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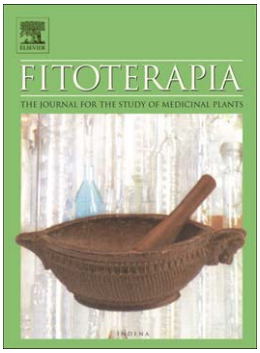

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Abstract

A new pentacyclic triterpenoid glucoside, together with fourteen known compounds, was isolated from the roots of *Combretum racemosum* (Combretaceae). The structure of the new compound was established as 28-*O*-*β*-D-glucopyranosyl-2*α*,3*β*,21*β*,23-tetrahydroxyolean-18-en-28-oate (**1**) on the basis of detailed spectroscopic data including MS, 1D, and 2D NMR. The inhibitory activity of compounds **1-15** against promyelocytic leukemia HL-60 and human erythromyeloblastoid leukemia K562 cell lines was evaluated. Compounds **11** (3-*O*-*β*-acetyl-ursolic acid), **14** (betulinic acid), and **15** (quadranoside II) exhibited significant cytotoxicity, with IC_{50} values of 13 to 50 µM. Among the isolated triterpenes, compounds **1**, **3** (arjungenin), **5** (terminolic acid), and **11** exhibited moderate antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Enterococcus faecalis* (MICs within a range of 64 and 256 µg/mL).

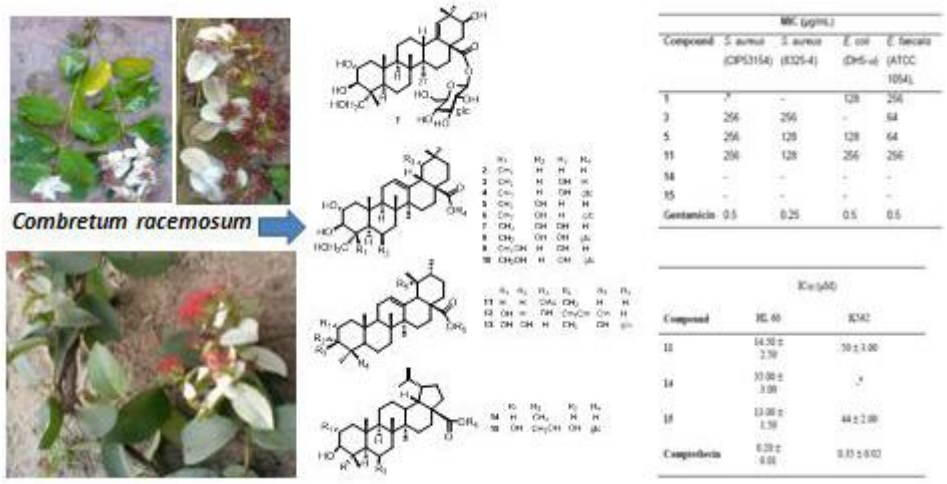
Highlights

►A new pentacyclic triterpenoid glucoside was isolated from *Combretum racemosum*.

►Four triterpenes isolated from this plant showed antibacterial activity.

►Three triterpenes showed cytotoxic activity against HL-60 and K562 cells.

Graphical abstract



Keywords: *Combretum racemosum*; Combretaceae; triterpenes; antibacterial activity; cytotoxic activity.

1. Introduction

The Combretaceae family includes more than 600 species distributed among about 20 genera, with pantropical distribution. *Combretum* is one of the two most commonly occurring genera with about 370 species and is widely used in Africa [1, 2]. Species of *Combretum* genus have been of interest in the last two decades thanks to the isolation of some compounds with highly significant activities in anticancer and anti-infectious models [3, 4, 5]. This has made the *Combretum* genus a very important group to search for bioactive compounds. *C. racemosum* is a straggling shrub widespread across Africa that bears a mass of crimson flowers which is very spectacular, named Christmas rose in the Southern Nigeria local English [6]. The plant has been used for several years in African traditional medical practices and as a condiment in soups. In addition to its anthelmintic [4], trypanocidal [7], and antimicrobial properties for genito-urinary and gastrointestinal infections [8], the plant is also used for the treatment of haemorrhoids, convulsive coughing, tuberculosis, toothache and male sterility [6, 9]. Furthermore, the anti-inflammatory, antiulcer, vasorelaxant, and trypanocidal properties of the *C. racemosum* extracts have been validated in recent pharmacological studies [10, 11, 12, 13, 14]. Water or organic extracts of *C. racemosum* leaves also showed significant antimicrobial activities against various pathogenic microorganisms [4, 8, 15]. However, little is known about the active components conferring therapeutic properties to these extracts. Previous phytochemical analysis of *C. racemosum* extracts revealed the presence of triterpenes [8, 16]. Up to now, the chemical constituents of the roots of *C. racemosum* have not yet been studied, which prompted us to investigate their active constituents. Thus, the present study focused on

the isolation and purification of a new pentacyclic triterpenoid glucoside (**1**), together with fourteen known compounds (**2-15**), from the 80% MeOH extract of the roots of *C. racemosum*. Their cytotoxicity against promyelocytic leukemia HL-60 and human erythromyeloblastoid leukemia cells K562 was evaluated. Additionally, their antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Enterococcus faecalis* was also evaluated.

2. Results and Discussion

The 80% hydromethanol extract of the roots of *C. racemosum* was concentrated and then partitioned successively with $CHCl_3$ and *n*-butanol (*n*-BuOH). The $CHCl_3$ soluble fraction was subjected to vacuum liquid chromatography (VLC) over silica gel followed by semiprep. HPLC to afford compounds **1, 3**, **5**, **11**, **12**, and **14** whereas the *n*-BuOH soluble fraction was fractionated by VLC over RP-C_{18} followed by semiprep. HPLC to obtain compounds **1**, **4**, **6-10**, and **15** (Fig. 1). Their structures were determined by extensive spectroscopic analysis, including HSQC, HMBC, and COSY, together with chemical evidences.

Compound **1** was obtained as a colorless powder with the molecular formula $C_{36}H_{58}O_{11}$ as deduced from the HR-ESIMS, which exhibited an ion peak at *m*/*z* 689.3873 ($[M + Na]^+$, calc for 689.3877). Analysis of the ^1H and ^{13}C NMR spectra of **1** revealed the presence of an anomeric proton at δ_H 5.54 (d, *J*=8.2 Hz) correlated in the HSQC spectrum with an anomeric carbon at δ_C 95.9 (Table 1). Analysis of the COSY and HSQC experiments of **1** allowed complete assignment of the glycoside proton and carbon system leading to a β-D-glucopyranoside unit [17] (Table 1). The ^1H NMR spectrum of **1** showed also the presence of six tertiary methyl groups (δ_H 0.70, 0.85, 0.94, 1.01, 1.03 and 1.06), one olefinic proton (δ_H 5.11, d, *J*=1.5 Hz), one oxymethylene group (δ_H 3.27, and 3.52, each d, *J*=11.2 Hz), and three oxymethine groups [δ_H 3.72 (td, *J*=9.1,4.4 Hz), 3.36 (d, *J*=9.1 Hz) and 3.60 (dd, *J*=13.2,3.6

Hz)]. Its ^{13}C NMR spectrum exhibited signals for 36 carbons, including the six glucosidic carbons, one carboxyl (δ_C 176.5), one double bond (δ_C 134.3 and 137.8), one oxygenated methylene (δ_C 66.3), and three oxygenated methines (δ_C 70.0, 72.7 and 78.5), and the other 23 carbons had chemical shifts from 13.7 to 52.5. These spectral data suggested that compound **1** was an oleanane-type triterpenoid glucoside [18]. In the HMBC spectrum, the cross-peaks observed between the methyl at δ_H 0.70 (s, H-24) and signals at δ_C 78.5 (C-3) and δ_C 66.3 (C-23) placed a secondary OH at C-3 and a hydroxymethylene at C-4. The location of this primary hydroxyl group at C-23 was deduced from the chemical shift of C-24 at δ_C 13.7 characteristic of an axial position, and by comparison of the ^{13}C NMR spectrum with that of arjunolic acid (OH at C-23) and hyptatic acid A (OH at C-24) [18]. The ^{1}H-^{1}H COSY correlation observed between δ_H 3.36 (H-3) and δ_H 3.72 suggested that a hydroxyl group was located at C-2. The large vicinal coupling constant value between H-2 and H-3 ($J_{2\text{-}3}$= 9.1 Hz) suggested a quasi *trans*-diaxial relationship, like in arjunolic acid [19]. In the ROESY spectrum (Fig 2), correlations between H-3 and H-5 (δ_H 1.25) confirmed the α-axial orientation of the two protons. The ROESY correlations observed between H-2 and protons of the methyl angular groups at δ_H 0.70 (s, H-24), and δ_H 1.01 (s, H-25), and between H_3-25 and δ_H 1.03 (s, H-26), confirmed the β-axial orientation of these CH_3 and H-2. The HMBC correlation between the carbon methyl signal of C-27 at δ_C 15.6 and the proton at δ_H 2.32 (*br*d (J=10.4 Hz) attributed to the angular methyne proton H-13, suggested that the aglycone was not a Δ^{12}-unsaturated oleanene as in arjunolic acid [19]. The HMBC correlations between this proton H-13 (δ_H 2.32) and the two ethylenic carbons at δ_C 137.8 (C-18) and 134.3 (C-19) suggested a Δ^{18}-unsaturated oleanene skeleton as in 2α,3α,24-trihydroxyolean-18-en-28-oic acid [20]. The long range ^4J ^{1}H-^{1}H COSY correlation observed between the olefinic proton at δ_H 5.11 (H-19) and the proton H-13 and the HMBC correlations between the olefinic proton H-19 and carbons C-13, C-17, C-20, C-21, C-29, and C-30 confirmed the position of the

double bond at C-18 (Fig 2). This data was supported by the ROESY experiment that showed rOe effects between H-19/H-12, H-19/H_3-29, and H-19/H_3-30.

Fig. 2. Key ROESY and HMBC relationships of compound **1**.

The comparison of ^{13}C NMR data of compound **1** with those of 2*α*,3*α*,24-trihydroxyolean-18-en-28-oic acid [20], an oleanane-type triterpenoid obtained from *Eucalyptus exserta*, indicated that these compounds differ by the presence of a hydroxyl group at C-21 and the configuration of C-2 and C-3. This was supported by the downfield shifts of C-20 (δ_C 38.2), C-21 (δ_C 72.7), and C-22 (δ_C 41.1). The position of the hydroxyl group at C-21 was confirmed by the HMBC correlations observed between H-21 (δ_H 3.60) and C-17, C-19, C-22, C-20, C-29, and C-30. The *α*-axial orientation of H-21 was justified by the value of the vicinal coupling constant H-21/H-22ax (J = 13.2 Hz) and the ROESY correlations observed between H-21/H_3-29 and H-21/H-22eq (δ_H 2.06, dd, J=13.2, 3.6). Full assignments of the proton and carbon resonances of the aglycone were achieved by analysis of the COSY, HSQC and HMBC spectra (Table 1). The triterpene skeleton of **1** was identified as 2*α*,3*β*,21*β*, 23-tetrahydroxyolean-18-en-28-oic acid. The chemical shifts of the signals due to C-3 and C-28 of aglycone, in ^{13}C NMR spectra ($\delta_{C\text{-}3}$ 78.5 and $\delta_{C\text{-}28}$ 176.5), suggested the C-28 glycosylation of **1** [18]. This was confirmed by the HMBC correlation observed between the anomeric proton of the *β*-D-glucopyranoside and the C-28 of aglycone. Therefore, compound **1** was assigned as 28-*O*-*β*-D-glucopyranosyl-2*α*,3*β*,21*β*,23-tetrahydroxyolean-18-en-28-oate.

Fig.1. Chemical structures of triterpenes **1-15**, isolated from *Combretum racemosum.*

Table 1 ^{1}H, ^{13}C NMR, HMBC, and ROESY spectral data of compound **1**.

Position	^{1}H-NMR	^{13}C-NMR	DEPT	HMBC correlation	ROESY correlation
	δ_H m (*J* in Hz)	δ_C			
1	0.89 m	48.1	CH_2	C-2, C-10, C-25	
	2.08 m				
2	3.72 td (9.1,4.4)	70.0	CH		H-25
3	3.36 d (9.1)	78.5	CH	C-2, C-23, C-24	H-23, H-5
4	-	44.1	C		
5	1.25 m	49.3	CH	C-23, C-24, C-25	H-9
6	1.40 dd (12.8,7.9)	22.3	CH_2		
	1.65 dm (12.8)				
7	1.45 m	35.1	CH_2	C-26	
8		41.9	C	H-26, H-27	
9	1.43 m	52.5	CH	C-25, C-26	H-27
10	-	39.3	C	H-1, H-25	
11	1.07 m	19.0	CH_2	C-13	
	1.45 m				
12	1.27 m	27.5	CH_2	C-13	
	1.63 m				
13	2.32 *br* d (10.4)	41.8	CH	H-11, H-12	H-26
14	-	43.8	C	H-13, H-26, H-27	
15	1.22 m	30.3	CH_2	C-27	
	1.74 m				
16	1.45 m	35.0	CH_2	C-17, C-28	
	2.31 dd (12.4, 2.0)				
17	-	51.8	C	H-13, H-22	
18	-	137.8	C	H-13, H-16	
19	5.11 d (1.5)	134.3	CH	C-13, C-17, C-21, C-29, C-30	H-12, H-29, H-30
20	-	38.2	C	H-29, H-30	
21	3.60 dd (13.2, 3.6)	72.7	CH	C-30, C-29, C-20, C-17, C-22	H-29
22	1.67 t (13.2)	41.1	CH_2	C-17, C-28	H-18
	2.06 dd (13.2, 3.6)				H-21
23	3.27 d (11.2)	66.3	CH_2	C-3, C-4, C-5	H-3
	3.52 d (11.2)				
24	0.70 s	13.7	CH_3		H-25
25	1.01 s	18.8	CH_3	C-1, C-9, C-10	H-24
26	1.03 s	16.6	CH_3	C-8, C-14	H-13
27	0.85 s	15.6	CH_3	C-8, C-14	H-9
28	-	176.5	**C**	H-1′, H-16, H-22	
29	1.06 s	28.3	CH_3	C-20, C-30	H-21
30	0.94 s	22.1	CH_3	C-20, C29	
28-*O*-glc					
1′	5.54 d (8.2)	95.9	CH	C-3′, C-28	H-3′, H-5′
2′	3.33 m	74.1	CH	C-4′	
3′	3.36 t (9.6)	78.2	CH	C-1′, C-5′	H-1′, H-5′
4′	3.39 t (9.6)	71.1	CH	C-2′, C-6′	
5′	3.40 m	78.8	CH	C-3′, C-1′	H-1′, H-3′
6′	3.73 dd (11.6,2.1)	62.4	CH_2	C-4′, C-5′	
	3.85 dd (11.5,0.8)				

Compounds **2-14** were known and their structural assignments were made by ESI-MS and 1D, and 2D NMR analysis. Their spectroscopic data (Tables 4 and 5) were in good agreement with those reported in the literature for arjunolic acid (**2**) [19], arjungenin (**3**) [21]**,**

arjunglucoside I (**4**) [21], terminolic acid (**5**) [22], chebuloside II (**6**) [23], combregenin (**7**) [22], combreglucoside (**8**) [22], bellericagenin B (**9**) [24], bellericaside B (**10**) [24], 3-*O*-*β*-acetyl-ursolic acid (**11**) [25, 26], 24-hydroxy-tormentic acid (**12**) [27], kaji-ichigoside F1 (**13**) [28]**,** betulinic acid (**14**) [29], and quadranoside II (**15**) [30].

Table 4
^{1}H and ^{13}C NMR data of compound **2-8**.

	2		**3**		**4**		**5**		**6**		**7**		**8**	
	δ_H m (*J* in Hz)	δ_C	δ_H m (*J* in Hz)	δ_C	δ_H m (*J* in Hz)	δ_C	δ_H m (*J* in Hz)	δ_C	δ_H m (*J* in Hz)	δ_C	δ_H m (*J* in Hz)	δ_C	δ_H m (*J* in Hz)	δ_C
1	0.91 m	47.5	0.92 br d (12.5)	47.7	0.92 t (12.5)	47.8	0.88 t (11.9)	50.1	0.89 t (12.6)	50.1	0.90 t (11.8)	49.9	0.90 t (12.1)	49.9
	1.95 m		1.93 dd (12.5,4.5)		1.93 dd (12.5,4.5)		1.91 dd (11.9 (4.4)		1.91 dd (12.4,4.6)		1.88 dd (11.9,5.0)		1.88 dd (12.1,4.8)	
2	3.70 m	69.7	3.72 td (9.4,4.5)	69.7	3.72 ddd (12.5,9.7,4.5)	69.7	3.76 td (11.9, 4.4)	69.7	3.76 td (10.9,4.5)	69.7	3.76 ddd (11.1,9.5,5.0	69.7	3.76 td (10.1, 4.8)	69.7
3	3.37 d (9.5)	78.2	3.38 d (9.4)	78.3	3.37 d (9.7)	78.7	3.32 d (11.9)	78.2	3.31 d (10.9)	78.2	3.32 d (9.5)	78.3	3.32 d (10.1)	78.3
4		44.1		44.1		44.1		44.8		44.8		44.8		44.8
5	1.31 m	48.2	1.33 m	48.4	1.32 d (6.5)	48.4	1.29 m	48.8	1.30 *br* s	49.0	1.33 d (1.8)	49.3	1.33 d (1.8)	49.2
6	1.43 m	18.6	1.50 m	19.3	1.45 m	19.3	4.40 *br* s	68.5	4.40 *br* s	68.5	4.42 *br* s	68.7	4.42 *br* s	68.8
	1.49 m		1.48 m		1.48 m									
7	1.31 m	33.6	1.28 dd (10.1,2.9)	33.3	1.30 dd (13.0,6.5)	33.3	1.51 dd (14.0,1.8)	41.1	1.51 dd (14.3,2.1)	41.0	1.51 dd (14.4,2.5)	41.3	1.51 dd (14.4,2.5)	41.2
	1.63 m		1.63 m		1.62 m		1.79 dt (14.0,3.3)		1.77 dd (14.3,3.9)		1.81 dd (14.4,4.5)		1.79 dd (14.4,4.5)	
8		40.6		40.7		40.9		39.8		39.9		40.0		40.1
9	1.71 m	49.2	1.86 t (8.8)	49.1	1.85 t (8.7)	49.0	1.73 m	49.3	1.73 dd (11.1,3.9)	49.2	1.88 m	49.5	1.87 m	49.5
10		39.1		39.2		39.2		38.6		38.6		38.7		38.7
11	2.02 m	24.2	2.02 m	24.9	2.02 m	24.9	11.98 m	24.6	1.97 m	24.0	2.04 m	24.8	2.04 m	24.8
	2.02 m		2.02 m		2.02 m		2.11 m		2.10 m		2.11 dd (11.0,3.9)		2.11 dd (10.9,3.5)	
12	5.27 *br* s	123.3	5.35 t (3.4)	124.7	5.36 t (3.5)	124.8	5.31 t (3.4)	123.7	5.33 t (3.5)	123.9	5.39 t (3.5)	125.0	5.39 t (3.5)	125.0
13		144.8		144.8		144.5		144.7		144.3		144.0		143.8
14		43.1		42.7		42.8		43.5		43.5		43.2		43.2
15	1.09 m	28.8	1.02 m	29.4	1.02 m	29.4	1.08 m	28.8	1.11 m	28.8	1.03 dd (11.0,3.7)	29.4	1.02 m	29.4
	1.80 m		1.62 m		1.74 m		1.87 td (13.8,3.0)		1.85 dd (13.9,3.4)		1.78 m		1.78 m	
16	1.97 m	24.7	1.62 m	28.6	1.74 m	28.4	1.61 *br* d (13.1)	24.6	1.75 m	24.6	1.63 m	28.6	1.75 br d (11.0)	28.5
	2.30 m		2.31 td (14.6,5.0)		2.35 0		2.01 m		2.07 td (13.9,3.4)		2.30 td (13.4,3.7)		2.34 m	
17		46.9		46.7		47.1		48.5		48.0		46.7		47.1
18	2.88 m	41.9	3.08 *br* s	45.2	3.08 br s	45.1	2.90 dd (13.7,3.6)	42.8	2.90 dd (13.8,4.2)	42.6	3.10 *br* s	45.2	3.09 *br* s	45.0
19	1.15 m	47.3	3.28 d (4.4)	82.5	3.29 m	82.4	1.15 m	47.3	1.19 dd (11.3,4.4)	47.2	3.29 d (3.9)	82.5	3.31 d (3.8)	82.4
	1.71 m						1.72 m		1.75 t (11.3)					
20		31.6		36.0		36.0		31.6		31.5		36.0		36.0
21	1.22 m	34.9	1.62 m	29.5	1.68 dd (9.8,3.5)	29.5	1.22 *br* d (16.2)	34.9	1.24 m	34.9	1.02 dd (13.7,3.4)	29.6	1.05 m	29.6
	1.41 m		1.78 td (9.9,3.0)		1.80 td (9.8,3.5)		1.39 td (16.2,3.9		1.43 td (13.7,4.0)		1.75 m		1.75 m	
22	1.56 m	34.0	1.62 m	34.0	1.68 dd (9.8, 3.5)	33.3	1.56 *br* d (13.7)	33.9	1.64 dt (13.7,3.2)	33.1	1.64 m	34.0	1.69 dd (21.2,7.8)	33.2
	1.76 m		1.78 td (9.9,3.0)		1.80 dd (9.8,3.5)		1.76 m		1.75 dd (13.7,3.2)		1.79 m		1.80 dd (21.2,4.1)	
23	3.29 d (11.0)	66.3	3.29 d (12.2)	66.4	3.29 d (11.1)	66.5	3.46 d (11.1)	65.9	3.46 d (11.1)	66.9	3.48 d (11.0)	66.0	3.48 d (11.1)	65.9
	3.53 d (11.0)		3.38 d (12.2)		3.38 d (11.1)		3.61 d (11.1)		3.60 d (11.2)		3.61 d (11.0)		3.61 d (11.1)	
24	0.72 s	13.9	0.72 s	13.8	0.73 s	13.8	1.09 s	15.2	1.08 s	15.2	1.09 s	15.2	1.09 s	15.2
25	1.06 s	17.5	1.05 s	17.4	1.05 s	17.5	1.40 s	18.9	1.41 s	18.9	1.40 s	18.4	1.40 s	18.9
26	0.85 s	17.5	0.79 s	17.8	0.77 s	17.8	1.12 s	19.1	1.09 s	19.1	1.05 s	18.2	1.05 s	18.6
27	1.20 s	26.5	1.33 s	25.1	1.33 s	25.0	1.17 s	26.5	1.16 s	26.4	1.30 s	25.1	1.30 s	25.0
28		180.2		182.4		178.6		181.9		178.1		182.3		178.6
29	0.93 s	33.7	0.96 s	28.7	0.96 s	28.6	0.93 s	33.6	0.94 s	33.5	0.96 s	28.7	0.97 s	28.6
30	0.97 s	23.7	0.99 s	25.1	0.97 s	25.2	0.97 s	24.0	0.96 s	24.0	0.99 s	25.2	0.98 s	25.2
glc														
1′					5.40 d (8.2)	95.8			5.40 d (8.1)	95.8			5.39 d (8.1)	95.9
2′					3.34 dd (8.9,8.2)	73.9			3.35 dd (9.1,8.1)	73.9			3.35 dd (9.1,8.1)	73.9
3′					3.42 t (8.9)	78.3			3.42 t (9.1)	78.3			3.43 t (9.1)	78.3
4′					3.37 t (8.9)	71.1			3.39 t (9.1)	71.4			3.39 t (9.1)	71.1
5′					3.37 m	78.4			3.37 m	78.6			3.37 m	78.7
6′					3.70 dd (12.2,4.2)	62.4			3.70 dd (12.1,4.4)	62.4			3.71 dd (12.1,4.2)	62.4
					3.84 dd (12.2,1.6)				3.84 dd (12.1,1.6)				3.84 dd (12.1,1.3)	

Table 5
^{1}H and ^{13}C NMR data of compound **9-15**.

	9		**10**		**11**		**12**		**13**		**14**		**15**	
	δ_H m (*J* in Hz)	δ_C	δ_H m (*J* in Hz)	δ_C	δ_H m (*J* in Hz)	δ_C	δ_H m (*J* in Hz)	δ_C	δ_H m (*J* in Hz)	δ_C	δ_H m (*J* in Hz)	δ_C	δ_H m (*J* in Hz)	δ_C
1	0.91 dd (12.4,5.9)	47.6	0.93 m	47.6	1.11 m	38.3	0.94 t (12.9)	47.8	1.30 m	42.6	0.91 m	38.9	0.83 t (11.8)	50.2
	1.93 dd (12.4,4.5)		1.94 m		1.67 m		1.98 dd (12.9,4.5)		1.60 dt (12.1,4.0)		1.69 m		1.99 m	
2	3.85 ddd (12.5,9.7,4.5)	69.8	3.86 m	69.8	1.65 m	23.6	3.80 ddd (12.9,9.5,4.5)	69.6	3.96 m	67.2	1.61 td (11.4,3.2)	27.4	3.75 td (9.6,4.7)	70.0
3	3.48 d (9.7)	79.5	3.48 d (9.7)	79.5	4.52 d (9.2)	80.9	3.08 d (9.5)	86.0	3.34 m	80.1	3.21 d (11.4)	79.0	3.30 d (9.6)	78.2
4		48.2		48.2		37.7		44.4		39.5		38.7		44.8
5	1.45 m	48.4	1.43 m	48.5	0.87 m	55.3	0.99 m	57.2	1.28 m	49.2	0.70 m	55.4	1.23 *br* s	49.3
6	1.44 dd (9.1, 6.6)	19.8	1.43 m	19.8	1.39 m	18.2	1.30 m	27.3	1.45 dd (12.1,4.0)	19.3	1.40 m	18.3	4.36 *br* s	68.7
	1.63 m		1.61 m		1.55 m		1.65 m				1.54 m			
7	1.28 m	33.7	1.69 m	33.3	1.36 m	32.8	1.35 m	34.4	1.34 m	34.0	1.39 m	34.3	1.60 dd (12.1,2.3)	42.4
	1.63 m		1.80 m		1.51 m		1.55 m		1.60 dd (12.1,4.0)		1.69 m		1.69 m	
8		40.7		40.9		39.5		41.1		41.4		40.7		43.8
9	1.85 t (9.1)	49.9	1.84 m	49.3	1.56 m	47.5	1.76 t (11.9)	49.2	1.86 m	48.2	1.29 m	50.9	1.48 m	52.6
10		39.0		39.0		36.9		39.1		39.4		37.0		38.9
11	2.02 m	25.1	2.02 m	25.2	1.94 m	23.3	2.04 m	25.0	2.03 m	24.8	1.29 m	20.9	1.48 m	22.3
	2.02 m				1.94 m		2.04 m				1.45 m		1.55 m	
12	5.34 t (4.4)	124.6	5.35 t (3.5)	124.7	5.26 t (3.4)	125.8	5.32 t (3.5)	129.1	5.34 t (3.5)	129.6	1.05 dd (12.7,8.3)	25.5	1.10 dd (12.8,6.3)	26.9
											1.72 m		1.78 m	
13		144.7		144.5		138.0		140.2		139.7	2.21 td (12.6,3.6)	38.4	2.45 td (11.7,2.2)	38.4
14		42.6		42.7		41.9		42.6		42.8		42.5		41.2
15	1.01 m	29.4	1.04 m	29.4	1.11 m	28.0	1.02 m	29.6	1.03 m	29.7	1.21 dt (13.5,2.7)	29.4	1.20 m	30.9
	1.78 td (11.1,2.8)		1.80 m		1.89 m		1.83 m		1.85 m		1.54 m		1.64 dd (11.2,3.3)	
16	1.63 m	28.6	1.75 m	28.4	1.68 m	24.1	1.26 m	27.3	1.65 dd (13.4,3.7)	26.5	1.44 m	32.2	1.47 dd (9.6,4.0)	32.7
	2.32 td (11.1,2.8)		2.35 m		1.68 m		1.75 m		2.64 m		2.29 dt (12.8,3.2)		2.37 dt (9.7,3.2)	
17		46.7		47.1		47.9		48.7		49.3		56.3		57.9
18	3.07 d (3.6)	45.2	3.07 *br* s	45.1	2.21 d (11.0)	52.6	2.53 *br* s	55.1	2.54 *br* s	55.0	1.63 t (11.2)	50.5	1.70 t (11.1)	50.7
19	3.27 d (3.6)	82.4	3.29 d (3.6)	82.4	1.36 m	39.0		73.6		73.6	3.02 td (11.2,5.1)	49.3	3.04 td (11.1,5.0)	48.3
20		36.0		36.0	1.04 m	38.8	1.37 m	43.1	1.37 m	43.0	1.43 m	150.4		151.8
21	1.01 m	29.5	1.04 m	29.5	1.34 m	30.6	1.31 dd (9.0,6.8)	30.8	1.26 m	27.2	1.49 m	30.6	1.40 m	31.4
	1.63 m		1.75 m		1.54 m				1.75 m		2.00 m		1.94 m	
22	1.63 m	34.0	1.30 m	33.6	1.65 m	36.7	1.65 m	39.1	1.65 dd (13.4,3.7)	38.3	0.99 m	37.2	1.49 m	37.5
	1.80 td (11.1,3.4)		1.60 m		1.75 dt (12.6,3.4)		1.75 dd (12.5,6.5)		1.80 m		1.99 m		2.02 m	
23	3.54 d (11.6)	64.5	3.53 d (11.6)	64.5	0.89 s	28.1	1.26 s	23.8	1.01 s	29.2	0.78 s	28.0	3.46 d (11.1)	65.9
	4.08 d (11.6)		4.08 d (11.6)										3.59 d (11.1)	
24	3.65 d (11.5)	62.7	3.65 d (11.4)	62.7	0.87 s	16.7	3.41 d (11.2)	66.2	0.89 s	22.5	0.84 s	15.4	1.06 s	15.0
	4.07 (11.5)		4.07 d (11.4)				4.06 d (11.2)							
25	1.04 s	17.3	1.04 s	17.4	0.98 s	15.5	1.01 s	17.5	1.02 s	17.0	0.96 s	16.1	1.30 s	19.8
26	0.78 s	17.7	0.75 s	17.7	0.80 s	17.0	0.80 s	17.5	0.80 s	17.7	1.00 s	16.0	1.29 s	17.2
27	1.33 s	25.1	1.32 s	25.2	1.10 s	23.6	1.36 s	24.8	1.37 s	24.8		14.7	1.00 s	15.3
28		182.3		178.6		183.1		180.0		178.6		179.8		176.2
29	0.96 s	28.7	0.96 s	28.6	0.88 d (5.2)	17.1	1.21 s	27.1	1.23 s	27.1	4.63 *br* s	109.7	4.63 *br* s	110.3
											4.75 *br* s		4.75 *br* s	
30	0.99 s	25.1	0.97 s	25.0	0.96 d (5.7)	21.3	0.95 d (6.7)	16.6	0.96 s	16.6	1.73 s	19.4	1.73 s	19.5
glc					Ac.									
1′			5.39 d (8.1)	95.8	2.07 s	21.2			5.34 d (8.2)				5.52 d (8.2)	95.2
2′			3.35 dd (9.2,8.1)	73.9		171.0			3.34 dd (8.6,8.2)				3.34 dd (8.5,8.1)	74.1
3′			3.42 t (9.2)	78.3					3.42 t (8.6)				3.45 t (8.5)	78.4
4′			3.38 t (9.2)	71.1					3.37 t (8.6)				3.40 t (8.5)	71.1
5′			3.37 m	78.7					3.35 m				3.37 m	78.7
6′			3.70 m	62.4					3.70 dd (11.9,4.7)				3.72 dd (12.1,4.4)	62.4
			3.84 dd (12.7,1.5)						3.82 dd (11.9,2.0)				3.86 dd (12.1,1.1)	

A serial dilution technique using 96-well microtiter plates was used to check the MIC of the pure compounds (**1**-**15**) against *Staphylococcus aureus* (CIP 53154), *S. aureus* (8325-4) and *Enterococcus faecalis* (ATCC 1054), and *Escherichia coli* (DH5-α) (Table 2). The four compounds **1**, **3**, **5** and **11** showed antimicrobial activity against the four bacteria. Terminolic acid (**5**) was the most active with MIC values of 64 µg/mL against *E. faecalis* and 128 µg/mL against *E. coli* and *S. aureus* (8325-4). Compound **1** showed a low antibacterial activity against *E. faecalis* (MIC 256 µg/mL) and *E. coli* (128 µg/mL). Arjungenin (**3**) showed also moderate activity against *E. faecalis*, whereas compound **11** was the less active compound. Comparison of the relation structure activity of the compound with antibacterial activity (compounds **3** and **5**) with compounds **4** and **6** suggested that glucosylation at C-28 of terminolic acid and arjungenin was not favorable for the antibacterial activity.

Table 2
MIC values of compounds **1**-**15** against four bacteria strains.[a]

	MIC (µg/mL)			
Compound	*S. aureus* (CIP53154)	*S. aureus* (8325-4)	*E. coli* (DH5-α)	*E. faecalis* (ATCC 1054),
1	-[b]	-	128	256
3	256	256	-	64
5	256	128	128	64
11	256	128	256	256
14	-	-	-	-
15	-	-	-	-
Gentamicin	0.5	0.25	0.5	0.5

[a] No microbial growth inhibition at 512 µg/mL for compounds **2**, **4**, **6**-**10**, **12**, and **13**.
[b] -: No microbial growth inhibition at 256 µg/mL.

Additionally, compounds **1**-**15** were evaluated for their inhibitory effects on the proliferation of the promyelocytic leukemia (HL-60) and human erythromyeloblastoid leukemia (K562) cells (Table 3). Only compounds **11**, **14** and **15** showed moderate cytotoxic activity against HL-60 with IC_{50} values of 14.5, 35 and 13 µM, respectively. Compounds **11** and **15** also displayed a moderate cytotoxic activity against K562 with IC_{50} values of 50 and 44 µM, respectively.

Table 3
Cytotoxic activities of compounds **1-15** against HL 60 and K562 cell lines.[a]

	IC_{50} (µM)	
Compound	HL 60	K562
11	14.50 ± 2.50	50 ± 3.00
14	35.00 ± 3.00	-[a]
15	13.00 ± 1.50	44 ± 2.00
Camptothecin	0.20 ± 0.01	0.35 ± 0.02

[a] 50% inhibition not achieved at 50 µM for compounds **1-10**, **12**, and **13**.

The overall results may support the use of this plant in traditional medicine for the treatment of gastric disorders and microbial infections [4, 6, 8, 9].

3. Experimental

3.1. General experimental procedures

NMR spectra were carried in CD_3OD on Bruker Avance DRX III 500 instruments (^1H at 500 MHz and ^{13}C-*J*mod at 125 MHz). HR-ESI-MS experiments were performed using a Micromass Q-TOF micro instrument (Manchester, UK). Optical rotations were determined in MeOH with a Perkin-Elmer 341 polarimeter. TLC was performed on pre-coated silica-gel 60 F_{254} 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. C_{18} reversed phase column (Phenomenex 250x15 mm, Luna 5µ) was used for semi-preparative HPLC with binary gradient eluent (H_2O (pH 2.4 with TFA); MeOH) and a flow rate of 3 mL/min; the chromatogram was monitored at 205, 225, 250, and 350 nm.

3.2. Plant material

The roots of *Combretum racemosum* P. Beauv. were collected in Grand-Alépé (South-East of Yamoussoukro, Ivory Coast), in October 2009. The plant was identified by Pr. Laurent AKE-ASSI of FHB University and a voucher specimen (No Aké-Assi-Sn) has been deposited in the

herbarium of the National Center of Floristic of University of FHB University of Abidjan-Cocody (Ivory Coast).

3.3. Extraction and isolation

The dried and powdered roots of *C. racemosum* (750 g) were macerated for 24 h with 15 L of EtOAc to afford 5 g of EtOAc extract. After drying, the resulting powdered material was macerated with MeOH 80% (15 L) for 24 h and then boiled for 3 h. After filtration, the organic phase was concentrated to 1 L under reduced pressure to remove MeOH. The resulting aqueous solution was sequentially extracted three times with $CHCl_3$ and *n*-BuOH to give the $CHCl_3$ soluble fraction (A) (14.5 g) and the *n*-BuOH soluble fraction (B) (22.1 g). Fraction A was subjected to vacuum liquid chromatography (VLC) on silica gel (9 × 5 cm) eluted successively with 1 L of 0%, 1%, 2%, 3%, 5%, 10%, 15%, 20%, 30%, and 50% MeOH in $CHCl_3$ to give subfractions A_1-$A_{10,}$ respectively. The fraction A_2 (1.125 g) was fractionated by silica gel CC, using a gradient of $CHCl_3$-MeOH (1:0 to 95:5) to give compound **14** (21 mg). Fractions eluted with $CHCl_3$-MeOH (98:2) were further purified by silica gel CPP using $CHCl_3$-MeOH (98:2) as eluent to give **11** (7 mg) and **16** (10 mg). The fractions A_7 and A_8 (3.3 g) were fractionated by RP-18 CC (3.5 x 26 cm), using a gradient of MeOH-H_2O (5:5 to 8:2) to give compound **7** (76 mg) eluted with MeOH-H_2O (55:45), and **3** (255 mg) eluted with MeOH-H_2O (7:3). Fractions eluted with MeOH-H_2O (75:25).were purified by semipreparative HPLC using a gradient of MeOH (from 73% to 80% in 30 min) to give compounds **12** (*R*t 13.5 min; 6 mg), **5** (*R*t 15.5 min; 6 mg), and **2** (*R*t 18.5 min; 6 mg).

Fraction B was subjected to VLC on RP18 (9 × 5 cm), eluted successively with1 L of 20, 40, 60, 80, and 100% MeOH in H_2O, to give subfractions B_1 to B_5, respectively. The fraction B_3 (5.5 g) was fractionated by RP_{18} CC (4.5 x 20 cm), using a gradient of MeOH-H_2O (4:6 to 8:2). Fractions eluted with MeOH-H_2O (5:5) (1.7 g) were purified by silica gel CC using a gradient of $CHCl_3$-MeOH-H_2O (9:1:0 to 14:6:1). Fractions eluted with $CHCl_3$-MeOH (85:15)

(92 mg) were purified by semipreparative HPLC using the gradient of MeOH (from 62% to 68% in 30 min) to give compounds **1** (*R*t 16.0 min; 6 mg) and **4** (*R*t 17.3 min; 14 mg). Fractions eluted with $CHCl_3$-MeOH (8:2) (626 mg) were purified by silica gel CC using $CHCl_3$-MeOH (9:1 to 8:2) and then fraction eluted with $CHCl_3$-MeOH (85:15) was further purified by silica gel CPP using $CHCl_3$-MeOH-H_2O (75:25:2) as eluent to give **8** (28 mg) and **10** (10 mg). Fractions eluted with MeOH-H_2O (6:4) were fractionated by CC on silica gel using a gradient of $CHCl_3$-MeOH (1:0 to 8:2) to give **13** (5 mg). Fractions eluted with $CHCl_3$-MeOH (9:1) were purified by semiprep. HPLC using MeOH increasing from 62% to 68% in 30 min to give compounds **7** (*R*t 14.5 min; 9 mg) and **9** (*R*t 16.5 min; 5 mg) whereas frs eluted with $CHCl_3$-MeOH (8:2) were purified by semiprep. HPLC using MeOH increasing from 60% to 65% in 30 min to give compounds **6** (*R*t 21.5 min; 19 mg) and **15** (*R*t 18.3 min; 35 mg).

3.4. Sugar analysis and determination of absolute configuration

A part of the fractions A and B (200 mg each) was refluxed with TFA 2N (25 mL) for 4 h. After filtration, the mixture was extracted with EtOAc (3 x 25 mL) and the acid aq layer was evaporated. The residue was purified by prep. HPLC on a Rezex ROA column with H_2SO_4 2.5 mM as solvent to yield glucose (*R*t 9.74). The glucose fraction was then neutralized with NaOH 50 mM and freeze-dried. The residues were solubilized in pyridine and soln. were filtrated and then evaporated. Glucose was dissolved in hexane-EtOH-TFA (50:50:1) by ultrasonication. The solutions were analyzed by chiral HPLC with a Chiralpak IC using a mixture of hexane-EtOH-TFA (80:20:0.1) as solvent. By comparison with authentic D or L monosaccharide samples, the configurations were identified as D-glucose (*R*t 19.3 and 24.5).

3.5. 28-*O*-*β*-D-glucopyranosyl-2*α*,3*β*,21*β*,23-tetrahydroxyolean-18-en-28-oate **(1)**

White amorphous powder, $[\alpha]^{20}_{D}$ + 21.2 (*c* 0.11, MeOH); ^{1}H and ^{13}C NMR (MeOH-d_4) spectroscopic data, see Table 1; HR-ESI-MS *m/z*: 689.3873 $[M + Na]^+$ (calcd for $C_{36}H_{58}O_{11}Na$, 689.3877).

3.6. Broth diffusion antibacterial assays.

The liquid microdilution growth inhibition method was used to determine the MIC values of the actives compounds **1**, **3**, **5**, **9** and **11** against standard strains of *S. aureus*, *E. faecalis* and *E. coli* as described in [31]. 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 µL of Muller-Hinton medium. This was serially diluted 2-fold with Muller-Hinton medium to obtain concentration ranges of 4 to 256 µg/mL. Fifty microliters of each concentration was added in a well (96-well microplate) containing 150 µL of Mueller-Hinton medium and 5 µL of the standard inoculum. The final concentration of DMSO in the well was less than 5% (preliminary analysis with 5% (v/v) DMSO/Mueller-Hinton medium affected neither the growth of the test organisms nor the change of color due to this growth). The negative control well consisted of 12.5 µL of DMSO, 187.5 µL of Mueller-Hinton medium, and 5 µL of the standard 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, and each time the MIC values were identical. Gentamicin was used as positive control in the same conditions.

3.7. Cell proliferation assay.

Promyelocytic leukemia HL-60 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 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 IC_{50} values were determined. The values represent averages of three independent experiments.

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Supporting Information.

HR-ESIMS and NMR data for compound **1**-**15**.

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