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Triterpene glycosides from the aerial parts of *Gouania longipetala*

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Abstract

Six previously undescribed triterpenoid saponins, gouaniaside I-VI, were isolated from the aerial parts of *Gouania longipetala* Hemsl. (Rhamnaceae), in addition to four known triterpenes. The structure elucidation of these compounds was based on analyses of spectroscopic data including 1D- and 2D-NMR and HR-ESI-MS techniques. The inhibitory activity of isolated compounds against promyelocytic leukemia HL60 and human erythromyeloblastoid leukemia K562 cell lines was evaluated and jujuboside I exhibited moderate cytotoxicity, with IC₅₀ values of 13.5 and 21.0 μM, respectively. Among the isolated triterpenes, alphitolic acid exhibited moderate antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli* (MICs 32, 64 and 128 μg/mL, respectively).

Keywords: *Gouania longipetala*; Rhamnaceae; triterpenoids; antibacterial activity; cytotoxic activity.

1. Introduction

Gouania longipetala Hemsl. (Rhamnaceae) is a scandent shrub mainly present in closed-forests and jungle regrowths (Buerki et al., 2011, Burkill et al., 1985). In traditional medicine, decoction or maceration of *G. longipetala* stem bark in local alcoholic drink is used to treat gastro-intestinal problems and gynecological complaints related to gestation and post-delivery hemorrhages (Ezeja et al., 2014). The stem bark extract has been shown to possess antibacterial, anti-inflammatory and antioxidant activities (Ekuadzi et al., 2012, Ezeja et al., 2014). We previously reported the isolation and structural elucidation of two new acylated flavonol glycosides and seven known flavonoids from the methanol extract of *G. longipetala* (Gossan et al., 2015). We continued our investigation of the constituents of the aerial parts of this plant, and isolated six previously undescribed triterpenoid saponins (**1-6**), together with four known compounds (**7-10**) from the MeOH and EtOAc extracts. Compounds **1-10** were evaluated for their inhibitory activity against promyelocytic leukemia HL60 and human erythromyeloblastoid leukemia K562 cells, as well as their antibacterial activity against *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli*.

2. Results and discussion

The ethyl acetate and methanol extracts of the aerial parts of *Gouania longipetala* Hemsl. (Rhamnaceae) were fractionated and purified separately by combination of chromatographic methods to obtain seven triterpenoid glycosides (**1-7**) from the methanol extract and three triterpenes (**8-10**) from the ethyl acetate extract (Figure 1).

The known compounds were elucidated as jujuboside I (**7**) (Wu et al., 2013), terminolic acid (**8**) (Jossang et al., 1996), alphitolic acid (**9**) (Kuang et al., 1989, Suksamaran et al., 2006) and gouanic acid B (**10**) (Giacomelli et al., 2007) (Figure 1). Their structural assignments were made by HR-ESI-MS, 1D-, and 2D-NMR analysis. Their spectroscopic data were in perfect agreement with those reported in the literature. In order to determine the sugar composition in these compounds, acid hydrolysis of a part of the saponin mixture generated four sugar units in the aqueous layer, identified after purification and measurement of their optical rotation as D-glucose, D-galactose, L-arabinose and L-rhamnose.

Gouaniaside I (**1**) was obtained as a white amorphous powder. The positive HR-ESI-MS showed a molecular ion peak at m/z 789.4409 [M + Na]⁺ (calcd for C₄₁H₆₆O₁₃Na, 789.4401), corresponding to the molecular formula C₄₁H₆₆O₁₃. The ¹H NMR spectrum of the aglycone part displayed signals of a dammarane triterpenoid characterized by seven tertiary methyl groups at δ_H 0.87, 0.89, 1.04, 1.15, 1.16, 1.71, and 1.74, three oxygenated methines at δ_H 3.13 (dd, $J = 12.1, 4.8$ Hz, H-3), 3.68 (dd, $J = 12.7, 3.4$ Hz, H-7), 4.70 (ddd, $J = 11.2, 8.6, 1.5$ Hz, H-23), an oxygenated methylene at δ_H 4.02 and 4.06 (each d, $J = 7.0$ Hz, H₂-18), and a vinyl proton at δ_H 5.18 (dt, $J = 8.6, 1.5$ Hz, H-24). Its ¹³C NMR spectrum exhibited 30 signals including seven methyl groups [δ_C 13.4 (C-30), 16.5 (C-19), 16.8 (C-29), 18.4 (C-27), 25.8 (C-26), 28.5 (C-28), and 29.7 (C-21)], three oxymethine carbons [δ_C 69.6, 76.0 and 90.1], an oxymethylene carbon [δ_C 66.9], two olefinic carbons [δ_C 126.3 (C-24), and 136.8 (C-25)], an acetal carbon [δ_C 111.2] and a quaternary oxygenated carbon [δ_C 69.4] (Table 1).

These data indicated that the aglycone of **1** was similar to the aglycone of jujuboside I (**7**) and suggested that **1** was a jujubogenin derivative (Wu et al., 2013). The only variation concerned the signal for C-7 (δ_C 36.8) in **7** which was replaced by an oxygenated methine at δ_C 76.0 in **1**. The position of the hydroxyl group at C-7 (B ring) was confirmed by the HMBC correlations observed between H₃-30 and C-7 and between H-7 and C-5. In the ROESY spectrum, correlations observed between H-3/H-5, H₃-28/H-5, H-5/H-9, H-5/H-7 and H-9/H-7 confirmed the α -axial orientation of these protons and the β orientation of the hydroxyl group at C-7. Similarly, the cross-peaks, observed on the ROESY spectrum, between H₃-29/H₃-19 and H₃-19/H₃-30 confirmed the β -axial orientation of these methyl groups. The aglycone structure of **1** was assigned as 7 β -hydroxy-jujubogenin on the basis of the ¹H and ¹³C NMR spectra and correlations observed in COSY, HSQC and HMBC experiments. Most of the ¹³C NMR signals were assigned through ²J_{H-C} and ³J_{H-C} couplings of the seven methyls and are in agreement with literature data except for the B ring (Table 1). The magnitude of the coupling constants between H-13 and H-17 ($J = 6.9$ Hz) and H-23 (β) and H-22 (β) ($J = 11.2$ Hz), and the ROESY correlations observed between H-17, H₃-21, and H-22 (α), between H-22 (β) and H-23, and between H-13/H-15 (β) and H-23/H-15 (β) are in agreement with the configuration of rings D-F of jujubogenin (Renault et al., 1997). The downfield shift of C-3 (δ_C 90.1) suggested a monodesmosidic saponin. Analysis of the ¹H and ¹³C NMR spectra of **1** revealed the presence of two anomeric protons at δ_H 4.55 and 5.12 correlated in the HSQC spectrum with the anomeric carbons at δ_C 104.9 and 102.0, respectively (Table 2). Starting from the anomeric proton at δ_H 4.55 (d, $J = 4.7$ Hz), the NMR signals belonging to the same system were assigned to a C-2 monosubstituted α -L-arabinopyranose (ara) (Table 2) by interpretation of COSY, HSQC and HMBC spectra and by comparing the ¹³C NMR chemical shifts with those of related systems reported in the literature (Alabdul Magid et al., 2015, Wu et al., 2013). The L-arabinopyranose unit was determined to be in an α -configuration on the basis of the J_{H1-H2} value (4.7 Hz) and this was confirmed by the observation of ROESY correlations between the α -axial protons H-1/H-3 and H-1/H-5. The second sugar unit with its anomeric proton at δ_H 5.12 (d, $J = 1.5$ Hz) was identified as a terminal α -L-rhamnopyranose (rha) with its methyl group at δ_{H6} 1.24 (d, $J = 6.2$ Hz) (Table 2) (Alabdul Magid et al., 2015). The observed small coupling constant ($J_{H1,H2}$) of the rhamnopyranose and the chemical shift of its C-5 (δ_C 70.2) indicated an α configuration (Backinowsky et al., 1980). The HMBC correlations observed between rha-H-1 and ara-C-2 indicated that the rhamnose residue was 2-*O*-linked to the arabinose unit, whereas the HMBC correlations between ara-H-1 and C-3 of aglycone showed that the diglycosidic chain [rha-(1→2)-ara-] was linked to the aglycone at C-3 position. Thus, the structure of **1** was established as 7 β -hydroxy-jujubogenin-3-*O*- α -L-rhamnopyranosyl-(1→2)- α -L-arabinopyranoside.

The molecular formula of gouaniaside II (**2**) was determined as C₄₁H₆₄O₁₂ by HR-ESI-MS. The ¹H NMR spectrum of the aglycone of **2** displayed signals for seven methyl groups at δ_H 0.83, 1.16, 1.75, 1.73, 1.04, 0.97, and 1.78 (all s, H₃-19, H₃-21, H₃-26, H₃-27, H₃-28, H₃-29 and H₃-30), an oxygenated methine at δ_H 3.15 (dd, $J = 11.8, 3.8$ Hz, H-3), an oxygenated methylene (δ_H 3.40 and 4.23 (each d, $J = 7.0$ Hz, H₂-18) and a vinyl proton at δ_H 5.20 (td, $J = 8.5, 1.5$ Hz, H-24). A comparison of the ¹³C NMR spectra of **2** with that of **7** revealed a similar aglycone. The main difference was the presence of signals of a second double bond (δ_C 128.3 and 130.2) (Table 1). Its NMR spectroscopic data differed from those of compound **7** only in ring B. The second double bond was placed at C-7/C-8 as deduced from the HMBC correlations between H-9/C-8, H-11/C-8 and H-13/C-8 and between H-5/C-7 and H-9/C-7. H₃-30 (δ_H 1.78, δ_C 20.7) was located at C-7 as confirmed by HMBC correlations from H₃-30 to C-5, C-6, C-7 and C-8. The relative configurations of methyl groups and the other asymmetric carbons were verified as in compound **1** by the ROESY correlations and were identical to those of jujubogenin. Thus, the aglycone of **2** was identified as 7-methyl,7,8-didehydro,30-*nor*-jujubogenin. Furthermore, the NMR data suggested that **2** possessed the same sugar moieties as **1** and the linkages of sugar moieties were also established by analysis of the HMBC correlations. Thus, compound **2** was concluded to be 7-methyl,7,8-didehydro,30-*nor*-jujubogenin-3-*O*- α -L-rhamnopyranosyl-(1→2)- α -L-arabinopyranoside.

The molecular formula of gouaniaside III (**3**) was established as C₄₇H₇₄O₁₇ (HR-ESI-MS m/z 933.4836 [M+Na]⁺). Comparison of the NMR data of compounds **2** and **3** indicated that they possessed the same aglycone but one supplementary sugar moiety in **3** (Tables 1 and 2). Analysis of COSY and HSQC spectra, allowed assignment of this supplementary monosaccharide as a terminal glucopyranose unit (glc), whose anomeric proton and carbon were assigned at δ_H 4.52 (d, $J = 7.7$ Hz) and δ_C 104.3. The deshielded signals of ara-C-3 (δ_C 82.2) indicated that the additional glucopyranose moiety was attached to C-3 of the arabinosyl unit. This was confirmed by the HMBC correlation between glc-H-1 (δ_H 4.52) and ara-C-3 (δ_C 82.2). Thus, the structure of **3** was elucidated as 7-methyl,7,8-didehydro,30-*nor*-jujubogenin-3-*O*- β -D-glucopyranosyl-(1→3)-[α -L-rhamnopyranosyl-(1→2)]- α -L-arabinopyranoside.

The positive HR-ESI-MS spectrum of gouaniaside IV (**4**) showed a pseudomolecular ion peak [M + Na]⁺ at m/z 975.4918 (C₄₉H₇₆O₁₈Na) suggesting the presence of a supplementary acetyl group (42 amu) compared to **3**. The ¹H and ¹³C NMR data of **4** were closely comparable to those of **3**, except for the signals of the glucose moiety and the presence of signals for an acetyl group (δ_H 2.08, δ_C 20.8, 172.7) (Table 2). The β -D-glucopyranose possessed two deshielded protons H₂-6 at δ_H 4.22 and 4.44, indicating the position of the acetyl group. This was readily confirmed by the HMBC correlation between glc-H₂-6 and the carbonyl signal (δ_C 172.7) of the acetyl group. Extensive elucidation

of its 1D and 2D NMR data led to the structure of **4** as 7-methyl,7,8-didehydro,30-nor-jujubogenin-3-O-(6-O-acetyl-

β -D-glucopyranosyl-(1 \rightarrow 3))- [α -L-rhamnopyranosyl-(1 \rightarrow 2)]- α -L-arabinopyranoside.

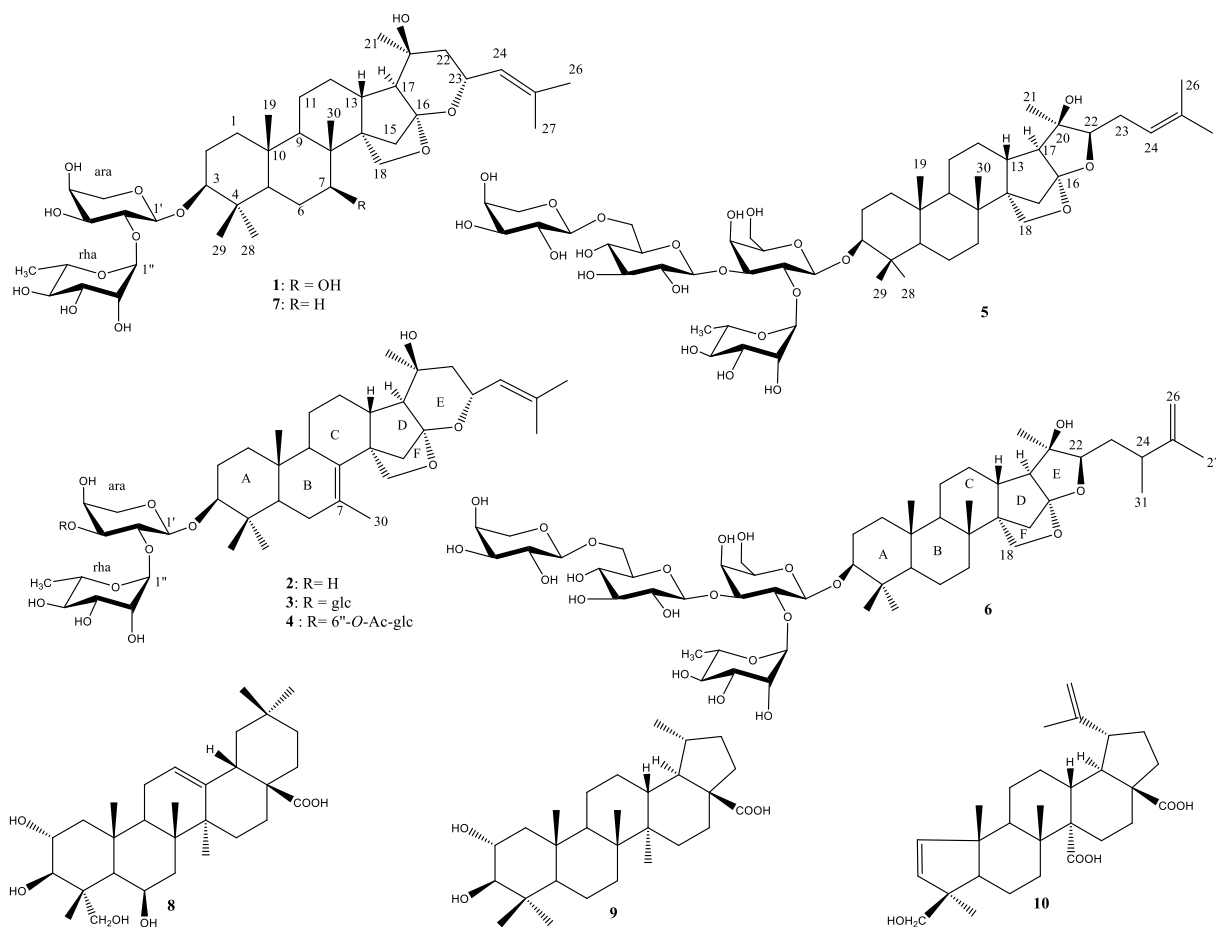


Figure 1. Chemical structures of triterpenes **1-10**, isolated from the aerial parts of *Gouania longipetala*.

The molecular formula of gouaniaside V (**5**) was established as $C_{53}H_{86}O_{22}$ by HR-ESI-MS (m/z 1097.5519 [$M + Na$] $^+$). The 1H NMR spectrum of **5** displayed for the aglycone signals for seven methyl groups, an oxygenated methine [δ_H 3.18 (dd, $J = 11.4, 4.1$ Hz, H-3)], an oxygenated methylene [δ_H 3.95 and 3.97 (each d, $J = 9.1$ Hz, H₂-18)], and a vinylic proton [δ_H 5.22 (t, $J = 6.5$ Hz, H-24)] (Table 1). Full assignment of the ^{13}C NMR signals was achieved through $^2J_{H-C}$ and $^3J_{H-C}$ correlations in the HMBC spectra which allow the identification of the known aglycone (20*R*,22*R*)-16 β ,22:16 α ,18-diepoxydammar-24-ene-3 β ,20-diol (Maciuk et al, 2004, Yoshikawa et al., 1992). The terminal tetrahydrofuran ring structure was assumed on the basis of the deshielded acetal carbon C-16 at δ_C 118.9, of an oxygenated methine C-22 (δ_C 95.5) correlated in the HSQC spectrum with a proton at δ_H 4.14 (*brt*, $J = 6.5$ Hz), and of a methylene C-23 (δ_C 28.7) reliable to two equivalent protons at δ_H 2.30 (t, $J = 6.5$ Hz). The spatial ROE correlations between H₃-21/H-22 and between H-17/H₂-18 confirmed the configuration at C-16, C-20, and C-22 (Yoshikawa et al., 1992). The 1H and ^{13}C -NMR spectra revealed the presence of four sugar units with the anomeric carbons at δ_C 105.9, 105.4, 104.9, and 101.0 and the corresponding anomeric protons at δ_H 4.44 (d, $J = 7.0$ Hz), 4.28 (d, $J = 6.4$ Hz), 4.52 (d, $J = 7.0$ Hz) and 5.38 (d, $J = 1.4$ Hz) (Table 2). Analysis of COSY, HSQC, and HMBC spectra allowed identification

of a β -D-galactopyranosyl (gal) disubstituted at gal-C-2 and gal-C-3 positions, a terminal α -L-arabinopyranosyl, a β -D-glucopyranosyl substituted at C-6 and a terminal α -L-rhamnopyranosyl, respectively (Table 2). The HMBC correlations between gal-H-1/C-3 (δ_C 89.9) of aglycone, rha-H-1/gal-C-2 (δ_C 75.5), glc-H-1/gal-C-3 (δ_C 85.6) and ara-H-1/glc-C-6 (δ_C 70.7) indicated that gouaniaside V (**5**) was (20*R*,22*R*)-16 β ,22:16 α ,18-diepoxydammar-24-ene-3 β ,20-diol-3-O-(α -L-arabinopyranosyl-(1 \rightarrow 6))- β -D-glucopyranosyl-(1 \rightarrow 3))- [α -L-rhamnopyranosyl-(1 \rightarrow 2)]- β -D-galactopyranoside.

The molecular formula of gouaniaside VI (**6**) was established as $C_{54}H_{88}O_{22}$ by HR-ESI-MS (m/z 1111.5669 [$M + Na$] $^+$, calcd for $C_{54}H_{88}O_{22}Na$). The 1H NMR spectrum of **6** showed six singlets for six tertiary methyl groups at δ_H 0.87, 0.89, 1.06, 1.15, 1.21 and 1.66, and a methyl doublet at δ_H 1.06 ($J = 2.8$ Hz) (Table 1). The presence of two other doublets at δ_H 4.74 and 4.76 (each $J = 1.0$ Hz) assigned to two exomethylene protons, suggested that **6** possessed an isopropyl side chain with terminal methylene, as observed with C₃₁-type derivatives (Brandao et al., 1993). The 1H NMR and ^{13}C NMR spectra of **6** showed signals superimposable to those of **5** except for the side chain suggesting a dammarane derivative possessing a C₃₁ skeleton (Table 1) (Brandao et al., 1993, Perera et al., 1993). The COSY correlations between H-22/H-23, H-23/H-24 and

Compounds **1-10** were evaluated for their effects on promyelocytic leukemia HL60 and human erythromyeloblastoid leukemia K562 cell lines with camptothecin as the positive control (Table 3). It was found that only jujuboside I (**7**) showed a moderate cytotoxicity, with IC₅₀ value of 13.5 μM. In addition, the antibacterial

activity of these compounds was evaluated against *Staphylococcus aureus*, *Escherichia coli* and *Enterococcus faecalis* and only aliphatic acid (**9**) was found to exhibit significant inhibitory activity against *S. aureus*, *E. faecalis* and *E. coli* with MIC of 32 μg/mL, 64 μg/mL and 128 μg/mL respectively (Table 3).

Table 3. Antimicrobial and cytotoxic activities of compounds **1-10**.^{a,b}

Compound	MIC (μg/mL)			Anti-proliferative activity (μM)	
	<i>S. aureus</i> (CIP53154)	<i>E. coli</i> (DH5-α)	<i>E. faecalis</i> (ATCC 1054)	HL60 (IC ₅₀)	K562 (IC ₅₀)
7	- ^a	- ^a	- ^a	13.5 ± 0.12	13.5 ± 0.11
9	32	128	64	- ^b	- ^b
Camptothecin ^c	-	-	-	0.25 ± 0.03	0.30 ± 0.05
Gentamicin ^c	0.5	0.5	0.5		

^a No microbial growth inhibition at 512 μg/mL for compounds **1-6** and **8-10**.

^b No anti-proliferative activity at 50 μM for compounds **1-8** and **10**.

^c Used as positive controls.

3. Experimental

3.1. General experimental procedures

NMR spectra were carried in CD₃OD on Bruker Avance DRX III 500 instruments (¹H at 500 MHz and ¹³C-*J*mod at 125 MHz). HR-ESI-MS experiments were performed using a Micromass Q-TOF micro instrument (Manchester, UK) equipped with an electrospray source. The samples were introduced by direct infusion in a solution of MeOH at a rate of 5 mL min⁻¹. The capillary voltage was 3000V, the cone voltage was 35V, and the temperature was 80 °C. Optical rotations were determined in MeOH with a Perkin-Elmer 341 polarimeter. TLC was performed on pre-coated silica-gel 60 F₂₅₄ Merck. CC was carried out on Kieselgel 60 (63-200 mesh) or LiChroprep RP-18 (40-63 μm) Merck. HPLC was performed on a Dionex apparatus equipped with an ASI-100 autosampler, an Ultimate 3000 pump, a diode array detector UVD 340S and Chromeleon software. C18 reversed phase column (Phenomenex 250x15 mm, Luna 5μ) was used for semi-preparative HPLC with binary gradient eluent (H₂O (pH 2.4 with TFA); MeOH), a flow rate of 4 mL/min and the chromatogram was monitored at 205, 225, 250 and 350 nm.

3.2. Plant material

The aerial parts of *Gouania longipetala* Hemsl. (Rhamnaceae) were collected at Bayota town, near Gagnoa city in the south-central Ivory Coast, in September 2009. The plant was identified by Pr. Laurent AKE-ASSI of FHB University and a voucher specimen (No Aké-Assi S.N-02) has been deposited in the herbarium of the National Center of Floristic of FHB University of Cocody (Ivory Coast).

3.3. Extraction and isolation

The dried powdered aerial parts of *G. longipetala* (950 g) were successively extracted with petroleum ether (PE), EtOAc and MeOH after maceration at room temperature for 24 h (19 L of each solvent). After evaporation of the solvents, 7.9 g of PE extract, 4.7 g of EtOAc extract and 26.5 g of MeOH extract were obtained.

The MeOH extract was subjected to VLC over RP-18 (9 x 5 cm) eluted successively with 20, 40, 60, 80 and 100% MeOH in H₂O, to give five fractions (A₁-A₅, respectively). Fraction A₂ (2.3 g) was applied to a RP-18 CC (2 x 16 cm) eluted with a gradient of MeOH:H₂O (2:8→5:5) to afford 130 fractions, each 30 mL. Frs [55-80] (367 mg), eluted with MeOH:H₂O (35:65), were purified by silica gel CC eluted with a gradient of CHCl₃:MeOH (9:1→6:4) to yield compound **2** (14 mg). Fraction A₃ (5.5 g) was applied to a silica gel VLC (9 x 5 cm) eluted with CHCl₃:MeOH:H₂O (9:1:0→60:40:7) to give six fractions (B₁-B₆, respectively), each 280 mL. Fraction B₅ (1.8 g), eluted with CHCl₃:MeOH (9:1→7:3) was further purified over silica gel CC (2.8 x 24 cm, 40 fractions, each 100 mL) to afford 64 mg of compound **6** (eluted with 85:15 of CHCl₃:MeOH). Frs [5-9] (83 mg), eluted with CHCl₃:MeOH (9:1) were purified by semi-prep HPLC using 50% of MeOH for 30 min yielding compound **4** (*rt* 17.5 min, 16 mg). Frs [13-17] (331 mg), eluted with CHCl₃:MeOH (85:15) were applied to a silica gel CC eluted with a gradient of CHCl₃:MeOH (9:1→7:3) and further purified by semi-prep HPLC using 50% of MeOH for 45 min yielding compounds **5** (*rt* 12.5 min, 9 mg), **3** (*rt* 13.5 min, 8 mg), **1** (*rt* 21.1 min, 10 mg), and **7** (*rt* 32.5 min, 24 mg).

The EtOAc extract was subjected to VLC over silica gel (9 x 5 cm) eluted successively with 1, 2, 3, 5, and 10% MeOH in CHCl₃. Fraction eluted with 5% MeOH in CHCl₃ (520 mg) was applied to a RP-18 CC (1.8 x 12.8 cm) eluted with a gradient of MeOH:H₂O (6:4→9:1). Fractions eluted with MeOH:H₂O (7:3) were further purified by semi-prep HPLC using a gradient from 70 to 75% of MeOH in 20 min yielding compound **10** (*rt* 14.0 min, 6 mg). Fraction eluted with 10% MeOH in CHCl₃ (275 mg) was applied to a silica gel CC (1.8 x 12.8 cm) eluted with a gradient of CHCl₃:MeOH (10:0→9:1) yielding compounds **8** (14 mg) and **9** (8 mg).

3.4. Acid hydrolysis

An aliquot of the crude saponin mixture (A₂ and A₃ 500 mg each) was refluxed (90 °C) with 50 mL of 2M TFA for 4 h. After extraction with ethyl acetate (3 x 30 mL), the aqueous layer was evaporated to furnish the monosaccharides residue (300 mg). Four sugars were identified as arabinose, glucose, galactose and rhamnose by comparison with authentic samples on TLC in MeCOEt:*iso*-PrOH:acetone:H₂O (20:10:7:6). A part of the monosaccharides residue (50 mg) was subjected to a preparative TLC using the same solvent. The optical rotation of each purified sugar was measured to reveal rhamnose ($[\alpha]_D^{20} + 2.4$ (*c* 1, H₂O)), glucose ($[\alpha]_D^{20} + 27.6$ (*c* 0.75, H₂O)), L-arabinose ($[\alpha]_D^{20} + 15.1$ (*c* 0.95, H₂O)) and galactose ($[\alpha]_D^{20} + 41.2$ (*c* 1, H₂O)).

3.5. Broth diffusion antibacterial assays.

A serial dilution technique using 96-well microtiter plates was used to check the MIC of the pure compounds (**1-10**) against *Staphylococcus aureus* (CIP 53154), *S. aureus* (8325-4) and *Enterococcus faecalis* (ATCC 1054), for Gram positive and *Escherichia coli* (DH5- α) for Gram negative, as previously described (Yao-Kouassi et al., 2008). Briefly, the mother compound solutions (10 mg/mL) were prepared by dissolving the compound in DMSO. Fifty microliters of each solution was added to 950 μ L of Muller-Hinton medium. This was serially diluted 2-fold with Muller-Hinton medium to obtain concentration ranges of 4 to 256 μ g/mL. Fifty microliters of each concentration was added in a well (96-well microplate) containing 150 μ L of Mueller-Hinton medium and 5 μ L of the standard bacterial inoculum. The negative control well consisted of 12.5 μ L of DMSO, 187.5 μ L of Mueller-Hinton medium and 5 μ L of the standard bacterial inoculum. The plates were covered with a sterile plate sealer, then agitated and incubated at 37 °C for 18 h. Microbial growth was determined by observing the deposit of the bacteria at the bottom of the wells. The lowest concentration inhibiting bacterial deposit was considered as the MIC. The experiments were run in triplicate for each compound on each strain, and each time the MIC values were identical. Gentamicin was used as positive control of bacterial growth inhibition in the same conditions.

3.6. Cell proliferation assay.

Promyelocytic leukemia HL60 and human erythromyeloblastoid leukemia K562 cells were spread onto 96-well flat-bottom plates at a density of 2500 cells per well, and then incubated for 24 h in RPMI 1640 Medium

Supporting Information

HR-ESI-MS spectra and 1D and 2D NMR of **1-6**.

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supplemented with 10% fetal bovine serum and antibiotics. After culture, the cells were treated with saponins for 72 h. The cell cultures were then analyzed using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner salt (MTS) according to the manufacturer's instructions (Promega Corporation, Charbonnières, France). Camptothecin was used as positive control with the same conduction. MTS is reduced by cells into a colored formazan product. Absorbance was analyzed at a wavelength of 540 nm with a Multiskan Ex microplate absorbance reader (Thermo Scientific, Paris, France). The results of these assays were used to obtain the dose-response curves from which IC₅₀ values were determined. The values represent averages of three independent experiments.

3.7. Gouaniaside I (1): $[\alpha]_D^{20} - 49.5$ (*c* 0.69, MeOH); ¹H and ¹³C NMR of the aglycone part (CD₃OD, 500 MHz), see Table 1; ¹H and ¹³C NMR of the glycosidic part, see Table 2; HR-ESI-MS *m/z*: 789.4409 [M + Na]⁺ (calcd for C₄₁H₆₆O₁₃Na, 789.4401).

3.8. Gouaniaside II (2): $[\alpha]_D^{20} - 55.8$ (*c* 0.17, MeOH); ¹H and ¹³C NMR of the aglycone part (CD₃OD, 500 MHz), see Table 1; ¹H and ¹³C NMR of the glycosidic part, see Table 2; HR-ESI-MS *m/z*: 771.4288 [M + Na]⁺ (calcd for C₄₁H₆₄O₁₂Na, 771.4295).

3.9. Gouaniaside III (3): $[\alpha]_D^{20} - 62.3$ (*c* 0.55, MeOH); ¹H and ¹³C NMR of the aglycone part (CD₃OD, 500 MHz), see Table 1; ¹H and ¹³C NMR of the glycosidic part, see Table 2; HR-ESI-MS *m/z*: 933.4836 [M + Na]⁺ (calcd for : C₄₇H₇₄O₁₇Na, 933.4824).

3.10. Gouaniaside IV (4): $[\alpha]_D^{20} - 45.2$ (*c* 0.27, MeOH); ¹H and ¹³C NMR of the aglycone part (CD₃OD, 500 MHz), see Table 1; ¹H and ¹³C NMR of the glycosidic part, see Table 2; HR-ESI-MS *m/z*: 975.4918 [M + Na]⁺ (calcd for : C₄₉H₇₆O₁₈Na, 975.4929).

3.11. Gouaniaside V (5): $[\alpha]_D^{20} - 20.5$ (*c* 0.34, MeOH); ¹H and ¹³C NMR of the aglycone part (CD₃OD, 500 MHz), see Table 1; ¹H and ¹³C NMR of the glycosidic part, see Table 2; HR-ESIMS *m/z*: 1097.5519 [M + Na]⁺ (calcd for : C₅₃H₈₆O₂₂Na, 1097.5508).

3.12. Gouaniaside VI (6): $[\alpha]_D^{20} - 30.5$ (*c* 0.31, MeOH); ¹H and ¹³C NMR of the aglycone part (CD₃OD, 500 MHz), see Table 1; ¹H and ¹³C NMR of the glycosidic part, see Table 2; HR-ESI-MS *m/z*: 1111.5669 [M + Na]⁺ (calcd for : C₅₄H₈₈O₂₂Na, 1111.5665).

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