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Manniosides B-F, five new triterpenoid saponins from the leaves of *Schefflera mannii* (Hook.f.) Harms

Beaudelaire K. Ponou [a,b,*], Chiaki Tanaka [b], Rémy B. Teponno [a], Azefack L. Tapondjou [a], Tomofumi Miyamoto [b,**]

[a] Research Unit of Environmental and Applied Chemistry, Department of Chemistry, Faculty of Science, University of Dschang, Box 183, Dschang, Cameroon
[b] Department of Natural Products Chemistry, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, 812-8582, Japan

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ABSTRACT

Fifteen triterpenoid saponins including five new compounds (Mannioside B: 3β-[(β-D-glucopyranosyl)oxy]urs-12-en-28-oic acid α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**1**), mannioside C: 3β-[(β-D-glucopyranosyl)23-dioxy]urs-12-en-28-oic acid α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**2**), mannioside D: 3β,23-dihydroxyurs-12-en-28-oic acid β-D-glucopyranosyl-(1 → 6)- β-D-glucopyranosyl ester (**3**), mannioside E: 3β-hydroxy-23-oxolup-20(29)-en-28-oic acid α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (**4**) and mannioside F: (22S)-27β-[(β-D-glucopyranosyl)oxy]-22-hydroxyprotosta-12,24-dien-3β-yl β-D-glucopyranoside (**5**)) were isolated from the leaves of *Schefflera mannii* (Hook.f.) Harms. Their structures were established on the basis of 1D and 2D NMR data, mass spectrometry and chemical methods. The major isolated compounds were tested for their antiproliferative activity on human malignant epithelial (HeLa) cells but were not efficient at the concentration of 33 mM.

1. Introduction

The Araliaceae family is known as one of the most medically important plant families [1]. Several chemical and pharmacological studies revealed triterpenoid saponins to be the main bioactive components present in Araliaceae species [2–7]. Saponins isolated from Araliaceae are reported to exhibit various pharmacological activities including anti-inflammatory, antiproliferative, antifungal, antioxidant, hepatoprotective, neuroprotective, wound healing and cancer preventing effects [8]. The genus *Schefflera* (Araliaceae) comprises about 600 described species found in the tropics and subtropics [9] where they are used as folk medicine for the treatment of inflammation, rheumatism, fever, pain, and as a general tonic [1,10,11]. They are also known as a rich source of pentacyclic triterpenoids and their glycoside derivatives, mainly with oleanane, ursane, and lupane type skeletons [1,4,10,12]. *Schefflera mannii* (Hook.f.) Harms is found in Cameroon, Equatorial Guinea, Nigeria, and São Tomé and Príncipe [13]. No previous phytochemical studies on this plant have been reported.

As part of our continuous effort to isolate bioactive saponins from Cameroonian medicinal plants [7,14–20], we have examined the methanol extract from the leaves of *S. mannii.* In the present paper, we describe the isolation and structure elucidation of five new triterpenoid saponins (**1–5**) along with ten known compounds (**6–15**). Although compound **14** was previously identified [21], its NMR data are herein reported for the first time. Furthermore, the major isolated compounds were tested for their antiproliferative activity on human malignant epithelial cells (HeLa).

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on a JASCO DIP-370 Digital Polarimeter. ^{1}H and ^{13}C NMR were performed in C_5D_5N/D_2O (20 : 1) on a Varian INOVA-600 NMR Spectrometer (600 MHz for ^{1}H, 150 MHz for ^{13}C). All chemical shifts (δ) are given in ppm with reference to

* Corresponding author. Research Unit of Environmental and Applied Chemistry, Department of Chemistry, Faculty of Science, University of Dschang, Box 183, Dschang, Cameroon.
** Corresponding author.
E-mail addresses: beaudelaireponou@yahoo.fr (B.K. Ponou), miyamoto@phar.kyushu-u.ac.jp (T. Miyamoto).

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tetramethylsilane (TMS) as internal standard and coupling constants (*J*) are in Hz. ESI-TOF-MS were recorded on a Bruker Micro TOF II LC/MS Spectrometer. Centrifugation was done on Mini Centrifuge model NG002B. Column chromatography was performed using Sephadex LH-20 (eluted with MeOH) and silica gel 60 (0.040–0.063 mm, Merck) (eluted with $CHCl_3$/MeOH/H_2O (60 : 37: 7, 70 : 30: 0.5, 80 : 20: 0.5, 90 : 10: 5)). MPLC was done with a KISO Power Tool E5305T pump and an Advantec SF-160 fraction collector. TLC were carried out on precoated Kieselgel 60 F254 (Merck) plates developed with $CHCl_3$/MeOH/H_2O (60 : 37: 7, 70 : 30: 1, 80 : 20: 1, 90 : 10: 5) and on Kieselgel 60 RP-8 F254S (Merck KGaA 64271 Darmstadt, Germany) developed with MeOH/H_2O and CH_3CN/H_2O mixtures. They were visualised by spraying with 5% methanolic H_2SO_4 followed by heating. Preparative HPLC was performed with a JASCO PU-2080 Plus Intelligent HPLC pump connected to a 5C_{18}-AR-II column (Nacalai Tesque Inc., Japan) and JASCO UV-2075 Inteligent UV/VIS detector, λ_{max} 210 nm. 300 μL, 40 μL and 20 μL of solution were injected each time with flow rates of 2.5 mL/min (on 20 mm × 250 mm), 1.5 mL/min (on 10 mm × 250 mm) and 1 mL/min (on 4.6 mm × 250 mm) column, respectively. GC-MS was performed with GCMS-QP2010SE (Shimadzu, Japan) with Inert Cap 5MS/Sil i.d. 0.25 × 30 m (GL Sciences Inc., Japan) [Column temperature: 100–280 °C, rate of temperature increase: 10 °C/min]. The following sugar samples were commercially obtained: D-glucose, L-rhamnose, L-glucose (Aldrich Chem. Co., Japan), D-rhamnose (Carbosynth Ltd., United Kingdom), L-cysteine methyl ester hydrochloride (Kanto Chemical Co., Inc., Japan), *N*-trimethylsilylimidazole (TMS-imidazole) (Tokyo Kasei Kogyo Co., Ltd., Japan). Fetal bovine serum (FBS) was purchased from Nichirei Bioscience Inc. (Tokyo, Japan) and heat-inactivated at 56 °C for 30 min for cell culture.

2.2. Plant material

The leaves of S. mannii *were collected in November 2017 in Dschang (*5°27′0″ N et 10°4′0″ E*), West Region of Cameroon, and identified at the Cameroon National Herbarium in Yaoundé by Mr. NANA Victor* in comparison with a voucher specimen deposited under the reference N° 35063/HNC.

2.3. Extraction and isolation

The air dried and pulverized plant material (3 kg) was extracted at room temperature (24 °C) three times (each time for 24 h) with 15 L of MeOH (95%). The filtrate obtained was concentrated under reduced pressure at 40 °C to give 405 g of crude extract using rotator evaporator (yield 13.5%). Part of the extract (1 g) was dissolved in MeOH, centrifuged to eliminate non soluble particles (9.1 mg) and the MeOH soluble portion was subjected to Sephadex column to afford five fractions A (158.3 mg), B (377.7 mg), C (154.7 mg), D (151.5 mg) and E (66.9 mg). MPLC of fraction B on normal phase silica gel column using $CHCl_3$/MeOH/H_2O (70 : 30: 0.5) as eluent gave seven subfractions B1 (46.5 mg), B2 (18.3 mg), B3 (112.1 mg), B4 (60.6 mg), B5 (19.5 mg), B6 (29.6 mg) and B7 (43.2 mg). HPLC of subfraction B3 eluted with MeOH–H_2O (80 : 20) on 20 mm × 250 mm column gave compound **12** (t_R 44.0 min, 11.2 mg), subfractions B32 (48.7 mg), B33 (9.0 mg) and B34 (5.3 mg). Further HPLC purifications of B32 on a cholester 4.6 mm × 250 mm column eluted with MeOH/H_2O (65 : 35) afforded **8** (t_R 25.0 min, 25.2 mg), and **13** (t_R 28.6 min, 9.2 mg). When the same conditions applied to B33, compounds **3** (t_R 20.6 min, 3.5 mg) and **14** (t_R 24.1 min, 1.7 mg) were isolated. Subfraction B6 was submitted to HPLC on 20 mm × 250 mm column eluted with MeOH/H_2O (80 : 20) to give **1** (t_R 42.5 min, 1.2 mg) while in the same conditions, B7 led to the isolation of **11** (t_R 28.2 min, 6.3 mg), **2** (t_R 30.2 min, 7.7 mg), and **6** (t_R 34.8 min, 8.0 mg). Based on the similarities observed on TLC profiles of subfraction B2 and fraction C, they were combined and the resulting fraction was subjected to MPLC on normal phase silica gel using $CHCl_3$/MeOH/H_2O (90 : 10: 5 then 70 : 30: 0.5) as elution systems to obtain six subfractions named C1 (52.4 mg), C2 (8.9 mg), C3 (23.7 mg), C4 (3.4 mg), C5 (34 mg) and C6 (34.5 mg). HPLC purifications of the combined C3, C4 and C5 on 10 mm × 250 mm column eluted with MeOH/H_2O (75 : 25) allowed the isolation of **9** (t_R 75.1 min, 1.2 mg), **7** (t_R 55.7 min, 5.4 mg), **15** (t_R 60.4 min, 1.5 mg), and **10** (t_R 101.8 min, 1.1 mg). Given the relative small amount of subfraction B34 which showed compounds not yet isolated from the previous fractions, and the complexity to separate its constituents using MeOH/H_2O mixtures as mobile phase, we have repeated the experiments in similar conditions starting with 1.2 g of the MeOH extract. The second round of the experiments allowed to obtain several fractions including one, namely Br33 (24.1 mg) comparable to B34 on TLC profiles. Both Br33 and B34, were combined and the resulting fraction was submitted to HPLC on 4.6 mm × 250 mm column eluted with CH_3CN/H_2O (35 : 65) giving **4** (t_R 17.8 min, 1.7 mg), **5** (t_R 9.1 min, 1.7 mg) in addition to **8** (t_R 11.0 min, 1.8 mg) and **3** (t_R 15.4 min, 3.5 mg) initially isolated.

2.3.1. Mannioside B (1)

White amorphous powder; $[\alpha]^{24}_D$ = +1.66° (c = 0.0006 g/mL, MeOH); ^{1}H NMR (C_5D_5N: D_2O (20 : 1), 600 MHz) and ^{13}C NMR (C_5D_5N: D_2O (20 : 1), 150 MHz) data: see Table 1; HRESIMS: *m/z* 1111.5371 $[M+Na]^+$ (calcd for $C_{54}H_{88}O_{22}Na$, 1111.5665).

2.3.2. Mannioside C (2)

White amorphous powder; $[\alpha]^{23}_D$ = −13.94 (c = 0.0038 g/mL, MeOH); ^{1}H NMR (C_5D_5N: D_2O (20 : 1), 600 MHz) and ^{13}C NMR (C_5D_5N: D_2O (20 : 1), 150 MHz) data: see Table 1; HRESIMS: *m/z* 1127.5289 $[M+Na]^+$ (calcd for $C_{54}H_{88}O_{23}Na$, 1127.5614).

2.3.3. Mannioside D (3)

White amorphous powder; $[\alpha]^{24}_D$ = +9.13 (c = 0.0023 g/mL, MeOH); ^{1}H NMR (C_5D_5N: D_2O (20 : 1), 600 MHz) and ^{13}C NMR (C_5D_5N: D_2O (20 : 1), 150 MHz) data: see Table 1; HRESIMS: *m/z* 819.4386 $[M+Na]^+$ (calcd for $C_{42}H_{68}O_{14}Na$, 819.4507).

2.3.4. Mannioside E (4)

White amorphous powder; $[\alpha]^{24}_D$ = −8.07 (c = 0.0026 g/mL, MeOH); ^{1}H NMR (C_5D_5N: D_2O (20 : 1), 600 MHz) and ^{13}C NMR (C_5D_5N: D_2O (20 : 1), 150 MHz) data: see Table 2; HRESIMS: *m/z* 963.4726 $[M+Na]^+$ (calcd for $C_{48}H_{76}O_{18}Na$, 963.4929).

2.3.5. Mannioside F (5)

White amorphous powder; $[\alpha]^{23}_D$ = −7.00 (c = 0.0010 g/mL, MeOH); ^{1}H NMR (C_5D_5N: D_2O (20 : 1), 600 MHz) and ^{13}C NMR (C_5D_5N: D_2O (20 : 1), 150 MHz) data: see Table 2; HRESIMS: *m/z* 805.4671 $[M+Na]^+$ (calcd for $C_{42}H_{70}O_{13}Na$, 805.4714).

2.3.6. Echinocystic acid 28α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester (14)

White amorphous powder; $[\alpha]^{24}_D$ = −5.00 (c = 0.0010 g/mL, MeOH); ^{1}H NMR (C_5D_5N: D_2O (20 : 1), 600 MHz) and ^{13}C NMR (C_5D_5N: D_2O (20 : 1), 150 MHz) data: see Table 2. HRESIMS: *m/z* 965.4944 $[M+Na]^+$ (calcd for $C_{48}H_{78}O_{18}Na$, 965.5986).

2.4. Acid hydrolysis and GC analysis

Each saponin (*ca.* 0.5 mg) was heated in 1 M HCl (0.1 mL) at 90 °C for 3 h. The reaction mixture was dried *in vacuo* and dissolved in pyridine (0.2 mL). TMS-imidazole (50 μL) was added to the part of solution (0.1 mL) then heated at 50 °C for 30 min. The reaction mixture was diluted with H_2O (0.2 mL) and extracted with hexane (0.1 mL) then analysed by GC-MS by comparison with standard samples derivated in the same conditions. L-Cysteine methyl ester hydrochloride (*ca.* 1.0 mg) was added to the remaining pyridine solution (0.1 mL) and heated at 60 °C for 1 h then the TMS derivative was prepared the same manner mentioned above and analysed by GC-MS. D-glucose (t_R 19.51 min) and

Table 1
^{1}H and ^{13}C NMR data of compounds **1–3** (C_5D_5N: D_2O (20 : 1), 600 MHz, 150 MHz).

	1		2		3	
Position	^{13}C	^{1}H (multiplicity, *J* in Hz)	^{13}C	^{1}H (multiplicity, *J* in Hz)	^{13}C	^{1}H (multiplicity, *J* in Hz)
1	38.8	0.86 (m), 1.44 (m)	38.8	0.94 (m), 1.50 (m)	38.7	1.00 (m), 1.57 (m)
2	26.2	1.85 (m), 2.27 (m)	25.8	1.96 (m), 2.34 (m)	27.1	1.94 (m)
3	88.8	3.42 dd (11.7, 4.5)	82.2	4.25, (o)	73.0	4.19 (o)
4	39.8	–	43.0	–	42.5	–
5	55.6	0.78 (br d)	47.2	1.63 (br d)	48.2	1.49 (br d)
6	18.2	1.27 (m), 1.44 (m)	17.9	1.32 (m), 1.65 (m)	18.3	1.37 (m), 1.60 (m)
7	32.9	1.35 (m), 1.49 (m)	32.9	1.35 (m), 1.71 (m)	32.9	1.36 (m), 1.66 (m)
8	39.2	–	39.8	–	39.9	–
9	47.8	1.54 (m)	47.6	1.65 (m)	47.8	1.67 (m)
10	36.6	–	36.6	–	36.8	–
11	23.8	1.92 (m)	23.6	1.92 (m)	23.5	1.95 (m)
12	125.8	5.46 (br s)	125.9	5.46 (br s)	125.8	5.47
13	138.2	–	138.5	–	138.1	–
14	42.2	–	42.3	–	42.3	–
15	28.5	1.20 (m)	28.5	1.14 (m)	28.5	1.15 (m), 2.39 (m)
16	24.3	2.00 (m)	24.0	2.04 (m)	24.3	2.04 (m)
17	48.1	–	48.2	–	48.2	–
18	53.0	2.51 (d, 11.4)	53.0	2.49 (d, 11.1)	53.0	2.51 (d, 11.3)
19	38.7	1.42 (m)	39.1	1.39 (m)	38.9	1.38 (m)
20	39.1	0.91 (m)	38.7	0.88 (m)	38.7	0.85 (m)
21	30.5	1.28 (m), 1.38 (m)	30.5	1.23 (m), 1.37 (m)	30.5	1.23 (m), 1.36 (m)
22	36.0	1.77 (m), 1.92 (m)	36.4	1.76 (m), 1.91 (m)	36.5	1.79 (m), 1.93 (m)
23	28.0	1.31 (s)	64.1	3.68 (d, 10.9), 4.31(o)	67.2	3.68 (d, 10.9), 4.14 (o)
24	16.8	1.01 (s)	13.4	0.95 (s)	12.8	1.03 (s)
25	15.5	0.86 (s)	16.5	0.94 (s)	16.0	1.00 (s)
26	17.2	1.12 (s)	17.5	1.14 (s)	17.6	1.17 (s)
27	23.5	1.21 (s)	23.5	1.16 (s)	23.5	1.13 (s)
28	176.1	–	176.4	–	176.3	–
29	16.8	0.97 (d, 6.4)	16.5	0.94 (d, 5.9)	17.1	0.92 (d, 6.4)
30	21.1	0.93 (d, 6.2)	21.8	0.89 (d, 5.6)	21.0	0.86 (d, 5.6)
1'	106.5	4.94 (d, 7.8)	105.4	5.15 (d, 7.8)		
	75.2	4.06 (o)	75.3	4.06 (o)		
	78.2	4.33 (o)	78.1	4.24 (o)		
	71.5	4.20 (o)	71.2	4.20 (o)		
	76.1	4.12 (o)	77.8	3.95 (o)		
	62.5	4.37 (o), 4.59 (dd, 11.8, 2.3)	62.3	4.35 (o), 4.59 (dd, 11.8; 2.3)		
1''	95.1	6.16 (d, 8.2)	95.3	6.14 (d, 8.2)	95.3	6.17 (d, 8.2)
	73.4	4.14 (o)	73.4	4.14 (o)	73.3	4.16 (o)
	78.0	4.26 (o)	78.0	4.26 (o)	78.0	4.26 (o)
	70.3	4.32 (o)	70.3	4.32 (o)	70.4	4.32 (o)
	77.5	4.09 (o)	77.5	4.09 (o)	77.5	4.10 (m)
	68.7	4.31 (o), 4.65 (o)	69.0	4.31 (o), 4.65 (o)	69.0	4.31 (o), 4.71 (dd, 11.2, 1.8)
1'''	104.3	4.97 (d, 7.6)	104.3	4.97 (d, 7.6)	104.7	5.01 (d, 7.1)
	74.7	3.96 (dd, 9.0; 8.0)	74.7	3.95 (dd, 9.0; 8.0)	74.7	4.01 (dd, 8.8, 8.0)
	76.1	4.13 (o)	76.1	4.12 (o)	77.8	4.18 (o)
	78.1	4.32 (o)	78.1	4.32 (o)	71.1	4.15 (o)
	76.5	3.64 (m)	76.7	3.63 (m)	78.0	3.90 (m)
	60.7	4.06 (o), 4.20 (o)	60.9	4.06 (o), 4.19 (o)	62.2	4.31 (o), 4.47 (dd, 11.9; 2.3)
1'''''	102.3	5.79 (br s)	102.3	5.77 (br s)		
	72.0	4.67 (o)	72.0	4.67 (o)		
	72.1	4.54 (dd, 9.3; 3.4)	72.1	4.54 (dd, 9.3; 3.4)		
	73.4	4.34 (o)	73.3	4.34 (o)		
	70.8	4.88 (m)	70.0	4.88 (m)		
	18.2	1.68 (d, 6.2)	18.2	1.68 (d, 6.0)		

L-rhamnose (t_R 18.28 min) were characterized for compounds **1, 2, 4** and **14** while only D-glucose was present in **3** and **5**.

Standard TMS-Sugars (t_R, min).

TMS-glucose, $t_R = 14.27$; TMS-rhamnose, $t_R = 11.20$.

Standard TMS-Thiazolidine Derivatives (t_R, min).

D -glucose, $t_R = 19.51$; L-glucose, $t_R = 19.64$; L-rhamnose, $t_R = 18.29$; D-rhamnose, $t_R = 18.43$.

2.5. Antiproliferative assay

Human malignant epithelial cells (HeLa) were cultured in Eagle's minimum essential medium (EMEM) supplemented with 10% fetal bovine serum (FBS) kept in an incubator at 37 °C in a humidified air containing 5% CO_2. FBS was purchased from Nichirei Bioscience Inc. (Tokyo, Japan). Cell viability was determined by a Cell-Titer 96 Aqueous Non-Radioactive Cell Proliferation (MTS) Assay (Promega, WI, U.S.A.) according to the manufacturer's protocol. HeLa cells (1×10^4 cells/well) were seeded in 96 well plates and incubated for 24 h, subsequently grown with compounds for additional 48 h, and then cell proliferation assay was performed [16]. Cells were counted after 24h then, after 48h. Cisplatin was used as a reference drug.

3. Results and discussion

The crude methanol extract of the leaves of *S. mannii* was repeatedly subjected to column chromatography to afford fifteen compounds

Table 2
^{1}H and ^{13}C NMR data of compounds **4, 5** and **14** (C_5D_5N: D_2O (20 : 1), 600 MHz, 150 MHz).

	4		5		14	
Position	^{13}C	^{1}H (multiplicity, *J* in Hz)	^{13}C	^{1}H (multiplicity, *J* in Hz)	^{13}C	^{1}H (multiplicity, *J* in Hz)
1	38.5	1.02 (m), 1.64 (m)	36.6	1.03 (m), 1.54 (m)	40.3	0.96 (m), 1.52 (m)
2	26.8	1.92 (m), 1.99 (m)	26.5	1.86 (m), 2.35 (m)	24.7	1.96 (o)
3	71.5	4.12 (o)	89.1	3.49 (dd, 11.7; 3.9)	79.3	4.17
4	56.2	–	39.3	–	40.5	–
5	47.5	1.38 (o)	50.8	1.38 (o)	57.1	0.78
6	20.9	0.94 (m), 1.33 (m)	18.0	1.47 (m)	19.6	1.44
7	33.6	1.20 (m), 1.36 (m)	29.5	2.43 (m)	34.7	1.34, 1.51
8	41.3	–	33.7	–	41.3	–
9	50.5	1.42 (dd, 9.1, 3.6)	50.0	1.38 (o)	48.5	1.74
10	36.9	–	35.4	–	37.1	–
11	21.0	1.15 (m), 1.47 (m)	23.9	2.10 (m)	25.1	1.95 (o)
12	25.8	1.15 (m), 1.86 (m)	118.3	5.30 (br s)	124.0	5.56 (br s)
13	38.1	2.62 (m)	145.8	–	145.6	–
14	42.6	–	43.6	–	42.5	–
15	29.8	1.18 (m), 1.97 (m)	30.0	2.44 (m)	37.1	1.76 (o), 2.45 (br d)
16	32.0	1.52 (m), 2.66 (m)	26.2	1.85 (m)	75.3	5.26 (brs)
17	56.7	–	49.5	3.60 (br s)	50.4	–
18	49.5	1.74 (m)	21.4	0.92 (s)	42.5	3.44 (dd, 14.4; 4.2)
19	47.2	3.38 (td, 11.0, 5.0)	12.9	0.74 (s)	48.5	1.29 (o), 2.71 (m)
20	150.7	–	48.6	2.21 (m)	32.0	–
21	30.6	1.43 (m), 2.16 (m)	12.2	1.22 (d, 6.7)	33.2	2.06 (m), 2.35 (m)
22	36.5	1.51 (m), 2.23 (m)	72.8	4.07 (m)	29.0	1.82 (m)
23	207.7	9.63 (s)	29.7	2.44 (m), 2.58 (m)	29.9	1.14 (s)
24	9.2	1.34 (s)	128.9	5.88 (t, 7.3)	17.7	0.97 (s)
25	16.1	1.10 (s)	133.2	–	16.9	0.89 (s)
26	16.2	0.82 (s)	21.7	2.01 (s)	18.8	1.08 (s)
27	14.6	1.03 (s)	67.6	4.66 (d, 11.6), 4.75 (d, 11.6)	28.4	1.73 (s)
28	174.9	–	27.4	1.30 (s)	177.4	–
29	109.7	4.77 (br s), 4.89 (br s)	15.6	1.12 (s)	34.3	0.91 (s)
30	19.2	1.76 (s)	27.2	1.07 (s)	25.9	0.98 (s)
1'			106.4	4.98 (d, 7.8)		
			74.3	4.08 (o)		
			77.9	4.28 (o)		
			71.2	4.21 (o)		
			77.9	3.95 (m)		
			62.0	4.39 (o), 4.56 (o)		
1''	94.9	6.32 (d, 8.2)	103.0	4.92 (d, 7.8)	96.9	6.15 (d, 8.3)
	73.6	4.11 (o)	74.3	4.08 (o)	74.9	4.01 (o)
	78.2	4.29 (o)	77.9	4.29 (o)	79.2	4.18 (o)
	70.2	4.34 (o)	71.2	4.22 (o)	71.6	4.23 (o)
	77.9	4.10 (o)	77.9	4.03 (m)	79.6	4.17 (o)
	68.9	4.30 (o), 4.67 (o)	62.0	4.39 (o), 4.56 (o)	70.2	4.25 (o), 4.59 (o)
1'''	104.4	4.95 (d, 7.9)			105.4	4.91 (d, 7.9)
	74.7	3.93 (dd, 9.0; 8.0)			76.2	3.87 (dd, 9.0; 8.0)
	76.0	4.11 (o)			77.5	4.06 (o)
	77.9	4.10 (o)			79.4	4.25 (o)
	76.6	3.61 (m)			78.1	3.56 (m)
	60.9	4.06 (dd, 12.2; 3.7), 4.18 (o)			62.3	4.00 (o), 4.12
1''''	102.4	5.77 (brs)			103.8	5.72 (brs)
	72.0	4.67 (o)			73.5	4.61 (o)
	72.1	4.54 (dd, 9.3; 3.3)			73.6	4.48 (dd, 9.3; 3.4)
	73.3	4.33 (o)			74.8	4.27 (o)
	70.1	4.87 (m)			71.5	4.83 (m)
	18.2	1.68 (d, 6.2)			19.6	1.62 (d, 6.2)

including five new triterpenoid saponins, mannioside B (**1**), mannioside C (**2**), mannioside D (**3**), mannioside E (**4**), mannioside F (**5**), and ten known compounds, scheffursoside C (**6**) [10], 3*β*,23-dihydroxyurs-12-en-28-oic acid *β*-D-glucopyranosyl ester (**7**) [22], 3*β*,23-dihydroxyurs-12-en-28-oic acid *α*-L-rhamnopyranosyl-(1 → 4)-*β*-D-glucopyranosyl-(1 → 6)-*β*-D-glucopyranosyl ester (**8**) [23], bafouoside B (**9**) [3], bafouoside A (**10**) [3], cirenshenoside S (**11**) [24], pulsatilloside C (**12**) [25], cussonoside A (**13**) [2], echinocystic acid *α*-L-rhamnopyranosyl-(1 → 4)-*β*-D-glucopyranosyl-(1 → 6)-*β*-D-glucopyranosyl ester (**14**) [21] and 3*β*,23-dihydroxyolean-12-en-28-oic acid *β*-D-glucopyranosyl ester (**15**) [26]. Their structures were determined using MS, 1D and 2D NMR experiments (^{1}H,^{1}H COSY, TOCSY, ROESY, HSQC, HMBC) and chemical methods.

3.1. Structure elucidation

Compound **1** was isolated as a white amorphous powder. The molecular formula $C_{54}H_{88}O_{22}$ was deduced from its HRESIMS spectrum which showed a pseudo molecular ion peak at *m/z* 1111.5371 $[M+Na]^+$ (calcd for $C_{54}H_{88}O_{22}Na$, 1111.5665). On the basis of the ^{1}H and ^{13}C NMR spectral data (Table 1), compound **1** was identified as an urs-12-ene type pentacyclic triterpene saponin, and this was further confirmed by comparison of its NMR data with those of known urs-12-ene derivatives [27].

The aglycone of **1** was identified as 3*β*-hydroxyurs-12-en-28-oic acid and all the ^{1}H and ^{13}C NMR spectral data were in good agreement with literature values [28]. Signals of four anomeric protons at *δ* 4.94 (d, *J* = 7.8, H-1′), 6.16 (d, *J* = 8.2, H-1″), 4.97 (d, *J* = 7.6, H-1‴) and 5.79 (brs,

H-1‴') were also observed, giving HSQC correlations with four anomeric carbons at δ 106.5, 95.1, 104.3 and 102.3, respectively. The identification of protons belonging to each sugar unit was achieved *via* TOCSY experiment and their assignment to the respective carbon atoms was deduced from $^{1}H,^{1}H$ COSY and HSQC experiments starting from anomeric protons. The analysis of the chemical shifts of the sugar part allowed the identification of three glucopyranosyl (Glc) and one rhamnopyranosyl (Rha) units. The β anomeric configuration of the glucopyranosyl units was deduced from their coupling constants ranged between 7 and 8 Hz, while the α configuration was assigned to the rhamnopyranosyl unit because its anomeric proton appeared as a broad singlet. Extensive survey of bidesmosidic pentacyclic triterpenoid saponins from Araliaceae species showed that, the sugar chains are preferably attached at C-3 and C-28 [4,6,7,28–30]. In the case of triterpenoid saponins having betulinic, oleanolic or ursolic acid as aglycone, C-3 of the aglycone resonates at about 88.8 ppm [28,31,32]. The downfield shift observed for C-3 (δ 88.8) and the upfield shift for C-28 (δ 176.2) (Table 1) reflected the bidesmosidic nature of compound **1**. Furthermore, the HMBC correlations observed between the anomeric protons at δ 4.94 (H-1′) and 6.16 (H-1″) and carbons at δ 88.8 (C-3) and 176.2 (C-28), respectively evidenced the linkage positions of the sugar chains. The interglycosidic linkage sites of the sugar chain at C-28 were deduced from additional HMBC correlations observed between the anomeric protons at δ 4.97 (H-1‴) and 5.79 (H-1‴'), and carbons at δ

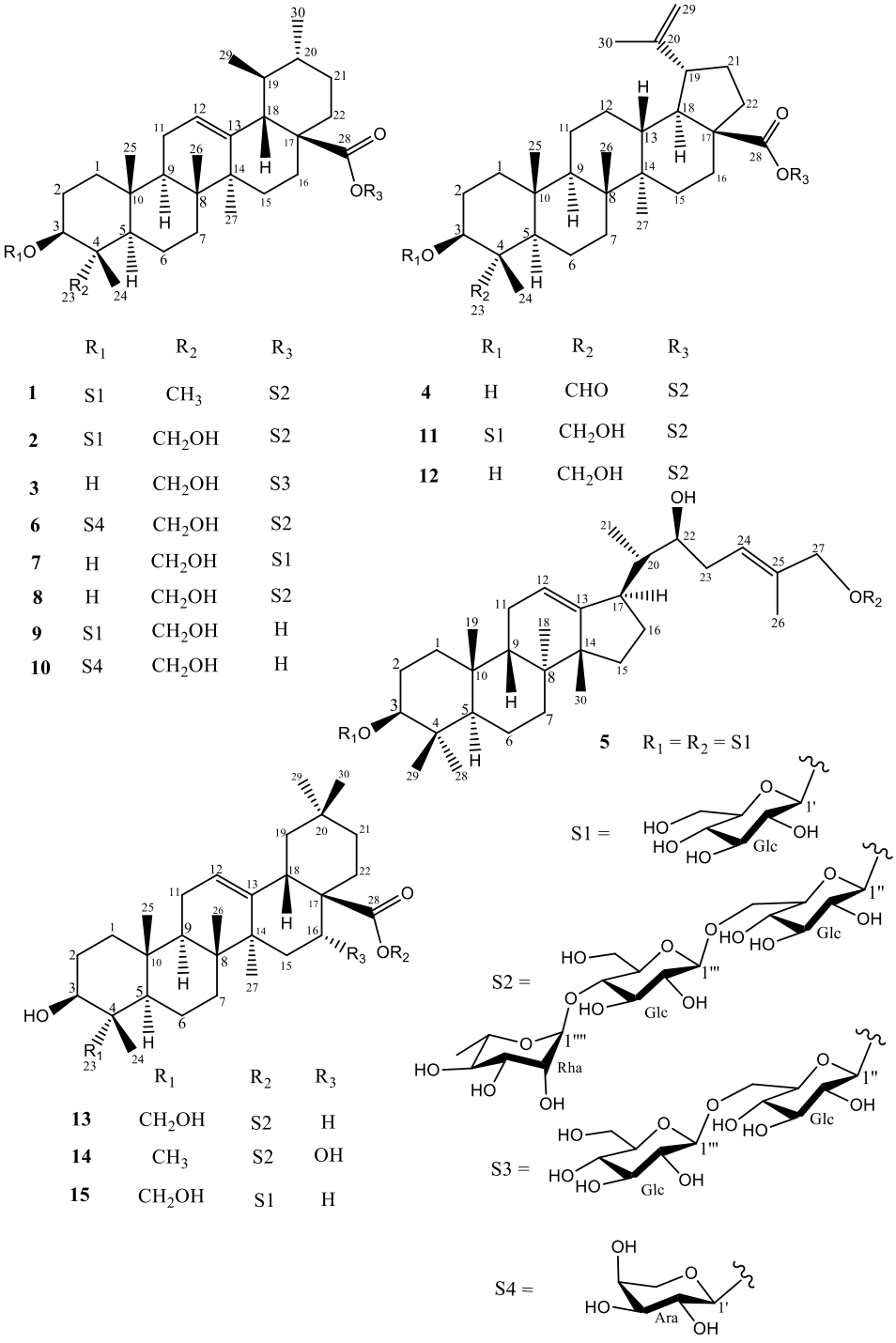

Fig. 1. Structures of compounds **1**–**15**.

68.7 (C-6″) and δ 78.1 (C-4‴), respectively. This sugar chain is commonly found in triterpenoid saponins from Araliaceae species [23,28,33]. The remaining glucopyranosyl unit was then attached to C-3 of the aglycone. The absolute configuration was determined to be D for glucose and L for rhamnose by GC analysis (see experimental). Thus, the structure of compound **1** was elucidated as 3β-[(β-D-glucopyranosyl) oxy]urs-12-en-28-oic acid α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester, a new triterpenoid saponin trivially named mannioside B.

Compound **2** was obtained as a white amorphous powder. Its HRESIMS showed the ion cluster at *m/z* 1127.5289 $[M+Na]^+$ (calcd for $C_{54}H_{88}O_{23}Na$, 1127.5614). Compared to **1**, compound **2** has an additional oxygen atom. Both ^1H and ^{13}C NMR data (Table 1) revealed compound **2** to be an oxidized form of **1** in which one of the methyl groups was converted to a hydroxymethylene. The upfield shift observed for C-3 (δ 82.2) in **2** with respect to **1** (δ 88.8) in addition to the HMBC cross peak correlation observed between H_3C-24 (δ 0.95, s) and the hydroxymethylene group signal at δ 64.1 as well as the C-3 (δ 82.2) chemical shift confirmed the hypothesis and ascertained the position of the hydroxymethylene at C-23. Thus the structure of compound **2** was established as 3β-[(β-D-glucopyranosyl)23-dioxy]urs-12-en-28-oic acid α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester, a new triterpenoid saponin trivially named mannioside C. Compound **2** is an ursane-type isomer of tanguticoside A (oleanane-type) previously isolated from *Clematis tangutica* (Ranunculaceae) [34].

Compound **3** was isolated as a white amorphous powder. Its HRESIMS showed the pseudomolecular ion at *m/z* 819.4386 $[M+Na]^+$ (calcd for $C_{42}H_{68}O_{14}Na$, 819.4507). The difference of 308.0903 a.m.u observed between **2** and **3** suggested the loss of two sugars units, including one hexose and one deoxyhexose from **2**. The above suggestion was further confirmed by ^1H and ^{13}C NMR spectral data, showing compounds **2** and **3** to have the same aglycone. Nevertheless, the main difference appeared in the sugar parts where compound **3** displayed signals of only two anomeric protons at δ 6.17 (d, J = 8.2, H-1′) and 5.01 (d, J = 7.8, H-1″) giving HSQC correlations with two anomeric carbons at 95.3 and 104.7, respectively. Full inspection of the sugar part allowed to identify two β-glucopyranosyl units. The HMBC correlations observed from the anomeric protons at δ 6.17 (H-1′) and 5.01 (H-1″), to carbons at δ 176.3 (C-28) and 69.0 (C-6′) allowed to attach the sugar chain as shown in Fig. 1. The structure of compound **3** was then established as 3β,23-dihydroxyurs-12-en-28-oic acid β-D-glucopyranosyl-(1 → 6)- β-D-glucopyranosyl ester, a new monodesmosidic triterpenoid saponin trivially named mannioside D.

Compound **4** was obtained as a white powder and its HRESIMS exhibited a pseudomolecular ion peak at *m/z* 963.4726 $[M+Na]^+$, corresponding to the molecular formula $C_{48}H_{76}O_{18}Na$ (calcd for $C_{48}H_{76}O_{18}Na$, 963.4929). Taken together the ^1H and ^{13}C NMR data (Table 2), compound **4** was deduced to be a lupane-type triterpenoid glycoside [1,25,29,35]. Based on the 1D and 2D NMR data, the aglycone of **4** was identified as 3β-hydroxy-23-oxolup-20(29)-en-28-oic acid [35]. The β orientation of the hydroxy group at C-3 was firstly suggested by the upfield shifts observed for C-3 (δ 71.5) and C-24 (δ 9.2) with respect to the α orientation in which C-3 and C-24 resonates at about δ 73.0 and 15.0 [35,36], respectively. The above suggestion was further confirmed by the ROESY spectrum through the cross peak correlation observed between H-3 and H-5, since H-5 is biogenetically α oriented in pentacyclic triterpenoids (Fig. 2). The sugar sequence was established as α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl, commonly found in triterpenoid saponins from Araliaceae species [23,28,33] and its location at C-28 of the aglycone was deduced from the HMBC correlation between H-1′ and C-28. The structure of **4** was then determined as 3β-hydroxy-23-oxolup-20(29)-en-28-oic acid α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl ester, a new monodesmosidic triterpenoid saponin trivially named mannioside E. However, an epimer of **4** at C-3 trivially named wujiapioside A was previously reported from *Acanthopanax gracilistylus*

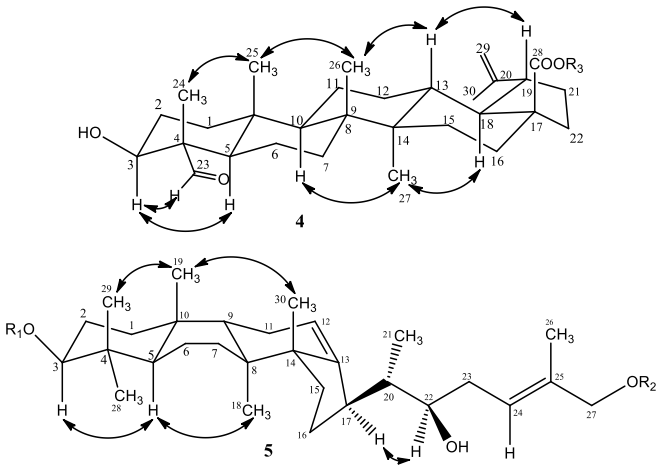

Fig. 2. Important ROESY correlations of the aglycones of **4** and **5**.

[35] and *Acanthopanax koreanum* [37]. Important ROESY correlations of the aglycone of **4** are shown in Fig. 2.

Compound **5** was isolated as a white amorphous powder. Its molecular formula $C_{42}H_{70}O_{13}$ was deduced from its HRESIMS spectrum which exhibited a pseudo molecular ion peak at *m/z* 805.4671 $[M+Na]^+$ (calcd for $C_{42}H_{70}O_{13}Na$, 805.4714). On the basis of ^1H and ^{13}C NMR spectral data (Table 2), compound **5** was identified as a protostane type-triterpenoid. Its ^1H NMR spectrum showed six tertiary methyl groups at δ 0.74, 0.92, 1.07, 1.12, 1.30 and 2.01, one secondary methyl group at δ 1.22 (d, J = 6.7), two anomeric protons at δ 4.98 (d, J = 7.8, H-1′) and 4.92 (d, J = 7.8, H-1″) attributable to two sugar units based on cross peak correlations observed on the HSQC spectrum. Two olefinic proton signal were depicted at δ 5.30 (br s, H-12) and 5.88 (t, J = 7.3, H-24). The ^{13}C NMR data of the aglycone of compound **5** include four olefinic carbon atoms at δ 118.3 (C-12), 145.8 (C-13), 128.9 (C-24) and 133.2 (C-25), a hydroxymethylene carbon signal at δ 67.6 (C-27) as well as two hydroxymethines at δ 89.1 (C-3) and δ 72.8 (C-22). The HSQC correlations observed between the anomeric protons at δ 4.98 and 4.92 and carbons at δ 106.4 and 103.0, respectively confirmed the presence of two sugar units. Based on 1D and 2D NMR data of **5** in comparison with those of related compounds [38], the aglycone part was established as a protostane type-triterpenoid. The positions of the olefinic carbons were obtained through HMBC correlations observed from the olefinic methyl group at δ 2.01 (Me-26) to C-24 (δ 128.9), C-25 (δ 133.2) and C-27 (δ 67.6) and from Me-30 (δ 1.07) to C-14 (δ 145.8) while those of the hydroxymethines were deduced from cross peak correlations observed from Me-28 (δ 1.30) and 29 (δ 1.12) to C-3 (δ 89.1), and from Me-21 (δ 1.22) to C-22 (δ 72.8). The C-3, C-8 and C-14 configurations were evidenced by the ROESY spectrum in which correlations were observed between Me-19 (δ 0.74) which is biogenetically β oriented and Me-30 (δ 1.07), H-5 (α oriented) with H-3 and Me-18 (Fig. 2). The stereochemistry at C-22 was determined *via* the ROESY correlation depicted between H-17, Me-21 (which are biogenetically α oriented) and H-22. From the above information, the aglycone was recognized to be (22S)-protosta-12, 24-diene-3β,22,27-triol. The ring proton of each monosaccharide residue was assigned starting from the readily identifiable anomeric protons using ^1H,^1H COSY, HSQC and TOCSY spectra. Extensive analysis of ^1H,^1H coupling constants as well as 2D experiments followed by comparison of ^1H and ^{13}C NMR data (Table 2) with those reported in the literature revealed the presence of β-glucopyranosyl moieties. The absolute configuration was determined to be D by GC analysis. The linkage sites of the sugars were determined by HMBC correlations observed between the anomeric protons at δ 4.98 (H-1′), 4.92 (H-1″) and at δ 89.1 (C-3), 67.6 (C-27), respectively. Thus, the structure of **5** was elucidated as (22S)-27β-[(β-D-glucopyranosyl)oxy]-22-hydroxyprotosta-12,24-dien-3β-yl β-D-glucopyranoside, a new bidesmosidic triterpenoid saponin trivially

named mannioside F.

The isolation of triterpenoid saponins from this plant is in good agreement with results previously obtained from *Schefflera* species with the prevalence of triterpenoid saponins having the sugar sequence α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl at C-28 [4,10,12]. Protostane type triterpenoids were isolated from plants belonging to the genera *Alisma* (Alismataceae) [39–42] and *Garcinia* (Guttiferae) [38]. To the best of our knowledge, this is the first report on the isolation of a protostane type triterpenoid from a plant of the Araliaceae family. However, their stereoisomers (Dammarane) were previously isolated from *Panax* species (Araliaceae) [43–45].

3.2. Biological activity

Given the fact that saponins from Araliaceae species have been shown to be antiproliferative against human malignant epithelial cells (HeLa) [46,47], the major isolated compounds were tested for their antiproliferative activity on HeLa cells. All the compounds were not efficient at the concentration of 33 mM. These observations are in good agreement with other screening results for *anti*-inflammatory, cytotoxic, antimicrobial and molluscicidal activities of triterpenoid derivatives which established that the presence of sugar(s) bound to the 28-COOH group considerably decreases their activity [48–51] when sugar chains attached at C-3 and having at least three sugar units increase the anti-proliferative activity of saponins [52].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.carres.2021.108279.

References

[1] N. De Tommasi, C. Pizz, A. Bellino, P. Venturella, Triterpenoids saponins from *Schefflera divaricata*, J. Nat. Prod. 60 (1997) 663–668, https://doi.org/10.1021/np9605807.

[2] M.-A. Lacaille-Dubois, M. Ilyas, H. Wagner, Cussonosides A and B, two triterpene-saponins from *Cussonia barteri*, Planta Med. 52 (1986) 80–83, https://doi.org/10.1055/s-2007-969084.

[3] L.A. Tapondjou, D. Lontsi, B.L. Sondengam, F. Shaheen, M.I. Choudhary, A.-U. Rahman, F.R. Heerden, H.-J. Park, K. Lee, Saponins from *Cussonia bancoensis* and their inhibitory effects on nitric oxide production, J. Nat. Prod. 66 (2003) 1266–1269, https://doi.org/10.1021/np020412g.

[4] L.A. Tapondjou, A.C. Mitaine-Offer, T. Miyamoto, H. Lerche, J.F. Mirjolet, G. N. Nicolas, M.-A. Lacaille-Dubois, Triterpene saponins from *Schefflera abyssinica*, Biochem. Systemat. Ecol. 34 (2006) 887–889, https://doi.org/10.1016/j.bse.2006.05.018.

[5] A.-C. Mitaine-Offer, L.A. Tapondjou, D. Lontsi, B.L. Sondengam, M.I. Choudharyd, A.-U. Rahman, M.-A. Lacaille-Dubois, Constituents isolated from *Polyscias fulva*, Biochem. Systemat. Ecol. 32 (2004) 607–610, https://doi.org/10.1016/j.bse.2003.10.008.

[6] G.B. Kougan, T. Miyamoto, J.F. Mirjolet, O. Duchamp, B.L. Sondengam, M.-A. Lacaille-Dubois, Arboreasides A-E, triterpene saponins from the bark of *Cussonia arborea*, J. Nat. Prod. 72 (2009) 1081–1086, https://doi.org/10.1021/np8008094.

[7] B.K. Ponou, R.N. Nono, R.B. Teponno, A.L. Tapondjou, M.-A. Lacaille-Dubois, L. Quassinti, M. Bramucci, L. Barboni, Bafouoside C, a new triterpenoid saponin from the roots of *Cussonia bancoensis* Aubrev. & Pellegr, Phytochem. Lett. 10 (2014) 255–259, https://doi.org/10.1016/j.phytol.2014.10.007.

[8] S.G. Sparg, M.E. Light, J.V. Staden, Biological activities and distribution of plant saponins, J. Ethnopharmacol. 94 (2004) 219–243, https://doi.org/10.1016/j.jep.2004.05.016.

[9] G.M. Plunkett, P.P. Lowry II, D.G. Frodin, J. Wen, Phylogeny and geography of *Schefflera*: pervasive polyphyly in the largest genus of araliaceae, Ann. Mo. Bot. Gard. 92 (2005) 202–204. https://www.jstor.org/stable/3298514.

[10] C. Maeda, K. Ohtani, R. Kasai, K. Yamasaki, M.N. Due, T.N. Nhamt, Q.K.N. Cu, Oleanane and ursane glycosides from Schefflera octophylla, Phytochemistry 37 (1994) 1131–1137, https://doi.org/10.1016/S0031-9422(00)89543-6.

[11] L. Chen, Q.H. Zhu, R.C. Lin, Chemical constituents and biological activity of *Schefflera* plant, J. Chin. Med. Mater. 25 (2002) 363–366.

[12] C. Wu, Y.-H. Duan, M.-M. Li, W. Tang, X. Wu, G.-C. Wang, W.-C. Ye, G.-X. Zhou, Y.-L. Li, Triterpenoid saponins from the stem barks of *Schefflera heptaphylla*, Planta Med. 79 (2013) 1348–1355, https://doi.org/10.1055/s-0033-1350674.

[13] M. Cheek, Schefflera mannii. The IUCN red list of threatened species, e. T45425A10997220, https://www.iucnredlist.org/species/45425/10997220, 2004. (Accessed 17 July 2020).

[14] L.A. Tapondjou, L.B.T. Nyaa, P. Tane, M. Ricciutelli, L. Quassinti, M. Bramucci, G. Lupidi, B.K. Ponou, L. Barboni, A.-C. Parkioside, Cytotoxic and antioxidant triterpene saponins from Butyrospermum parkii (Sapotaceae), Carbohydr. Res. 346 (2011) 2699–2704, https://doi.org/10.1016/j.carres.2011.09.014.

[15] R.T. Fouedjou, R.B. Teponno, L. Quassinti, M. Bramucci, D. Petrelli, L.A. Vitali, D. Fiorini, L.A. Tapondjou, L. Barboni, Steroidal saponins from the leaves of Cordyline fruticosa (L.) A. Chev. and their cytotoxic and antimicrobial activity, Phytochem. Lett. 7 (2014) 62–68, https://doi.org/10.1016/j.phytol.2013.10.001.

[16] R.B. Teponno, C. Tanaka, B. Jie, L.A. Tapondjou, T. Miyamoto, A.-J. Trifasciatosides, Steroidal saponins from *Sansevieria trifasciata*, Chem. Pharm. Bull. 64 (2016) 1347–1355, https://doi.org/10.1248/cpb.c16-00337.

[17] R.B. Teponno, J.P. Dzoyem, N.N. Raymond, U. Kauhl, L.P. Sandjo, L.A. Tapondjou, U. Bakowsky, T. Opatz, Cytotoxicity of secondary metabolites from *Dracaena viridiflora* Engl & Krause and their semisynthetic analogues, Record Nat. Prod. 11 (2017) 421–430, https://doi.org/10.25135/rnp.54.17.03.050.

[18] B.K. Ponou, L. Barboni, R.B. Teponno, M. Mbiantcha, T.B. Nguelefack, H.-J. Park, K.T. Lee, L.A. Tapondjou, Polyhydroxyoleanane-type triterpenoids from *Combretum molle* and their anti-inflammatory activity, Phytochem. Lett. 1 (2008) 183–187, https://doi.org/10.1016/j.phytol.2008.09.002.

[19] B.K. Ponou, R.B. Teponno, M. Ricciutelli, L. Quassinti, M. Bramucci, G. Lupidi, L. Barboni, L.A. Tapondjou, Dimeric antioxidant and cytotoxic triterpenoid saponins from *Terminalia ivorensis*, A. Chev. Phytochemistry 71 (2010) 2108–2115, https://doi.org/10.1016/j.phytochem.2010.08.020.

[20] B.K. Ponou, R.B. Teponno, L.A. Tapondjou, M.-A. Lacaille-Dubois, L. Quassinti, M. Bramucci, L. Barboni, Steroidal saponins from the aerial parts of *Cordyline fruticosa* L. var. *strawberries*, Fitoterapia 134 (2019) 454–458, https://doi.org/10.1016/j.fitote.2019.03.019.

[21] Y.-G. Xia, G.-Y. Li, J. Liang, C.A. Ortori, B.-Y. Yang, H.-X. Kuang, D.A. Barrett, A strategy for characterization of triterpene saponins in *Caulophyllum robustum* hairy roots by liquid chromatography with electrospray ionization quadrupole time-of-flight mass spectrometry, J. Pharmaceut. Biomed. 100 (2014) 109–122, https://doi.org/10.1016/j.jpba.2014.07.024.

[22] H. Yang, E.J. Jeong, J. Kim, S.H. Sung, Y.C. Kim, Antiproliferative triterpenes from the leaves and twigs of *Juglans sinensis* on HSC-T6 cells, J. Nat. Prod. 74 (2011) 751–756, https://doi.org/10.1021/np1008202.

[23] I.I. Dovgii, V.I. Grishkovets, V.V. Kachala, A.S. Shashkov, Tripterpene glycosides A, B1, B2, C, D, G2, H1 and H2 from leaves of *Cussonia paniculata*, Chem. Nat. Compd. 41 (2005) 200–204, https://doi.org/10.1007/s10600-005-0111-1.

[24] G. Wang, J. Xu, Structures of four new triterpenoid saponins from the leaves of Oplopanax elatus Nakai, VII. Studies in, Plant Sci. 6 (1999) 52–57, https://doi.org/10.1016/S0928-3420(99)80008-1.

[25] W. Ye, A. He, S. Zhao, C.-T. Che, Pulsatilloside C from the roots of *Pulsatilla chinensis*, J. Nat. Prod. 61 (1998) 658–659, https://doi.org/10.1021/np9704821.

[26] D.A. Panov, V.I. Grishkovets, V.V. Kachala, A.S. Shashkov, Triterpene glycosides from *Kalopanax septemlobum*. VII. Minor glycosides from stems of *Kalopanax septemlobum* Var. *maximowiczii* and *Kalopanax septemlobum* Var. *typicum*, Chem. Nat. Compd. 42 (2006) 61–66, https://doi.org/10.1007/s10600-006-0036-3.

[27] S.B. Mahato, A.P. Kundu, ^{13}C-NMR spectra of pentacyclic triterpenoids. A compilation and some salient features, Phytochemistry 37 (1994) 1517–1575, https://doi.org/10.1016/S0031-9422(00)89569-2.

[28] L. Harinantenaina, R. Kasai, K. Yamasaki, Cussosaponins A-E, triterpene saponins from the leaves of *Cussonia racemosa*, a Malagasy endemic plant, Chem. Pharm. Bull. 50 (2002) 1290–1293, https://doi.org/10.1248/cpb.50.1290.

[29] C.-S. Yook, X.-Q. Liu, S.-Y. Chang, S.-Y. Park, T. Nohara, Lupane-triterpene glycosides from the leaves of *Acanthopanax gracilistylus*, Chem. Pharm. Bull. 50 (2002) 1383–1385, https://doi.org/10.1248/cpb.50.1383.

[30] F.R. Melek, T. Miyase, S.M.A. Khalik, M.R. El-Gindi, Triterpenoid saponins from *Schefflera arboricola*, Phytochemistry 63 (2003) 401–407, https://doi.org/10.1016/S0031-9422(03)00117-1.

[31] V.D. Huan, S. Yamamura, K. Ohtani, R. Kasai, K. Yamasaki, N.T. Nham, H.M. Chau, Oleanane saponins from *Polyscias fruticosa*, Phytochemistry 47 (1998) 451–457, https://doi.org/10.1016/S0031-9422(97)00618-3.

[32] T. Nguyen, T. Nguyen, H. Nguyen, S. Nguyen, P. Nguyen, Oleanane saponins from *Polyscias guilfoylei* BAIL. (Araliaceae), Sci. Technol. Dev. J. 12 (2009) 21–28, https://doi.org/10.32508/stdj.v12i10.2295.

[33] V. Mshvildadze, R. Elias, R. Faure, L. Derbrauwer, G. Dekanosidze, E. Kemertelidze, G. Balansard, Triterpenoids saponins from berries of *Hedera colchica*, Chem. Pharm. Bull. 49 (2001) 752–754, https://doi.org/10.1248/cpb.49.752.

[34] H.M. Zhong, C.X. Chen, X. Tian, Y.X. Chui, Y.Z. Chen, Triterpenoid saponins from *Clematis tangutica*, Planta Med. 67 (2001) 484–488, https://doi.org/10.1055/s-2001-15803.
[35] X.-Q. Liu, S.-Y. Chang, S.-Y. Park, T. Nohara, C.-S. Yook, A new lupane-triterpene glycoside from the leaves of *Acanthopanax gracilistylus*, Arch Pharm. Res. (Seoul) 25 (2002) 831–836, https://doi.org/10.1007/BF02977000.
[36] H.-S. Choi, H.J. Kim, S.-G. Nam, I.-S. Kim, K.-T. Lee, C.-S. Yook, Y.S. Lee, Lupane glycosides from the leaves of A*canthopanax koreanum*, Chem. Pharm. Bull. 56 (2008) 1613–1616, https://doi.org/10.1248/cpb.56.1613.
[37] L.D. Dat, N.P. Thao, B.T.T. Luyen, B.H. Tai, M.H. Woo, Z. Manzoor, I. Ali, Y.S. Koh, Y.H. Kim, A new saponin from *Acanthopanax koreanum* with anti-inflammatory activity, Arch Pharm. Res. (Seoul) 40 (2017) 311–317, https://doi.org/10.1007/s12272-016-0879-5.
[38] V. Rukachaisirikul, P. Pailee, A. Hiranrat, P. Tuchinda, C. Yoosook, J. Kasisit, W. C. Taylor, V. Reutrakul, Anti-HIV-1 triterpenes and digeranylbenzophenone from trunk bark and stems of *Garcinia speciosa*, Planta Med. 69 (2003) 1141–1146, https://doi.org/10.1055/s-2003-818006.
[39] Z.-P. Mai, K. Zhou, G.-B. Ge, C. Wang, X.-K. Huo, P.-P. Dong, S. Deng, B.-J. Zhang, H.-L. Zhang, S.-S. Huang, X.-C. Ma, Protostane triterpenoids from the rhizome of *Alisma orientale* exhibit inhibitory effects on human carboxylesterase 2, J. Nat. Prod. 78 (2015) 2372–2380, https://doi.org/10.1021/acs.jnatprod.5b00321.
[40] C. Wang, X.-K. Huo, Z.-L. Luan, F. Cao, X.-G. Tian, X.-Y. Zhao, C.-P. Sun, L. Feng, J. Ning, B.-J. Zhang, X.-C. Ma, Alismanin A, a triterpenoid with a C34 skeleton from *Alisma orientale* as a natural agonist of human pregnane X receptor, Org. Lett. 19 (2017) 5645–5648, https://doi.org/10.1021/acs.orglett.7b02738.
[41] H.-M. Li, D. Liu, W.-F. Dai, X.-Q. Chen, R.-T. Li, A new protostane-type triterpenoid from *Alisma plantago-aquatica* subsp. *orientale* (Sam.) Sam, Nat. Prod. Res. 33 (2018) 3083–3088, https://doi.org/10.1080/14786419.2018.1519710.
[42] Q. Jin, J. Zhang, J. Hou, M. Lei, C. Liu, X. Wang, Y. Huang, S. Yao, B.Y. Hwang, W. Wu, D. Guo, Novel C-17 spirost protostane-type triterpenoids from *Alisma plantago-aquatica* with antiinflammatory activity in Caco-2 cells, Acta Pharm. Sin. B. 9 (2019) 809–818, https://doi.org/10.1016/j.apsb.2019.04.002.
[43] H. Zhao, L. Shi, J.Q. Cao, W. Li, X. Wen, Y.Q. Zhao, A new triterpene saponin from P*anax japonicus* C. A. Meyer var m*ajor* (Burk.) C. Y. Wu et K. M. Feng,, Chin. Chem. Lett. 21 (2010) 1216–1228, https://doi.org/10.1016/j.cclet.2010.06.010.
[44] T.-L. Tran, Y.-R. Kim, J.-L. Yang, D.-R. Oh, T.-T. Dao, W.-K. Oh, Dammarane triterpenes from the leaves of *Panax ginseng* enhance cellular immunity, Bioorg. Med. Chem. 22 (2014) 499–504, https://doi.org/10.1016/j.bmc.2013.11.002.
[45] Y. Cen, X. Fa-Xiang, H. Xiao-Jun, L. Shao-Ping, Z. Qing-Wen, A novel 12, 23-epoxy dammarane saponin from *Panax notoginseng*, Chin. J. Nat. Med. 13 (2015) 303–306, https://doi.org/10.1016/S1875-5364(15)30019-4.
[46] M. Takaya, M. Nomura, T. Takahashi, Y. Kondo, K.-T. Lee, S. Kobayashi, 23-hydroxyursolic acid causes cell growth-inhibition by inducing caspase-dependent apoptosis in human cervical squamous carcinoma HeLa cells, Anticancer Res. 29 (2009) 995–1000.
[47] J. Quetin-Leclercq, R. Elias, G. Balansard, R. Bassleer, L. Angenot, Cytotoxic activity of some triterpenoid saponins, Planta Med. 58 (1992) 279–281, https://doi.org/10.1055/s-2006-961456.
[48] D. Timon, P.J. Julien, M. Gasquet, G. Balansard, P. Bernard, Research of antifungal from several active principe extracts from climbing-ivy: *Hedera helix*, Ann. Pharm. Fr. 38 (1980) 545–552.
[49] K. Hostettmann, H. Kizu, T. Tominori, Molluscicidal properties of various saponins, Planta Med. 44 (1982) 34–35, https://doi.org/10.1055/s-2007-971396.
[50] Y.K. Kim, R.G. Kim, S.J. Park, J.H. Ha, J.W. Choi, H.J. Park, K.T. Lee, In vitro anti-inflammatory activity of Kalopanaxsaponin A isolated from *Kalopanax pictus* in murine macrophage raw 264.7 cells, Biol. Pharm. Bull. 25 (2002) 472–476, https://doi.org/10.1248/bpb.25.472.
[51] K.T. Lee, I.C. Sohn, H.J. Park, D.W.W. Kim, G.O. Jung, K.Y. Park, Essential moiety for antimutagenic and cytotoxic activity of hederagenin monodesmosides and bidesmosides isolated from the stem bark of *Kolopanax pictus*, Planta Med. 66 (2000) 329–332, https://doi.org/10.1055/s-2000-8539.
[52] L.A. Tapondjou, K.J. Siems, S. Böttger, M.F. Melzig, Steroidal saponins from the mesocarp of the fruits of Raph*ia Farinifera (*Arecaceae) and their cytotoxic activity, Nat. Prod. Commun. 10 (2015) 1941–1944, https://doi.org/10.1177/1934578X1501001134.