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Isolation and Identification of Nicotiflorin and Narcissin from the Aerial Parts of *Peucedanum aucheri* Boiss.

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Abstract: *Peucedanum aucheri* Boiss. (Apiaceae) is a herbaceous wild plant native to Iran and is used in Iranian folk medicine as a diuretic and for the treatment of kidney disorders. Phytochemical investigation of different extracts prepared from the aerial part *of P. aucheri* Boiss. resulted to the isolation of two main flavonol glycosides from methanolic extract. Using comprehensive spectroscopic methods, including 1D and 2D nuclear magnetic resonance (NMR) spectroscopy and mass spectroscopy, chemical structure of isolated compounds were determined as kaempfrol-3-o-rutinoside (nicotiflorin) and isorhamnetin-3-o-rutinoside (narcissin). Although narcissin has previously been isolated from *P. ruthenicum*, to the best of our knowledge, isolation of nicotiflorin from *Peucedanum* genus is reported for the first time.

Key words: *Peucedanum aucheri*, Apiaceae, flavonoid, nicotiflorin, narcissin.

1. Introduction

The genus *Peucedanum* with more than 100 species in the world is one of the biggest genera in Apiaceae family, and the plants could be found in South, Western and Central Asia, Europe and mediterranean region [1]. These plants have medicinal uses and some species have been used traditionally in the treatment of cold [2], angina and as anti-tussive and anti-asthma [3]. The medicinal plant *P. pastinacifolium* Boiss. has been shown to decrease lipids in hypercholesterolemic rats [4] and posses antispasmodic activity on rat's ileum [5], while the antimicrobial activity of *P. ruthenicum* M. Bieb was shown against gram-positive and gram-negative bacteria [6]. A variety of other medicinal effects, such as antiplatelet aggregation [7], antioxidant [8], antimycobacterial [9] and antimutagenic activity [10], have also been demonstrated for different plants of this genus in other studies.

Coumarins, flavonoids, butenolides, chromones, terpenes and sterols are the main natural compounds that have been isolated from different *Peucedanum* species through the last phytochemical studies [11]. Among them, flavonoids are more investigated and interested compounds, and many flavonoids have been isolated from different species of this genus. Isolation of quercetin, rutin, isorhamnetin, kaempferol and quercetin-3-o-rutinoside from different species of *Pecudanum* are the most important examples [11, 12].

Flavonoids are of pharmacological importance, and their antiatherosclerotic, anti-inflammatory, antitumor, antithrombogenic, anti-angiogenesis, antiviral, antibacterial and antifungal effects have been demonstrated through numerous scientific studies [13]. Inverse correlation between flavonoid intake and total plasma cholesterol concentration [14], inverse correlation of flavonoids intake and incidence of dementia [15] and decreasing the risk of death from coronary heart disease in elderly patients [16] are other important medicinal benefits of flavonoid intake in humans.

P. aucheri Boiss., which is called in Kurdish "Ba-reh-za", is a herbaceous wild plant native to Iran [17] and considerably used as a condiment in the west provinces of Iran, especially in Kurdistan and Kermanshah. *P. aucheri* is also a medicinal plant, and in Iranian folk medicine, it has been generally used as

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a diuretic and for the treatment of kidney disorders. This study aimed to investigate the chemical structure of main flavonoids existing in the aerial parts of *P. aucheri* Boiss..

2. Materials and Methods

2.1 Plant Material

Aerial parts of *P. aucheri* Boiss. were collected in May 2014 from Marivan city, Kurdistan province, Iran. The plant was characterized by Mrs Farahnaz Houshidari, botanical expert of Research Center for Agriculture and Natural Resources in Kurdistan and a voucher specimen (No. 3487) has been registered and kept in Department of Pharmacognosy, Faculty of Pharmacy, Isfahan University of Medical Science, Isfahan, Iran.

2.2 General Methods

H nuclear magnetic resonance (HNMR) and C nuclear magnetic resonance (CNMR) spectra were recorded by Bruker 400 MHz (H at 400 MHz and C at 100 MHz) spectrometer, using CD_3OD as solvent and also for signal calibration (δ_H = 3.31, δ_C = 49.0). One-bond heteronuclear ^{1}H-^{13}C connectivities were determined with 2D heteronuclear single-quantum coherence (HSQC) pulse sequence with an interpulse delay set for $^{1}J_{CH}$ of 130 Hz, while two and three bond heteronuclear ^{1}H-^{13}C connectivities, determined with 2D heteronuclear multiple bond correlation (HMBC), were optimized for $^{2\text{-}3}J_{CH}$ of 8 Hz.

Medium pressure liquid chromatography (MPLC) was performed by Buchi Gradient System C-605 apparatus using glass columns of LiChroprep® RP-18 (25-40 μm) and C-660 Buchi fraction collector. Thin layer chromatography (TLC) was done when necessary on silicagel 60 F254 plates using $BuOH:H_2O:CH_3CO_2H$ 60:25:15 (BAW) as mobile phase and cerium sulfate in 2 N H_2SO_4 and natural product (NP) as reagents for visualizing the spots.

High-performance liquid chromatography (HPLC) was performed by Waters 515 apparatus equipped with a refractive index detector (Waters 2414) and semipreparative C18 column (Novapak® 3.9 mm × 300 mm), in isocratic mode.

2.3 Extraction, Isolation and Identification

Air-dried powdered aerial parts of *P. aucheri* Boiss. (1.1 kg) were extracted at room temperature in a three step extraction method with the following solvents at increasing polarity: hexane, dichloromethane (DCM) and methanol (MeOH). Each step was conducted for 1 day under stirring and repeated three times using 5 L of solvent. All the extracts were concentrated to dryness using rotary evaporator and checked with TLC (SiO_2, BAW 60:15:25, v/v/v) for interesting phenolic compounds.

The methanol extract (78 g) was subjected to column chromatography by MPLC instrument on a glass column (36 mm × 460 mm), filled by silicagel (40-63 μm) using a linear gradient of MeOH:DCM with increasing polarity. Analyzing the eluates by TLC (SiO_2, BAW 60:15:25, v/v/v), the 10th fraction was subjected to reverse column chromatography on a RP-18 glass column (36 mm × 460 mm, Lichroprep®, 25-40 μm) using a linear gradient solvent system of H_2O to MeOH. Based on TLC (SiO_2, BAW 60:15:25, v/v/v) and preliminary NMR analysis of the eluates, two fractions were considered to be rich on phenolic compounds and selected for final purification by HPLC.

The first fraction was subjected to purification by HPLC using a semi preparative C18 column and H_2O-MeOH (50:50) isocratic mobile phase, resulting the compound 1 (5 mg, t_R = 11 min) as a pure flavonoid glycoside.

The second fraction was first re-fractionated by MPLC instrument on a RP-18 column (15 mm × 460 mm) with a linear gradient of $MeOH:H_2O$ (15:85) to pure MeOH. An aliquot of the main sub-fraction was then subjected to purification by recrystallization, which finally yielded the compound 2 (7 mg) as a pure flavonoid glycoside.

3. Results and Discussion

Two flavonol glycosides were isolated and purified from the methanolic extract of the aerial parts of *P. aucheri*.

Compound 1 was isolated as a yellowish powder. High resolution analysis of compound 1 on the molecular ion peak in the negative ion mode gave a peak at m/z = 593.25 M-H, indicating the molecular formula as $C_{27}H_{30}O_{15}$. CNMR spectrum in agreement with the molecular formula showed 27 carbon signals, while, HNMR spectrum of compound 1 revealed the characteristic signals of flavonol glycosides. Two singlet proton signals at δ_H 6.23 (s, 1 H, H-6) and δ_H 6.43 (s, 1 H, H-8), followed by two doublet proton signals at δ_H 6.91 (d, *J* = 8.9 Hz, 2 H, H-3' and H-5') and δ_H 8.09 (d, *J* = 8.9 Hz, 2 H, H-2' and H-6') revealed the kaempferol-like nature of the aglycon part of the compound (Table 1). There were also two anomeric proton signals at δ_H 5.15 (d, *J* = 7.5 Hz, 1 H, Glu-1) and δ_H 4.54 (bs, 1 H, Rha-1), which together with overlapped proton signals at δ_H 3-4, demonstrating the glycosilated nature of compound 1 as

Table 1 ^{1}H and ^{13}C NMR data of flavonol glycosides isolated from the aerial parts of *P. aucheri*.

Position	Compound 1 (kaempferol- 3-O-rutinoside)		Compound 2 (isorhamnetin-3-o-rutinoside)	
	δ_H (mult., int., *J* (Hz))	δ_C	δ_H (mult., int., J (Hz))	δ_C
2	-	158.30	-	157.80
3	-	134.50	-	134.65
4	-	178.80	-	178.50
5	-	161.70	-	162.40
6	6.23 (s, 1 H)	99.98	6.12 (s, 1 H)	99.95
7	-	165.80	-	165.32
8	6.43 (s, 1 H)	94.95	6.31 (s, 1 H)	94.86
9	-	158.30	-	158.40
10	-	104.80	-	104.94
1′	-	122.60	-	123.16
2′	8.08 (d, 1 H, 8.9 Hz)	132.40	7.55 (s, 1 H)	115.71
3′	6.90 (d, 1 H, 8.9 Hz)	116.09	-	148.92
4′	-	160.40	-	149.81
5′	6.92 (d, 1 H, 8.9 Hz)	116.09	6.78 (d, 1 H, 8.0 Hz)	116.06
6′	8.10 (d, 1 H, 8.9 Hz)	132.40	7.57 (d, 1 H, 8.0 Hz)	123.55
3′-OCH_3	-	-	3.25 (s, 1 H)	55.02
1″	5.15 (d, 1 H, 7.5 Hz)	104.61	5.16 (d, 1 H, 7.5 Hz)	104.70
2″	3.21-3.85[a]	75.77	3.21-3.72[a]	75.72
3″	3.21-3.85[a]	78.14	3.21-3.72[a]	77.19
4″	3.21-3.85[a]	71.44	3.21-3.72[a]	71.39
5″	3.21-3.85[a]	77.21	3.21-3.72[a]	78.17
6″	3.21-3.85[a]	68.56	3.21-3.72[a]	68.54
1‴	4.54 (bs, 1 H)	102.21	4.42 (bs, 1 H)	102.43
2‴	3.21-3.85[a]	72.10	3.21-3.72[a]	72.11
3‴	3.21-3.85[a]	72.29	3.21-3.72[a]	72.22
4‴	3.21-3.85[a]	73.89	3.21-3.72[a]	73.92
5‴	3.21-3.85[a]	69.75	3.21-3.72[a]	69.72
6‴	1.14 (d, 1 H, 6.0 Hz)	17.94	1.02 (d, 1 H, 6.0 Hz)	17.90

Mult.: multiplicity of the NMR signal; int: integral of the NMR signal (showing the number of H); *J*: coupling constant (*J* coupling); s: singlet; bs: broad singlet; d: doublet; [a] overlapped with other signals.

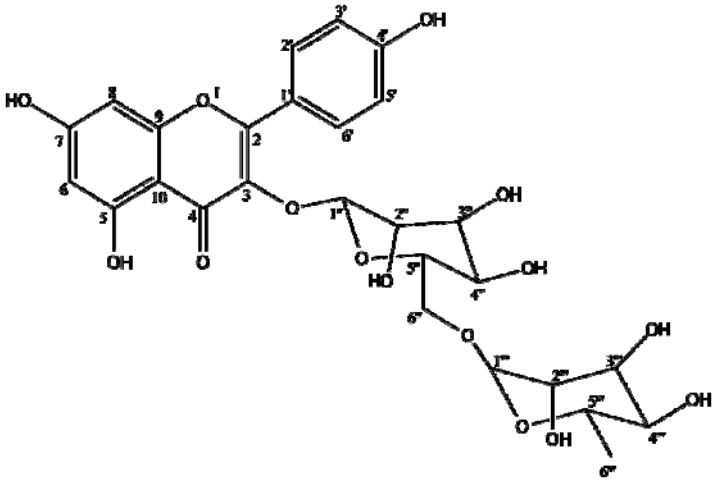

Fig. 1 Chemical structure of compound 1 (kaempferol 3-O-rutinoside) isolated from the aerial parts of *P. aucheri.*

a rutinoside unit. Observing a big singlet at δ_H 1.14 (d, 1 H, J = 6 Hz, Rha-CH3), typical of rhamnose methoxyl group, was also used to confirm the presence of rhamnose in the chemical structure of compound 1. According to these data and by comparing them with the NMR data of different flavonol glycosides reported in the literature [18], the structure of compound 1 was defined as kaempferol 3-O-rutinoside (nicotiflorin) (Fig. 1).

Compound 2 was also isolated as a yellowish powder. Comparing the NMR spectrum of this compound with that of compound 1 exhibited a great amount of similarity between the two compounds, except for the presence of a 3'-methoxyl group in the compound 2. Two doublet proton signals at δ_H 6.12 (d, 1 H, H-6, J = 2.5 Hz) and δ_H 6.31 (d, 1 H, H-8, J = 2.5 Hz), together with a doublet proton signal at δ_H 6.78 (d, J = 8.0 Hz, 1 H, H-5') and an overlapped proton signal at about δ_H 7.57 (d, J = 8.0, 1 H, H-6' and s, 1 H, H-2') were observed in the HNMR spectra of compound 2, revealing the substituted-flavonol nature of the aglycon part of compound 2. A big singlet of methoxyl group existed at HNMR spectrum (δ_H 3.25; s, 3 H, 3'-OCH_3), which was supported by the methoxyl carbon signal at CNMR spectrum (δ_C 55.02), confirming the presence of a methoxyl group in the chemical structure of aglycon part of compound 2 (Table 1). According to these data and the NMR data reported in Ref. [1], the chemical structure of compound 2 was defined as isorhamnetin 3-O-rutinoside (narcissin) (Fig. 2).

Many secondary metabolites, including flavonoids and coumarins, have been isolated from various parts of many *Peucedanum* species [11]. In this study, two O-glycosilated flavonol derivatives, namely nicotiflorin and narcissin, were isolated from the aerial parts of *P. aucheri*, an important edible and medicinal plant, common to the west regions of Iran.

Nicotiflorin is a naturally occurred flavonol glycoside, which has been isolated from plant species, like *Astragalus armatus* [19], *Edgeworthia chrysantha* [20], *Caragana bungei* [21], Flos sophorae immaturus [22], *Carthamus tinctorius* [23] and *Acalypha hispida* [24]. It has been demonstrated to have many interesting pharmacological activities, such as decreasing arterial blood pressure and heart rate [25], hepatoprotective effects on CCl_4-induced liver injury [23], potent antioxidant, anti-inflammatory, antinociceptive [26], antihypertensive and antianaphylactic effects [27] and had a protective effect against cerebral ischemic damage [28]. It is also known that nicotiflorin have a protective effect against memory dysfunction and oxidative stress in multi-infarct dementia model rats [29].

Narcissin is also a natural flavonol glycoside and has been isolated from plant species, like *P. ruthenicum* [1],

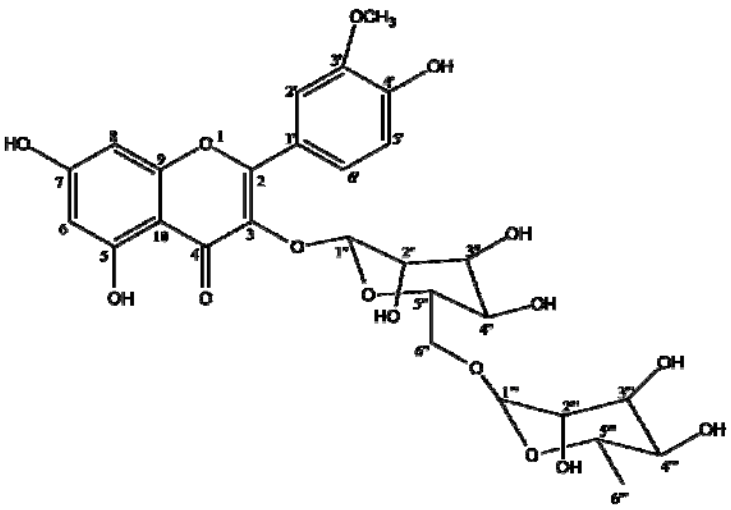

Fig. 2 Chemical structure of compound 2 (isorhamnetin-3-O-rutinoside) isolated from the aerial parts of *P. aucheri.*

Caragana bungei [21], *Avicennia marina* [30], *Calotropis gigantean* [31] and *Aristolochia kankauensis* [32]. It has been proved to have important medicinal activities, including induction of apoptosis in human myelogenous erythroleukaemia cells [33], markedly increasing the coronary flow and decreasing the arterial blood pressure, the oxygen consumption and the myocardial contractile force (MCF) in isolated hearts [34], antiprotozoal activity that showing selectivity against *Entamoeba histolytica* [35] and anthelmintic effect against gastrointestinal nematodes [36].

4. Conclusions

Phytochemical investigation of *Peucedanum aucheri* Boiss. showed the isolation of nicotiflorin and narcissin as the main phenolic constituents from aerial parts of the plant. Although narcissin has been previously isolated from *P. ruthenicum*, isolation of nicotiflorin from *Peucedanum* species is reported for the first time by this study and could be used to investigate and explain the medicinal properties of the plant.

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