbons (37%) and only 3.3% sesquiterpene hydrocarbons. It is difficult to use this difference as a taxonomic distance indicator or just a habitant variation.

Plant Material. Aerial parts of *P. pastinacifolium* at the full flowering stage were collected from Isfahan Province, Iran, in May 2010 at an altitude of 1530 m. The plant identity was confirmed by the Botany Department of Isfahan University. A voucher specimen of the plant was deposited in the herbarium of the School of Pharmacy and Pharmaceutical Sciences (No. 1124). The air dried plant material was roughly cut and ground to a coarse powder.

Extraction and Isolation. 500 g of aerial parts was extracted with acetone for two days (5 L × 4). The extract was concentrated to a dark green viscous mass (EXT1 = 30 g), which was then filtered on a bed of TLC grade silica gel using EtOAc to yield 15 g extract (EXT2). The latter was fractioned by VLC (RP 18) using a gradient of MeOH–H_2O from 60 to 90% to afford four fractions (F1–F4).

Fraction F2 (1.75 g) was separated by VLC (Si gel 0.015–0.04 column) using a gradient of EtOAC in heptane from 20 to 100% to obtain four fractions (F2a–d). Fraction F2c was recrystallized to give a mass of crystals, which was a mixture of three furanocoumarins. It was further purified by DCM–MeOH (98:2) to give a pure coumarin **1** and a binary mixture of two coumarins **2** and **3**.

Fraction F2a and part of F3 were combined and separated by VLC (Si-gel 0.015–0.04 μm) using heptane–EtOAc (9:1) to obtain four fractions (F2a1–a4). Fraction F2a4 after evaporation resulted in compound **4**. Some fatty acid mixtures were obtained that were not further analyzed.

Essential Oil Isolation. Plant material was hydrodistilled in a Clevenger-type apparatus for 3 h according to the method recommended in the British Pharmacopeia [18]. The volatile oil was dried over anhydrous sodium sulfate and stored in a sealed vial at 4°C until analysis. The yield of oil was calculated based on dry weight of the plant material.

GC-MS Analysis. Gas chromatography combined with mass spectrometry (GC-MS) was used for identification of the components. The analysis was performed on an Agilent 5975C mass selective detector coupled with an Agilent 7890A GC, equipped with an HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm). The oven temperature was programmed from 60°C to 280°C at 4°C/min. Helium was used as carrier gas at a flow rate of 2 mL/min. Injector and detector temperatures were 280°C. The MS operating parameters were: ionization voltage 70 eV; ion source temperature 230°C.

Identification of the Components of the Essential Oil. Identification of components of the oil was based on GC retention indices relative to *n*-alkanes and computer matching with NIST and Wiley275.L libraries, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature [19, 20].

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