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 flora of Turkey. [1-2]

Heracleum species have been traditionally used as spice and food additive 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 i.e. *H. crenatifolium* as vegetable and condiment [4]; *H. trachyloma* against asthma and bronchitis [5]; *H. spondylium* L. subsp. *ternatum* as galactagogue [6]; *H. persicum* and *H. platytaenium* for gastritis, epilepsy and as sedative [7] in Turkey.

There are plenty of phytochemical studies on *Heracleum* species 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] Although the phytochemical studies and bioactivity studies are mostly conducted with the coumarin compounds of the genus, we would like to examine the flavonoid content of *H. pastinaca* Fenzl. (Figure 1) which is a rare endemic tiny plant, mainly distributed in the inner and the southwest region of Anatolia. [20]

Conventional chromatographic purification procedures were carried out to isolate the compounds of the *H. pastinaca*. The structures of the compounds were elucidated by extensive 1D and 2D NMR and ESI-MS experiments confirmed by the relevant literature data. Chemotaxonomic significance of the compounds was discussed.

EXPERIMENTAL

General

NMR (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 LH-20 (SP LH-20) (General Electrics Healthcare) was used for Gel Permeation Chromatography (GPC). LiChroprep C_{18} (Merck, 40-63 μm) was used for Medium Pressure Liquid Chromatography (MPLC) (Buchi Pump Module: C-601, UV-Photometer: C-640, control Unit: C-620, Fr. Collector: C-660). 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.

Plant material

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

Extraction and isolation

The dried and powdered whole parts of *H. pastinaca* (90 g) was extracted with MeOH (500 mL x 4) at 37 °C. After the evaporation of the solvent (yield 18%), the crude MeOH extract (17 g) was first dissolved in water than partitioned between *n*-hexane and *n*-BuOH respectively. n-BuOH (4.5 g) extract was first submitted to column chromatography on Sephadex LH-20 (2,5 x 60 cm) and eluted with MeOH. Four main fractions (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 reverse phase column (1.5 cm x 15 cm) and eluted with 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 sub fractions (Fr.3 a-d).

Fr. 3d gave compound **3** (4 mg). Further purification of Fr. 3a (116 mg) with reverse phase column (1.5 cm x 15 cm) and eluted with gradient H_2O:MeOH solvent system (20%→ 30%; 10 mL/min; 4-5 mbar) yielded two sub fractions. These two fractions were submitted to TLC plate (20x20 cm) separately and eluted with 70:30:3 ($CHCl_3$: MeOH: H2O) to yield compounds **1** (22 mg) and compound **2** (32 mg) respectively. Fr. 3c (63 mg) was submitted to two different TLC plates (20x20 cm) and eluted with 70:30:3 ($CHCl_3$: MeOH: H2O). After elution, the bands that belong 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 are confirmed by 2D HMBC experiments. Together with ESI-MS data and comparison with relevant literature the compounds are 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‴).

RESULTS AND DISCUSSION

The present work reports for the first time the characterization of seven flavonoid glycosides **1-7** from the whole parts of *H. pastinaca.* To 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. i.e. isoquercetin (**1**) from *H. napalense* [26] and *H. mollendorfii* [15]; astragalin from *H. mollendorfii* [15] and rutin from *H. sphondylium* [27-28] before. 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 etc. [29-31] The habitat of the samples were about 2600 m altitudes where the plants were exposed to a high UV radiation. This fact should effect the production of different type and quantities of flavonoids of the plant. The phytochemical investigation of the *Heracleum* species are mostly focused on the linear and angular type furanocoumarins and different biological activities of the genus such as, insecticidal, antibacterial, antiviral and antifungal were may be attributed to the these coumarin type compounds.[3] There are limited phytochemical studies about the isolation of the flavonoids from *Heracleum* species. Few flavonoids i.e. kaempferol, quercetin, isorhamnetin [16] rutin [28], astragalin [15], flavantaside and epirutin[32] were reported from different *Heracleum* species. In this study, the isolated and elucidated flavonols are mainly kaempferol, quercetin and isorhamnetin glycosides. Flavonoids posseses many important biological activities such as antimicrobial [33], antioxidant [34], antiviral [35] etc. The presence of those valuable flavonoids in *Heracleum* species definitely enriches the chemical diversity and

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

CONCLUSION

The first phytochemical study of *Heracleum pastinaca* led to the isolation and structure identification of seven flavonoid glycosides. The structure of 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 should be a possible fact that; flavonoids can also play an important role to contribute the bioactivity and traditional uses of the *Heracleum* species.

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