Article Type: Full Paper

New Cytotoxic Triterpenoid Saponins from the Roots of *Albizia gummifera* C.A. Smith

Line Made Simo [a,b], Olivier Placide Noté* [a,b], Joséphine Ngo Mbing[b], Sarah Ali Aouazou[a], Dominique Guillaume[c], Christian Dominique Muller[a], Dieudonné Emmanuel Pegnyemb[b], and Annelise Lobstein[a]

[a] Laboratoire d'Innovation Thérapeutique, UMR 7200, Faculté de Pharmacie, CNRS-Université de Strasbourg, 74 route du Rhin, F-67401 Illkirch Cedex, France

[b] Laboratoire de Pharmacochimie des Substances Naturelles, Département de Chimie Organique, Faculté de Sciences, Université de Yaoundé I, BP 812, Yaoundé, Cameroun(oliviernote1@yahoo.fr (Olivier Noté))

[c]Laboratoire de Chimie Thérapeutique, UMR7312, Université de Reims, 51 rue Cognacq-Jay, 51100 Reims, France

Abstract

As part of our search of new bioactive saponins from Cameroonian medicinal plants, two new oleanane-type saponins, named gummiferaosides D–E (**1**–**2**), along with one known saponin, julibroside J_8 (**3**), were isolated from the roots of *Albizia gummifera*. Their structures were established on the basis of extensive 1D and 2D NMR (^{1}H-, ^{13}C-NMR, DEPT, COSY, TOCSY, NOESY, HSQC, HSQC-TOCSY and HMBC) and HRESIMS studies, and by chemical evidence. The apoptotic effect of saponins **1**–**3** was evaluated on the A431 human epidermoid cancer cell. Flow cytometric analyses showed that saponins **1**–**3** induced apoptosis of human epidermoid cancer cell (A431) in a dose-dependent manner.

Keywords:*Albizia gummifera;* Mimosaceae ; Triterpenoid saponins; apoptosis of human epidermoid cancer cell A431.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/cbdv.201700260

Introduction

The genus *Albizia* comprises about 150 species widely distributed in the tropics, with the greatest diversity in Africa and South America [1]. Triterpenoid glycosides commonly described in *Albizia* genus are complex acacic acid-type saponins, some of which have been reported to inhibit the growth of tumor cells, and thus appear as a new potential class of anticancer natural triterpenoid saponins [2]. However, the proapoptotic function of acacic acid-type saponins isolated from *Albizia* genus has only been reported for adianthifoliosides A and D, isolated from *Albizia adianthifolia* [3], and very recently for zygiaosides A-B isolated from *Albizia zygia* [4]. These saponins share some structural features: they all have acacic acid asaglycone unit, oligosaccharide moieties at C-3 and C-28, and an acyl group at C-21.

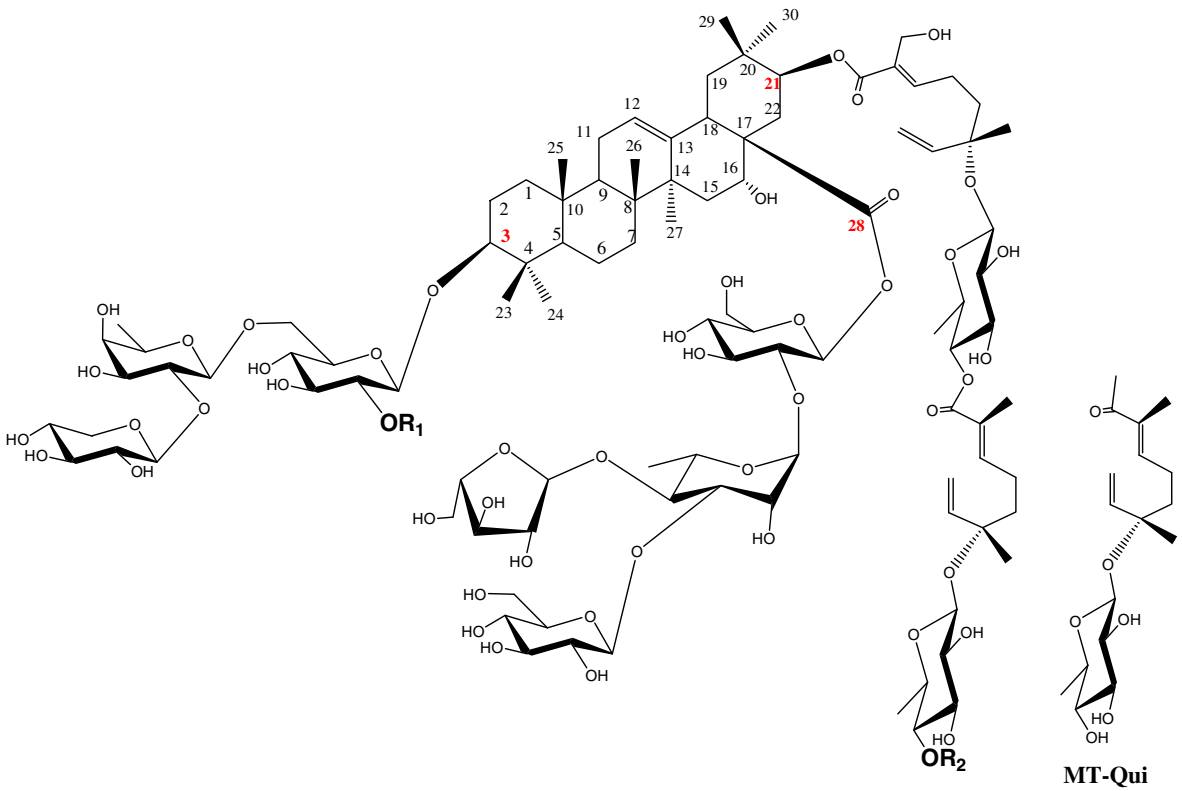
As part of our continuing search of new apoptosis-inducing triterpenoid saponins from Cameroonian *Albizia* genus [4, 5], we investigated the saponins content of the roots of *Albizia gummifera.* It is an umbrella-like crown tree about 50 m high of the legume family growing naturally in Nigeria, Cameroon, Democratic Republic of Congo, Angola, Ethiopia, Kenya, and Madagascar where plant parts are used in traditional medicine [6]. In Kenya a bark infusion is taken to treat malaria and the pounded bark is used as a snuff to treat headache, while the pounded roots are added to a bath to treat skin diseases, and an extract of crushed pods is drunk to treat stomach-ache. In eastern DR Congo a bark maceration is used as a body wash and drink to treat psoriasis. The roots and leaves are purgative and are used in Madagascar to treat diarrhoea and eye troubles. Its leaf decoction is reputed to have antitussive activity and is administered to treat asthma [6]. The *In vitro* antimalarial activity of the extracts of *Albizia gummifera* has also been reported [7]. Previous phytochemical studies reported the presence of triterpenoid saponins from stem

bark [8] and roots [9] of *A. gummifera*growing in Ethiopia and Madagascar, respectively.

In the present investigation of the roots of *A. gummifera* growing in Cameroon, we report the isolation and structural characterization of two new oleanane-type saponins, named gummiferaosides D–E (**1**–**2**), along with one known saponin, julibroside J_8 (**3**). The isolated saponins (**1**–**3**) were evaluated for their pro-apoptotic effect on the A431 human epidermoid cancer cell, results are reported herein.

Results and Discussion

The air-dried powdered roots of *A. gummifera* (300 g) were extracted with aq-EtOH 70% using a soxhlet apparatus. After evaporation of the solvent, the resulting brown residue was partitioned between water and water-saturated *n*-BuOH. The *n*-BuOH fraction was then submitted to vacuum-liquid chromatography (VLC) on reversed-phase silica gel yielding three main fractions which were subjected to VLC on silica gel. Purification of the eluated subfractions by Semprep-HPLC afforded two new triterpenoid saponins, named gummiferaosides D–E (**1**–**2**), together with known julibroside J_8 (**3**) (**Scheme 1)**.



	R_1	R_2
(**1**)	Glc	MT-Qui
(**2**)	H	MT-Qui
(**3**)	H	H

Scheme 1. Structures of the isolated saponins (**1**–**3**)

Gummiferaoside D (**1**) was obtained as a white, amorphous powder. Its positive HR-ESI-MS gave a pseudo-molecular ion peak at m/z 2663.2630 $[M + NH_4]^+$(calcd 2663.2552), consistent with a molecular formula of $C_{124}H_{196}O_{60}$. Upon acid hydrolysis with 2.0 M HCl, **1** gave an acacic acid lactone unit, identified with an authentic sample, together with glucose (Glc), xylose (Xyl), arabinofuranose (Araf), fucose (Fuc), rhamnose (Rha), and quinovose (Qui), which were identified by co-TLC with authentic samples. The absolute configuration of these sugar residues was determined to be D for Glc, Xyl, Qui, and Fuc, and L for Araf and Rha based on GC analysis of their trimethylsilyl thiazolidine derivatives [10]. ^{1}H-NMR spectrum of **1** showed seven angular methyl groups as singlets at δ (H) 0.99, 1.06, 1.07, 1.11,

1.18, 1.30, and 1.90 (each 3H, s), one olefinic proton at δ (H) 5.65 (1H, *brs*), and sugar proton signals at δ (H) 4.87–6.30. Its ^{13}C-NMR spectrum showed two olefinic carbon signals at δ (C) 122.8 and 143.1, suggesting that **1** was an oleanane type triterpenoid saponin. 1D (^{1}H-, ^{13}C-NMR, DEPT) and 2D (COSY, HSQC, NOESY, HSQC-TOCSY, and HMBC) NMR techniques permitted the unambiguous assignment of all ^{1}H- and ^{13}C-NMR signals of the aglycone of **1** (Table 1). This aglycon was thus recognized to be acacic acid (3*β*, 16*α*, 21*β*-trihydroxyolean-12-ene-28-oic acid) by comparison of its ^{1}H- and ^{13}C-NMR signals with those reported in the literature [11–20].

Table 1. *^{13}C-NMR (125 MHz) and ^{1}H-NMR (500 MHz) data of the Aglycone Part of Compounds **1**–**2** (in pyridine-d_5; δ in ppm)[a].*

DEPT	**1**		**2**	
	δ (C)	*δ* (H) (*multiplicity*)	*δ* (C)	*δ* (H) (*multiplicity*)
CH_2 (1)	38.6	1.61; 1.19	38.6	1.58; 1.19
CH_2 (2)	26.6	2.29; 1.96	26.6	1.93; 2.30
CH(3)	88.3	3.54	88.1	3.62
C (4)	39.4	-	39.4	-
CH(5)	55.8	0.99	55.7	0.99
CH_2 (6)	18.4	1.70; 1.48	18.4	1.71; 1.53
CH_2 (7)	33.4	1.70; nd	33.3	1.74; nd
C (8)	39.9	-	39.9	-
CH(9)	46.9	1.93	46.9	1.93
C (10)	36.8	-	36.8	-
CH_2 (11)	23.2	2.08; nd	23.3	2.08; nd
CH(12)	122.8	5.65 (*brs*)	122.8	5.64 (*brs*)
C (13)	143.1	-	144.9	-
C (14)	41.8	-	41.8	-
CH_2 (15)	35.7	2.27; 2.05	35.7	2.26; 2.05
CH(16)	73.6	5.25	73.6	5.24
C (17)	51.4	-	51.4	-
CH(18)	40.5	3.45	40.7	3.45
CH_2 (19)	47.6	2.98; 1.45	47.6	2.96; 1.42
C (20)	35.2	-	35.2	-
CH(21)	76.9	5.35	76.7	5.35
CH_2 (22)	36.2	2.75; 2.20	36.2	2.72; 2.19
CH_3 (23)	27.9	1.30 (*s*)	28.0	1.33 (*s*)
CH_3 (24)	16.7	1.18 (*s*)	17.1	1.18 (*s*)
CH_3 (25)	15.6	0.99 (*s*)	15.7	0.99 (*s*)
CH_3 (26)	17.1	1.07 (*s*)	16.9	1.05 (*s*)
CH_3 (27)	27.0	1.90s	27.0	1.89 (*s*)
C (28)	174.2	-	174.2	-
CH_3 (29)	28.9	1.06 (*s*)	28.9	1.05 (*s*)
CH_3 (30)	18.9	1.11 (*s*)	18.9	1.10 (*s*)

Assignments based on the HMBC, HSQC, COSY, HSQC-TOCSY, NOESY, and DEPT experiments. nd, not determined

[a] Overlapped proton NMR signals are reported without designated multiplicity.

Substitutions at C-3 and C-28 of acacic acid were evidenced by the observed glycosylation-induced shifts of C-3 at δ 88.3 and C-28 at δ 174.2. Substitution at C-21 was ascertained from the acylation-induced shift observed for C-21 at δ 76.9. All of these data established that **1** was a 21-acyl 3, 28-bidesmosidic acacic acid derivative; sugar chains being linked to C-3 and C-28 through an ether and ester bond, respectively.

The ^{1}H-NMR spectrum of **1** showed eleven anomeric protons at δ (H) 4.91 [d, *J* = 7.7 Hz, glucose (GlcI)], 4.99 [d, *J* = 8.1 Hz, fucose (Fuc)], 5.45 [d, *J* = 8.0 Hz, glucose (GlcII)], 5.08 [d, *J* = 7.3 Hz, xylose (Xyl)], 6.09 [d, *J* = 8.1 Hz, glucose (GlcIII)], 5.89 [brs, rhamnose (Rha)], 5.36 [d, *J* = 7.7 Hz, glucose (GlcIV)], 6.30 [d, *J* = 6.8 Hz, arabinofuranose (Ara_f)], 4.90 [d, *J* = 7.7 Hz, quinovose (QuiI)], 4.87 [d, *J* = 7.7 Hz, quinovose (QuiII)], and 4.96 [d, *J* = 8.1 Hz, quinovose (QuiIII)], which correlated with eleven anomeric carbon atom signalsat δ (C) 104.7, 103.2, 105.5, 106.6, 95.5, 101.8, 105.6, 110.8, 99.1, 99.1, and 96.7, respectively, in the HSQC spectrum. The ^{1}H- and ^{13}C-NMR data (Tables 2-3) of the monosaccharide residues were assigned starting, either from the readily identifiable anomeric proton of each hexosyl or pentosyl unit, or from the CH_3-proton doublet of each 6-deoxyhexosyl unit, by means of COSY, HSQC-TOCSY, HSQC, NOESY and HMBC spectra. Data indicated that these eleven sugar residues were in their pyranose form. Based on the coupling constants of the anomeric protons and the chemical shifts of the anomeric carbons, the anomeric configuration of the sugar moieties were determined as *β* for the glucose, xylose, fucose, and quinovose moieties and *α* for the rhamnose and arabinofuranose moieties.

Table 2. *^{13}C-NMR (125 MHz) and ^{1}H-NMR (500 MHz) Data of the Sugar Moieties Attached at C-3 and C-28 of Compounds **1**–**2**(in pyridine-d_5; δ in ppm)[a].*

Position	DEPT	1		2	
		δ (C)	δ (H) (*J* in Hz)	δ (C)	δ (H) (*J* in Hz)
3-O-sugars					
Glcl			````		
	CH (1)	104.7	4.91 (*d, J= 7.7*)	106.7	4.93 (*d, J= 7.7*)
	CH (2)	82.5	4.27	75.8	4.06
	CH (3)	78.3	4.15	78.3	4.19
	CH (4)	71.6	4.05	71.6	4.18
	CH (5)	76.3	4.08	77.1	4.10
	CH_2 (6)	69.9	4.74; 4.36	69.8	4.77; 4.39
Fuc					
	CH (1)	103.2	4.99 (*d, J= 8.1*)	103.3	5.03 (*d, J= 7.5*)
	CH (2)	81.8	4.46	82.0	4.47
	CH (3)	75.0	4.10	75.0	4.04
	CH (4)	71.9	3.97	71.3	4.16
	CH (5)	70.6	3.69	71.4	3.77
	CH_3 (6)	18.0	1.37 (*d, J= 6.6*)	17.2	1.51 (*d, J= 6.6*)
Glc II					
	CH (1)	105.5	5.45 (*d, J= 8.0*)		
	CH (2)	75.4	4.02		
	CH (3)	77.3	4.15		
	CH (4)	71.1	3.79		
	CH (5)	77.0	3.75		
	CH_2 (6)	62.5	4.44; 4.30		
Xyl					
	CH (1)	106.6	5.08 (*d, J= 7.3*)	106.9	5.10 (*d, J= 7.0*)
	CH (2)	75.4	4.05	75.8	4.04
	CH (3)	78.1	4.00	78.2	4.08
	CH (4)	70.7	4.10	70.6	4.18
	CH_2 (5)	67.0	4.48; 3.60	67.0	4.49; 3.61
28-O-sugars					
Glc III					
	CH (1)	95.5	6.09 (*d, J= 8.1*)	95.6	6.08 (*d, J= 7.9*)
	CH (2)	76.0	4.05	76.8	4.09
	CH (3)	78.1	4.16	77.2	4.16
	CH (4)	75.1	4.10	71.0	4.18
	CH (5)	79.2	3.96	78.5	3.98
	CH_2 (6)	62.5	4.50; 4.29	61.6	4.35; 4.18
Rha					
	CH (1)	101.8	5.89 (*brs*)	101.7	5.91 (*brs*)
	CH (2)	70.5	5.21	70.4	5.19
	CH (3)	81.8	4.96	82.0	4.92
	CH (4)	79.2	4.51	79.0	4.50
	CH (5)	69.1	4.58	69.0	4.57
	CH_3 (6)	18.4	1.63 (*d, J= 6.5*)	18.9	1.78 (*d, J= 5.6*)
Glc VI					
	CH (1)	105.6	5.36 (*d, J= 7.7*)	105.7	5.36 (*d, J= 7.0*)
	CH (2)	75.2	4.00	75.3	3.99
	CH (3)	77.4	4.15	78.1	4.23
	CH (4)	71.0	4.12	71.6	4.09
	CH (5)	78.2	3.92	78.4	3.99
	CH_2 (6)	62.3	4.50; 4.28	62.5	4.51; 4.19
Araf					
	CH (1)	110.8	6.30 (*d, J= 6.8*)	111.0	6.29 (*d, J= 7.7*)
	CH (2)	84.3	5.01	84.3	5.00
	CH (3)	78.2	4.83	78.3	4.82
	CH (4)	85.2	4.77	85.2	4.76
	CH_2 (5)	61.7	4.27; 4.18	62.3	4.27; 4.17

Assignments were based on the HMBC, HSQC, COSY, TOCSY, NOESY, and DEPT experiments. nd, not determined. [a)]Overlapped proton NMR signals are reported without designated multiplicity.

Table 3. *^{13}C-NMR (125 MHz) and ^{1}H-NMR (500 MHz) Data of the Quinovosyl Moieties attached at C-21 of compounds **1**–**2** (in pyridine-d_5; δ in ppm)[a].*

Quinovose (Qui)	DEPT	**1**		**2**	
		δ (C)	δ (H) (*J* in Hz)	δ (C)	δ (H) (*J* in Hz)
QuiI					
	CH (1)	99.1	4.90 (*d, J = 7.7*)	99.1	4.90 (*d, J = 7.7*)
	CH (2)	75.4	4.02	75.4	4.03
	CH (3)	76.2	4.21	76.2	4.21
	CH (4)	77.3	5.36	77.1	5.35
	CH (5)	70.2	3.70	70.2	3.70
	CH_3 (6)	18.7	1.37 (*d, J = 6.5*)	18.7	1.37 (*d, J = 6.6*)
QuiII					
	CH (1)	99.1	4.87 (*d, J = 7.7*)	99.1	4.86 (*d, J = 7.7*)
	CH (2)	75.5	4.02	75.5	4.03
	CH (3)	76.2	4.23	76.2	4.21
	CH (4)	77.4	5.37	77.4	5.37
	CH (5)	72.6	3.75	72.6	3.75
	CH_3 (6)	18.9	1.63 (*d, J = 6.5*)	18.9	1.62 (*d, J = 6.2*)
QuiIII					
	CH (1)	96.7	4.96 (*d, J = 8.1*)	96.7	4.97 (*d, J = 7.9*)
	CH (2)	75.6	4.08	75.6	4.02
	CH (3)	75.7	4.23	75.8	4.24
	CH (4)	76.9	3.75	76.9	3.75
	CH (5)	72.4	3.73	72.4	3.73
	CH_3 (6)	18.7	1.60 (*d, J = 6.5*)	18.7	1.60 (*d, J = 6.2*)

Assignments based on the HMBC, HSQC, COSY, TOCSY, NOESY, and DEPT experiments. nd, not determined. [a)]Overlapped proton NMR signals are reported without designated multiplicity

In addition, the ^{1}H-NMR spectrum of compound **1** exhibited three olefinic proton signals at δ (H) 7.06 (d, *J* = 7.3 Hz), 7.00 (d, *J* = 7.7 Hz), and 7.06 (d, *J* = 7.3 Hz), and three groups of one-substituted olefinic proton signals, one group at δ (H) 6.24 (dd, *J* = 11.1; 17.6Hz), 5.24 (d, *J* = 11.1 Hz) and 5.44 (d, *J* = 17.6 Hz), another group at δ (H) 6.27 (dd, *J* = 11.1; 17.6 Hz), 5.25 (d, *J* = 11.1 Hz) and 5.48 (d, *J* = 17.6 Hz), and the last group at δ (H) 6.21 (dd, *J* = 11.1; 17.9 Hz), 5.30 (d, *J* = 11.1 Hz) and 5.38 (d, *J* = 17.9 Hz), indicating that compound **1** had three monoterpenoid units [16] (Table 4).

Table 4. *^{13}C-NMR (125 MHz) and ^{1}H-NMR (500 MHz) Data of the Monoterpene Moieties Attached at C-21 of Compounds **1**–**2** (in pyridine-d_5; δ in ppm)[a].*

Monoterpene (MT)	DEPT	**1**		**2**	
		δ (C)	δ (H) (*J* in Hz)	δ (C)	δ (H) (*J* in Hz)
MT_1					
	C (1)	167.5	-	167.4	-
	C (2)	133.6	-	128.6	-
	CH (3)	142.6	7.06 (*t, J= 7.3*)	142.6	7.05 (*t, J= 7.7*)
	CH_2 (4)	23.5	2.46; nd	23.5	2.68; 2.45
	CH_2 (5)	40.8	1.78; nd	40.3	1.82; 1.73
	C (6)	79.2	-	79.2	-
	CH(7)	145.0	6.24 (*dd, J= 11.1; 17.6*)	144.9	6.24 (*dd, J= 11.2; 17.8*)
	CH_2 (8)	114.7	5.44 (*d, J= 17.6*); 5.24 (*d, J= 11.1*)	114.7	5.44 (*d, J= 17.8*); 5.25 (*d, J= 11.2*)
	CH_2 (9)	56.0	4.74 (*brs*)	56.0	4.72 (*brs*)
	CH_3 (10)	23.6	1.51(*s*)	23.6	1.51 (*s*)
MT_2					
	C (1)	167.3	-	167.3	-
	C (2)	127.9	-	127.9	-
	CH (3)	142.8	7.00 (*t, J= 7.7*)	142.7	7.00 (*t, J= 7.7*)
	CH_2 (4)	23.6	2.45; nd	23.7	2.68; 2.45
	CH_2 (5)	40.6	1.76; nd	40.6	1.82; 1.73
	C (6)	79.3	-	79.3	-
	CH(7)	143.8	6.27 (*dd, J= 11.1; 17.6*)	143.8	6.22 (*dd, J= 11.1; 17.8*)
	CH_2 (8)	115.0	5.43 (*d, J= 17.6*); 5.25 (*d, J= 11.1*)	114.9	5.42 (*d, J= 17.8*); 5.29 (*d, J= 11.1*)
	CH_3 (9)	12.5	1.90 (*s*)	12.5	1.92 (*s*)
	CH_3 (10)	23.6	1.54 (*s*)	23.7	1.54 (*s*)
MT_3					
	C (1)	167.0	-	167.0	-
	C (2)	127.7	-	127.9	-
	CH (3)	143.0	7.06 (*t, J= 7.3*)	143.0	7.07 (*t, J= 7.3*)
	CH_2 (4)	23.5	2.45; nd	23.6	2.66; 2.44
	CH_2 (5)	40.3	1.80; nd	40.8	1.83; 1.73
	C (6)	79.5	-	79.5	-
	CH(7)	143.6	6.21(*dd, J= 11.1; 17.9*)	143.7	6.00 (*dd, J= 6.9; 17.4*)
	CH_2 (8)	115.3	5.38 (*d, J= 17.9*); 5.30 (*d, J= 11.1*)	115.3	5.42 (*d, J= 17.4*); 5.29 (*d, J= 6.9*)
	CH_3 (9)	12.5	1.96 (*s*)	12.5	1.95 (*s*)
	CH_3 (10)	23.6	1.58 (*s*)	23.4	1.57 (*s*)

Assignments based on the HMBC, HSQC, COSY, TOCSY, NOESY, and DEPT experiments. nd, not determined.
[a)]Overlapped proton NMR signals are reported without designated multiplicity

Extensive analysis of 1D and 2D NMR spectra of **1**, revealed that the sugar moiety at C-3 was identical to that of albizoside B [12], julibroside J_{31} [15], albizoside D [16], and julibroside J_{32} [17], and that the sugar moiety at C-28 was identical to that of julibrosides J_{16} and J_{17} [13], J_{14} and J_{28} [14], J_{32}, J_{35}, and J_{36} [15], adianthifoliosides A–F [18,19], and coriarioside A [20]. Hence, the units attached at C-3 and C-28 of the aglycon of **1** were establishedas *β*-D-xylopyranosyl-(1→2)-*β*-D-fucopyranosyl-

(1→6)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranoside, and α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside, respectively. By comparing the ^{13}C NMR data of **1** with those published in the literature for related compounds, it was found that the ^{13}C NMR data of **1** were almost superimposable with those of albizoside D [16] and julibroside J_{36} [17], except for the signals of the monoterpene-quinovosyl moiety. Indeed, the difference was the observation of the signals of one additional monoterpene-quinovosyl moiety in compound **1** in comparison to albizoside D [16] and julibroside J_{36} [17]. This was supported by the mass spectrum of **1**, which displayed 312 mass units more than that of albizoside D [16] and julibroside J_{36} [17], accounting for the mass of one quinovose and one monoterpene unit. In addition, the monoterpene-quinovosyl moiety of **1**, differs from that of zygiaoside A [5], gummiferaoside C [9], lebbeckoside A [11], coriariosides A [20], C and D [21], only by the additional presence of one hydroxyl group attached to C-9 of the monoterpenoid unit directly attached to C-21 of the aglycon as evidenced by the signals of C-2 (δ 133.6) and of C-9 (δ 56.0) of a 9-hydroxy-monoterpenoid unit. Furthermore, the ^{13}C NMR data of compound **1** showed that the signals of C-5 and C-10 of the monoterpene moieties in **1** were quite similar to those of the outer monoterpene moiety of albizoside C [12] and of Julibroside III [14] indicating the (*S*) absolute configuration at C-6 of the three monoterpene moieties. Extensive analysis of 1D and 2D NMR spectra of **1** allowed to identify the monoterpene-quinovosyl moiety attached at C-21 of **1** as {(2*E*,6*S*)-2-hydroxymethyl-6-methyl-6-*O*-{4-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-*O*-(β-D-quinovopyranosyl)octa-2,7-dienoyl]-4-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-*O*-(β-D-quinovopyranosyl)octa-2,7-dienoyl]-β-D-quinovopyranosyl}-2,6-dimethylocta-2,7-dienoyl}. Accordingly, the structure of gummiferaoside D (**1**) was elucidated as 3-*O*-[

β-D-xylopyranosyl-(1→2)-*β*-D-fucopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-glucopyranosyl]-21-*O*-{(2*E*,6*S*)-2-hydroxymethyl-6-methyl-6-*O*-{4-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-*O*-(*β*-D-quinovopyranosyl)octa-2,7-dienoyl]-4-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-*O*-(*β*-D-quinovopyranosyl)octa-2,7-dienoyl]-*β*-D-quinovopyranosyl}-2,6-dimethylocta-2,7-dienoyl}acacic acid 28-*O*- *α*-L-arabinofuranosyl-(1→4)-[*β*-D-glucopyranosyl-(1→3)]-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester.

Gummiferaoside E (**2**), obtained as a white amorphous powder, exhibited in its HRESIMS spectrum (positive-ion mode) a pseudo-molecular ion peak at m/z 2502.1972 $[M + NH_4]^+$ (calcd 2502.2135), consistent with a molecular formula of $C_{118}H_{186}O_{55}$. As already observed for **1**, acid hydrolysis of **2** also afforded an acacic acid lactone unit, identified by co-TLC with authentic sample, together with D-glucose, D-xylose, D-fucose, D-quinovose, L-arabinofuranose and L-rhamnose units which were identified by GC analysis of their trimethylsilyl thiazolidine derivatives (Experimental Section). Comparison of 1D and 2D NMR data of **2** with those of **1** indicated that **2** had also acacic acid as aglycon (Table 1).The observation of glycosylation- and acylation-induced shifts at δ (C) 88.1 (C-3 of aglycon), 76.7 (C-21 of aglycon), and 174.2 (C-28 of aglycon) in the ^{13}C-NMR spectrum of **2** suggested that it should be also a 21-acyl 3,28-bidesmosidic acacic acid derivative with sugar chains linked to C-3 and C-28 through an ether and ester bond, respectively, and with an acyl group attached at C-21. The ^{1}H-NMR spectrum of **2** showed ten anomeric protons signals at δ (H) 4.93 [d, *J* = 7.7 Hz, glucose (GlcI)], 5.03 [d, *J* = 7.5 Hz, fucose (Fuc)], 5.10 [d, *J* = 7.0 Hz, xylose (Xyl)], 6.08 [d, *J* = 7.9 Hz, glucose (GlcII)], 5.91 [brs, rhamnose (Rha)], 5.36 [d, *J* = 7.0 Hz, glucose (GlcIII)], 6.29 [d, *J* = 7.7 Hz, arabinofuranose (Araf)], 4.90 [d, *J* = 7.7 Hz, quinovose (QuiI)], 4.86 [d, *J* = 7.7 Hz, quinovose (QuiII)], and 4.97 [d, *J* = 7.9 Hz, quinovose (QuiIII)], which

correlated with ten anomeric carbon atom resonances at δ (C) 106.5, 103.1, 106.9, 95.6, 101.7, 105.7, 111.0, 99.1, 99.1, and 96.7, respectively in the HSQC spectrum (Tables 2-3). Comparison of their NMR data revealed that the units attached at C-21 and C-28 of the aglycon of **2** were identical to those of **1**, and the two saponins differ only by the sugar chain attached at C-3 of the aglycon, as suggested by the mass spectrum of **2**, which displayed 162 mass units less than that of **1**, accounting for the mass of one hexose unit, assigned to one glucopyranosyl moiety to account for the similarities between their ^{1}H and ^{13}C NMR spectra.Therefore, the moieties at C-21 and C-28 of **2** were established as {(2*E*,6*S*)-2-hydroxymethyl-6-methyl-6-*O*-{4-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-*O*-(*β*-D-quinovopyranosyl)octa-2,7-dienoyl]-4-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-*O*-(*β*-D-quinovopyranosyl)octa-2,7-dienoyl]-*β*-D-quinovopyranosyl}-2,6-dimethylocta-2,7-dienoyl}and*α*-L-arabinofuranosyl-(1→4)-[*β*-D-glucopyranosyl-(1→3)]-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside moiety, respectively. Extensive analysis of 1D and 2D NMR spectra of **2** allowed us to identify the unit attached at C-3 as *β*-D-xylopyranosyl-(1→2)-*β*-D-fucopyranosyl-(1→6)-*β*-D-glucopyranoside, a unit identical to that of lebbeckosides A and B [11]. Consequently, the structure of gummiferaoside E (**2**) was established as 3-*O*-[*β*-D-xylopyranosyl-(1→2)-*β*-D-fucopyranosyl-(1→6)-*β*-D-glucopyranosyl]-21-*O*-{(2*E*,6*S*)-2-hydroxymethyl-6-methyl-6-*O*-{4-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-*O*-(*β*-D-quinovopyranosyl)octa-2,7-dienoyl]-4-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-*O*-(*β*-D-quinovopyranosyl)octa-2,7-dienoyl]-*β*-D-quinovopyranosyl}-2,6-dimethylocta-2,7-dienoyl}acacic acid 28-*O*-*α*-L-arabinofuranosyl-(1→4)-[*β*-D-glucopyranosyl-(1→3)]-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester.

Compound **3**, isolated as an amorphous powder, was identified as 3-*O*-[*β*-D-xylopyranosyl-(1→2)-*β*-D-fucopyranosyl-(1→6)-*β*-D-glucopyranosyl]-21-*O*-{(2*E*,6*S*)-2-hydroxymethyl-6-methyl-6-*O*-{4-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-*O*-(*β*-D-quinovopyranosyl)octa-2,7-dienoyl]-4-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-*O*-(*β*-D-quinovopyranosyl)octa-2,7-dienoyl} acacic acid 28-*O*-*α*-L-arabinofuranosyl-(1→4)-[*β*-D-glucopyranosyl-(1→3)]-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester, named Julibroside J_8 by comparison of its spectra data with those reported in the literature [22].

The pro-apoptotic activity of compounds **1**-**3** was evaluated using Annexin V-FITC binding assay on the A431 human epidermoid cancer cell. In result, percentage of apoptotic cells following 24 h of treatment was increased in a concentration-dependent manner for A431 cell (Figure1). Compounds **2** and **3** displayed the most promising proapoptotic activity since computed concentration of each compound to induced half-maximal effects (EC_{50}) on cell apoptosis were determined as being 158 µM, 13 µM, and 23 µM for compounds **1**-**3**, respectively.

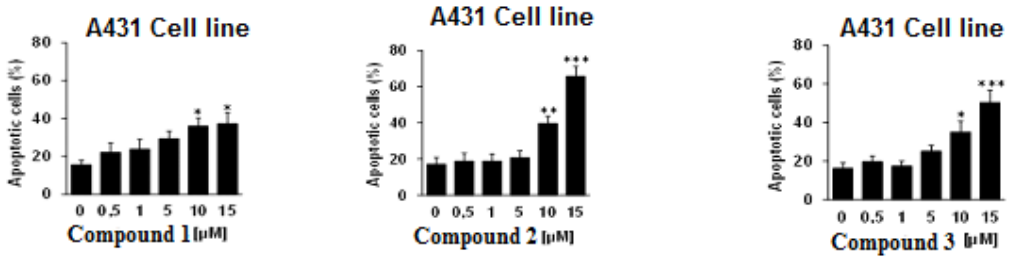


Figure 1. Effects of compounds **1- 3** on the apoptosis rate.
Cells were exposed to compounds at the indicated concentrations and incubated for 24h. Cell apoptosis rate was assessed by flow cytometry using the Annexin V-FITC/PI staining assay (n=3).
**P*<0.05 and ****P*<0.001 *vs.* vehicle-treated cells.

Conclusions

This study unveiled the presence, in the roots of *Albizia gummifera* growing in Cameroon, of two new acacic acid-type saponins, among which one molecule, gummiferaoside E (**2**), having a promising proapoptotic function towards the A431 human epidermoid cancer cell. It is worthy to note that the proapoptotic function of acacic acid-type saponins from *Albizia* genus had been, so far, only reported for adianthifoliosides A and D [3] and zygiaosides A and B [4]. Our study confirms that acacic acid, oligosaccharide moieties at C-3 and C-28 and an acyl group at C-21 constitute a pharmacophore compatible with proapoptotic function. Interestingly, substitution of C-2 of GlcI seems to inhibit proapoptotic function since compound **1** (EC_{50} 158 µM), having a tetrasaccharide moiety at C-3 of aglycone, with a substituted GlcI at its C-2 position, is less active than compound **2** (EC_{50}13 µM), which has a linear trisaccharide moiety at the same position of the aglycone. Nevertheless, further studies are necessary to support this conclusion. Gummiferaoside E (**2**), being the most active compound, might therefore be highly useful to further decipher its mechanism of action and to obtain new structure-activity relationship data of the related triterpenoid saponins towards the A431 human epidermoid cancer cell. It will be also of high interest to evaluate the effect of Gummiferaoside E (**2**) on other types of cancer cells.

Experimental Section

General

Optical rotations were measured on a Jasco P-2000 polarimeter. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded at room temperature in pyridine-d_5 using a Bruker 500 MHz spectrometer. Chemical shifts are given in δ

(ppm) value relative to TMS as internal standard. HRESIMS spectra were recorded on a microTOF ESI-TOF mass spectrometer (Agilent) operating in positive mode. Analytical HPLC was performed on Varian 920-LC apparatus equipped with an autosampler, a pump, a diode array detector (DAD), and Galaxie software. Semipreparative HPLC was performed on a Gilson apparatus equipped with Trilution LC software using a Nucleodur 100-5 C18ec (21 x 250 mm, 5 µm) column purchased from Machery-Nagel (Germany). Thin layer chromatography (TLC) was performed on precoated silica gel plates (60 F_{254}, Merck) using the system solvent *n*-BuOH-AcOH-H_2O, 65:15:25 as eluent. The spots were observed after spray with Komarowsky reagent, a (5:1) mixture of *p*-hydroxybenzaldehyde (2% in MeOH) and (Ethanolic H_2SO_4 50%). Vacuum-liquid chromatography (VLC) was carried out using RP-18 silica gel 60 (25-40 µm) and silica gel 60 (15-40 µm and 40-63 µm).

Plant material.

The roots of *Albizia gummifera* were harvested at Nkolbisson, Yaoundé peripheral quarter, in Cameroon in November 2013 under the guidance of Mr. Victor Nana, botanist of the National Herbarium of Cameroon (NHC), where a voucher specimen (31315/HNC) was deposited.

Extraction and isolation.

Air-dried and finely powdered roots of *A. gummifera* (300 g) were extracted with 70% EtOH in soxhlet apparatus. The hydroalcoholic solution was then evaporated to dryness under reduced pressure to yield a brown residue (6.80 g). The residue was suspended in 200 mL of water and partitioned between H_2O and satured *n*-BuOH (3x 300 mL). The *n*-BuOH phase was evaporated to dryness affording 4.90 g of a

brown gum which was taken in a minimum amount of water (10 mL) and then submitted to vacuum-liquid chromatography (VLC) using RP-18 (25-40 μm) eluting with H_2O, 50% MeOH, and 100% MeOH. The 100% MeOH extract was evaporated to dryness affording 3.90 g crude saponin mixture that was then submitted to VLC using silica gel 60 (15-40 μm), eluted with $CHCl_3$-MeOH-H_2O (80:20:2, 70:30:5, 60:33:7, and 60:40:10) to give three main fractions (G1- G3). Fraction G2 (700.1 mg) was subjected to VLC on silica gel 60 (15-40 μm), eluted with $CHCl_3$-MeOH-H_2O (80:20:2, 70:30:5, 60:33:7, and 60:40:10) affording three main subfractions (G21-G23). Subfraction G23 (175.4 mg) was purified by semipreparative HPLC using gradient system of CH_3CN-H_2O (20 ml/min) to yield compounds **1** (t_R, 9.30 min, 5.8 mg) and **2** (t_R, 17.10 min, 6.1 mg), while subfraction G21 (130.5 mg), purified in the same conditions, yielded compound**3** (t_R, 14.97 min, 6.7 mg).

Gummiferaoside D (= 3-*O*-[*β*-D-xylopyranosyl-(1→2)-*β*-D-fucopyranosyl-(1→6)-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-glucopyranosyl]-21-*O*-{(2*E*,6*S*)-6-*O*-{4-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-*O*-(*β*-D-quinovopyranosyl)octa-2,7-dienoyl]-4-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-*O*-(*β*-D-quinovopyranosyl)octa-2,7-dienoyl]-*β*-D-quinovopyranosyl}-2,6-dimethylocta-2,7-dienoyl}acacic acid 28-*O*- *α*-L-arabinofuranosyl-(1→4)-[*β*-D-glucopyranosyl-(1→3)]-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester (**1**). White amorphous powder.^{1}H- and ^{13}C-NMR (*pyridine-d_5*): for aglycon, sugars and monoterpenoid moieties, see Tables 1-4. Positive HR-ESI-MS: m/z 2663.2630 [M + NH_4]$^+$ (calcd 2663.2552) *Gummiferaoside E* (=3-*O*-[*β*-D-xylopyranosyl-(1→2)-*β*-D-fucopyranosyl-(1→6)-*β*-D-glucopyranosyl]-21-*O*-{(2*E*,6*S*)-6-*O*-{4-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-*O*-(*β*-D-quinovopyranosyl)octa-2,7-dienoyl]-4-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-*O*-(*β*-D-quinovopyranosyl)octa-2,7-dienoyl]-*β*-D-quinovopyranosyl}-2,6-dimethylocta-2,7-dienoyl} acacic acid 28-*O*-*α*-L-arabinofuranosyl-(1→4)-[*β*-D-glucopyranosyl-

(1→3)]-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranosyl ester (**2**).White amorphous powder.^{1}H- and^{13}C-NMR (*pyridine-d_5*): for aglycon, sugars and monoterpenoid moieties, see Tables 1-4. Positive HR-ESI-MS: m/z 2502.1972 $[M + NH_4]^+$ (calcd 2502.2135)

Acidic hydrolysis of isolated saponins.

Each saponin (2 mg) was hydrolyzed with 2 ml of 2 M HCl at 85 °C during 2 h. After cooling, the solvent was removed under reduced pressure. The sugar mixture was extracted from the aqueous phase (10 ml) and washed with CH_2Cl_2 (3x 5 ml). Combined CH_2Cl_2 extracts were washed with water to give after evaporation the aglycon moiety, which was compared with authentic standard sample by Co-TLC. The sugars were first analyzed by TLC over silica gel ($CHCl_3$–MeOH–H_2O, 8:5:1) by comparison with standard samples. The absolute configuration of each monosaccharide was determined from GC–MS analysis of their trimethylsilylated derivatives by comparison with authentic samples using the method previously described [10]. The following sugars were detected: D-glucose, D-xylose, D-fucose, D-quinovose, L-arabinofuranose and L-rhamnose.

Cell lines and culture conditions.

The A431 human epidermoid cancer cell, was purchased from ATCC (LGC Standards, Molsheim, France), and cultivated in DMEM-based media (Sigma-Aldrich, Saint-Quentin-Fallavier, France), supplemented with 10% (v/v) fetal bovine serum (BioWhittaker, Verviers, Belgium), 2 mMultraglutamine, 50 µM non-essential amino acids, 50 U/ml penicillin and 50 µg/ml streptomycin (Sigma-Aldrich). The culture was

kept at 37°C in a humidified incubator equilibrated with 5% CO_2. Before confluency adherent cells were trypsinized and subcultured twice a week.

Pro-apoptotic evaluation.

Cells were treated with the three different compounds (**1**–**3**) in ranging concentrations (1 to 15µM) then collected for apoptosis induction estimation. A minimum of 5,000 cells was acquired per sample and analyzed on the InCyte software (Guava/Millipore/Merck, CA, USA). Apoptosis rates were assessed by capillary cytometry (Guava EasyCyte Plus, Millipore Merck) using Annexin V-FITC (ImmunoTools, Germany) and PI (MiltenyiBiotec Inc., USA) according to the manufacturer's recommendations. Gates were drawn around the appropriate cell population using a forward scatter (FSC) versus side scatter (SSC) acquisition dot plot to exclude debris. Final concentration of DMSO applied to cells during incubation with tested drugs was always 0.5%. In the tested setup that concentration had no adverse effects on cell viability, nor cell morphology. To discriminate between negative and positive events in the analysis, a non-stained control sample from each culture condition always accompanied acquisition of the stained cells to define their cut off. Negative control, i.e. sample with cells without compounds but with the same amount of DMSO as for diluted compounds, as well as positive control with 50µM Celastrol, a natural pentacyclic triterpenoid (Enzo Life Sciences, Farmingdale, US), were included in each experiment. Cytometers performances are checked weekly using the Guava easy Check Kit 4500-0025 (Merck/Millipore/Guava Hayward, CA, USA).

Statistical analysis

Data, presented as bar graphs, were expressed as means ± S.E.M. of at least three independent experiments. Statistical evaluation was performed with the one-way ANOVA test followed by the post-hoc Bonferroni test using GraphPad Prism software (Prism version 5.04 for Windows, GraphPad Software, CA, USA); a *p*-value less than 0.05 was considered as significant (*), less than 0.01 very significant (**) and less than 0.001 highly significant (***).

Acknowledgements

We thank the *World Academy of sciences* (*TWAS*) (grant no: 3240277733) for financial support of this work. The authors are also grateful to Mr. Victor Nana of the *National Herbarium of Cameroon* (NHC) for the identification and collection of plant.

Author Contribution Statement

Conceived and designed the experiments: *Line Made Simo, Olivier Placide Noté, Joséphine Ngo Mbing,* Sarah Ali Aouazou

Performed the experiments: *Line Made Simo, Olivier Placide Noté,* Sarah Ali Aouazou

Analyzed the data: *Line Made Simo, Olivier Placide Noté, Joséphine Ngo Mbing*, Sarah Ali Aouazou

Wrote the paper: *Line Made Simo, Olivier Placide Noté, Joséphine Ngo Mbing,* Sarah Ali Aouazou

Revised the article for intellectual content: *Dominique Guillaume*, *Christian Dominique Muller*, *Dieudonné Emmanuel Pegnyemb*, and *Annelise Lobstein*

Final approval of the version to be published: *Dominique Guillaume*, *Christian Dominique Muller*, *Dieudonné Emmanuel Pegnyemb*, and *Annelise Lobstein*

References

[1]. M. Abdel-Kader, J. Hoch, J.M. Berger, R. Evans, J.S. Miller, J.H. Wisse, S.W. Mamber, J.M. Dalton, D.G. Kingston, Two bioactive saponins from *Albizia subdimidiata* from the Suriname rainforest, *J. Nat. Prod*. **2001**, *64*, 536–539.

[2]. M.A. Lacaille-Dubois, D.E. Pegnyemb, O.P. Noté, A.C. Mitaine-Offer, A review of acacic acid-type saponins from Leguminosae-Mimosoideae as potent cytotoxic and apoptosis inducing agents, *Phytochem Rev.* **2011**, *10*, 565–584.

[3]. M. Haddad, V. Laurens, M.A. Lacaille-Dubois, Induction of apoptosis in a leukemia cell line by triterpene saponins from *Albizia adianthifolia*, *Bioorg. Med. Chem.* **2004,***12,*4725–4734.

[4]. O.P. Noté, L. Simo, J.N. Mbing, D. Guillaume, S.A. Aouazou, C.D. Muller, D.E. Pegnyemb, A. Lobstein, Two new triterpenoid saponins from the roots of *Albizia zygia* (DC.) J.F. Macbr, *Phytochem. Lett*. **2016**, *18*, 128–135.

[5]. O.P. Noté, S.A. Azouaou, L. Simo, C. Antheaume, D. Guillaume, D.E. Pegnyemb, C.D. Muller, A. Lobstein*,* Phenotype-specific apoptosis induced by three new triterpenoid saponins from *Albizia glaberrima* (Schumach. & Thonn.) Benth. *Fitoperapia,* **2016**, *109*, 80–86.

[6]. M. Arbonnier, in Arbres, arbustes et lianes des zones d'Afrique de l'Ouest. Ed. Quæ MNHN, Paris, **2009**, p 383.

[7]. A.V. Ofulla, G.M. Chege, G.M. Rukunga, F.K. Kiarie, J.I. Githure, M.W. Kofi-Tsekpo, In vitro antimalarial activity of extracts of *Albizia gummifera*, *Aspilia mossambicensis, Melia azedarach* and *Azadirachta indica* against *Plasmodium falciparum.Afr. J. Health Sci.* **1995**, *2,*309–311.

[8]. A. Debella, E. Haslinger, M.G. Schmid, F. Bucar, G. Michl, D. Abebe, O. KunertTriterpenoid saponins and sapogenin lactones from *Albizia gummifera*, *Phytochemistry* **2000**, *53*, 885–892.

[9]. S. Cao, A. Norris, J.S. Miller, F. Ratovoson, J. Razafitsalama, R. Andriantsiferana, V.E. Rasmison, K. Tendyke, T. Suh, D.G.I. Kingston, Cytotoxic triterpenoid saponins of *Albizia gummifera* from the rain forest. *J. Nat. Prod.* **2007**, *70*, 361–366.

[10]. M. Chaabi, P. Chabert, C. Vonthron-Sénécheau, B. Weniger, M. Ouattara, H.Corstjens, I. Sente, L. Declercq, A. Lobstein, Acylated flavonol pentaglycosides from *Baphianitida* leaves, *Phytochem. Lett*. **2010**, *3*, 70–74.

[11]. O.P. Noté, D. Jihu, C. Antheaume, M. Zeniou, D.E. Pegnyemb, D. Guillaume, H. Chneiwess, M.C. Kilhoffer, A. Lobstein, Triterpenoid saponins from *Albizia lebbeck* (L.) Benth and their inhibitory effect on the survival of high grade human brain tumor cells, *Carbohydr. Res*. **2015**, *404*, 26–33.

[12]. R. Liu, S. Ma, S. Yu, Y. Pei, S. Zhang, X. Chen, J. Zhang, Cytotoxic oleanane triterpene saponins from *Albizia chinensis*, *J. Nat. Prod.* **2009**, *72*, 632–639.

[13]. K. Zou, Q.Y. Zhang, B. Wang, J.R. Cui, Y.Y. Zhao, R.Y. Zhang, Cytotoxic triterpernoid saponins acetylated with monoterpenoid acid from *Albizia julibrissin*, *Helv. Chim. Acta*. **2010**, *93*, 2100–2106.

[14]. H. Liang, W.Y. Tong, Y.Y. Zhao, J.R. Cui, G.U. Tu, An antitumor compound julibroside J_{28} from *Albizia julibrissin*, *Bioorg.Med. Chem. Lett.* **2005**, *15*, 4493–4495.

[15]. L. Zheng, J. Zheng, Y. Zhao, B. Wang, L. Wu, H. Liang, Three anti-tumor saponins from *Albizia julibrissin*, *Bioorg.Med. Chem. Lett.* **2006**,*16*, 276–2768.

[16]. R. Liu, S. Ma, Y. Liu, S. Yu, X. Chen, J. Zhang, Albizosides D and E, two new cytotoxic triterpene saponins from *Albizia chinensis*, *Carbohydr. Res*. **2010**, *345*, 1877–1881.

[17]. L. Zheng, J. Zheng, Q. Zhang, B. Wang, Y. Zhao, L. Wu, Three new oleanane triterpenoid saponins acetylated with monoterpenoidacid from *Albizia julibrissin*, *Fitoterapia* **2010**, *81*, 859–863.

[18]. M. Haddad, T. Miyamoto, V. Laurens, M. A. Lacaille-Dubois, Two new biologically active triterpenoidal saponins acylated with salicylic acid from *Albizia adianthifolia*. *J. Nat. Prod*. **2003**, *66*, 372–377.

[19]. M. Haddad, T. Miyamoto, M.A. Lacaille-Dubois, New triterpenoidal saponins acylated with monoterpenic acid from *Albizia adianthifolia*. *Helv.Chim. Acta.* **2004**, *87*, 1228–1238.

[20]. O.P. Noté, A.C. Mitaine-offer, T. Miyamoto, T. Paululat, J.F. Mirjolet, O. Duchamp, D.E. Pegnyemb, M.A. Lacaille-Dubois, Cytotoxic acacic acid glycosides from the roots of *Albizia coriaria*, *J. Nat. Prod*. **2009**, *72*, 1725–1730.

[21]. O.P. Noté, P. Chabert, D.E. Pegnyemb, B. Weniger, M.A. Lacaille-Dubois, A. Lobstein, Structure elucidation of new acacic acid-type saponins from *Albizia coriaria, Magn. Reson. Chem.* **2010**, *48*, 829–836.

[22]. K. Zou, W.Y. Tong, H. Liang, J.R. Cui, G.Z. Tu, Y.Y. Zhao, R.Y. Zhan, Diastereoisomeric saponins from *Albizia julibrissin*, *Carbohydr. Res.* **2005**, *340*, 1329–1334.