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Triterpenoid saponins from the roots of two *Gypsophila* species

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ABSTRACT

Two triterpenoid saponins with two known ones have been isolated from the roots of *Gypsophila arrostii* var. *nebulosa*, and two new ones from the roots of *Gypsophila bicolor*. Their structures were established by extensive NMR and mass spectroscopic techniques as 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosylquillaic acid 28-*O*-β-D-xylopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-β-D-fucopyranosyl ester (**1**), 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosylgypsogenin 28-*O*-β-D-xylopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-β-D-fucopyranosyl ester (**2**), 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosylgypsogenin 28-*O*-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[(4-*O*-acetyl)-β-D-quinovopyranosyl-(1→4)]-β-D-fucopyranosyl ester (**3**), gypsogenic acid 28-*O*-β-D-glucopyranosyl-(1→3)-{6-*O*-[3-hydroxy-3-methylglutaryl]-β-D-glucopyranosyl-(1→6)}-β-D-galactopyranosyl ester (**4**). Three compounds were evaluated against one human colon cancer cell line SW480 and one rat cardiomyoblast cell line H9c2.

Introduction

The genus *Gypsophila* (Caryophyllacae) represented by small perennial herbs comprises more than 150 species and some of these species have long been used as pharmaceutical and ornamental plants (Nie et al., 2010a). Some of them are a rich source of saponins having a pharmaceutical and commercial importance as medicines, detergents, adjuvants, and cosmetics (Jia et al., 2002). A great diversity of saponins has been reported in several species such as *Gypsophila pilulifera* (Arslan et al., 2012), *Gypsophila oldhamania* (Luo et al., 2008), *Gypsophila repens* (Elbandy et al., 2007) and *Gypsophila arrostii* (Frechet et al., 1991; Hostettmann and Marston, 1995). In our continuing study on saponins from *Gypsophila* genus (Elbandy et al., 2007), we have examined the saponin fraction of the roots of *G. arrostii* var. *nebulosa* (Boiss. & Heldr.) Bark. and *Gypsophila bicolor* (Freyn & Sint.) Grossh collected in the Southwestern of Turkey. In the present paper, we report the isolation and structure elucidation of two new triterpenoid saponins (**1**, **2**) together with two known ones from *G. arrostii* var. *nebulosa*, and two new triterpenoid saponins (**3**, **4**) from *G. bicolor*. The cytotoxicity of **1**, **3**, and 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosylgypsogenin 28-*O*-β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranosyl ester was evaluated against a human colon cancer cell line (SW 480) and a rat cardiomyoblast cell line (H9c2). Their structures were elucidated by spectroscopic methods including 600 MHz 1D and 2D NMR experiments (^{1}H, ^{13}C, COSY, TOCSY, NOESY, HSQC, HMBC) in combination with HR-ESI-MS and by comparaison of their physical and spectral data with literature values.

Results and discussion

The *n*-BuOH fractions obtained from the MeOH/H_2O (7:3) extract of the roots of *G. arrostii* var. *nebulosa* and *G. bicolor*, Ga and Gb respectively, were fractionated by vacuum-liquid chromatography (VLC) and purified by repeated medium-pressure liquid chromatography (MPLC) on normal- and RP18 silica gel yielding compounds **1**, **2** from Ga and **3**, **4** from Gb (Fig. 1). Their structures

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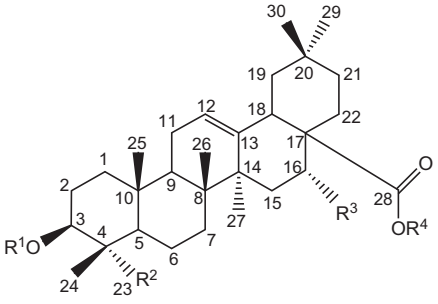

	R^1	R^2	R^3	R^4
1	S1	CHO	OH	S2
2	S1	CHO	H	S2
3	S1	CHO	H	S3
4	H	COOH	H	S4

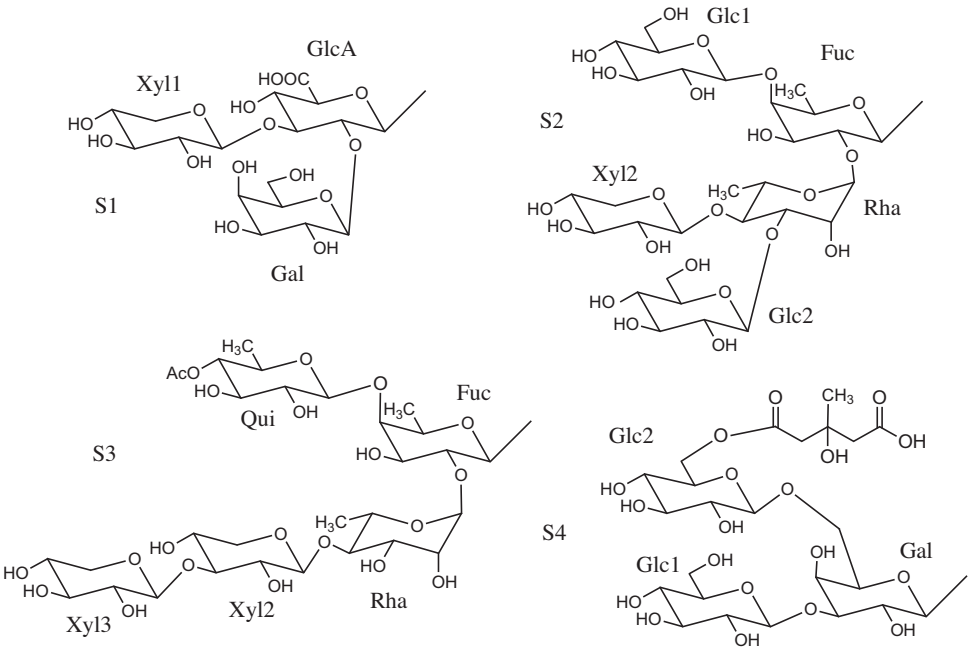

Fig. 1. Saponins from the roots of *G. arrostii* var. *nebulosa* and *G. bicolor*.

were established mainly by spectroscopic methods including 600 MHz NMR experiments and mass spectrometry. Futhermore two known saponins isolated from Ga were identified by comparaison of their spectral data with literature values as 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosylgypsogenin 28-*O*-β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranosyl ester (Nie et al., 2010b) and gypsogenin 28-*O*-[β-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl-(1→3)]-[β-D-glucopyranosyl-(1→6)]-β-D-galactopyranosyl ester (Elgamal et al., 1995).

Compounds **1**–**4** were isolated as white amorphous powders. The monosaccharides obtained by acid hydrolysis of each compound were identified by comparaison on TLC with authentic samples as glucuronic acid, xylose, galactose, glucose, fucose, rhamnose for **1**, **2**, glucuronic acid, xylose, galactose, fucose, quinovose, rhamnose for **3** and galactose, glucose for **4**. The absolute configurations were determined by GC analysis (Hara et al., 1987) to be D for all the sugars excepted for the rhamnose (L-configuration). The $^3J_{H\text{-}1,H\text{-}2}$ values in the ^{1}H NMR spectrum of the glucuronic acid, xylose, galactose, glucose, fucose, quinovose in their pyranose form (6.2–8.5 Hz) indicated their β anomeric configuration and the large $^1J_{H\text{-}1,C\text{-}1}$ value of the rhamnose (168 Hz) confirmed that the anomeric proton was equatorial (α-pyranoid form).

Compound **1** exhibited in the HR-ESI-MS a quasi-molecular ion peak at *m/z* 1727.7149 $[M+Na]^+$ (calcd. 1727.7152) compatible with the molecular formula $C_{76}H_{120}O_{42}$. Compound **1** showed in ESI-MS spectrum (positive-ion mode) an ion peak at *m/z* 1727 indicating a molecular weight of 1704. The ^{1}H and ^{13}C NMR spectra of **1** displayed resonances due to the triterpene part characteristic of quillaic acid aglycon (Table 1), with six angular methyl groups at δ_H 1.14 (s, H_3-24), 1.00 (s, H_3-25), 0.76 (s, H_3-26), 1.38 (s, H_3-27), 0.86 (s, H_3-29) and 0.95 (s, H_3-30) showing correlations in the HSQC spectrum with their corresponding carbon at δ_C 10.8 (C-24), 16.4 (C-25), 17.9 (C-26), 27.3 (C-27), 33.3 (C-29) and 24.9 (C-30). Futhermore, other characteristic signals were observed

Table 1
^{13}C NMR and ^{1}H NMR spectroscopic data of the aglycon moieties of compounds **1–4**.[a,b]

Position	**1**		**2**		**3**		**4**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	39.3	1.06 m 1.68 m	37.6	0.97 m 1.56 m	37.7	0.80 1.29	38.8	1.51 1.06
2	25.6	1.76 2.04	24.1	1.60 1.96 m	24.6	1.82 2.21	24.2	1.85 1.95 m
3	85.9	3.86 m	82.7	3.68 m	83.9	3.99	75.2	4.53
4	56.4	–	53.9	–	54.9	–	54.0	–
5	49.1	1.32 m	47.3	1.26	47.3	1.57	51.2	nd
6	21.3	0.92 nd	19.7	0.83 m 1.38 m	20.5	1.08 1.36	23.8	1.88 nd
7	33.7	1.26 1.53 br d (10.0)	31.8	nd 1.41 m	32.3	1.41 nd	32.0	1.60 br d (10.9) nd
8	41.1	–	39.2	–	39.9	–	39.9	–
9	48.0	1.73	45.9	1.61	45.9	1.61	47.2	1.68 m
10	37.1	–	35.4	–	35.7	–	36.5	–
11	24.4	1.75 1.90	22.8	1.82 m nd	23.2	1.73 br s nd	22.7	1.84 1.88
12	123.2	5.30 br t (3.6)	121.1	5.23 br s	122.8	5.31 br t	123.1	5.35 br t
13	144.6	–	143.1	–	143.9	–	143.9	–
14	42.6	–	41.0	–	41.8	–	41.9	–
15	36.5	1.37 1.72	26.9	nd	28.1	1.40 1.82	28.1	1.50 2.10 br t (11.6)
16	74.9	4.45	22.7	1.86 m nd	23.6	1.78 nd	20.8	1.47 m nd
17	49.9	–	47.5	–	47.3	–	46.7	–
18	42.3	2.92 dd (14.4, 4.0)	40.6	2.82 m	41.5	2.99 br d (13.6)	41.3	3.07 br d (14.3)
19	48.0	1.04 dd (13.6, 5.2) 2.28 t (13.6)	46.6	1.00 m 2.23 m	45.7	1.10 nd	46.2	1.15 1.62 m
20	31.3	–	30.2	–	30.9	–	30.3	–
21	36.5	1.16 1.94 m	34.6	1.28 m nd	33.6	1.18 nd	33.5	1.06 1.24
22	31.9	1.81 dd (14.0, 5.2) 1.89	28.8	1.24 m nd	31.9	nd	32.6	1.24 nd
23	211.0	9.42 s	209.7	9.40 s	210.5	9.78 s	181.5	–
24	10.8	1.14 s	10.2	1.09 s	10.5	1.34 s	12.1	1.50 s
25	16.4	1.00 s	15.4	0.91 s	15.2	0.69 s	15.8	0.89 s
26	17.9	0.76 s	16.5	0.68 s	17.5	0.90 s	17.1	1.02 s
27	27.3	1.38 s	25.4	1.11 s	26.1	1.17 s	25.7	1.10 s
28	177.2	–	174.6	–	176.4	–	175.8	–
29	33.3	0.86 s	32.6	0.84 s	32.7	0.79 s	32.8	0.77 s
30	24.9	0.95 s	24.1	0.90 s	23.2	0.74 s	23.3	0.79 s

[a] Overlapped signals are reported without designated multiplicity; nd: not determined.
[b] In CD_3OD for **1**, DMSO-d_6 for **2** and pyridine-d_5 for **3**, **4**, δ in ppm, J in parentheses in Hz.

such as one olefinic proton at δ_H 5.30 (1H, br t, H-12) showing HSQC correlation with δ_C 123.2 (C-12), one aldehydic proton at δ_H 9.42 (s) and two oxygen bearing methine proton signals at δ_H 3.86 (H-3), 4.45 (H-16). The NOESY correlation between δ_H 0.76 (s, H_3-26) and δ_H 1.72 ($H_{\beta\text{-ax}}$-15), and between δ_H 1.72 ($H_{\beta\text{-ax}}$-15) and δ_H 4.45 (H-16), suggested a β-equatorial orientation of the H-16 and thus a α-axial orientation of the OH group. Extensive 2D NMR analysis confirmed the structure of the aglycon to be quillaic acid, which is in good agreement with literature data (Guo et al., 2000; Nord and Kenne, 1999). The chemical shift values at δ_C 85.9 (C-3) and δ_C 177.2 (C-28), suggested that the saponin was a bisdesmosidic glycoside with saccharide units attached to these positions. The presence of eight sugar moieties in **1** was evidenced by the ^{1}H NMR spectrum which displayed eight anomeric protons at δ_H 4.36 (d, J = 7.2 Hz), 4.78 (d, J = 7.6 Hz), 4.60 (d, J = 7.9 Hz), 5.27 (d, J = 7.2 Hz), 5.16 (d, J = 1.6 Hz), 4.45 (d, overlapped), 4.67 (d, J = 7.6 Hz) and 4.53 (d, J = 7.6 Hz), giving correlations with eight anomeric carbons at δ_C 104.3, 103.8, 104.7, 95.2, 101.9, 106.3, 105.1 and 105.1, respectively in the HSQC spectrum. Complete assignments of each sugar were achieved by extensive 1D and 2D NMR analyses, allowing the identification of one β-glucuronopyranosyl (GlcA), one β-galactopyranosyl (Gal), two β-xylopyranosyl (Xyl1, Xyl2), one β-fucopyranosyl (Fuc), one α-rhamnopyranosyl (Rha), two β-glucopyranosyl (Glc1, Glc2) units, respectively. HMBC correlations between δ_H 4.36 (GlcA H-1) and δ_C 85.9 (Agly C-3), between δ_H 4.60 (Xyl1 H-1) and δ_C 86.6 (GlcA C-3), and between δ_H 4.78 (Gal H-1) and δ_C 78.6 (GlcA C-2) showed that the sequence linked at C-3 was identified as 3-*O*-Gal-(1→2)-[Xyl-(1→3)]-GlcA, which is commonly encountered in the genus *Gypsophila* (Gevrenova et al., 2006; Nie et al., 2010a; Arslan et al., 2012; Elbandy et al., 2007; Frechet et al., 1991). After subtraction of the anomeric signals of the sugars linked at the C-3 position from the total HSQC spectrum, the signals of five sugars linked to the aglycon through an ester linkage remained (Fuc, Rha, Glc1, Glc2 and Xyl2). In the HSQC spectrum the deshielded anomeric ^{1}H NMR signal of Fuc at δ_H 5.27 giving a correlation with the shielded anomeric carbon signal at δ_C 95.2 suggested that the Fuc residue was linked at C-28 through an ester linkage. This was confirmed by an HMBC correlation between δ_H 5.27 (Fuc H-1) and δ_C 177.2 (Agly C-28). The COSY cross peak δ_H 5.27 (Fuc H-1)/δ_H 3.73 (Fuc H-2) and the HSQC correlation δ_H/δ_C 3.73/76.4 suggested the substitution of Fuc at the position 2. This was confirmed by the HMBC cross peak δ_H/δ_C 5.16/76.4 (Rha H-1/Fuc C-2) and by the NOESY cross peak at δ_H/δ_H 5.16/3.73 (Rha H-1/Fuc H-2). Futhermore, the HMBC correlation at δ_H/δ_C 1.26/78.6 (Rha H_3-6/Rha C-4), suggested Rha C-4 to be substituted. This was confirmed by the HMBC cross peak at δ_H/δ_C 4.67/78.6 (Xyl2 H-1/Rha C-4) and by the NOESY cross peak δ_H/δ_H 4.67/3.66 (Xyl2

H-1/Rha H-4) (Gevrenova et al., 2006). At this stage, the partial sequence of the glycosyl ester chain at C-28 was characterized as Xyl-(1→4)-Rha-(1→2)-Fuc-(1→28)-Agly, commonly encountered in the genus of *Gypsophila* (Nie et al., 2010a; Arslan et al., 2012; Luo et al., 2008; Elbandy et al., 2007; Frechet et al., 1991). The two other sugar units were two terminal glucopyranosyl units (Glc1 and Glc2) as ascertained from the analysis of 2D NMR spectra. The HMBC correlation at δ_H/δ_C 1.23/83.6 (Fuc H-6/Fuc C-4), confirmed that Fuc C-4 was substituted. The HMBCs at δ_H/δ_C 4.45/83.6 (Glc1 H-1/Fuc C-4) and 4.53/82.7 (Glc2 H-1/Rha C-3) suggested that Glc1 was linked at Fuc C-4 and Glc2 at Rha C-3, respectively. These linkages were confirmed by NOESY cross peaks at δ_H/δ_H 4.45/3.74 (Glc1 H-1/Fuc H-4), and δ_H/δ_H 4.53/3.95 (Glc2 H-1/Rha H-3). This extensive 2D NMR analysis allowed the complete sequencing of the glycosidic ester chain linked at C-28 as Xyl2-(1→4)-[Glc2-(1→3)]-Rha-(1→2)-[Glc1-(1→4)]-Fuc. Thus the structure of **1** was elucidated as 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosylquillaic acid 28-*O*-β-D-xylopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-β-D-fucopyranosyl ester.

Compound **2** exhibited in the HR-ESI-MS a quasi-molecular ion peak at *m/z* 1711.7210 $[M+Na]^+$ (calcd. 1711.7203) corresponding to the molecular formula $C_{76}H_{120}O_{41}$. The ESI-MS spectrum (negative-ion mode) of **2** exhibited a quasi-molecular ion peak at *m/z* 1687 $[M-H]^-$ indicating a molecular weight of 1688 followed by a significant fragment ion peak at *m/z* 1527 $[(M-H)-162]^-$, corresponding to the loss of one hexosyl moiety. Extensive 2D NMR analysis (Table 1) showed that compounds **1** and **2** differed only in the aglycon part at C-15 and C-16. Namely the characteristic ^{13}C NMR signals of a methylenic and secondary alkoholic functions in **1** at δ_C 36.5 (C-15) and δ_C 74.9 (C-16) were replaced by two methylenic signals at δ_C 26.9 (C-15) and δ_C 22.7 (C-16) in **2**, which allowed to identify the aglycon of **2** as gypsogenin (Lacaille-Dubois et al., 1999; Gaidi et al., 2000). Thus the structure of **2** was elucidated as 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosylgypsogenin 28-*O*-β-D-xylopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-β-D-fucopyranosyl ester.

Compound **3** exhibited in the HR-ESI-MS a quasi-molecular ion peak at *m/z* 1707.7252 $[M+Na]^+$ (calcd. 1707.7254) corresponding to the molecular formula $C_{77}H_{120}O_{40}$ and in the ESI-TOF-MS spectrum (positive-ion mode) a quasi-molecular ion peak at *m/z* 1707 $[M+Na]^+$ indicating a molecular weight of 1684. The ^1H and ^{13}C NMR data of **3** assigned by extensive 2D NMR analysis allowed the identification of gypsogenin as aglycon (Table 1 and Table 2) (Nie et al., 2010a,b). The deshielded chemical shift at δ_C 83.9 (C-3) and the shielded chemical shift at δ_C 176.4 (C-28) suggested that **3** was a bisdesmosidic saponin. The presence of eight monosaccharide moieties in **3** was evidenced by eight anomeric proton resonances at δ_H 4.64 (d, overlapped), 5.33 (d, J = 7.8 Hz), 5.18 (d, J = 7.6 Hz), 5.82 (d, J = 8.1 Hz), 4.93 (d, J = 6.2 Hz), 6.21 (br s), 4.95 (d, J = 6.2 Hz) and 4.99 (d, J = 8.5 Hz), which were HSQC-correlated with δ_C 102.9, 103.2, 103.9, 94.0, 105.7, 100.7, 106.0 and 104.9, respectively. Extensive 2D NMR spectroscopic analysis allowed the identification of three Xyl, one Rha, one Fuc and one quinovopyranosyl (Qui) units. Additionally, the HSQC cross peak at δ_H/δ_C 2.06/20.6 and the carboxyl signal at δ_C 170.5 allowed the identification of one acetyl group. Comparaison of the 2D-NMR spectra of **3** with those of **2** revealed that they share the same trisaccharide sequence at C-3 of the gypsogenin (Elbandy et al., 2007) and differed only by the tetrasaccharide chain at C-28, which was identified by 2D NMR analysis as one Fuc, one Rha, one Qui and two Xyl units (Xyl2 and Xyl3). The partial sequence characterized by HMBC correlations as Xyl2-(1→4)-Rha-(1→2)-Fuc-(1→28)-Agly was already observed in **1** and **2** and was in good agrement with literature data (Elbandy et al., 2007). The position of the sugar unit Xyl3 was determined by observation of an HMBC correlation between δ_H 4.99 (Xyl3 H-1) and δ_C 86.5 (Xyl2 C-3) and by NOESY cross peak at δ_H/δ_H 4.99/3.89 (Xyl3 H-1/Xyl2 H-3) proving the terminal Xyl3 to be linked at the C-3 position of Xyl2. The position of the sugar unit Qui was determined by observation of NOESY cross peak at δ_H/δ_H 4.93/4.04 (Qui H-1/Fuc H-4). Therefore, the complete sequence at C-28 was characterized as Xyl3-(1→3)-Xyl2-(1→4)-Rha-(1→2)-[Qui-(1→4)]-Fuc- which was previously characterized in *Acanthophyllum pachystegium* (Haddad et al., 2004) and in *A. glandulosum* (Gaidi et al., 2004). In the ^1H NMR spectrum, the deshielded signal at δ_H 4.99 (Qui H-4) correlating with δ_C 76.1 in the HSQC spectrum suggested the 4-position of Qui to be acetylated. This was confirmed by the presence of a HMBC cross peak between δ_H 4.99 (Qui H-4) and δ_C 170.5. On the basis of the above results, the structure of **3** was established as 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosylgypsogenin 28-*O*-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[(4-*O*-acetyl)-β-D-quinovopyranosyl-(1→4)]-β-D-fucopyranosyl ester.

Compound **4** exhibited in the HR-ESI-MS a quasi-molecular ion peak at *m/z* 1139.5254 $[M+Na]^+$ (calcd. 1139.5250) corresponding to the molecular formula $C_{54}H_{84}O_{24}$. The ESI-TOF-MS spectrum (positive-ion mode) of **4** exhibited a quasi-molecular ion peak at *m/z* 1139 $[M+Na]^+$, indicating a molecular weight of 1116. The ^1H and ^{13}C NMR chemical shifts of the aglycon of **4** assigned from 2D NMR spectra were characteristic of gypsogenic acid (Elbandy et al., 2007; Timité et al., 2010), with six singlet methyl signals at δ_H 1.50 (H_3-24), 0.89 (H_3-25), 1.02 (H_3-26), 1.10 (H_3-27), 0.77 (H_3-29) and 0.79 (H_3-30) showing HSQC correlations with their corresponding carbon at δ_C 12.1 (C-24), 15.8 (C-25), 17.1 (C-26), 25.7 (C-27), 32.8 (C-29), and 23.3 (C-30), respectively. Other characteristic signals were observed in the HSQC spectrum such as one olefinic proton signal at δ_H/δ_C 5.35/123.1 (H-12, br t/C-12) and one oxygen bearing methine proton signal at δ_H/δ_C 4.53/75.2 (H-3/C-3). The signals at δ_C 75.2 (C-3) and 175.8 (C-28) suggested that **4** was a monodesmosidic saponin with a glycosyl ester linkage at C-28. The presence of three sugar moieties in **4** was evidenced by the ^1H NMR spectrum which displayed three anomeric protons at δ_H 6.09 (d, J = 8.3 Hz), 5.22 (d, J = 7.8 Hz) and 4.86 (d, J = 7.8 Hz), giving correlations in the HSQC spectrum with three anomeric carbons at δ_C 94.5, 104.8 and 104.2 respectively. Extensive 1D and 2D NMR anlyses allowed the identication of one Gal and two Glc (Glc1, Glc2) respectively. The correlation in the HSQC spectrum at δ_H/δ_C 6.09/94.5 showed that the Gal residue was attached to the carboxylic acid function at C-28 of the gypsogenic acid through an ester linkage. This was confirmed by the HMBC correlation between δ_H 6.09 (Gal H-1)/δ_C/175.8 (Agly C-28). The cross peak in the HMBC spectrum between δ_H 5.22 (Glc1 H-1) and δ_C 87.2 (Gal C-3) proved the Glc1-(1→3)-Gal linkage. Futhermore, the HMBC correlation between δ_H 4.21 (Gal H-6) and δ_C 104.2 (Glc2 C-1) suggested a Glc2-(1→6)-Gal linkage. This was confirmed by NOESY cross peaks at δ_H/δ_H 4.86/4.58 (Glc2 H-1/Gal H-6), and δ_H/δ_H 5.22/4.23 (Glc1 H-1/Gal H-3). The deshielded signals of Glc2 (C-6) at δ_C 64.3 and H_2-6 at δ_H 4.53 and 4.90 observed in HSQC spectrum suggested an acylation at this position. The presence of a dicrotalic (=3-hydroxy-3-methylpentanedioic acid) acyl group was ascertained by the observation of a set of additional signals in the 1D- and 2D-NMR spectra corresponding to a 4-carboxy-3-hydroxy-3-methyl-1-oxobutyl moiety, which were in good agreement with literature data (Mihci-Gaidi et al., 2010; Koike et al., 1998). This was confirmed by the HMBC cross peak δ_H/δ_C 4.53/171.6 Glc2 H-6/dicrotalic acid C-1. Thus, the structure of **4** was elucidated as gypsogenic acid 28-*O*-β-D-glucopyranosyl-(1→3)-{6-*O*-[3-hydroxy-3-methylglutaryl]-β-D-glucopyranosyl-(1→6)}-β-D-galactopyranosyl ester.

Several triterpene and steroidal saponins were reported to be cytotoxic against a large panel of cancer cells (Lacaille-Dubois, 2005).

Table 2
^{13}C NMR and ^{1}H NMR spectroscopic data of the sugar moieties of compounds **1**–**4**.[a,b]

1			**2**			**3**			**4**		
	δ_C	δ_H		δ_C	δ_H		δ_C	δ_H		δ_C	δ_H
3-*O*-											
GlcA-1	104.3	4.36 d (7.2)	GlcA-1	101.6	4.15 d (7.1)	GlcA-1	102.9	4.64			
2	78.6	3.65	2	77.6	3.40 t (8.2)	2	77.5	4.20			
3	86.6	3.65	3	82.7	3.56 t (8.8)	3	85.2	4.15			
4	72.0	3.53 t (8.4)	4	70.5	3.21	4	71.3	4.12			
5	77.7	3.57 d (10.0)	5	76.4	3.17	5	76.7	4.14			
6	176.1	–	6	171.5	–	6	nd	–			
Gal-1	103.8	4.78 d (7.6)	Gal-1	102.3	4.54 d (7.3)	Gal-1	103.2	5.33 d (7.8)			
2	73.6	3.44 m	2	73.6	3.25	2	72.6	4.25			
3	75.2	3.42 dd (9.6, 3.2)	3	73.5	3.20	3	74.5	3.98			
4	70.7	3.81	4	68.1	3.66	4	69.9	4.29			
5	76.4	3.30	5	74.4	3.27	5	76.1	3.82			
6	62.6	3.69	6	59.7	3.47	6	61.4	4.20			
		3.84			3.60			4.32			
Xyl1-1	104.7	4.60 d (7.9)	Xyl1-1	102.3	4.60 d (7.6)	Xy1-1	103.9	5.18 d (7.6)			
2	75.2	3.22	2	73.5	3.01	2	74.4	3.83			
3	78.2	3.30	3	76.4	3.12	3	77.3	4.03			
4	70.7	3.50 m	4	69.7	3.28	4	70.2	4.04			
5	67.0	3.22	5	65.7	3.01	5	66.4	3.57			
		3.88 dd (11.6, 5.6)			3.69			4.19			
28-*O*-											
Fuc-1	95.2	5.27 d (7.2)	Fuc-1	93.5	5.28	Fuc-1	94.0	5.82 d (8.1)	Gal-1	94.5	6.09 d (8.3)
2	76.4	3.73	2	73.3	3.56 t (8.8)	2	71.8	4.46	2	72.5	4.12
3	76.5	3.48	3	76.1	3.52 br d (8.8)	3	76.1	4.16	3	87.2	4.23 m
4	83.6	3.74	4	82.3	3.60	4	84.3	4.04	4	68.5	4.17 (br s)
5	72.0	3.68 dd (8.4, 3.2)	5	69.8	3.66	5	70.9	3.94	5	76.7	4.02 m
6	17.0	1.23 d (6.4)	6	16.3	1.11 d (6.4)	6	16.2	1.44	6	68.8	4.21
											4.58 dd (10.7, 2.0)
Rha-1	101.9	5.16 d (1.6)	Rha-1	100.0	5.01 br s	Qui-1	105.7	4.93 d (6.2)	Glc1-1	104.8	5.22 d (7.8)
2	71.1	4.24 br t (2.7)	2	68.9	4.05 m	2	74.7	3.86	2	74.8	3.98 br t (8.5)
3	82.7	3.95 dd (9.2, 2.8)	3	80.2	3.81 dd (9.3, 2.8)	3	74.3	3.94	3	77.4	4.10
4	78.6	3.66	4	76.6	3.07	4	76.1	4.99	4	71.1	3.96
5	69.2	3.80	5	67.1	3.67	5	70.2	nd	5	77.9	3.90
6	18.8	1.26 d (6.0)	6	17.8	1.14 d (6.4)	6	17.4	1.18	6	61.8	4.11
											4.44 dd (10.2, 1.9)
						Ac-CH_3	20.6	2.06 s			
						COO	170.5	–			
Glc1-1	106.3	4.45	Glc1-1	104.9	4.30 d (7.6)	Rha-1	100.7	6.21 br s	Glc2-1	104.2	4.86 d (7.8)
2	75.2	3.30	2	73.3	3.10	2	70.9	4.65	2	74.6	3.90
3	76.8	3.00 lt (9.2)	3	76.6	3.12	3	71.8	4.46	3	77.4	4.09
4	71.4	3.32	4	71.1	3.20	4	84.0	4.20	4	71.1	3.93
5	78.2	3.32	5	76.7	3.12	5	68.0	4.29	5	74.7	3.89
6	62.0	3.74	6	60.8	3.46	6	17.9	1.58 d (5.7)	6	64.3	4.53
		nd			3.64						4.90 dd (11.2, 2.1)
Glc2-1	105.1	4.53 d (7.6)	Glc2-1	103.4	4.40 d (7.6)	Xyl2-1	106.0	4.95 d (6.2)			
2	74.9	3.29	2	74.2	3.10	2	74.5	3.88			
3	78.0	3.32	3	76.6	3.11	3	86.5	3.89			
4	71.4	3.30	4	71.2	3.22	4	68.3	3.96			
5	78.2	3.28	5	76.8	3.09	5	66.1	3.42 t (10.2)			
								4.10			
6	62.0	3.71 m	6	60.8	3.47						
		nd			3.66						
Xyl2-1	105.1	4.67 d (7.6)	Xyl2-1	104.5	4.54 d (7.6)	Xyl3-1	104.9	4.99 d (8.5)			
2	75.6	3.10 dd (8.5, 7.6)	2	73.6	2.88 m	2	74.7	3.87			
3	78.6	3.25	3	76.6	3.07	3	77.3	4.02			
4	71.4	3.46	4	69.7	3.26	4	70.1	4.04			
5	67.0	3.15 t -like(10.8)	5	65.7	3.01	5	66.4	3.56			
		3.82			3.66			4.18			
Dicrotalic acid-1										171.6	–
2										47.0	2.84 m
											2.86 m
3										69.9	–
4										47.2	2.72 d (15.9)
											2.96 d (15.0)
5										179.5	–
6										27.2	1.49 s

[a] Overlapped signals are reported without designated multiplicity; nd: not determined.
[b] In CD_3OD for **1**, DMSO-d_6 for **2** and pyridine-d_5 for **3**, **4**, δ in ppm, J in parentheses in Hz.

Therefore, the new compounds **1**, **3** and the known 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosylgypsogenin 28-*O*-β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranosyl ester were evaluated for their cytotoxic activities against one human cancer cell line (colorectal SW480 used for the determination of anticancer agent activity, Finlay et al., 1986) and an embryonic rat heart-derived cell line (cardiomyoblast H9c2 used for the study

of doxorubicin cytotoxicity, Wattanapitayakul et al., 2005) by XTT assay (Jost et al., 1992). We used etoposide (topoisomerase inhibitor from natural origin, Orlikova et al., 2014), methotrexate (antifolate anticancer agent, Kodidela et al., 2014) and doxorubicin (a DNA interacting agent with topoisomerase inhibition, Rao, 2013) as reference drugs. The IC_{50} was 20 μM for etoposide and methotrexate on SW480 and H9c2 cells, while doxorubicin showed an IC_{50} of 1 μM on both cell lines. In the same experimental conditions, compound **1** exhibited cytotoxicity against the two cell lines with IC_{50} values of 9.97 μM (SW480) and 14.67 μM (H9c2). However, compound **3** and the known one were inactive (IC_{50}>20 μM).

Conclusion

We isolated two new triterpenoid saponins from *G. arrostii* var. *nebulosa* roots (**1**, **2**) and two new ones from *G. bicolor* roots (**3**, **4**). Literature survey revealed that the sequence 3-*O*-Gal-(1→2)-[Xyl-(1→3)]-GlcA gypsogenin 28-*O*-Xyl-(1→4)-Rha-(1→2)-Fuc ester occurs in several *Gypsophila* species and in some more plants of the Caryophyllaceae family such as *Acanthophyllum* (Timité et al., 2010), *Psammosilene* (Zhong et al., 2002), *Arenaria* (Gaidi et al., 2001), and seems to represent a chemotaxonomic marker for this family. Additionnal glycosylations and acylations contribute to the great diversity of these saponins. Saponin **1**, having quillaic acid as aglycon showed a potential cytotoxic activity against SW 480 and H9c2 cell lines, higher than the positive controls etoposide and methotrexate.

Experimental

General

The 1D and 2D NMR spectra (^{1}H, ^{1}H COSY, TOCSY, NOESY, HSQC and HMBC) were performed using a UNITY-600 spectrometer at the operating frequency of 600 MHz on a Varian INOVA 600 instrument equipped with a Sun-4-L-X computer system (600 MHz for ^{1}H and 150 MHz for ^{13}C NMR spectra), for details, see (Gaidi et al., 2000). Some spectra were recorded on a Varian VNMR-S 600 MHz spectrometer equipped with 3 mm triple resonance inverse and 3 mm dual broad band probeheads. All chemical shifts (δ) are given in ppm, and the samples were solubilized in CD_3OD (**1**), DMSO-d_6 (**2**) pyridine-d_5 (**3**, **4**). Solvent signals were used as internal standards. Spectra in DMSO-d_6 were recorded at T = 36 °C. The carbon type (CH_3, CH_2, CH, C) was determined by DEPT experiments. ESI- (negative or positive) and ESI-MS (positive): Q-TOF-1-Micromass spectrometer in *m*/*z* for **1**, **2**, and a MicroTOF for **3**, **4**. The HR-ESI-MS was conducted in the positive-ion mode on a micromass Quattro LS instrument. Optical rotations were taken with a Perkin-Elmer-241 polarimeter. GC analysis was carried out on a thermoquest gas chromatograph using a DB-1701 cap.column (30 m × 0.25 mm, i.d) (J and W Scientific); detection by FID; detector temperature, 250°, injection temperature, 230°, initial temperature was maintened at 80° for 5 min and then raised to 270° at 15°/min; carrier gas, He. TLC and HPTLC employed precoated Si gel plates 60 F_{254} (Merck). The spray reagent for saponins was vanilin reagent (2% mixture of conc. H_2SO_4 soln. and 1% vanilin in EtOH). Isolations were carried out using a medium-pressure liquid chromatography (MPLC) system [Alltech pump, Büchi column (460 × 15 mm and 230 × 15 mm), Büchi precolumn (110 × 15 mm)].

Plant material

The roots of *G. arrostii* var. *nebulosa* (Boiss. & Heldr.) Bark. were collected from Isparta- İslamköy (800–1200 m) in August 2002, and identified by Dr. Necattin TÜRKMEN (Çukurova University, Turkey, Science Faculty, Biology Department). The roots of *G. bicolor* (Freyn & Sint.) Grossh were collected from Van- Özalp Yeni Tulgalı village (2100 m) in July 2002, and identified by Prof. Dr. Hasan ÖZÇELİK (Sütçü İmam University Turkey Science Faculty, Biology Department). The voucher specimens (N°22092013 for *G. arrostii* and N° 30092013 for *G. bicolor*) have been deposited in the herbarium of the University of Mugla-Marmaris (Turkey).

Extraction and isolation

Dried powdered roots of *G. arrostii* var. *nebulosa* (720 g) were extracted by reflux with MeOH (2 × 4 l). After evaporation of the solvents under vacuum, the residue was suspended in H_2O and partitioned three times against *n*-BuOH (3 × 300 ml) yielding 12 g of *n*-BuOH extract. An aliquot of 600 mg of this extract was subjected to VLC over RP-18 (MeOH–H_2O, 0/1, 1/1, 1/0, each 150 ml) to give three fractions (Fr.A: 128 mg, Fr.B: 109 mg, Fr.C: 300 mg). 140 mg of Fr.C was purified by MPLC (3 ml/min) over silica gel using $CHCl_3$–MeOH–H_2O (60/32/7) as eluent to give **1** (8.6 mg) and **2** (5.2 mg).

Dried powdered roots of *G. bicolor* (423 g) were extracted by reflux with MeOH (2 × 2 l). After evaporation of the solvents under vacuum, the residue was suspended in H_2O and partitioned three times by *n*-BuOH (3 × 200 ml) yielding 18 g of *n*-BuOH extract. An aliquot of this (550 mg) was subjected to VLC over RP-18, eluted with MeOH–H_2O (0/1, 1/1, 1/0, each 140 ml) to give three fractions (Fr.D: 101 mg, Fr.E: 270 mg, Fr.F: 170 mg). 140 mg of fraction Fr.E was fractionated by MPLC (3 ml/min) over silica gel using a $CHCl_3$–MeOH–H_2O solvent (60/32/7) to afford twelve fractions Fr.E1–Fr.E12. The fraction Fr.E7 (21 mg) was purified by MPLC over silica gel using $CHCl_3$–MeOH–H_2O (60/32/7) as eluent to give **4** (3.2 mg). The fraction Fr.F (170 mg) was fractionated by MPLC over silica gel using a $CHCl_3$–MeOH–H_2O solvent (60/32/7) to afford seventeen fractions Fr.F1–Fr.F17. The fractions Fr.F10–Fr.F12 (46 mg) were purified by MPLC over silica gel in the same conditions to give **3** (5.2 mg).

Compound characterization

*3-O-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosylquillaic acid 28-O-β-D-xylopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-β-D-fucopyranosyl ester (**1**)*

White, amorphous powder; $[\alpha]_D^{25}$ +35 (*c* 0,08, MeOH); ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), see Tables 1 and 2; ESI-MS (positive-ion mode) *m*/*z* 1727 $[M+Na]^+$, HR-ESI-MS (positive-ion mode) *m*/*z* 1727.7149 $[M+Na]^+$ (calcd. for 1727.7152).

*3-O-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosylgypsogenin 28-O-β-D-xylopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-β-D-fucopyranosyl ester (**2**)*

White, amorphous powder; $[\alpha]_D^{25}$ +21 (*c* 0.10, MeOH); ^{1}H NMR (DMSO-d_6, 600 MHz) and ^{13}C NMR (DMSO-d_6, 150 MHz), see Tables 1 and 2; ESI-MS (negative-ion mode) *m*/*z* 1687 $[M-H]^-$, HR-ESI-MS (positive-ion mode) *m*/*z* 1711.7210 $[M+Na]^+$ (calcd. for 1711.7203).

*3-O-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosylgypsogenin 28-O-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[(4-O-acetyl)-β-D-quinovopyranosyl-(1→4)]-β-D-fucopyranosyl ester (**3**)*

White, amorphous powder; $[\alpha]_D^{25}$ +7 (*c* 0.18 MeOH); ^{1}H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2; ESI-TOF-MS (positive-ion mode) *m*/*z* 1707

$[M+Na]^+$, HR-ESI-MS (positive-ion mode) *m/z* 1707.7252 $[M+Na]^+$ (calcd. for.1707.7254).

*Gypsogenic acid 28-O-β-D-glucopyranosyl-(1→3)-{6-O-[3-hydroxy-3-methylglutaryl]-β-D-glucopyranosyl-(1→6)}-β-D-galactopyranosyl ester. (**4**)*

White, amorphous powder; $[\alpha]_D^{25}$ +4 (*c* 0.18, MeOH); ^1H NMR (pyridine-d_5, 600 MHz) and ^{13}C NMR (pyridine-d_5, 150 MHz), see Tables 1 and 2; ESI-TOF-MS (positive-ion mode) *m/z* 1139 $[M+Na]^+$, HR-ESI-MS (positive-ion mode) *m/z* 1139.5254 $[M+Na]^+$ (calcd. for.1139.5250).

Acid hydrolysis and GC analysis

Each compound (3 mg) was hydrolyzed with 2 N aq. CF_3COOH (5 ml) for 3 h at 95 °C. After extraction with CH_2Cl_2 (3 × 5 ml), the aq. layer was repeatly evaporated to dryness with MeOH until neutral, and then analysed by TLC over silica gel ($CHCl_3$–MeOH–H_2O 8/5/1) by comparaison with authentic samples. Futhermore, the residue of sugars was dissolved in anhydrous pyridine (100 µl), and L-cysteine methyl ester hydrochloride (0.06 mol/l) was added. The mixture was stirred at 60 °C for 1 h, then 150 µl of HMDS-TMCS (hexamethyldisilazane-trimethylchlorosilane 3:1) was added, and the mixture was stirred at 60 °C for another 30 min. The precipitate was centrifuged off, and the supernatant was concentrated under N_2 stream. The residue was partioned between *n*-hexane and H_2O (0.1 ml each), and the hexane layer (1 µl) was analysed by GC (Hara et al., 1987). The absolute configurations were determined by comparing the retention times with thiazolidine derivatives prepared in a similar way from standard sugars (Sigma-Aldrich): D-glucuronic acid, D-xylose, D-galactose, D-glucose, D-fucose, L-rhamnose for **1**, **2**; D-glucuronic acid, D-xylose, D-galactose, D-fucose, D-quinovose, L-rhamnose for **3** and D-galactose, D-glucose for **4** were characterized by co-injection of the hydrosylate with standard silylated samples having t_R 15.2 min (D-glucuronic acid), 13.4 min (D-xylose), 19.6 min (D-galactose), 18.6 min (D-glucose), 12.1 min (D-fucose), 13.1 min (L-rhamnose) and 11.1 min (D-quinovose).

XTT cytotoxicity assay

Compounds **1**, **3** and 3-*O*-β-D-galactopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucuronopyranosylgypsogenin 28-*O*-β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranosyl ester were tested for cytotoxicity against one human cancer cell line SW480 (colorectal adenocarcinoma) and one embryonic heart-derived cell line H9c2 (rat cardiomyoblast), by means of the XTT method as described in the literature (Jost et al., 1992). The cell lines were provided by the Cohiro company, Dijon, France.

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References

Arslan, I., Celik, A., Chol, J.H., 2012. A cytotoxic triterpenoid saponin from underground parts of *Gypsophila pilulifera* Boiss. & Heldr. Fitoterapia 83, 699–703.

Elbandy, M., Miyamoto, T., Lacaille-Dubois, M.A., 2007. New triterpenoidal saponins from *Gypsophila repens*. Helv. Chim. Acta 90, 260–270.

Elgamal, M.H.A., Soliman, H.S.M., Karawya, M.S., Mikhova, B., Duddeck, H., 1995. Isolation of triterpene saponins from *Gypsophila capillaris*. Phytochemistry 38, 1481–1485.

Finlay, G.J., Wilson, W.R., Baguley, B.C., 1986. Comparison of in vitro activity of cytotoxic drugs towards human carcinoma and leukaemia cell lines. Eur. J. Cancer Clin. Oncol. 22, 655–662.

Frechet, D., Christ, B., Monegier, Du., Sorbier, B., Fischer, H., Vuilhorgne, M., 1991. Four triterpenoid saponins from dried roots of *Gypsophila* species. Phytochemistry 30, 927–931.

Gaidi, G., Miyamoto, T., Rustaiyan, A., Laurens, V., Lacaille-Dubois, M.A., 2000. Two new biologically active triterpene saponins from *Acanthophyllum squarrosum*. J. Nat. Prod. 63, 1497–1502.

Gaidi, G., Miyamoto, T., Lacaille-Dubois, M.A., 2001. Junceosides A–C, new triterpene saponins from *Arenaria juncea*. J. Nat. Prod. 64, 1533–1537.

Gaidi, G., Miyamoto, T., Ramezani, M., Lacaille-Dubois, M.A., 2004. Glandulosides A–D, Triterpene Saponins from *Acanthophyllum glandulosum*. J. Nat. Prod. 67, 1114–1118.

Gevrenova, R., Voutquenne-Nazabadioko, L., Harakat, D., Prost, E., Henry, M., 2006. Complete ^1H- and ^{13}C NMR assignements of saponins from roots of *Gypsophila trichotoma* Wend. Magn. Reson. Chem. 44, 686–691.

Guo, S., Falk, E., Kenne, L., Rönnberg, B., Sundquist, B.G., 2000. Triterpenoid saponins containing an acylated branched D-fucosyl residue from *Quillaja saponaria* Molina. Phytochemistry 53, 861–868.

Haddad, M., Miyamoto, T., Ramezani, M., Lacaille-Dubois, M.A., 2004. New triterpene saponins from *Acanthophyllum pachystegium*. Helv. Chim. Acta 87, 73–81.

Hara, S., Okabe, H., Mihashi, K., 1987. Gas-liquid chromatographic separation of aldose enantiomers as trimethylsilyl ethers of methyl 2-(polyhydroxyalkyl)thiazolidine-4(R)-carboxylates. Chem. Pharm. Bull. 35, 501–506.

Hostettmann, K., Marston, A., 1995. Saponins. In: Philipon, J.D., Ayres, D.C., Baxter, H. (Eds.), Chemistry and Pharmacology of Natural Products. Cambridge University Press, Cambridge, pp. 326–327.

Jia, Z., Koike, K., Sahu, N.P., Nikaido, T., 2002. Triterpenoid saponins from *Caryophyllaceae* family. In: Atta-ur-Rahman (Ed.), Studies in Natural Products Chemistry, vol. 26, Elsevier, Amsterdam, pp. 3–61.

Jost, L.M., Kikwood, J.M., Whiteside, T.L., 1992. Improved short- and long-term XTT-based colorimetric cellular cytotoxicity assay for melanoma and other tumor cells. J. Immunol. Methods 147, 153–165.

Kodidela, S., Suresh Chandra, P., Dubashi, B., 2014. Pharmacogenetics of methotrexate in acute lymphoblastic leukaemia: why still at the bench level? Eur. J. Clin. Pharmacol. 70, 253–260.

Koike, K., Jia, Z., Nikaido, T., 1998. Triterpenoid saponins from *Vaccaria segetalis*. Phytochemistry 47, 1343–1349.

Lacaille-Dubois, M.-A., 2005. Bioactive saponins with cancer related and immunomodulatory activity: recent developments. In: Atta-ur-Rahman (Ed.) Studies in Natural Products Chemistry, vol. 32, Elsevier, Amsterdam, pp. 209–246.

Lacaille-Dubois, M.-A., Hanquet, B., Cui, Z.H., Lou, C.H., Wagner, H., 1999. A new biologically active acylated triterpene saponin from *Silene fortunei*. J. Nat. Prod. 62, 133–136.

Luo, J.G., Ma, L., Kong, L.Y., 2008. New triterpenoid saponins with strong α-glucosidase inhibitory activity from the roots of *Gypsophina oldhamania*. Bioorg. Med. Chem. 16, 2912–2920.

Mihci-Gaidi, G., Ozbey, S., Orhan, I., Sener, B., Miyamoto, T., Mirjolet, J.F., Duchamp, O., Mitaine-Offer, A.C., Lacaille-Dubois, M.A., 2010. Triterpene saponins from *Cyclamen trocopteranthum*. Planta Med. 76, 1–4.

Nie, W., Luo, J.G., Kong, L.Y., 2010a. New triterpenoid saponins from the roots of *Gypsophila pacifica* Kom. Carbohydr. Res. 345, 68–73.

Nie, W., Luo, J.G., Kong, L.Y., 2010b. Triterpenoid saponins from roots of *Gypsophila pacifica*. Chin. Herbal Med. 2, 145–147.

Nord, L.I., Kenne, L., 1999. Separation and structural analysis of saponins in a bark extract from *Quillaja saponaria* Molina. Carbohydr. Res. 320, 70–81.

Orlikova, B., Legrand, N., Panning, J., Dicato, M., Diederich, M., 2014. Anti-inflammatory and anticancer drugs from nature. Cancer Treat. Res. 159, 123–143.

Rao, V.A., 2013. Iron chelators with topoisomerase-inhibitory activity and their anticancer applications. Antioxid. Redox Signal. 18, 930–955.

Timité, G., Mitaine-Offer, A.C., Miyamoto, T., Ramezani, M., Rustaiyan, A., Mirjolet, J.F., Duchamp, O., Lacaille-Dubois, M.-A., 2010. Structure elucidation of new oleanane-type glycosides from three species of *Acanthophyllum*. Magn. Reson. Chem. 48, 370–374.

Wattanapitayakul, S.K., Chularojmontri, L., Herunsalee, A., Charuchongkolwongse, S., Niumsakul, S., Bauer, J.A., 2005. Screening of antioxidants from medicinal plants for cardioprotective effect against doxorubicin toxicity. Basic Clin. Pharmacol. Toxicol. 96, 80–87.

Zhong, H., Ni, W., Hua, Y., Chen, Y., Chen, C., 2002. New triterpenoid saponins from *Psammosilene tunicoides*. Yunnan Zhiwu Yanjiu 24, 781–786.