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Short communication

Phenylacylated-flavonoids from *Peucedanum chryseum*

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ABSTRACT

Phytochemical investigation of the methanol extract of the aerial parts of *Peucedanum chryseum* (Boiss. & Heldr.) D.F.Chamb., Apiaceae, led to the isolation of a dihydrofuranochromone, cimifugin (**1**); a phloroacetophenone glucoside, myrciaphenone A (**2**); and a flavonoid glycoside, afzelin (**3**) along with two phenylacylated-flavonoid glycosides: rugosaflavonoid C (**4**), and isoquercitrin 6″-*O*-*p*-hydroxybenzoate (**5**). The structures of compounds **1**–**5** were elucidated by extensive 1D- and 2D-NMR spectroscopic analysis in combination with MS experiments and comparison with the relevant literature. All compounds are reported for the first time from this species and compounds **2**, **4**, and **5** from the genus *Peucedanum* and from Apiaceae.

Introduction

The genus *Peucedanum*, Apiaceae, comprises more than 120 species distributed in Europe, Asia, Africa, and North America and 21 species with 42% endemism in the Flora of Turkey (Davis, 1984; Sarkhail, 2014). *Peucedanum* species have been used in traditional folk medicine to treat various ailments such as cough, cramps, pain, rheumatism, asthma, gastrointestinal disorders, cardiovascular problems, and angina (Sarkhail, 2014). Due to their biological effects many different species have attracted attention in terms of their phytochemical contents. There are several reports on the chemistry of essential oil of the genus and different classes of coumarins, flavonoids, and simple phenolics were reported from this genus (Sarkhail, 2014). Coumarin compounds, especially pyrano- and furanocoumarins, were isolated from different *Peucedanum* species and usually the biological activities of the species were attributed mainly to these compounds (Chang-Yih et al., 1992; Kong et al., 2003). The chemical studies on *P. chryseum* (Boiss. & Heldr.) D.F.Chamb. only examined its monoterpene hydrocarbons and fatty acid composition (Ağalar et al., 2015).

The objective of the study is to isolate and characterize the secondary metabolites of *P. chryseum* which has not been phytochemically studied in detail before.

Materials and methods

NMR (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR, both used TMS as internal standard) were measured on a Bruker AM 400 spectrometer and MS spectra on a LC/MS High Resolution Time of Flight (TOF) Agilent 1200/6530 instrument. Kieselgel 60 (Merck, 0.063–0.200 mm) was used for open column chromatography (CC). Sephadex LH-20 (General Electric) was used for general permeation chromatography (GPC). LiChroprep C_{18} (Merck, 40–63 μm) was used for vacuum-liquid chromatography (VLC; vacuum by H_2O aspiration). TLC analyses were carried out on pre-coated Kieselgel 60 F_{254} aluminum plates (Merck). Compounds were detected by UV fluorescence and spraying 1% vanillin/H_2SO_4, followed by heating at 100 °C for 1–2 min.

Aerial parts (including flowers, leaves, and stems) of *Peucedanum chryseum* (Boiss. & Heldr.) D.F.Chamb., Apiaceae, which is endemic to Turkey, were collected from the roadside between Seydişehir and Akseki (about 2 km from Akseki, 1800 m) in July 2007. The plants were identified by Prof. Dr. Hayri Duman (Department of Biology, Faculty of Sciences, Gazi University). A voucher specimen (HUEF-08004) has been deposited in the Hacettepe University Faculty of Pharmacy Herbarium, Ankara, Turkey.

The dried powdered aerial parts of *P. chryseum* (1000 g) were extracted with methanol (3 l × 3) at 45 °C. After evaporation of the solvent (yield 8.2%), 82.6 g of MeOH extract was dissolved in 300 ml of water and successively partitioned with hexane (75 ml × 6), chloroform (200 ml × 4), and *n*-BuOH (150 ml × 3). After evaporation

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the *n*-BuOH (24.8 g) was applied to open CC using Sephadex LH-20 (SP-LH 20) with MeOH to give five subfractions (Fr. A–E). Fr. B (2.47 g) was subjected to open CC using normal-phase silica gel as stationary phase and eluted with EtOAc–MeOH–H_2O mixtures (100:10:5, 100:15:7.5, and 100:17:13) to provide 46 fractions. Further purification of Fr. B 11–16 with SP-LH 20 gave compound **1** (40 mg). Fr. C (1.18 g) was subjected to open CC using normal-phase silica gel as stationary phase and eluted with EtOAc–MeOH–H_2O mixtures (100:10:5 and 100:17:13) to provide twenty fractions. Fr. C 12–17 (80 mg) were combined and chromatographed over reverse-phase material (Lichroprep RP-18, 25–40 μm) eluting with decreasing polarity of MeOH:H_2O (0:100→50:50) mixtures to afford compound **2** (15 mg). Fr. D (1.9 g) was subjected to MPLC using a fraction collector (6–8 mbar, 10 ml/min) on reversed-phase silica gel and eluted with MeOH–H_2O 0:100→50:50 to give 67 subfractions. Fr. D 41–44 (36 mg) were combined and re-chromatographed over reversed-phase silica gel and eluted with MeOH:H_2O (0:100→50:50) to afford compounds **4** (12 mg) and **3** (3 mg). Fr. E (1.45 g) was fractionated over reversed-phase material eluting with different MeOH–H_2O (0:100→50:50) mixtures to afford 105 fractions. Fr. E 99–105 (35 mg) were further purified with SP-LH 20 and eluted with MeOH to give compound **5** (35 mg).

The structures of the isolated compounds were elucidated by 1D- and 2D-NMR analyses along with LC-MS and comparison with literature data: myrciaphenone A (**2**) (Yoshikawa et al., 1998), cimifugin (**1**) (Liu et al., 2008; Sasaki et al., 1982), isoquercitrin 6″-*O*-*p*-hydroxybenzoate (**5**) (Marzouk et al., 2006), rugosaflavonoid C (**4**) (Abou-Zaid and Nozzolillo, 1991; Hu et al., 2013), and afzelin (**3**) (Chen et al., 2004).

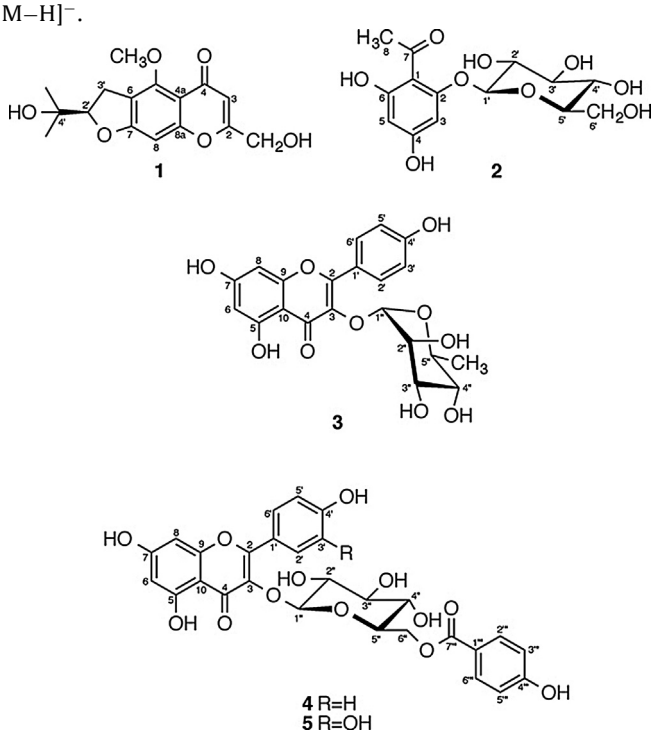
Cimifugin (**1**): ^{1}H NMR (400 MHz, CD_3OD) δ 6.61 (s, 1H, H-8), 6.24 (s, 1H, H-3), 4.77 (t, J = 8.6 Hz, 1H, H-2′), 4.45 (s, 2H, C$\underline{H}_2$OH), 3.94 (s, 3H, OC$\underline{H}_3$), 3.28 (d, J = 9.0 Hz, H-3′), 1.31 (s, 3H, gem (C$\underline{H}_3$)$_2$), 1.25 (s, 3H, gem (C$\underline{H}_3$)$_2$). ^{13}C NMR (100 MHz, MeOD) δ 178.36 (C-4), 167.28 (C-2), 165.70 (C-5), 159.73 (C-7), 155.62 (C-8a), 117.06 (C-6), 110.92 (C-4a), 107.85 (C-3), 93.11 (C-8), 91.21 (C-2′), 70.81 (C-4′), 59.74 (C$\underline{H}_2$OH), 59.62 (OC$\underline{H}_3$), 27.30 (C-3′), 23.98 (gem (C$\underline{H}_3$)$_2$), 23.92 (gem (C$\underline{H}_3$)$_2$) ESIMS *m/z* 308.15 $[M+2H]^+$.

Phloroacetophenone 2′-*O*-glucoside (myrciaphenone A) (**2**): ^{1}H NMR (400 MHz, CD_3OD) δ 6.17 (brs, 1H, H-3), 5.93 (d, J = 2.1 Hz, 1H, H-5), 5.03 (d, J = 7.6 Hz, 1H, H-1′), 3.92 (d, J = 12.0 Hz, 1H, H-6′α), 3.74 (dd, J = 12.0 Hz, 4.9 Hz, 1H, H-6′β), 3.57–3.39 (m, 4H, remaining sugar signals), 2.69 (s, 3H, H_3-8). ^{13}C NMR (100 MHz, CD_3OD) δ 203.15 (C-7), 166.33 (C-6), 165.74 (C-4), 161.21 (C-2), 105.05 (C-1), 100.52 (C-1′), 97.23 (C-5), 94.23 (C-3), 77.07 (C-3′/C-5′), 76.90 (C-5′/C-3′), 73.30 (C-2′), 69.62 (C-4′), 60.92 (C-6′), 32.03 (C-8) ESIMS *m/z* 329.15 $[M-H]^-$.

Kaempferol 3-*O*-α-rhamnopyranoside (afzelin) (**3**): ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 7.74 (d, J = 8.7 Hz, 2H, H-2′, H-6′), 6.91 (d, J = 7.3 Hz, 2H, H-3′, H-5′), 6.40 (brs, 1H, H-8), 6.19 (brs, 1H, H-6), 5.28 (brs, 1H, H-1″), 4.10–3.04 (m, 4H, remaining sugar signals), 0.77 (d, J = 5.8 Hz, 3H, H-6″); ESIMS *m/z* 431.15 $[M-H]^-$.

Kaempferol 3-*O*-β-(6″-*p*-hydroxybenzoyl)-glucoside (rugosaflavonoid C, astragalin 6″-*O*-*p*-hydroxybenzoate (**4**): ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 8.02 (d, J = 8.8 Hz, 2H, H-2′, H-6′), 7.52 (d, J = 8.7 Hz, 2H, H-2‴, H-6‴), 6.85 (d, J = 8.8 Hz, 2H, H-3′, H-5′), 6.66 (d, J = 8.6 Hz, 2H, H-3‴, H-5‴), 6.38 (brs, 1H, H-8), 6.20 (brs, 1H, H-6), 5.49 (d, J = 7.7 Hz, 1H, H-1″), 4.21 (dd, J = 11.3 Hz, 2.2 Hz, 1H, H-6″α), 4.10 (m, 1H, H-6″β), 3.72–3.76 (m, 1H, H-5″), 3.71–3.67 (brs, 1H, H-2″), 3.61–3.54 (m, 1H, H-3″), 3.48–3.44 (*, 1H, H-4″).*Overlapped with the solvent signal. ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ 177.89 (C-4), 165.69 (C-7‴), 162.40 (C-4‴), 162.37 (C-5), 160.46 (C-7), 160.41 (C-4′), 156.79 (C-9), 156.56 (C-2), 133.48 (C-3), 131.48 (C-2‴, C-6‴), 131.34 (C-2′, C-6′), 121.29 (C-1′), 120.48 (C-1‴), 115.57 (C-3‴, C-5‴), 115.53 (C-3′, C-5′), 104.06 (C-10), 101.74 (C-1″), 99.41 (C-6), 94.14 (C-8), 73.50 (C-3″/C-5″), 73.31 (C-5″/C-3″), 71.47 (C-2″), 68.74 (C-4″), 64.01 (C-6″) ESIMS *m/z* 567.10 $[M-H]^-$.

Isoquercitrin 6″-*O*-*p*-hydroxybenzoate (**5**): ^{1}H NMR (400 MHz, CD_3OD) δ 7.78 (brs, 1H, H-2′), 7.60 (d, J = 8.4 Hz, 2H, H-2‴, H-6‴), 7.57 (brd, J = 8.1 Hz, 1H, H-6′), 6.83 (d, J = 8.4 Hz, 1H, H-5′), 6.67 (d, J = 8.4 Hz, 2H, H-3‴, H-5‴), 6.35 (brs, 1H, H-8), 6.19 (brs, 1H, H-6), 5.22 (d, J = 7.7 Hz, 1H, H-1″), 4.37 (dd, J = 11.1 Hz, 2.1 Hz, 1H, H-6″α), 4.31 (dd, J = 10.8 Hz, 4.2 Hz, 1H, H-6″β), 3.97–3.78 (m, 3H, H-2″, H-3″, H-5″), 3.62 (brd, J = 9.2 Hz, 1H, H-4″). ^{13}C NMR (100 MHz, MeOD) δ 178.07 (C-4), 166.37-C-7‴), 164.64 (C-7), 161.96 (C-4‴), 161.51 (C-5), 157.25 (C-2), 156.91 (C-9), 148.50 (C-4′), 144.41 (C-3′), 134.07 (C-3), 131.20 (C-2‴, C-6‴), 121.58 (C-6′), 121.37 (C-1′), 120.43 (C-1‴), 116.21 (C-2′), 114.65 (C-5′), 114.60 (C-3‴, C-5‴), 104.04 (C-10), 103.59 (C-1″), 98.57 (C-6), 93.36 (C-8), 73.54 (C-3″/C-5″), 73.35 (C-5″/C-3″), 71.63 (C-2″), 68.92 (C-4″), 62.96 (C-6″) ESIMS *m/z* 584.15 $[M-H]^-$.



Result and discussion

The present work reports for the first time the characterization of five phenolic compounds (**1**–**5**) from the aerial parts of *P. chryseum*. Notably this is the first report of a phloroacetopheone glycoside, myrciaphenone A (**2**), and two acylated glycosyl flavonoids, rugosaflavonoid C (**4**) and isoquercitrin 6″-*O*-p-hydroxybenzoate (**5**), from the genus *Peucedanum* and family Apiaceae. The presence of flavonoids in higher plants has been associated with various environmental conditions to meet adapting and conflicting demands to various environmental pressures such as high-light/UV-stress, cold stress, nutritional deficiencies, and pathogen protection (Dixon and Paiva, 1995; Kusano et al., 2011; Roberts and Paul, 2006). Compounds **4** and **5**, with a benzoyl acylation pattern, are also proof of the UV stress mediated acylation of the flavonoids (Saito et al., 2013) since, the plant samples were collected from over 1800 m, where the plants were exposed to high UV radiation.

Several phytochemical studies on Apiaceae plants led to the isolation of mainly simple, psoralen-, and angelisin-type coumarins and their glycosides and there are many studies about the chemistry of *Peucedanum* also focusing on those compounds (Sarkhail, 2014). Cimifugin, a dihydrofuranochromone derivative, was first

isolated from *Peucedanum austriaca* (Stefanovic et al., 1984) and a glycoside of cimifugin (prim-*O*-glucosylcimifugin) was isolated from *P. japonicum* (Chang-Yih et al., 1992) along with afzelin from *P. oreoselinum* (Bodalski and Cisowski, 1971). This is the second report of cimifugin and afzelin isolated from a *Peucedanum* species with agreement in terms of Apiaceae chemistry.

Myrciaphenone A, a phloroglucinol glycoside, has been isolated previously from different plant sources, namely *Myrcia multiflora*, *Syzygium samarangens*, and *Corymbia maculate*, Myrtaceae (Yoshikawa et al., 1998; Sidana et al., 2013; Mamdouh et al., 2014); *Artemisia iwayomogi* and *A. stolonifera*, Asteraceae (Yan et al., 2014); *Curcuma comosa*, Zingiberaceae (Suksamrarn et al., 1997); and *Lawsonia inermis*, Lythraceae (Hsouna et al., 2011). Recently, more glucosylated forms (azerosides) have been reported from the roots of *Dorema glabrum*, Apiaceae (Delnavazi et al., 2015). Since phloroacetophenones possess a rather limited distribution within the genus *Peucedanum* as well as in the family Apiaceae, further studies are needed to better understand these compounds' contribution to the chemotaxonomy of this family. In conclusion, comparing the results of our research with those of other reports on the chemistry of the genus *Peucedanum* it is clear that nearly the same classes of secondary metabolites were present, except acylated glycosyl flavonoids. The geographical conditions, especially altitude, and the seasonable temperature variations may have led to this different and rare flavonoid pattern. The presence of those valuable phenolic compounds in *Peucedanum* species definitely enriches the chemical diversity and provides evidence for chemotaxonomic studies of *Peucedanum* species and the family Apiaceae as well.

Author's contribution

MYB and AKU carried out the phytochemical process for isolation. PG performed the NMR experiments, did the structure elucidation and peak assignments of the compounds. AKU, wrote the manuscript. ZG and LÖD contributed to the critical reading the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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