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Erythrosaponins A–J, triterpene saponins from the roots and stem bark of *Gardenia erythroclada*

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ABSTRACT

Ten undescribed triterpene saponins, named erythrosaponins A–J, along with one known analogue were isolated from the roots and stem bark of *Gardenia erythroclada*. Their structures were determined on the basis of extensive 1D and 2D NMR analyses. Absolute structure of erythrosaponin A was unequivocally affirmed by single-crystal X-ray crystallography. All isolated compounds were evaluated for their cytotoxicity against cancer cell lines (KB and HeLa S-3) and their anti-inflammatory activity based on the inhibition of NO production in RAW264.7 cells. Erythrosaponin D showed moderate cytotoxicity against KB and HeLa S-3 cells with IC_{50} values of 25.8 and 29.5 μM, respectively. Erythrosaponins D, F, G, I and J showed moderate anti-inflammatory with IC_{50} values in the range of 63.0–81.4 μM.

1. Introduction

Gardenia erythroclada Kurz (Rubiaceae) (synonym *Dioecrescis erythroclada*), also known as “Ma Khang Daeng” in Thai, is a tree widely distributed in the northeastern part of Thailand. It is used for traditional Thai medicine to relieve abdominal pain and stomachache, and for antipyretic purposes. Iridoid glycosides, aromatic glycosides and aliphatic glycosides have been reported from the leaves and branches of this plant (Kaewkrud et al., 2007). The genus *Gardenia* comprises more than 130 species distributed over tropical and subtropical regions of Asia, Africa, Australia, Madagascar and some Pacific Islands (Mai et al., 2016). This genus has interesting pharmacological activities such as anticancer, antibacterial, anti-implantation, antioxidant, anti-inflammatory and anti HIV properties (Youn et al., 2016). Previous phytochemical investigation on this genus revealed the presence of triterpenoids (Nuanyai et al., 2009), flavonoids (Bhandare and Laddha, 2016), iridoids (Xiao et al., 2017) and phenolic compounds (Wang et al., 2016). In this report, we discuss the isolation and structural elucidation of ten undescribed triterpene saponins, erythrosaponins A–J (**1**–**10**) and one known analogue (**11**) from the methanol crude extracts of two parts (roots and stem bark) of *G. erythroclada*. Cytotoxicity against KB (human epidermoid carcinoma) and Hela S–3 (human cervix adenocarcinoma) cells and anti-inflammatory activity based on the inhibition of NO production in RAW264.7 cells were evaluated for all isolated compounds.

2. Results and discussion

The methanol extracts of dried roots (10.0 kg) and stem bark (10.0 kg) of *G. erythroclada* were subjected to multiple chromatographic steps over Dianion HP-20, silica gel and Sephadex LH-20, yielding compounds **1**–**5**, **8**, **9** and **11** from the roots part and compounds **6**–**10** from the stem bark part. A total of eleven triterpene saponins were obtained, including ten undescribed compounds, erythrosaponins A–J (**1**–**10**) and one known compound, catunaroside G (**11**) (Gao et al., 2011). The structures of **1**–**11** (Fig. 1) were identified by physical data analyses, including 1D and 2D NMR, IR, HRESIMS and single-crystal X-ray crystallography. The sugar residues were identified by co-TLC, HPLC analyses and optical rotation after hydrolysis.

Erythrosaponin A (**1**) was obtained as colorless crystals with $[a]_D^{20}$ -39.7 (*c* 0.26, MeOH). Its molecular formula was determined as $C_{42}H_{68}O_{13}$ from the ion peak $[M+Na]^+$ at *m/z* 803.4567 (calcd. for $C_{42}H_{68}O_{13}Na$, 803.4558) in the positive HRESIMS. The IR spectrum showed absorption bands at 3428, 1727, and 1645 cm^{-1}, suggesting

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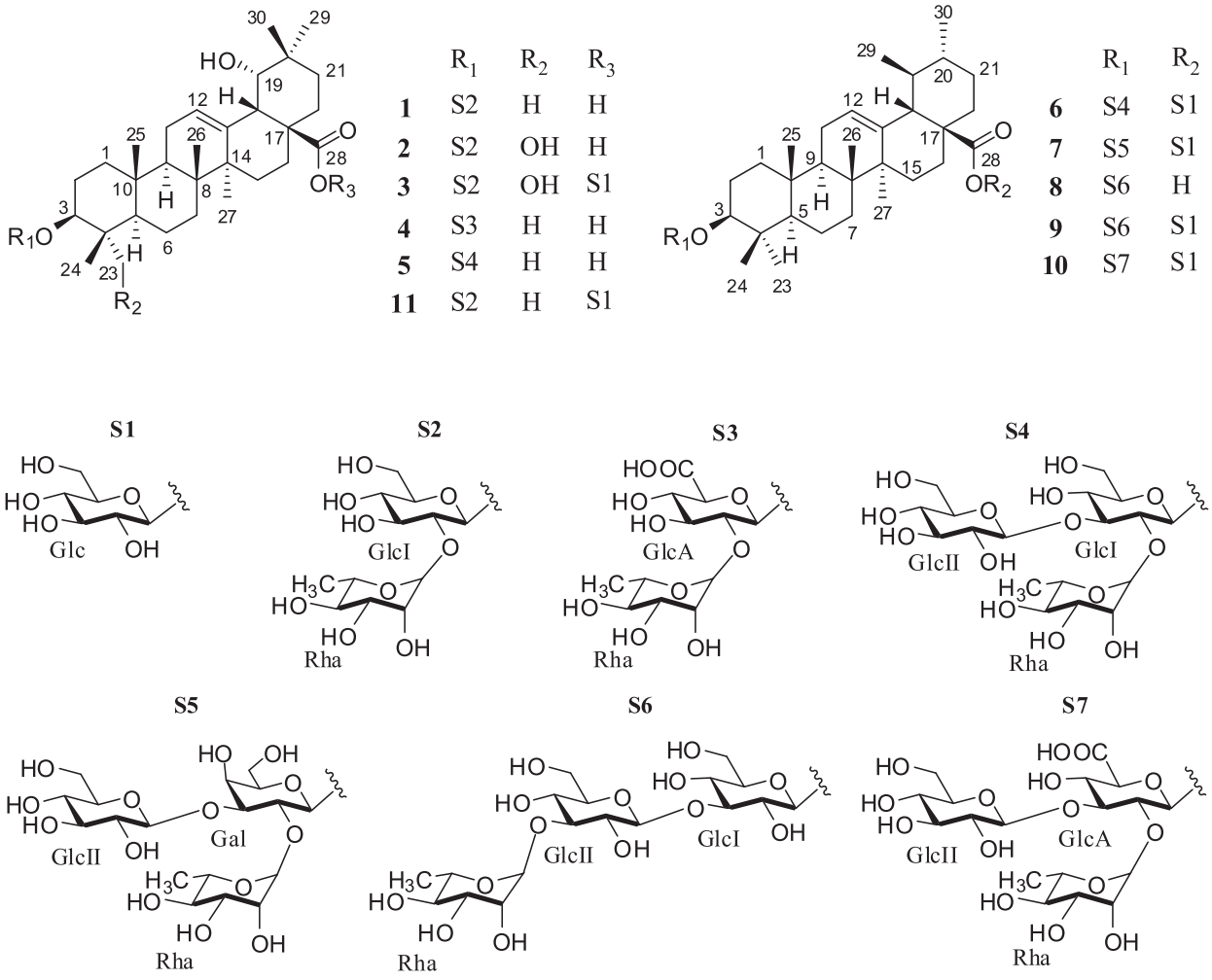

Fig. 1. Chemical structures of **1**–**11**.

the presence of hydroxyl, carbonyl, and olefinic groups, respectively. The ^{1}H and ^{13}C NMR data of **1** (Table 1) exhibited signals for seven methyl groups at δ_H 0.74 (3H, s, H-26), 0.85 (3H, s, H-24), 0.92 (6H, s, H-25, 29), 0.94 (3H, s, H-30), 1.04 (3H, s, H-23), and 1.28 (3H, s, H-27), one olefinic proton at δ_H 5.29 (1H, br s, H-12) with two typical olefinic carbon signals at δ_C 125.2 (C-12) and 144.8 (C-13), which were characteristic of an olean-12-ene skeleton. The spectroscopic data also showed signals of two oxymethines at δ_H 3.16 (1H, dd, J = 3.9, 11.6 Hz, H-3) with δ_C 90.6 (C-3) and at δ_H 3.23 (1H, d, J = 3.2 Hz, H-19) with δ_C 82.7 (C-19), and a carbon of carboxylic acid at δ_C 182.5 (C-28). The NOESY correlations (Fig. 2) between H-3 and H-23, and between H-19 and H-30 indicated the α-orientation of H-3 and β-orientation of H-19. Thus, the aglycone was identified as 3β,19α-dihydroxyolean-12-ene-28-oic acid (siaresinolic acid) (Barton et al., 1952). The presence of two sugar residues was confirmed from the observation of two anomeric protons at δ_H 4.39 (1H, d, J = 7.3 Hz, H-1-GlcI) and 5.35 (1H, br s, H-1-Rha) (Table 3), which were correlated in the HSQC spectrum with anomeric carbons at δ_C 105.8 and 102.0, respectively. After acid hydrolysis, the sugar units were confirmed to be D-glucose and L-rhamnose, which were identified by co-TLC, HPLC analyses and optical rotation. The configuration of the glucose and rhamnose glycosidic bonds was established as β-glucose and α-rhamnose, respectively by the coupling constants (Table 3) and the NOESY correlations. The HMBC correlations (Fig. 2) between the anomeric proton at δ_H 4.39 and the carbon at δ_C 90.6 (C-3), the anomeric proton at δ_H 5.35 and the carbon at δ_C 79.2 (C-2-GlcI), and between the oxymethine proton at δ_H 3.41 (1H, m, H-2-GlcI) and the carbons at δ_C 105.8 (C-1-Glc) and δ_C 102.0 (C-1-Rha) confirmed the glucose residue was linked to C-3 of aglycone and the terminal rhamnose was substituted at 2-OH of the glucopyranosyl unit. The comparison of the ^{1}H and ^{13}C NMR spectroscopic data (Table 1) of **1** showed similar resonances to those of **11**, apart from the lack of a glucopyranosyl unit at C-28 of aglycone. Absolute structure of **1** based on its β-D-glucose moiety was also affirmed by single-crystal X-ray crystallography (Fig. 3). On the basis of the above results, the structure of compound **1** was identified as 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-3β,19α-dihydroxyolean-12-en-28-oic acid.

Erythrosaponin B (**2**) was obtained as an amorphous powder with $[a]_D^{20}$ -33.3 (*c* 0.20, MeOH). Its molecular formula of $C_{42}H_{68}O_{14}$ was established by the positive HRESIMS $[M+Na]^+$ ion peak at *m*/*z* 819.4500 (calcd. for $C_{42}H_{68}O_{14}Na$, 819.4507). Comparing the ^{1}H and ^{13}C NMR data (Tables 1 and 3) of **2** with those of **1** showed the same sugar components and sequence of sugar chains. The difference was in the aglycone part; the methyl group at C-23 of aglycone in **1** was replaced to a hydroxymethyl group. The ^{1}H and ^{13}C NMR spectra showed two anomeric protons at δ_H 4.51 (1H, d, J = 7.7 Hz, H-1-GlcI) and 5.34 (1H, br s, H-1-Rha), which were correlated in the HSQC spectrum with anomeric carbons at δ_C 104.6 and 101.7, respectively. The NMR spectra of **2** showed two proton signals at δ_H

Table 1
^{1}H and ^{13}C NMR Spectroscopic Data for the Aglycones of **1**–**5** (MeOD-d_4).

Position	**1**		**2**		**3**		**4**		**5**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	1.00, m; 1.56, m	40.1	0.98, m; 1.55, m	39.5	1.03, m; 1.61, m	39.7	1.03, m; 1.58, m	40.1	1.03, m; 1.58, m	39.9
2	1.69, m; 1.93, m	27.4	1.73, m; 1.97, m	26.6	1.75, m; 1.99, m	26.7	1.70, m; 1.99, m	27.3	1.69, m; 1.93, m	27.2
3	3.16, dd (3.9, 11.6)	90.6	3.64, dd (3.6, 10.7)	82.2	3.68, dd (3.5, 12.1)	82.3	3.21, d (3.4, 11.6)	90.7	3.21, dd (3.4, 11.8)	90.1
4		40.4		43.9		43.9		40.5		40.2
5	0.79, m	57.6	1.26, m	48.6	1.32, m	48.7	0.81, m	57.6	0.79, m	57.4
6	1.42, m; 1.55, m	19.7	1.39, m; 1.49, m	18.9	1.42, m; 1.53, m	19.0	1.22, m; 1.58, m	19.7	1.42, m; 1.56, m	19.4
7	1.27, m; 1.50, m	34.2	1.22 m; 1.60, m	33.3	1.28, m; 1.64, m	33.3	1.31, m; 1.54, m	33.8	1.29, m; 1.51, m	34.0
8		40.9		40.6		40.8		41.0		40.7
9	1.72, m	49.2	1.77, m	49.2	1.81, m	49.3	1.75, m	49.5	1.71, m	49.4
10		38.2		37.7		37.7		38.2		38.0
11	1.92, m	25.0	1.95, m	24.7	1.98, m	24.8	1.95, m	25.0	1.93, m	24.8
12	5.29, br s	125.2	5.30, br s	124.9	5.36, br s	125.0	5.33, br s	125.2	5.31, br s	125.0
13		144.8		144.5		144.3		144.7		144.5
14		42.8		42.6		42.7		42.8		42.5
15	1.00, m; 1.72, m	29.7	1.00, m; 1.74, m	29.4	1.04, m; 1.74, m	29.4	1.02, m; 1.78, m	29.8	0.99, m; 1.76, m	29.5
16	2.27, m	28.7	2.27, m	28.6	2.35, m	28.5	2.31, m	28.7	2.25, m	28.5
17		46.9		46.6		47.1		47.0		46.7
18	3.03, br s	45.4	3.04, br s	45.1	3.08, br s	45.1	3.07, br s	45.4	3.04, br s	45.1
19	3.23, d (3.2)	82.7	3.24, d (3.6)	82.4	3.31, br s	82.5	3.27, d (3.4)	82.7	3.26, br s	82.5
20		36.2		35.9		35.9		36.2		36.0
21	1.60, m	29.7	1.59, m	29.4	1.68, m	29.5	1.61, m	29.7	1.62, m	29.4
22	1.60, m; 1.76, m	34.2	1.60, m; 1.75, m	34.0	1.67, m; 1.81, m	33.4	1.64, m; 1.79, m	34.3	1.61, m; 1.77, m	34.0
23	1.04, s	28.9	3.36, d (11.3); 3.59, d (11.3)	64.6	3.35, d (11.5); 3.63, d (11.5)	64.7	1.07, s	28.7	1.04, s	28.5
24	0.85, s	17.3	0.71, s	13.7	0.75, s	13.7	0.88, s	17.4	0.86, s	17.2
25	0.92. s	16.1	0.95, s	16.2	1.00, s	16.3	0.96, s	16.1	0.93, s	15.9
26	0.74, s	18.0	0.76, s	17.7	0.78, s	17.8	0.78, s	18.0	0.75, s	17.7
27	1.28, s	25.3	1.30, s	25.0	1.34, s	25.0	1.31, s	25.2	1.28, s	25.1
28		182.5		182.3		178.6		182.5		182.4
29	0.92, s	28.9	0.93, s	28.6	0.98, s	28.6	0.95, s	28.9	0.93, s	28.6
30	0.94, s	25.4	0.95, s	25.1	0.99, s	25.2	0.98, s	25.4	0.95, s	25.2

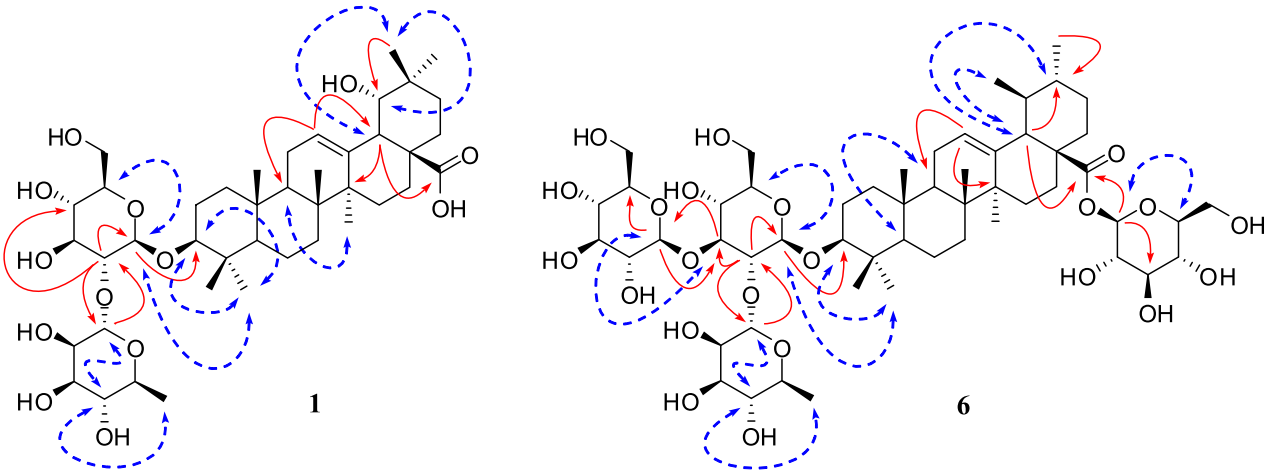

Fig. 2. Key HMBC (arrow curves) and NOESY (dashed curves) correlations of **1** and **6**.

3.36 (1H, d, J = 11.3 Hz, H-23a) and 3.59 (1H, d, J = 11.3, H-23b) with carbon signal at δ_C 64.6 (C-23) instead of a methyl signal in **1**. In addition, the HMBC correlations of H-23 with C-3 (δ_C 82.2), C-5 (δ_C 48.6), and C-24 (δ_C 13.7) also confirmed the presence of the hydroxymethyl group at C-23. Thus, the aglycone of **2** was identified as 3β,19α,23α-trihydroxyolean-12-en-28-oic acid (ilexosapogenin A) (Amimoto et al., 1993). Finally, the structure of **2** was assigned as 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl]-3β,19α,23α-trihydroxyolean-12-en-28-oic acid.

Erythrosaponin C (**3**) was obtained as an amorphous powder with $[a]_D^{20}$ -74.7 (c 0.54, MeOH). Its molecular formula was determined to be $C_{48}H_{78}O_{19}$ by HRESIMS $[M+Na]^+$ m/z 981.5044 (calcd. for $C_{48}H_{78}O_{19}Na$, 981.5035). According to the NMR analysis and hydrolysis results, compound **3** possessed the same aglycon and 3-*O*-sugar chain as **2**, except that the carboxylic acid group was substituted with a glucopyranosyl group. The ^{1}H and ^{13}C NMR spectra showed three anomeric protons at δ_H 4.55 (1H, d, J = 7.5 Hz, H-1-GlcI), 5.39 (1H, br s, H-1-Rha), and 5.41 (1H, d, J = 8.1 Hz, H-1-Glc), which were correlated in the HSQC spectrum with anomeric carbons at δ_C 104.6, 101.8, and 95.8, respectively. The HMBC spectrum showed cross-peak between the anomeric proton at δ_H 5.41 (1H, d, J = 8.1 Hz, H-1-Glc) and the carbon at δ_C 178.6 (C-28), suggesting that the β-D-glucose was located at C-28 of **2**. Accordingly, **3** was determined to be 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-

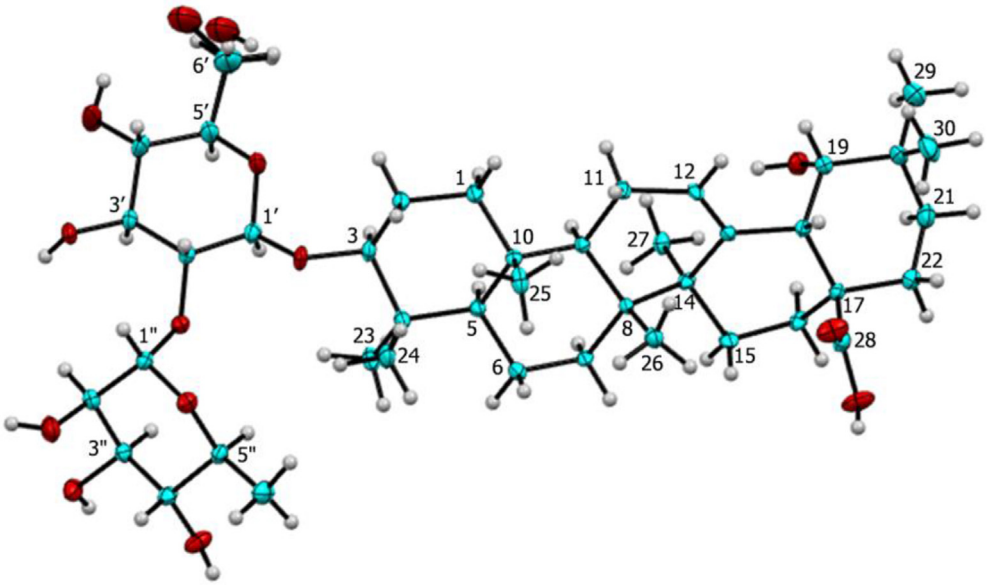

Fig. 3. ORTEP drawing of **1**, **Note** that the glucose moiety has a doubly disordered C6′ (H_2)–OH group with occupancy factors of 0.56 and 0.44.

glucopyranosyl]-28-*O*-(*β*-D-glucopyranosyl)-3*β*,19*α*,23*α*-trihydroxyolean-12-en-28-oic acid.

Erythrosaponin D (**4**) was obtained as an amorphous powder with $[a]_D^{20}$ -103.1 (*c* 0.32, MeOH). Its molecular formula of $C_{42}H_{66}O_{14}$ was suggested by the positive HRESIMS $[M+Na]^+$ ion peak at *m*/*z* 817.4349 (calcd. for $C_{42}H_{66}O_{14}$ Na, 817.4350). The ^1H and ^{13}C NMR data of **4** were similar to those of **1**, except for a sugar unit. The hydroxymethyl group of glucopyranosyl moiety was oxidized to become a carboxylic acid group (δ_C 176.0) in glucuronopyranosyl moiety. The HMBC correlations observed from δ_H 3.64 (1H, d, $J = 8.1$, H-5-GlcA) to δ_C 105.8 (C-1-GlcA), 79.3 (C-3-GlcA), and 176.0 (C-6-GlcA) confirmed the presence of a glucuronic acid unit. Two anomeric proton signals at δ_H 4.47 (1H, d, $J = 7.0$ Hz, H-1-GlcA) and 5.39 (1H, br s, H-1-Rha) were correlated in the HSQC spectrum with anomeric carbons at δ_C 105.8 and 102.1, respectively. On the basis of the above deductions, the structure of **4** was established as 3-*O*-[*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucuronopyranosyl]-3*β*,19*α*-dihydroxyolean-12-en-28-oic acid.

Erythrosaponin E (**5**) was obtained as an amorphous powder with $[a]_D^{20}$ -82.5 (*c* 0.20, MeOH). Its molecular formula was determined as $C_{48}H_{78}O_{18}$ from the positive HRESIMS $[M+Na]^+$ ion peak at *m*/*z* 965.5083 (calcd. for $C_{48}H_{78}O_{18}$Na, 965.5086). Comparison of the ^1H and ^{13}C NMR spectra of **5** with those of **1** indicated that **5** differed from **1** in the presence of an additional deoxyglucose unit. Three anomeric proton signals at δ_H 4.45 (1H, d, $J = 7.6$ Hz, H-1-GlcI), 4.53 (1H, d, $J = 7.8$ Hz, H-1-GlcII), and 5.44 (1H, br s, H-1-Rha) were correlated in the HSQC spectrum with anomeric carbons at δ_C 105.3, 103.9, and 101.7, respectively. The HMBC spectrum showed cross-peak between the anomeric proton at δ_H 4.53 and the carbon at δ_C 88.5 (C-3-GlcI), between the oxymethine proton at δ_H 3.60 (H-2-GlcI) and the carbons at δ_C 88.5, 101.7 (C-1-Rha), and 105.3 (C-1-GlcI) confirmed the presence of a *α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucuronopyranosyl chain located at C-3 of **5**. Thus, the structure of **5** was determined to be 3-*O*-[*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranosyl]-3*β*,19*α*-dihydroxyolean-12-en-28-oic acid.

Erythrosaponin F (**6**) was obtained as an amorphous powder with $[a]_D^{20}$ -97.5 (*c* 0.54, MeOH). Its molecular formula was determined to be $C_{54}H_{88}O_{22}$ from the ion peak $[M+Na]^+$ at *m*/*z* 1111.5660 (calcd. for $C_{54}H_{88}O_{22}$Na, 1111.5665) in the positive HRESIMS. The IR spectrum showed absorption bands at 3426, 1725, and 1642 cm^{-1}, suggesting the presence of hydroxyl, carbonyl, and olefinic groups, respectively. The ^1H and ^{13}C NMR data of **6** (Table 1) showed signals of seven methyl groups at δ_H 0.84 (3H, s, H-24), 0.84 (3H, s, H-25), 0.84 (3H, s, H-26), 0.93 (3H, br s, H-29), 0.94 (3H, br s, H-30), 1.02 (3H, s, H-23), and 1.09 (3H, s, H-27), one olefinic proton at δ_H 5.23 (1H, br s, H-12) with two typical olefinic carbon signals at δ_C 127.2 (C-12) and 139.1 (C-13), which were characteristic of an urs-12-ene skeleton. The NMR spectra also exhibited a signal for an oxymethine at δ_H 3.19 (1H, dd, $J = 3.2$, 11.4 Hz, H-3) with δ_C 90.6 (C-3), and one carbonyl carbon at δ_C 178.0 (C-28). The NOESY correlations (Fig. 2) showed cross-peak at H-3 with H-23, at H-18 (δ_H 2.21, d, $J = 11.2$ Hz) with H-20 (δ_H 0.94, m) and H-29 indicated the *α*-orientations of H-3 and H-19, and *β*-orientation of H-20. Thus, the aglycone part was identified as 3*β*-hydroxyurs-12-en-28-oic acid (ursolic acid) (Varanda et al., 1992). Four anomeric proton signals at δ_H 4.44 (1H, d, $J = 7.5$ Hz, H-1-GlcI), 4.52 (1H, d, $J = 7.8$ Hz, H-1-GlcII), 5.33 (1H, d, $J = 8.1$ Hz, H-1-Glc), and 5.44 (1H, br s, H-1-Rha) were observed, which were correlated in the HSQC spectrum with anomeric carbons at δ_C 105.3, 103.9, 95.6, and 101.7, respectively. After acid hydrolysis, the sugar units were confirmed to be D-glucose and L-rhamnose. The configuration of the glucose and rhamnose was established by the coupling constants as *β*-glucose and *α*-rhamnose (Table 4) and the NOESY experimental. The HMBC correlations (Fig. 2) between the anomeric proton at δ_H 4.44 (H-1-GlcI) with the carbon at δ_C 90.6 (C-3), the anomeric proton at δ_H 4.52 (H-1-GlcII) with the carbon at δ_C 88.5 (C-3-GlcI), at the anomeric proton δ_H 5.44 (H-1-Rha) with the carbon at δ_C 77.9 (C-2-GlcI), the anomeric proton at δ_H 5.33 (H-1-Glc) with the carbon at δ_C 178.0 (C-28), and between the oxymethine proton at δ_H 3.58 (1H, m, H-2-GlcI) with the carbon at δ_C 88.5 (C-3-GlcI), 101.7 (C-1-Rha), and 105.3(C-1-GlcI) confirmed the *α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranosyl chain at 3-OH and *β*-D-glucopyranosyl moiety at 28-COOH of aglycone. On the basis of the above results, the structure of **6** was identified as 3-*O*-[*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucopyranosyl]-28-*O*-(*β*-D-glucopyranosyl)-3*β*-hydroxyurs-12-en-28-oic acid.

Erythrosaponin G (**7**) was obtained as an amorphous powder with $[a]_D^{20}$ -101.8 (*c* 0.38, MeOH). Its molecular formula of $C_{54}H_{88}O_{22}$

Table 2
^{1}H and 13C NMR Spectroscopic Data for the Aglycones of **6**–**9** (MeOD-d_4) and **10** (DMSO-d_6).

Position	**6**		**7**		**8**		**9**		**10**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	1.02, m; 1.62, m	40.2	1.06, m; 1.60, m	37.8	1.02, m; 1.60, m	38.5	1.09, m; 1.70, m	37.8	0.86, m; 1.55, m	39.2
2	1.68, m; 1.91, m	27.2	1.71, m; 1.95, m	27.1	1.68, m; 1.90, m	27.4	1.73, m; 1.96, m	27.0	1.50, m; 1.93, m	25.8
3	3.19, dd (3.2, 11.4)	90.1	3.21, dd (3.3, 11.9)	90.1	3.18, dd (3.7, 11.4)	91.2	3.24, dd (3.0, 10.7)	90.9	3.07, m	88.2
4		42.5		41.0		41.2		40.0		41.9
5	0.75, m	57.3	0.76, m	57.3	0.77, m	57.4	0.81, m	57.0	0.71, m	55.6
6	1.37, m; 1.51, m	19.3	1.36, m; 1.53, m	19.3	1.37, m; 1.53, m	19.7	1.43, m; 1.57, m	19.3	1.45, m	18.0
7	1.31, m; 1.48, m	34.3	1.31, m; 1.53, m	34.3	1.32, m; 1.52, m	34.7	1.37, m; 1.55, m	34.3	1.23, m; 1.44, m	32.9
8		40.9		40.2		40.3		40.1		39.7
9	1.52, m	49.3	1.52, m	49.3	1.53, m	49.4	1.57, m	49.0	1.44, m	47.6
10		37.9		37.8		38.2		40.0		36.1
11	1.90, m	24.4	1.91, m	24.4	1.91, m	24.8	1.97, m	24.4	1.83, m	23.1
12	5.23, br s	127.2	5.23, br s	127.3	5.21, br s	127.2	5.29, br s	127.2	5.14, br s	125.2
13		139.1		139.0		140.0		139.0		137.9
14		43.2		43.2		43.6		43.2		41.9
15	1.08, m; 1.91, m	29.3	1.10, m; 1.93, m	29.3	1.10, m; 1.90, m	29.6	1.16, m; 1.98, m	29.2	0.89, m; 1.85, m	27.9
16	1.78, m; 2.05, m	25.2	1.76, m; 2.04, m	25.2	1.78, m; 2.02, m	25.7	1.79, m; 2.12, m	25.2	1.64, m; 1.96, m	23.4
17		49.5		49.5		48.9		49.2		47,4
18	2.21, d (11.2)	54.2	2.22, d (11)	54.2	2.18, d (11.2)	54.7	2.27, d (11.1)	54.1	2.10, dd (10.0)	52.6
19	1.37, m	40.3	1.39, m	40.4	1.35, m	40.7	1.43, m	40.3	1.33, m	38.6
20	0.94, m	40.2	0.97, m	40.2	0.96, m	40.5	1.01, m	40.2	0.92, m	38.4
21	1.31, m; 1.48, m	31.7	1.31, m; 1.52, m	31.7	1.32, m; 1.48, m	32.1	1.38, m; 1.55, m	31.7	1.24, m; 1.46, m	30.3
22	1.61, m; 1.73, m	37.8	1.62, m; 1.75, m	37.5	1.66, m; 1.78, m	38.2	1.68, m; 1.79, m	37.4	1.50, m; 1.64, m	36.5
23	1.02, s	28.5	1.04, m	28.6	1.05, s	29.0	1.12, s	28.6	0.95, s	27.7
24	0.84, s	17.6	0.82, m	17.7	0.83, s	18.1	0.88, s	17.7	0.82, s	17.0
25	0.84, s	17.3	0.82, m	17.3	0.83, s	17.5	0.88, s	17.1	0.76, s	16.6
26	0.84, s	17.9	0.82, m	17.9	0.83, s	18.2	0.88, s	17.9	0.72, s	17.3
27	1.09, s	24.0	1.10, m	24.0	1.10, s	24.6	1.16, s	24.1	1.02, s	23.4
28		178.0		178.0		182.0		177.9		175.4
29	0.93, br s	16.3	0.94, br s	16.3	0.94, br s	16.5	1.00, br s	16.2	0.87, br s	15.7
30	0.94, br s	21.6	0.96, br s	21.6	0.94, br s	22.0	1.00, br s	21.6	0.92, br s	21.3

Table 3
^{1}H and ^{13}C NMR Spectroscopic Data for the Sugar Units of **1**–**5** (MeOD-d_4).

Position	**1**		**2**		**3**		**4**		**5**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
3-O-	*β-D-GlcI*		*β-D-GlcI*		*β-D-GlcI*		*β-D-GlcA*		*β-D-GlcI*	
1	4.39, d (7.3)	105.8	4.51, d (7.7)	104.6	4.55, d (7.5)	104.6	4.47, d (70)	105.8	4.45, d (7.6)	105.3
2	3.41, m	79.2	3.36, m	78.7	3.42, m	78.8	3.51, m	79.1	3.60, m	78.0
3	3.21, m	77.8	3.23, m	77.4	3.29, m	78.3	3.51, m	79.3	3.71, m	88.5
4	3.27, m	72.3	3.27, m	72.0	3.34, m	72.0	3.51, m	74.2	3.28, m	71.5
5	3.43, m	79.7	3.46, m	79.3	3.51, m	79.3	3.64, d (8.1)	76.9	3.48, m	78.2
6	3.64, dd (5.1, 11.8)	63.0	3.64, dd (5.2, 11.7)	62.7	3.71, dd (5.2, 11.6)	62.8		176.0	3.66, dd (5.0, 11.5)	62.7
	3.83, dd (1.3, 11.8)		3.83, dd (1.3, 11.7)		3.87, dd (1.8, 11.6)				3.88, dd (1.5, 11.5)	
									β-D-GlcII	
1									4.53, d (7.8)	103.9
2									3.55, m	73.9
3									3.29, m	77.3
4									3.38, m	71.9
5									3.37, m	78.1
6									3.66, dd (5.0, 11.5)	62.5
									3.88, dd (1.5, 11.5)	
	α-L-Rha		*α-L-Rha*		*α-L-Rha*		*α-L-Rha*		*α-L-Rha*	
1	5.35, br s	102.0	5.34, br s	101.7	5.39, br s	101.8	5.39, br s	102.1	5.44, br s	101.7
2	3.93, m	72.2	3.94, m	71.8	4.01, m	71.9	3.98, m	72.3	3.98, m	72.0
3	3.73, dd (3.1, 9.5)	72.4	3.74, dd (3.3, 9.5)	72.1	3.79, dd 2.9, 9.5)	72.1	3.77, dd (3.2, 9.5)	72.4	3.74, m	72.1
4	3.37, m	74.2	3.37, m	73.9	3.37, m	73.9	3.40, t (9.6)	74.2	3.39, m	73.7
5	3.97, m	70.2	3.95, m	69.9	3.99, m	70.0	3.99, m	70.2	4.01, m	70.1
6	1.20, d (6.1)	18.2	1.21, d (6.2)	17.9	1.26, d (6.0)	17.9	1.23, d (6.1)	18.2	1.21, d (6.1)	18.0
28-O-					*β-D-Glc*					
1					5.41, d (8.1)	95.8				
2					3.41, m	74				
3					3.63, m	78.7				
4					3.38, m	71.1				
5					3.29, m	77.4				
6					3.71, dd (5.2, 11.6)	62.4				
					3.87, dd (1.8, 11.6)					

Table 4
^{1}H and ^{13}C NMR Spectroscopic Data for the Sugar Units of **6**–**9** (MeOD-d_4) and **10** (DMSO-d_6).

Position	**6**		**7**		**8**		**9**		**10**	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
3-O-	*β-D-GlcI*		*β-D-Gal*		*β-D-GlcI*		*β-D-GlcI*		*β-D-GlcA*	
1	4.44, d (7.5)	105.3	4.48, d (6.8)	105.3	4.37, d (7.8)	106.6	4.43, d (7.7)	106.2	4.32, d (5.5)	103.7
2	3.58, m	77.9	3.66, m	78.0	3.39, m	74.3	3.43, m	73.9	3.44, m	75.5
3	3.71, m	88.5	3.78, m	86.6	3.53, m	88.4	3.57, m	88.0	3.64, m	85.7
4	3.29, m	71.5	3.35, m	71.2	3.39, m	70.4	3.41, m	70.0	3.11, m	69.8
5	3.37, m	78.2	3.36, m	78.2	3.48, m	77.6	4.40, m	77.9	3.13, m	77.4
6	3.67, m	62.7	3.67, m	62.4	3.67, m	63.1	3.72, m	62.7		172.8
	3.83, m		3.79, m		3.86, m		3.89, m			
	β-D-GlcII		*β-D-GlcII*		*β-D-GlcII*		*β-D-GlcII*		*β-D-GlcII*	
1	4.52, d (7.8)	103.9	4.58, d (7.4)	103.4	4.58,d (7.8)	105.4	4.63, d (7.8)	105.0	4.43, d (5.5)	102.0
2	3.30, m	73.8	3.32, m	73.9	3.52, m	75.4	3.43, m	75.9	3.10, m	72.5
3	3.29, m	77.2	3.32, m	78.0	3.54, m	84.4	3.58, m	84.0	3.44, m	75.2
4	3.34, m	71.1	3.35, m	71.2	3.39, m	70.4	3.41, m	70.0	3.11, m	69.8
5	3.37, m	78.1	3.36, m	78.2	3.35, m	78.4	3.40, m	78.2	3.20, m	76.9
6	3.67, m	62.6	3.67, m	62.5	3.67, m	63.0	3.72, m	62.6	3.47, m	61.1
	3.83, m		3.86, m		3.86, m		3.89, m		3.71, m	
	α-L-Rha		*α-L-Rha*		*α-L-Rha*		*α-L-Rha*		*α-L-Rha*	
1	5.44, br s	101.7	5.42, br s	101.7	5.17, br s	103.0	5.22, br s	102.6	5.33, br s	100.4
2	3.97, m	72.0	3.99, m	71.9	3.95, m	72.7	3.99, m	72.2	3.77, m	70.5
3	3.73, m	72.1	3.74, m	72.1	3.71, m	72.6	3.75, m	72.1	3.47, m	70.6
4	3.38, m	73.7	3.37, m	73.7	3.53, m	74.3	3.56, m	73.8	3.19, m	72.2
5	4.01, m	70.1	3.99, m	70.2	3.98, m	70.5	4.03, m	70.1	3.86, m	68.5
6	1.20, d (6.1)	18.0	1.21, d (6.0)	18.0	1.24, d (6.2)	18.3	1.30, d (6.1)	17.9	1.05, d (4.7)	18.0
28-O-	*β-D-Glc*		*β-D-Glc*				*β-D-Glc*		*β-D-Glc*	
1	5.33, d (8.1)	95.6	5.34, d (8.1)	95.7			5.40, d (8.0)	95.6	5.18, d (8.1)	94.3
2	3.26, m	75.2	3.28, m	75.1			3.38, m	74.9	3.06, m	73.8
3	3.69, m	78.5	3.68, m	78.5			3.64, m	78.4	3.13, m	77.8
4	3.35, m	70.1	3.35, m	71.3			3.41, m	71.1	3.11, m	70.2
5	3.29, m	77.2	3.33, m	78.0			3.34, m	77.2	3.20, m	76.9
6	3.67, m	62.5	3.67, m	62.5			3.72, m	62.5	3.49, m	61.1
	3.83, m		3.86, m				3.89, m		3.60, m	

was established by the positive HRESIMS $[M+Na]^+$ ion peak at *m/z* 1111.5651 (calcd. for $C_{54}H_{88}O_{22}$ Na, 1111.5665). Careful comparison of the NMR spectroscopic data of **7** with those of **6** indicated no difference between two compounds, except for the replacement of the glucopyranosyl by galactopyranosyl unit. Acid hydrolysis of **7** yielded a sugar mixture containing D-glucose, D-galactose, and L-rhamnose. The ^{1}H and ^{13}C NMR spectra showed four anomeric protons at δ_H 4.48 (1H, d, $J = 6.8$ Hz, H-1-Gal), 4.58 (1H, d, $J = 7.4$ Hz, H-1-GlcII), 5.34 (1H, d, $J = 8.1$ Hz, H-1-Glc), and 5.42 (1H, br s, H-1-Rha, which were correlated in the HSQC spectrum with anomeric carbons at δ_C 105.3, 103.4, 95.7, and 101.7, respectively. The HMBC spectrum showed cross-peak between the anomeric proton at δ_H 4.48 and the carbon at δ_C 90.1 (C-3), the anomeric proton at δ_H 4.58 and the carbon atδ_C 86.6 (C-3-Gal), the anomeric proton at δ_H 5.42 and the carbon at δ_C 78.0 (C-2-Gal), and between the oxymethine proton at δ_H 3.66 (H-2-Gal) and the carbon at δ_C 86.6 (C-3-Gal), 101.7 (C-1-Rha), and 105.3 (C-1-Gal) confirmed the presence of *α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→3)-*β*-D-galactopyranosyl unit and the sugar chain was also compared with the NMR data of literature (Gupta et al., 2000). Thus, the structure of **7** was elucidated as 3-*O*-[*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→3)-*β*-D-galactopyranosyl]-28-*O*-(*β*-D-glucopyranosyl)-3*β*-hydroxyurs-12-en-28-oic acid.

Erythrosaponin H (**8**) was obtained as an amorphous powder with $[a]_D^{20}$ -115.2 (*c* 0.66, MeOH). Its molecular formula was determined to be $C_{48}H_{78}O_{17}$ by HRESIMS $[M+Na]^+$ *m/z* 949.5142 (calcd. for $C_{48}H_{78}O_{17}$Na, 949.5137). The comparison of the spectroscopic data of **8** with those of **6** (Tables 2 and 4) showed similarity, except for the absence of sugar unit at C-28 and the position of ramnopyranosyl in **6** was located from C-2-GlcI to C-3-GlcII in **8**. The ^{1}H and ^{13}C NMR spectra showed three anomeric protons at δ_H 4.37 (1H, d, $J = 7.8$ Hz, H-1-GlcI), 4.58 (1H, d, $J = 7.8$ Hz, H-1-GlcII), and 5.17 (1H, br s, H-1-Rha), which were correlated in the HSQC spectrum with anomeric carbons at δ_H 106.6, 105.4, and 103.0, respectively. The cross-peak between the anomeric protons at δ_H 4.37 (H-1-Glc1) and the carbon at δ_C 91.2 (C-3), the anomeric proton at δ_H 4.58 (H-1-GlcII) and the carbon at δ_C 88.4 (C-3-GlcI), and between the anomeric proton at δ_H 5.17 (H-1-Rha) and the carbon at δ_C 84.4 (C-3-GlcII) in the HMBC spectrum suggested that the *α*-L-rhamnopyranosyl-(1→3)-*O*-[*O*-*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranosyl was located at C-3 (δ_C 91.2) and the trisaccharide chain was also confirmed by comparison of the NMR data with the literature (Lemmich et al., 1995). Consequently, **8** was deduced to be 3-*O*-{*α*-L-rhamnopyranosyl-(1→3)-*O*-[*O*-*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranosyl}-3*β*-hydroxyurs-12-en-28-oic acid.

Erythrosaponin I (**9**) was obtained as an amorphous powder with $[a]_D^{20}$ -74.7 (*c* 0.50, MeOH). Its molecular formula of $C_{54}H_{88}O_{22}$ was suggested by the positive HRESIMS $[M+Na]^+$ ion peak at *m/z* 1111.5670 (calcd. for $C_{54}H_{88}O_{22}$ Na, 1111.5665). The ^{1}H and ^{13}C NMR data of **9** were similar to those of **8**, except for the presence of a 28-*O*-glucopyranosyl unit. The ^{1}H and ^{13}C NMR spectra showed four anomeric protons at δ_H 4.43 (1H, d, $J = 7.7$ Hz, H-1-GlcI), 4.63 (1H, d, $J = 7.8$ Hz, H-1-GlcII), 5.22 (1H, br s, H-1-Rha), and 5.40 (1H, d, $J = 8.0$ Hz, H-1-Glc), which were correlated in the HSQC spectrum with anomeric carbons at δ_C 106.2, 105.0, 102.6, and 95.6, respectively. In addition, acid hydrolysis of **9** also confirmed the presence of D-glucose and L-rhamnose. Thus, the structure of **9** was elucidated as 3-*O*-{*α*-L-rhamnopyranosyl-(1→3)-*O*-[*O*-*β*-D-glucopyranosyl-(1→3)]-*β*-D-glucopyranosyl}-28-*O*-(*β*-D-glucopyranosyl)-3*β*-hydroxyurs-12-en-28-oic acid.

Erythrosaponin J (**10**) was obtained as an amorphous powder with $[a]_D^{20}$ -24.1 (*c* 0.58, DMSO). Its molecular formula was determined to be $C_{54}H_{86}O_{23}$ from the positive HRESIMS $[M+Na]^+$ ion peak at *m/z* 1125.5468 (calcd. for $C_{54}H_{86}O_{23}$Na, 1125.5458). Analysis

of ^{1}H and ^{13}C NMR data (Tables 2 and 4) of **10** showed similarity to those of **6** except that the glucopyranosyl unit (GlcI) of **6** was replaced by a glucuronopyranosyl unit, the signal of carboxyl group shown at δ_C 172.8 (C-6-GlcA) and the lack of a hydroxymethyl group in **10**. The ^{1}H and ^{13}C NMR spectra showed four anomeric protons at δ_H 4.32 (1H, d, $J = 5.5$ Hz, H-1-GlcA), 4.43 (1H, d, $J = 5.5$ Hz, H-1-GlcII), 5.33 (1H, br s, H-1-Rha), and 5.18 (1H, d, $J = 8.1$ Hz, H-1-Glc), which were correlated in the HSQC spectrum with anomeric carbons at δ_C 103.7, 102.0, 100.4, and 94.3, respectively. Acid hydrolysis confirmed the presence of a sugar mixture containing D-glucose, D-glucoronic acid, and L-rhamnose. The NMR spectroscopic data (Table 4) of **10** confirmed the presence of β-glucoronic acid moiety. In addition, the sugar sequence was elucidated by the 2D NMR data. Thus, **10** was identified as 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→3)-β-D-glucuronopyranosyl]-28-*O*-(β-D-glucopyranosyl)-3β-hydroxyurs-12-en-28-oic acid.

All isolated compounds (**1**–**11**) were evaluated *in vitro* for their cytotoxicity against KB and HeLa S-3 cell lines and their anti-inflammatory activity based on the inhibition of NO production in RAW264.7 cells. Almost all compounds showed inactive cytotoxicity against two cancer cells, except that compound **4** showed moderate cytotoxicity against KB and HeLa S-3 cells with IC_{50} values of 25.8 and 29.5 μM, respectively. The cytotoxic effects of oleanane-type saponins have the 28-carboxylic acid based on the damage of cell membrane like a hemolytic action (Podolak et al., 2010). In this research suggested that disaccharide at C-3 of oleanane triterpenes, glucuronic acid-rhamnose (**4**) have more cytotoxic effect than glucose-rhamnose (**1**) and trisaccharide (**5**). Compounds **4**, **6**, **7**, **9** and **10** showed moderate anti-inflammatory with IC_{50} values of 76.8, 81.4, 69.4, 63.0 and 79.5 μM respectively. All isolated compounds have no affected to RAW264.7 cells at concentration lower than 300 μM. Doxorubicin (IC_{50} 0.16 and 0.03 μM for KB and HeLa S-3) was used for positive control of cytotoxicity and dexamethasone (IC_{50} 8.25 μM) was used for positive control of anti-inflammatory.

3. Experimental section

3.1. General experimental procedures

The UV–visible absorption spectra were recorded on a UV-2550 UV–vis spectrometer (Shimadzu, Kyoto, Japan). The IR spectra were measured on a Nicolet 6700 FT-IR spectrometer using KBr discs. Optical rotations were detected by a Jasco P-1010 polarimeter. NMR spectra were recorded on a Bruker 400 AVANCE spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). The HRESIMS were obtained using a Bruker MICROTOF model mass spectrometer. Single-crystal X-ray diffraction analysis was performed on a Bruker ×8 APEXII Kappa CCD area-detector diffractometer with MoKα radiation (λ = 0.71073 Å). Column chromatography was performed with Dianion HP-20, silica gel 60 (0.063–0.200 mm), and Sephadex LH-20 (25–100 μm, GE Healthcare).

3.2. Plant material

The roots of *Gardenia erythroclada* Kurz (synonym *Dioecrescis erythroclada*) (Rubiaceae) were collected in July 2015 and the stem bark of *G. erythroclada* was collected in January 2016 from Sahatsakhan district, Kalasin province, Thailand (16°43′35″ N 103°29′22″ E). The plant material was identified by Dr. Suttira Khumkratok, a botanist at the Walai Rukhavej Botanical Research Institute, Mahasarakham University, and a specimen retained as a reference (Khumkratok no. 5–12).

3.3. Extraction and isolation

The dried powder of *G. erythroclada* roots (10.0 kg) and stem bark (10.0 kg) were macerated for 3 days with MeOH (each 2 × 25 L). After evaporation of the solvent in vacuo, 450 g of root residue and 420 g of stem bark residue were obtained. Each of the two MeOH residue samples were suspended in H_2O and then separated with dianion HP-20, and eluted with H_2O and MeOH, respectively.

The MeOH-soluble residue (98 g) from the roots was chromatographed over silica gel and eluted with MeOH in EtOAc (0–100%, stepwise), yielding four fractions (A-D). Fraction A (5 g) was purified with sephadex LH-20, using CH_2Cl_2-MeOH (1:1) as eluent to give **1** (120 mg). Fraction B (12 g) was repeatedly subjected to silica gel, eluting with MeOH in CH_2Cl_2 (1:4), to provide two subfractions, and then each subfraction was further purified with sephadex LH-20, using CH_2Cl_2-MeOH (1:1) as eluent to afford **2** (95 mg) and **8** (25 mg). Compounds **5** (35 mg) and **11** (45 mg) were achieved from fraction C (10 g) by siliga gel, eluting with CH_2Cl_2-MeOH (4:1). Fraction D (15 g) was separated by silica gel and eluted with CH_2Cl_2-MeOH (4:1) to obtain two subfractions (Da and Db). Subfraction Da (5 g) was further purified with sephadex LH-20, eluting with CH_2Cl_2-MeOH (1:1), to yield **3** (15 mg) and **4** (11 mg). Compound **9** (15 mg) was purified from subfraction Db (2 g) with sephadex LH-20, eluting with CH_2Cl_2-MeOH (1:1).

The MeOH-soluble residue (85 g) from the stem bark was subjected to silica gel and eluted with MeOH in EtOAc (0–100%, stepwise), to provide three fractions (E-G). Compound **8** (10 mg) was purified from fraction E (3 g) with sephadex LH-20, eluting with CH_2Cl_2-MeOH (1:1). Fraction F (20 g) was chromatographed over silica gel and eluted with a gradient of CH_2Cl_2-MeOH (1:4 to 4:1), to give **6** (10 mg) and **9** (8 mg). Fraction G (17 g) was fractionated by siliga gel and eluted with CH_2Cl_2-MeOH (2:1), to give two subfraction (Ga and Gb). Compound **7** (12 mg) was purified from subfraction Ga (5 g) with sephadex LH-20, eluting with CH_2Cl_2-MeOH (1:1). Subfraction Gb was treated with sephadex LH-20, using CH_2Cl_2-MeOH (1:1) as eluent to yield **10** (9 mg).

3.3.1. Erythrosaponin A (*1*)

Colorless crystals; $[a]_D^{20}$ -39.7 (*c* 0.26, MeOH); IR (KBr) ν_{max} 3428, 1727, 1645 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; positive HRESIMS *m/z* 803.4567 $[M+ Na]^+$ (calcd. for $C_{42}H_{68}O_{13}Na$, 803.4558).

3.3.2. Erythrosaponin B (*2*)

Amorphous powder; $[a]_D^{20}$ -33.3 (*c* 0.20, MeOH); IR (KBr) ν_{max} 3425, 1729, 1647 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; positive HRESIMS *m/z* 819.4500 $[M+ Na]^+$ (calcd. for $C_{42}H_{68}O_{14}Na$, 819.4507).

3.3.3. Erythrosaponin C (*3*)

Amorphous powder; $[a]_D^{20}$ -74.7 (*c* 0.54, MeOH); IR (KBr) ν_{max} 3421, 1725, 1646 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; positive HRESIMS *m/z* 981.5044 $[M+ Na]^+$ (calcd. for $C_{42}H_{68}O_{13}Na$, 981.5035).

3.3.4. Erythrosaponin D (*4*)

Amorphous powder; $[a]_D^{20}$ -103.13 (*c* 0.32, MeOH); IR (KBr) ν_{max} 3423, 1727, 1642 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3; positive HRESIMS *m/z* 817.4349 $[M+ Na]^+$ (calcd. for $C_{42}H_{66}O_{14}Na$, 817.4350).

3.3.5. Erythrosaponin E (*5*)

Amorphous powder; $[a]_D^{20}$ -82.5 (*c* 0.20, MeOH); IR (KBr) ν_{max} 3431, 1730, 1648 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 3;

positive HRESIMS *m/z* 965.5083 [M+ Na]$^+$ (calcd. for $C_{48}H_{78}O_{18}Na$, 965.5086).

3.3.6. Erythrosaponin F (**6**)

Amorphous powder; $[a]_D^{20}$ -97.5 (*c* 0.54, MeOH); IR (KBr) ν_{max} 3426, 1725, 1642 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 4; positive HRESIMS *m/z* 1111.5660 [M+ Na]$^+$ (calcd. for $C_{54}H_{88}O_{22}Na$, 1111.5665).

3.3.7. Erythrosaponin G (**7**)

Amorphous powder; $[a]_D^{20}$ -101.8 (*c* 0.38, MeOH); IR (KBr) ν_{max} 3432, 1728, 1642 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 4; positive HRESIMS *m/z* 1111.5651 [M+ Na]$^+$ (calcd. for $C_{54}H_{88}O_{22}Na$, 1111.5665).

3.3.8. Erythrosaponin H (**8**)

Amorphous powder; $[a]_D^{20}$ -115.2 (*c* 0.66, MeOH); IR (KBr) ν_{max} 3426, 1724, 1640 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 4; positive HRESIMS *m/z* 949.5142 [M+ Na]$^+$ (calcd. for $C_{48}H_{17}O_{22}Na$, 949.5137).

3.3.9. Erythrosaponin I (**9**)

Amorphous powder; $[a]_D^{20}$ -74.7 (*c* 0.50, MeOH); IR (KBr) ν_{max} 3429, 1730, 1641 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 4; positive HRESIMS *m/z* 1111.5670 [M+ Na]$^+$ (calcd. for $C_{54}H_{88}O_{22}Na$, 1111.5665).

3.3.10. Erythrosaponin J (**10**)

Amorphous powder; $[a]_D^{20}$ -24.1 (*c* 0.58, DMSO); IR (KBr) ν_{max} 3429, 1727, 1649 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 2 and 4; positive HRESIMS *m/z* 1125.5468 [M+ Na]$^+$ (calcd. for $C_{54}H_{86}O_{23}Na$, 1125.5458).

3.4. X-ray crystallographic analysis of **1**

Colorless single crystals of **1** were obtained from the slow evaporation of a MeOH–ACN solution, as $C_{42}H_{68}O_{13}$ in the monoclinic, space group $P2_1$ (no. 4) with unit cell constants $a = 15.0656(11)$ Å, $b = 7.6106(5)$ Å, $c = 21.3562(15)$ Å, $\beta = 100.306(2)^\circ$, $V = 2409.2(3)$ Å^3, $Z = 2$, $D_{calc} = 1.077$ g/cm^3, $MW = 780.96$. A rod-like single crystal of **1** with dimensions $0.10 \times 0.12 \times 0.24$ mm was fixed on the tip of MiTeGen microloop using epoxy glue. With the help of a Bruker ×8 APEXII Kappa CCD area-detector diffractometer with MoKα radiation ($\lambda = 0.71073$ Å) and the APEX2 Software Suite, X-ray diffraction data were collected at 296(2) K. Data were then processed sequentially, i.e. integrated, reduced by SAINT+, corrected for Lorentz, polarization and absorption effects, and scaled by SADABS (SAINT+ and SADABS, 2008. Bruker AXS Inc., Madison, Wisconsin, USA) to yield 8819 unique reflections ($R_{int} = 0.067$). The structure was solved using the intrinsic phasing method with SHELXTL XT (Bruker, 2014b) and refined with full-matrix least squares on F^2 with SHELXTL XTLMP (Bruker, 2014a). The disordered solvents present in the large intermolecular voids were removed using PLATON SQUEEZE procedure (Spek, 2015). The refinement converged at the final $R(F^2) = 0.058$ and $wR(F^2) = 0.141$ for 5115 data with $F^2 > 2\sigma(F^2)$. The absolute structure of **1** was first established according to its β-D-glucose moiety with known absolute configuration (Fig. 1) and then directly re-confirmed by a Bruker ×8 PROSPECTOR KAPPA CCD diffractometer using an IμS X-ray microfocus source with CuKα radiation ($\lambda = 1.54178$ Å). Data processing and the removal of large intermolecular voids were accomplished according to standard procedure, as mentioned above. The refinement converged at the final $R(F^2) = 0.054$ and $wR(F^2) = 0.135$ for 6416 data with $F^2 > 2\sigma(F^2)$; the Flack parameter (Parsons et al., 2013) x = 0.14(10). The crystal data have been deposited with the Cambridge Crystallographic Data Centre (CCDC 1547305 and 1554048). Copies of these data can be obtained, free of charge, on application to the CCDC via www.ccdc.cam.ac.uk/conts/retrieving.html (or 12 Union Road, Cambridge CB2 1EZ, UK, fax: +44 1223 336033, e-mail: deposit@ccdc.cam.ac.uk).

3.5. Acid hydrolysis

A solution of erythrosaponins A–J (**1**–**10**) (5 mg) in 2 M HCl (1 mL) was heated at reflux for 24 h. The reaction mixture was neutralized with 2 M NaOH and extracted by partition with EtOAc (5×1 mL). The sugar residues were identified by co-TLC (TLC Silica gel 60 F_{254}) by comparison with standard sugar. The solvent system was CH_2Cl_2-MeOH-H_2O (2:1:0.2), and spots were visualized by spraying with EtOH-H_2SO_4-anisaldehyde (9:0.5:0.5, v/v), then heated at 150 °C. The R_f values of D-glucose, D-galactose, D-glucolonic acid and L-rhamnose by TLC were 0.30, 0.20, 0.04 and 0.56, respectively. In addition, the sugar were identified by HPLC analysis (column: RPM-Monosacc 300 × 7.8 mm, carrier: 100% H_2O 0.6 mL/min, retention time: 13.37, 15.42, 15.77 and 25.05 min) in comparison with the authentic D-glucose, L-rhamnose, D-galactose and D-glucolonic acid, respectively. The optical rotation value was measured after 24 h of dissolution in water, D-glucose $[a]_D^{20}$ +49.5 (*c* 0.1, H_2O), D-galactose $[a]_D^{20}$ +69.2 (*c* 0.1, H_2O), D-glucolonic acid $[a]_D^{20}$ +37.7 (*c* 0.1, H_2O) and L-rhamnose $[a]_D^{20}$ -12.8 (*c* 0.1, H_2O) (Tsukamoto et al., 1989), (Lehbili et al., 2017).

3.6. Cytotoxicity assay

All isolated compounds (**1**–**11**) were subjected to cytotoxic evaluation against KB (human epidermoid carcinoma) and HeLa S-3 (human cervical carcinoma) cell lines employing the colorimetric method (Kongkathip et al., 2003). Doxorubicin, which exhibits activity against two cancer cell lines, was used as the reference substance.

3.7. Anti-inflammatory assay

All isolated compounds (**1**–**11**) were subjected to anti-inflammatory activity based on the inhibition of NO production in RAW264.7 cells using a previously published protocol (Chen et al., 2017). Dexamethasone was used as the positive control.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.phytochem.2018.04.016.

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