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TRICETIN 4′-*O*-α-L-RHAMNOPYRANOSIDE: A NEW FLAVONOID FROM THE AERIAL PARTS OF *Erica arborea*

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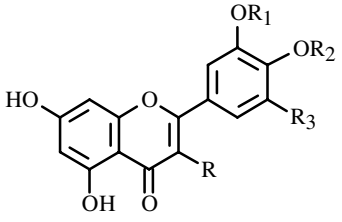
Reversed-phase preparative HPLC purification of the methanol (MeOH) extract of the aerial parts of Erica arborea *resulted in the isolation of a new flavonoid glycoside, tricetin 4′-*O-α-*L-rhamnopyranoside, together with the known flavonoid, isorhamnetin 3-*O-α-*L-rhamnopyranoside. The structures of these compounds were determined by spectroscopic means. The antioxidant properties of these compounds were assessed by the DPPH assay.*

Key words: *Erica arborea*, Ericaceae, flavonol, tricetin, glycoside, antioxidant activity, DPPH, chemical taxonomy.

The genus *Erica* (family Ericaceae) comprises about 100 species that are distributed in Europe, the Middle East, and Africa [1]. *Erica arborea* L., a shrub or small evergreen tree, commonly known as "tree heath", "briar root", or "funda", is native to a number of countries in Africa, Temperate Asia and Europe, and also naturalized in the British Isles, Australia, and New Zealand [2-4]. In Turkey, this species is widely distributed throughout West and North Anatolia and the Mediterranean basin, and its leaves and flowers have been used as diuretic, urinary antiseptic, diet tea, and laxative [5]. There is no report on any thorough and systematic phytochemical study on *E. arborea* available to date. However, the nonvolatile constituents of this plant were analyzed by GC-MS [6]. Monoterpenes and condensed tannin were also reported from this plant [7, 8]. As a part of our on-going phytochemical and pharmacological studies on Turkish and Iranian plants [9-12], we now report on the isolation, structure elucidation, and antioxidant activities of a new flavone rhamnoside (**1**) and a known flavonol rhamnoside (**2**) from the leaves of *E. arborea*.



1, 2

1: R = R_1 = H, R_2 = α-L-Rha*p*, R_3 = OH

2: R = *O*-α-Rha*p*, R_1 = Me, R_2 = R_3 = H

Reversed-phase preparative HPLC purification of the MeOH extract of the leaves of *E. arborea* afforded a new flavone rhamnoside, tricetin 4′-*O*-α-L-rhamnopyranoside (**1**), and a known flavonol rhamnoside, isorhamnetin 3-*O*-α-L-rhamnopyranoside (**2**). The structures of these compounds were determined by UV, MS, and NMR data analyses.

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TABLE 1. ^{1}H (Coupling Constant J/Hz in Parentheses) and ^{13}C NMR Data of **1**

C atom	Chemical shift in ppm	
	δ_H	δ_C
Aglycone (tricetin) moiety		
2	-	164.8
3	6.86 s	103.2
4	-	181.6
5	-	162.1
6	6.22 d (1.8)	95.3
7	-	164.8
8	6.38 d (1.8)	93.8
9	-	157.5
10	-	104.9
1′	-	120.9
2′	7.00 s	108.7
3′	-	145.8
4′	-	135.3
5′	-	145.8
6′	7.00 s	108.7
Rhamnosyl moiety		
1″	4.98 d (2.0)	102.3
2″	3.34-3.98*	70.8
3″	3.34-3.98*	70.9
4″	3.34-3.98*	72.1
5″	3.34-3.98*	70.2
6″	1.01 d (6.0)	16.9

*Overlapped peaks; 200 MHz for ^{1}H and 50 MHz for ^{13}C NMR (in CD_3OD)

TABLE 2. Antioxidant Properties of the MeOH Extract and Compounds **1** and **2** in the DPPH Assay

Compounds/Extract	RC_{50} value, mg/mL
MeOH extract	9.9×10^{-3}
Tricetin 4-*O*-α-L-rhamnopyranoside (**1**)	15.6×10^{-3}
Isorhamnetin 3-*O*-α-L-rhamnopyranoside (**2**)	17.2×10^{-3}
Positive control: quercetin	2.88×10^{-5}

Compound **1** was readily identified as a 5,7,3′,4′,5′-trioxygenated flavone from its UV spectra [13]. From the analyses with various shift reagents, it was found that compound **1** possessed a 4′-substituted tricetin (5,7,3′,4′,5′-pentahydroxyflavone) nucleus. The ESIMS mass spectrum of **1** revealed $[M+H]^+$ (positive ion mode) ion peak at *m/z* 449, suggesting M_r 448 and solving for $C_{21}H_{20}O_{11}$. The molecular formula was further confirmed from its HRESIMS spectrum, where the $[M+H]^+$ ion was observed at *m/z* 449.1084 ($C_{21}H_{21}O_{11}$ requires: *m/z* 449.1084). The ^{1}H and ^{13}C NMR spectra (Table 1) confirmed the identity of the aglycone unit as tricetin [14-16] and the sugar moiety as an α-L-rhamnose. On the basis of the results obtained from the UV analyses with various shift reagents, which confirmed that there was no free 4′-OH present in **1**, the rhamnosyl moiety could be placed at C-4′. The placement of the rhamnosyl unit at C-4 could also be further confirmed from the ^{1}H-^{13}C long-range correlation (^{3}J) from the anomeric proton of the rhamnosyl unit (H-1″, δ 4.98) to the oxygenated aromatic quarternary carbon (C-4′, δ 135.3), observed in the HMBC spectrum. Thus, compound **1** was identified unequivocally as tricetin 4′-*O*-α-L-rhamnopyranoside, which to the best of our knowledge is a new natural product. Compound **2** was readily identified as isorhamnetin 3-*O*-α-L-rhamnopyranoside by direct comparison of its UV, MS, ^{1}H, and ^{13}C NMR data with published data [14-16].

In the chemotaxonomic study on the family Ericaceae, gossypetin was defined as a taxonomic marker for this family [16]. Later, several studies revealed the presence of a number of other flavonoids and their glycosides in this family [17-20]. While flavonoid **2** occurs in many other plant genera from many families, the occurrence of tricetin or its derivatives is rather rare and sporadic [21]. The *O*-glycosidation at C-3 of flavonols is common in the genus *Erica* [17, 18]. It is interesting to note that 3′,4′,5′-oxygenation in the flavonoid skeletons (as in **1**) occurs in both *E. arborea* and *E. verticillata* [20]. In the same way, glycosylation at C-4′ (as in **1**) has also previously been observed in *E. cinerea* [19]. The new flavonoid **1** is, in fact, a 4′-rhamnoside of a known flavone, tricetin, which has previously been employed as a marker of *Eucalyptus* honey [22]. It is noteworthy that characters based on the distribution of flavonoids have successfully been used to address some taxonomic problems in the family Ericaceae [23]. Thus the isolation and identification of the flavonoid glycosides **1** and **2** from the *Erica arborea* might be chemotaxonomically significant.

The MeOH extract as well as the flavonoids (**1** and **2**) exhibited significant levels of antioxidant activity in the DPPH assay [24] (Table 2). The antioxidant activity of these flavonoids, like other natural phenolic antioxidants, is a consequence of the presence of phenolic moieties in the structures. The antioxidant activity of phenolic natural products is predominantly due to their redox properties, i.e., the ability to act as reducing agents, hydrogen donors, and singlet oxygen quenchers, and to some extent, could also be due to their metal chelation potential [25].

EXPERIMENTAL

UV spectra were obtained in MeOH using a Shimadzu UV 160A spectrometer. Optical rotations were measured on a Bellingham Stanley ADP220 polarimeter. NMR spectra were recorded in CD_3OD using a Bruker 200 MHz NMR Spectrometer (200 MHz for ^1H and 50 MHz for ^{13}C) and a Bruker 500 DRX NMR Spectrometer (COSY, HMBC and HSQC). Residual solvent peak was used as internal standard. HRMS analyses were performed on a Finnigan MAT95 spectrometer.

Plant Materials. The leaves of *Erica arborea* L. were purchased from local market in Chanakkale (western Anatolia) and their identity was confirmed by direct comparison with the herbarium sample (COMU-370) from the Dardanel Herbarium of the Biology Department in the Faculty of Sciences and Arts, Chanakkale Onsekiz Mart University.

Extraction. The dried and ground leaves of *Erica arborea* (200 g) were Soxhlet-extracted, successively, with *n*-hexane, dichloromethane (DCM) and methanol (MeOH) (1.1 L each). All these extracts were separately concentrated using a rotary evaporator at a maximum temperature of 45°C.

Isolation of Compounds. The MeOH extract (2 g×3) was subjected to SPE fractionation (Sep-Pak, C_{18} cartridge; 10 g) using a step gradient of MeOH-water mixtures (20:80, 40:60, 60:40, 80:20, 100:0). The resulting fractions were analyzed by preparative HPLC (CLC Shim-pack C_{18} column, 22×250 mm, 15m) eluted with a linear gradient of MeOH-water and monitored by a photo-diode-array detector at 220 and 280 nm. A portion of the 40% methanolic SPE fraction was analyzed by prep-HPLC (mobile phase: 0-50 min 35-45% MeOH in water, 50-62 min 45% MeOH in water, 62-64 min 45-35% MeOH in water, 64-75 min 35% MeOH, flow rate: 20 mL/min; detection at 220 and 280 nm) to afford the new flavonoid, tricetin 4′-*O*-α-L-rhamnopyranoside (**1**) (2.5 mg; t_R 12.7 min) and the known flavonol rhamnoside **2** (7.5 mg; t_R 22.0 min).

Tricetin 4′-*O*-α-L-rhamnopyranoside (1). Yellow amorphous solid, UV (MeOH, λ_{max}), nm: 254, 263, 294, 348; + NaOMe: 271, 321, 383; +$AlCl_3$: 272, 311 sh, 389, 415; +$AlCl_3$ + HCl: 272, 330.2, 350, 396; + NaOAc: 271, 319, 384; +NaOAc + H_3BO_3: 253, 263, 293, 348; ESIMS (positive ion mode) *m/z* 449 $[M+H]^+$. HRESIMS (positive ion mode) *m/z* 449.1084, $C_{21}H_{21}O_{11}$ requires: *m/z* 449.1084. ^1H NMR (200 MHz, CD_3OD) and ^{13}C NMR (50 MHz, CD_3OD): Table 1.

Isorhamnetin 3-*O*-α-L-rhamnopyranoside (2). Yellow amorphous solid, ESIMS *m/z* 463 $[M+H]^+$; UV, ^1H NMR and ^{13}C NMR as published data [15].

DPPH Assay. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), molecular formula $C_{18}H_{12}N_5O_6$, was obtained from Fluka Chemicals AG, Bucks. Quercetin was obtained from Avocado Research Chemicals Ltd, Shore road, Heysham, Lancs. The method used by Takao et al. [37] was adopted with suitable modifications [25, 26]. DPPH (4 mg) was dissolved in MeOH (50 mL) to obtain a concentration of 80 µg/mL.

Qualitative Assay. Test extract and compounds (**1** and **2**) were applied on a TLC plate and sprayed with DPPH solution using an atomizer. It was allowed to develop for 30 min. The color changes (purple on white) were noted.

Quantitative Assay. While the MeOH extract was dissolved in MeOH to obtain a concentration of 1 mg/mL, the concentration of the stock solution of the test compounds, **1** and **2**, in MeOH was 0.5 mg/mL. Dilutions were made to obtain

concentrations of 5×10^{-2}, 5×10^{-3}, 5×10^{-4}, 5×10^{-5}, 5×10^{-6}, 5×10^{-7}, 5×10^{-8}, 5×10^{-9}, 5×10^{-10} mg/mL. For the MeOH extract, the solution was diluted to obtain 1×10^{-1} mg/ mL and then the other dilutions as stated above. Diluted solutions (1 mL each) were mixed with DPPH (1 mL) and allowed to stand for half an hour for any reaction to occur. The UV absorbance was recorded at 517 nm. The experiment was performed in triplicate and the average absorption was noted for each concentration. The same procedure was followed for the positive control, quercetin.

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