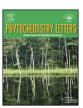


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Mini review

Dianthus erinaceus var. erinaceus: Extraction, isolation, characterization and antimicrobial activity investigation of novel saponins



Kiymet Mutlu^a, Nazli Boke Sarikahya^a, Ihsan Yasa^b, Suheyla Kirmizigul^{a,*}

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ABSTRACT

A phytochemical analysis of *Dianthus erinaceus* Boiss. var. *erinaceus* (Caryophyllaceae) has led to the isolation of two novel triterpenoid saponins, containing an oleane type skeleton, named dianosides K and L (1, 2), along with six known triterpenoid saponins (3–8). On the basis of chemical and spectrometric data, the structures of the new compounds were elucidated as 3–0-[β -D-glucopyranosyl (1 \rightarrow 3)]-[β -D-glucopyranosyl (1 \rightarrow 6)]- β -D-glucopyranosyl-olean-12-ene-23 α ,28- β -dioic acid 28-O- β -D-glucopyranosyl ester (1) and 3-O-[β -D-glucopyranosyl (1 \rightarrow 3)]-[β -D-glucopyranosyl (1 \rightarrow 6)]- β -D-glucopyranosyl-olean-12-ene-23 α ,28- β -dioic acid 28-O- α -L-mannopyranosyl (1 \rightarrow 6)- β -D-glucopyranosyl ester (2). All isolated natural compounds were structurally characterized by 1D- (¹H, ¹³C, DEPT); 2D- (COSY, HMQC, HMBC) NMR and HR-ESI/MS methods. The antimicrobial activity of compounds 1 and 2 were tested against four Gram-negative, three Gram-positive bacteria and the yeast *Candida albicans* by the MIC method

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1. Introduction

The plant genus *Dianthus* L. (fam. Caryophyllaceae) includes more than 300 species mainly originating in Eurasia. Generally, these species grow as wild weeds but some are cultivated as ornamental plants. The *Dianthus* genera has approximately

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67 species in Turkey, 42 of which are endemic. Dianthus erinaceus Boiss, var, erinaceus is a perennial endemic plant which grows only on Spil Mountain, Manisa, western Turkey (Davis, 1967). Several Dianthus species have been medicinally used to tackle infections and diseases in China, Iran and Mongolia for millennia. Previous chemical investigations of Dianthus species revealed that plants belonging to this genera are a rich source of triterpenoid saponins (Chen et al., 2010: Koike et al., 1994: Nakano et al., 2011: Oshima et al., 1984a,b,c). In addition, there have been isolation studies of flavonids (Obmann et al., 2012), pyrane type glycosides (Plouvier et al., 1986), macrocyclic anthocyanins (Nakayama et al., 2000) and cyclopeptides (Tong et al., 2012). Previous phytochemical and activity studies have demonstrated that isolated natural compounds from Dianthus species and the crude plant itself show antibacterial (Gou et al., 2011), antifungal (Galeotti et al., 2008), antioxidant (Durucasu et al., 2009), analgesic, antihepatotoxic (Hikino et al., 1984), cytotoxic (Yu et al., 2007) and proliferative (Tong et al., 2012) activities.

To the best of our knowledge, there have been no reports on the chemical composition or biological activity of *Dianthus erinaceus* var. *erinaceus*. Thus, this investigation focusses on *Dianthus erinaceus*, with the aim of analyzing its saponins. Purification procedures resulted in the isolation of two novel (dianosides K–L, **1–2**) and six known triterpenoid glycosides (**3-8**). The structures of the isolated compounds were identified using 1D (¹H, ¹³C, DEPT); 2D (COSY, HSQC, HMBC) NMR spectra and HR-ESI/MS. The antimicrobial effects of the new compounds (**1–2**) were examined against different microorganisms using MIC.

2. Results and discussion

Compound 1 was obtained as an amorphous, white powder. Its molecular formula was determined to be C54H86O25 from its pseudo-molecular ion peak at m/z 1157.5352 (calcd. m/z 1157.5350) [M+Na]⁺ in the HR-ESI/MS. Its IR spectrum revealed absorption bands at 3326 (OH), 1664 (C=O), 1657 (C=C) and 1020 cm⁻¹ (C=O-C). The ¹H NMR spectra of the aglycone section indicated the presence of six singlets at $\delta_{\rm H}$ 0.65, 0.82, 0.84, 0.85, 0.91, 1.07 of six tertiary methyl protons and a broad singlet at $\delta_{\rm H}$ 5.15 of an olefinic proton. The correlation in the heteronuclear single quantum coherence (HSQC) spectrum demonstrated the presence of six methyl carbons at δ_C 13.2, 16.0, 17.1, 23.9, 26.0, 33.2, a pair of olefinic carbons at δ_{C} 122.5 (CH) and δ_{C} 143.8 (C) and two carboxylic groups at $\delta_{\rm C}$ 175.0 and 181.0. In addition, the heteronuclear multiple bond correlation (HMBC) of H-24 ($\delta_{\rm H}$ 0.91) and H-3 ($\delta_{\rm H}$ 3.76) of $\delta_{\rm C}$ 181.0, showed that the carboxyl carbon at δ_{C} 181.0 could be assigned to C-23. Therefore, the other carboxyl carbon at $\delta_{\rm C}$ 175.0 was attributed to C-28. Thus, the aglycone was determined to be 3-β-hydroxyolean-12-en-23,28-dioicacid, which is also known as gypsogenic acid (Koike et al., 1994). The downfield 13 C NMR chemical shift at $\delta_{\rm C}$ 85.0 (C-3) and the upfield 13 C NMR chemical shift at δ_C 175.0 (C-28) proved that **1** is a bis-desmosidic saponin with glycosidic linkages at C-3 through an O-heterosidic bond and at C-28 through an ester bond. The ¹H and ¹³C NMR spectra of 1 contained four clear signals for anomeric protons and carbons at δ_H 4.12 (d, J = 7.2 Hz), 4.15 (d, J = 7.8 Hz), 4.29 (d, J = 7.2 Hz), 5.34 (d, J = 7.2 Hz) and δ_C 103.7, 104.0, 104.7, 93.7, respectively. All the proton signals for the sugar moieties were associated with one bond coupled with carbon signals using the HSQC spectrum. In the HMBC spectrum, the H-1 proton of glucose I at $\delta_{\rm H}$ 4.12 and the H-1 proton of glucose IV at $\delta_{\rm H}$ 5.34, showed long-range correlations with C-3 of the aglycone at $\delta_{\rm C}$ 85.0 and C-28 of the aglycone at $\delta_{\rm C}$ 175.0, respectively. On the other hand, long-range correlations between the H-1 proton of glucose II at $\delta_{\rm H}$ 4.15 and the C-6 carbon of glucose I at $\delta_{\rm C}$ 68.0; and the H-1 proton of glucose III at $\delta_{\rm H}$ 4.29 and the C-3 carbon of glucose I at $\delta_{\rm C}$ 88.6, showed the points at which the sugar molecules are linked to each other. All sugar residues were identified as $\beta\text{-D-glucose}$ by gas chromatography of the hydrolyzed product and by the coupling constant of their anomeric protons. Thus, compound 1 was confirmed to be 3-O-[$\beta\text{-D-glucopyranosyl}$ (1 \rightarrow 3)]-[$\beta\text{-D-glucopyranosyl}$ (1 \rightarrow 6)]- $\beta\text{-D-glucopyranosyl}$ -olean-12-ene-23\(\alpha\),28-\(\beta\)-dioic acid 28-O-\(\beta\)-D-glucopyranosyl ester, namely dianoside K.

Compound **2** was shown to have the molecular formula $C_{60}H_{96}O_{30}$ on the basis of the HR-ESI/MS data at m/z [M+Na]⁺= 1319.5885 (calcd. 1319.5879). Comparison of the 1H and ^{13}C NMR data (see Tables 1–2) of **2** with those of **1** showed considerable structural similarity except for the presence of one extra mannose moiety. The sugar part of **2** was found to consist of four glycose units and one mannose unit. The 1H NMR chemical shift at δ_H 4.16 (d, J=7.8 Hz), 4.19 (d, J=7.8 Hz), 4.33 (d, J=7.8 Hz), 4.65 (d, J=3.0 Hz), and 5.35 (d, J=7.8 Hz) are attributed to anomeric proton signals for the sugar part of the compound. The anomeric carbons

Table 1 ¹H NMR data for compounds **1** and **2**. ^{a-d}

3 3.76, m 3.75, m 1.52, s 5.18, s 5.44 0.91, s 0.92, s 0.82, s 0.84, s 0.65, s	position	1	2
5 1.50, m 1.52, m 12 5.15, s 5.18, s 24 0.91, s 0.92, s 25 0.82, s 0.84, s 26 0.65, s 0.65, s 27 1.07, s 1.10, s 29 0.85, s 0.88, s 30 0.84, s 0.86, s Glc I at C-3 4 4.12, d (7.2) 4.16, d (7.8) 2 2.92,m 2.97, m 3 3.40, m 3.44, m 4 2.90, m 2.94, m 5 3.16, m 3.15, m 6 3.60, 3.90, m 3.61, 3.90, m Glc II Glc II 1 4.15, d (7.8) 4.19, d (7.8) 2 2.80, m 2.84, m 3 3.06, m 3.12, m 4 3.02, m 3.10, m 5 3.00, m 3.10, m 4 3.02, m 3.05, m 3 3.00, m 3.12, m 4 3.02, m 3.05, m 3 3.10, m	3	3. 76. m	3.75. m
12			
24			
25			
26			
27	26		•
29	27		
Glc I at C-3 Glc I at C-3 1	29		
Glc I at C-3 1	30		
1			
2 2.92,m 3.40, m 3.44, m 3.44, m 4 2.90, m 2.94, m 3.15, m 3.15, m 3.60, 3.90, m 3.61, 3.90, m 3.00, m 3.10, m 3.21, m 3.10, m 3.21, m 3.10, m 3.21, m 3.20, m 3.12, m 3.294, m 2.95, m 3.42, 3.68, m 3.40, 3.70, m 3.44, m 3.04, m 3.06, m 3.44, m 3.04, m 3.06, m 3.18, m 3.20, m 3.31, m 3.20, m 3.31, m 3.20, m 3.31, m 3.20, m 3.31, m 3.20, m 3.32, m 3.33, m 3.33		Glc I at C-3	Glc I at C-3
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3 3.40, m 3.44, m 4 3.04, m 3.06, m 5 3.18, m 3.20, m 6 3.30, 3.64, m 3.08, 3.60, m Man 1 4.65, d (3.0) 2 3.74, m 3 3.68, m 4 3.58, m 5 3.35, m	1	5.34, d (7.2)	5.35, d (7.8)
4 3.04, m 3.06, m 5 3.18, m 3.20, m 6 3.30, 3.64, m 3.08, 3.60, m Man 1 4.65, d (3.0) 2 3.74, m 3 3.68, m 4 3.58, m 5 3.35, m	2	nd	3.23, m
5 3.18, m 3.20, m 6 3.30, 3.64, m 3.08, 3.60, m Man 1 4.65, d (3.0) 2 3.74, m 3 3.68, m 4 3.58, m 5 3.35, m	3	3.40, m	3.44, m
6 3.30, 3.64, m 3.08, 3.60, m Man 1 4.65, d (3.0) 2 3.74, m 3 3.68, m 4 3.58, m 5 3.35, m	4	3.04, m	3.06, m
Man 1 4.65, d (3.0) 2 3.74, m 3 3.68, m 4 3.58, m 5 3.35, m	5	3.18, m	3.20, m
1 4.65, d (3.0) 2 3.74, m 3 3.68, m 4 3.58, m 5 3.35, m	6	3.30, 3.64, m	3.08, 3.60, m
2 3.74, m 3 3.68, m 4 3.58, m 5 3.35, m			Man
3 3.68, m 4 3.58, m 5 3.35, m	1		4.65, d (3.0)
4 3.58, m 5 3.35, m			3.74, m
5 3.35, m			3.68, m
			3.58, m
6 3.45, 3.52, m	5		3.35, m
	6		3.45, 3.52, m

 $^{^{\}mathrm{a}}$ $^{\mathrm{1}}\mathrm{H}$ NMR data (δ) were measured in DMSO- d_{6} at 600 MHz.

^b Coupling constants (J) in Hz are given in parentheses.

^c The assignments are based on COSY, HSQC and HMBC experiments.

d nd: not detected.

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