Turk J Pharm Sci 2019;16(2):191-195
DOI: 10.4274/tjps.galenos.2018.53215

ORIGINAL ARTICLE

Flavonoid Glycosides from *Heracleum pastinaca* Fenzl

Heracleum pastinaca Fenzl'in Flavonoit Glikozitleri

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ABSTRACT

Objectives: The objective was to isolate and characterize the secondary metabolites of *Heracleum pastinaca*, which has not been previously investigated.

Materials and Methods: Conventional chromatographic procedures were carried out for isolation of the compounds. The structures of the compounds were elucidated by extensive 1D and 2D nuclear magnetic resonance spectroscopic analysis in combination with mass spectrometry experiments and comparison with the relevant literature data.

Results: This first phytochemical investigation on all parts of *H. pastinaca* Fenzl led to the isolation and identification of seven known flavonoid glycosides: isoquercetin (**1**), rutin (**2**), afzelin (**3**), astragalin (**4**), isorhamnetin 3-O-glucoside (**5**), nicotiflorin (**6**), and narcissoside (**7**).

Conclusion: This is the first report on the isolation of these flavonoid glycosides from *H. pastinaca* and compounds **3**, **5**, **6**, and **7** from the genus *Heracleum*.

Key words: *Heracleum*, Apiaceae, isorhamnetin, flavonoid glycosides, chemotaxonomy

ÖZ

Amaç: Bu çalışmanın amacı, üzerinde daha önce herhangi bir fitokimyasal çalışma bulunmayan *Heracleum pastinaca*'nın sekonder bileşiklerinin izolasyonu ve karakterizasyonudur.

Gereç ve Yöntemler: Maddelerin izolasyonları, klasik kromatografik prosedürlere göre yapılmıştır. Saf bileşiklerin yapıları, kütle spektrometresi deneyleri ile 1D ve 2D nükleer manyetik rezonans spektroskopik analizleri kullanılarak aydınlatılmış; literatür verileriyle de doğrulanmıştır.

Bulgular: *H. pastinaca* Fenzl'in tüm kısımlarının ilk defa fitokimyasal açıdan incelenmesinde, yedi adet bilinen; izokersetin (**1**), rutin (**2**), afzelin (**3**), astragalin (**4**), izoramnetin 3-O-glukozit (**5**), nikotiflorin (**6**) ve narkisozit (**7**) isimli flavonoit glikozitleri izole edilerek tanımlanmıştır.

Sonuç: Bu çalışma ile izole edilen flavonoitlerin tamamı, *H. pastinaca*'dan, **3**, **5**, **6**, **7** nolu bileşikler ise *Heracleum* cinsinden ilk defa tanımlanmıştır.

Anahtar kelimeler: *Heracleum*, Apiaceae, izoramnetin, flavonoit glikozitleri, kemotaksonomi

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Received: 16.01.2018, **Accepted:** 15.03.2018

INTRODUCTION

The genus *Heracleum*, which is known as hogweed, is one of the largest genera of Apiaceae, containing more than 120 species widely distributed in Central Europe and Asia as well as 17 species with 41% endemism in the flora of Turkey.[1,2]

Heracleum species have been traditionally used as spices and food additives as well as in the treatment of inflammation, flatulence, stomachache, epilepsy, and psoriasis. They also act as carminative, antiseptic, antimicrobial, analgesic, and anticonvulsant agents.[3] Some *Heracleum* species are used traditionally for different purposes in Turkey, i.e., *Heracleum crenatifolium* as a vegetable and condiment,[4] *Heracleum trachyloma* against asthma and bronchitis,[5] *Heracleum spondylium* L. subsp. *ternatum* as a galactagogue,[6] and *Heracleum persicum* and *Heracleum platytaenium* for gastritis and epilepsy and as a sedative.[7]

There are many phytochemical studies on *Heracleum* species that mainly focused on furanocoumarins[8,9] and furanocoumarin glycosides,[10,11] together with alkaloids,[12] polyacetylenes,[13] and flavonoids.[14-16] Essential oil compounds of the genus were also studied.[17-19] While the phytochemical studies and bioactivity studies were mostly conducted with the coumarin compounds of the genus, we wanted to examine the flavonoid content of *Heracleum pastinaca* Fenzl (Figure 1), which is a tiny rare endemic plant mainly distributed in the inner and southwest region of Anatolia.[20]

Conventional chromatographic purification procedures were carried out to isolate the compounds of *H. pastinaca*. The structures of the compounds were elucidated by extensive 1D and 2D nuclear magnetic resonance (NMR) and electrospray ionization (ESI)-mass spectrometry (MS) experiments confirmed by the relevant literature data. The chemotaxonomic significance of the compounds was discussed.

EXPERIMENTAL

General

NMR spectra (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR, both use TMS as internal standard) were measured on a Bruker AM 400 spectrometer and MS spectra on a LC/MS Shimadzu 8040 instrument. Kieselgel 60 (Merck, 0.063-0.200 mm) was used for open column chromatography (CC). Sephadex (SP) LH-20 (SP LH-20) (General Electrics Healthcare) was used for gel permeation chromatography. LiChroprep C_{18} (Merck, 40-63 µm) was used for medium pressure liquid chromatography (MPLC) (Buchi Pump Module: C-601, ultraviolet (UV)-Photometer: C-640, control unit: C-620, Fr. Collector: C-660). Thin layer chromatography (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.

Figure 1. *Heracleum pastinaca* Fenzl

Plant material

Whole parts (aerial parts and roots) of *H. pastinaca* were collected from the Maden-Kızıltepe region (Niğde-Ulukışla), at about 2600 m altitude from calcareous rock clefts on August 2017. A voucher specimen was deposited at the Herbarium of Hacettepe University Faculty of Pharmacy (code HUEF-17015).

Extraction and isolation

The dried and powdered whole parts of *H. pastinaca* (90 g) were extracted with MeOH (500 mL×4) at 37°C. After the evaporation of the solvent (yield 18%), the crude MeOH extract (17 g) was first dissolved in water and then partitioned between *n*-hexane and *n*-BuOH. *n*-BuOH (4.5 g) extract was first submitted to CC on Sephadex LH-20 (2.5×60 cm) and eluted with MeOH. Four main fractions (Fr) [Fr. 1 (1.7 g); Fr. 2 (2.2 g), Fr. 3 (332.6 mg), Fr. 4 (130 mg)] were obtained. Fr. 3 (332.6 mg) was submitted to a reverse phase column (1.5 cm×15 cm) and eluted with a gradient H_2O:MeOH solvent system (10%→ 50%; 10 mL/min; 4-5 mbar) with the MPLC system coupled with a fraction collector to give four subfractions (Fr. 3a-d).

Fr. 3d gave compound **3** (4 mg). Further purification of Fr. 3a (116 mg) with a reverse phase column (1.5 cm×15 cm) and elution with a gradient H_2O:MeOH solvent system (20%→ 30%; 10 mL/min; 4-5 mbar) yielded two subfractions. These two fractions were submitted to a TLC plate (20×20 cm) separately and eluted with 70:30:3 ($CHCl_3$:MeOH:H_2O) to yield compounds **1** (22 mg) and **2** (32 mg), respectively. Fr. 3c (63 mg) was submitted to two different TLC plates (20×20 cm) and eluted with 70:30:3 ($CHCl_3$:MeOH:H_2O). After elution, the bands belonging to the compounds were detected under UV_{254} light and scraped to obtain compounds **4** and **5** (24 mg) and compounds **6** and **7** (16 mg), respectively, as mixtures.

Structure elucidation

The structures of the compounds (Figure 2) were elucidated by 1D and 2D NMR experiments. The positions of the sugar units were confirmed by 2D HMBC experiments. Together with ESI-MS data and comparison with the relevant literature the compounds were elucidated as follows: isoquercetin (**1**),[21] rutin (**2**),[21,22] afzelin (**3**),[23] astragalin (**4**),[21,24] isorhamnetin 3-*O*-β-glucopyranoside (**5**),[25] kaempferol 3-*O*-rutinoside (**6**),[21,22] and isorhamnetin 3-*O*-rutinoside (**7**).[22]

Quercetin 3-O-β-glucopyranoside (Isoquercetin) ***(1)***

Yellow powder; Negative ESI/MS m/z: 463 [M-H]$^-$; ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.72 (d, *J*=2.1 Hz, 1H, H-2′), 7.58 (dd, *J*=8.5, 2.1 Hz, 1H, H-6′), 6.86 (d, *J*=8.5 Hz, 1H, H-5′), 6.24 (d, *J*=2.0 Hz, 1H, H-8), 6.08 (d, *J*=2.0 Hz, 1H, H-6), 5.11 (d, *J*=7.6 Hz, 1H, H-1″), 3.71 (dd, *J*=11.8, 2.3 Hz, 1H, H-6a″), 3.59 (dd, *J*=12.5, 4.6 Hz, 1H, H-6b″), 3.56–3.17 (m, 4H, remaining sugar signals).

Quercetin 3-O-α-rhamnopyranosyl (1→6)-β-glucopyranoside (Rutin) ***(2)***

Yellow powder; Negative ESI/MS m/z: 609 [M-H]$^-$; ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.68 (brs, 1H, H-2′), 7.64 (brd, *J*=8.0 Hz, 1H, H-6′), 6.87 (d, *J*=8.2 Hz, 1H, H-5′), 6.30 (brs, 1H, H-8), 6.13 (brs, 1H, H-6), 5.03 (d, *J*=7.6 Hz, 1H, H-1″), 4.53 (brs, 1H, H-1‴), 3.81 (brd, *J*=10.5 Hz, 1H, H-6a″), 3.70-3.20 (m, 9H, remaining sugar signals), 1.15 (d, *J*=6.2 Hz, 3H).

Kaempferol 3-O-α-rhamnopyranoside (Afzelin) ***(3)***

Yellow powder; Negative ESI/MS m/z: 431 [M-H]$^-$; ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.78 (d, *J*=8.9 Hz, 2H, H-2′, 6′), 6.95 (d, *J*=8.8 Hz, 2H, H-3′, 5′), 6.39 (d, *J*=2.1 Hz, 1H, H-8), 6.21 (d, *J*=2.1 Hz, 1H, H-6), 5.38 (d, *J*=1.6 Hz, 1H, H-1″), 4.23 (dd, *J*=3.3, 1.6 Hz, 1H, H-2″), 3.76-3.68 (m, 1H, H-3″), 3.53-3.40 (m, 2H, H-4″, 5″), 0.93 (d, *J*=5.7 Hz, 3H, H-6″).

Kaempferol 3-O-β-glucopyranoside (Astragalin) ***(4)***

Yellow powder; Negative ESI/MS m/z: 447 [M-H]$^-$; ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.04 (d, *J*=8.8 Hz, 2H, H-2′, 6′), 6.89 (d, *J*=8.2 Hz, 2H, H-3′, 5′), 6.24 (d, *J*=1.9 Hz, 1H, H-8), 6.09 (d, *J*=1.9 Hz, 1H, H-6), 5.27 (d, *J*=7.3 Hz, 1H, H-1″), 3.76-3.57 (m, 2H, H-6″), 3.56-3.16 (m, 4H, remaining sugar signals).

Isorhamnetin 3-O-β-glucopyranoside ***(5)***

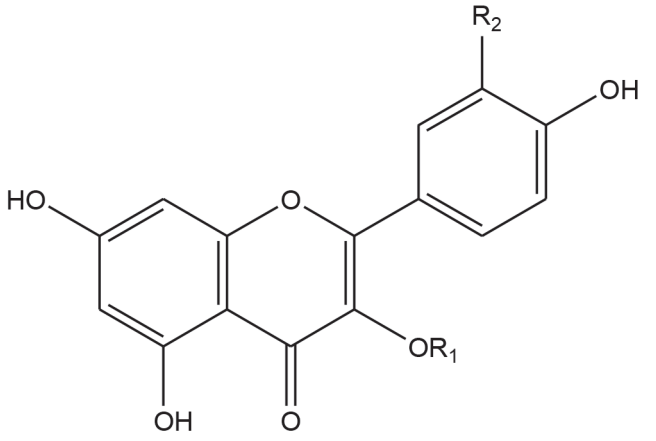
Yellow powder; Negative ESI/MS m/z: 477 [M-H]$^-$; ^{1}H NMR (400 MHz, MeOH-d_4) δ 7.91 (d, *J*=1.9 Hz, 1H, H-2′), 7.59 (dd, *J*=8.5, 1.9 Hz, 1H, H-6′), 6.89 (d, *J*=8.5 Hz, 1H, H-5′), 6.24 (d, *J*=1.9 Hz, 1H, H-8), 6.09 (d, *J*=1.9 Hz, 1H, H-6), 5.10 (d, *J*=7.4 Hz, 1H, H-1″), 3.94 (s, 3H, OC$\underline{H}_3$), 3.76-3.57 (m, 2H, H-6″), 3.56-3.16 (m, 4H, remaining sugar signals).

Kaempferol 3-O-rutinoside (Nicotiflorin) ***(6)***

Yellow powder; Negative ESI/MS m/z: 593 [M-H]$^-$; ^{1}H NMR (400 MHz, MeOH-d_4) δ 8.06 (d, *J*=8.8 Hz, 2H, H-2′, 6′), 6.89 (d, *J*=8.0 Hz, 2H, H-3′, 5′), 6.28 (brs, 1H, H-8), 6.12 (d, *J*=1.8 Hz, 1H, H-6), 5.14 (d, *J*=7.3 Hz, 1H, H-1″), 4.51 (brs, 1H, H-1‴), 3.86-3.62 (m, 2H, H-6″), 3.61-3.22 (m, 8H, remaining sugar signals), 1.15 (d, 6.2 Hz, 3H, H-6‴).

Isorhamnetin 3-O-rutinoside (Narcissoside) ***(7)***

Yellow powder; Negative ESI/MS m/z: 623 [M-H]$^-$; ^{1}H NMR (400 MHz, MeOH-d_4) 7.95 (d, *J*=1.8 Hz, 1H, H-2′), 7.62 (dd, *J*=8.5, 1.8 Hz, 1H, H-6′), 6.89 (d, *J*=8.0 Hz, 1H, H-5′), 6.28 (brs, 1H, H-8), 6.12 (d, *J*=1.8 Hz, 1H, H-6), 5.02 (d, *J*=7.3 Hz, 1H, H-1″), 4.52 (brs, 1H, H-1‴), 3.95 (s, 3H, OC$\underline{H}_3$) 3.86-3.62 (m, 2H, H-6″), 3.61-3.22 (m, 8H, remaining sugar signals), 1.12 (d, 6.2 Hz, 3H, H-6‴).



	R_1	R_2
1	Glucose	OH
2	Rutinose	OH
3	Rhamnose	H
4	Glucose	H
5	Glucose	OCH_3
6	Rutinose	H
7	Rutinose	OCH_3

Figure 2. Structures of compounds **(1-7)**

RESULTS AND DISCUSSION

The present work reports for the first time the characterization of seven flavonoid glycosides, **1-7**, from all parts of *H. pastinaca.* To the best of our knowledge, this is the first report of compounds **3, 5, 6**, and **7** from the genus *Heracleum*, while others were reported from different *Heracleum* species before, i.e., isoquercetin (**1**) from *H. napalense*[26] and *H. mollendorfii*,[15] astragalin from *H. mollendorfii*,[15] and rutin from *H. sphondylium*.[27,28] The presence of flavonoids in higher plants has been associated with various environmental conditions, such as high-light/UV stress, cold stress, nutritional deficiencies, and pathogen protection.[29-31] The habitats of the samples were at about 2600 m altitude, where the plants were exposed to high UV radiation. This fact should affect the production of different types and quantities of flavonoids in the plant. Phytochemical investigations of *Heracleum* species have mostly focused on the linear and angular type furanocoumarins, and different biological activities of the genus such as insecticidal, antibacterial, antiviral, and antifungal may be attributed to these coumarin-type compounds.[3] There are limited phytochemical studies about the isolation of flavonoids from *Heracleum* species. A number of flavonoids, i.e., kaempferol, quercetin, isorhamnetin,[16] rutin,[28] astragalin,[15] flavantaside, and epirutin[32] were reported from different *Heracleum* species. In the present study, the isolated and elucidated flavonols were mainly kaempferol, quercetin, and isorhamnetin glycosides. Flavonoids possess many important biological activities such as antimicrobial,[33] antioxidant,[34] and antiviral.[35] The presence of those valuable flavonoids in *Heracleum* species definitely

enriches their chemical diversity and provides evidence for chemotaxonomic studies of *Heracleum* species and the family Apiaceae as well.

CONCLUSIONS

This first phytochemical study of *H. pastinaca* led to the isolation and structure identification of seven flavonoid glycosides. The structures of the isolated compounds were elucidated by 1D and 2D NMR analyses, together with ESI-MS data and comparison with relevant literature data: isoquercetin (**1**),[21] rutin (**2**),[21,22] afzelin (**3**),[23] astragalin (**4**),[21,24] isorhamnetin 3-*O*-β-glucopyranoside (**5**),[25] nicotiflorin (**6**),[21,22] and narcissoside (**7**).[22] Notably this is the first report of these flavonol glycosides from *H. pastinaca* and compounds **3**, **5**, **6**, and **7** from the genus *Heracleum*. In conclusion, when considering the relationship between the bioactivities and the chemistry of *Heracleum* species, it is a possible that flavonoids can also play an important role in contributing to the bioactivity and traditional uses of *Heracleum* species.

ACKNOWLEDGEMENTS

The author is indebted to Dr. Şengül Dilem Doğan (Erciyes University) for her help with the structure elucidation and Dr. Ahmet Savran for identifying the plant material and providing the high-resolution photo of the plant.

Conflict of Interest: No conflict of interest was declared by the authors.

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