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Triterpenoids with antimicrobial activity from *Drypetes inaequalis*

Simon Suh Awanchiri [a], Hanh Trinh-Van-Dufat [c], Jovita Chi Shirri [a], Marlise Diane J. Dongfack [a], Guy Merlin Nguenang [b], Sabrina Boutefnouchet [c], Zacharias T. Fomum [a], Elisabeth Seguin [d], Philippe Verite [e], François Tillequin [c], Jean Wandji [a,*]

[a] Department of Organic Chemistry, University of Yaounde-1, Faculty of Science, P.O. Box 812, Yaounde, Cameroon
[b] Department of Vegetal Biology and Physiology, University of Yaounde-1, Faculty of Science, P.O. Box 812, Yaounde, Cameroon
[c] Laboratoire de Pharmacognosie, UMR/CNRS N° 8638, Université Paris Descartes, Faculté des Sciences Biologiques et Pharmaceutiques, 4-Avenue de l'Observatoire, 75006 Paris, France
[d] Laboratoire de Pharmacognosie, UFR de Médecine Pharmacie Rouen, 22-Bld Gambetta, 76183 Rouen Cedex 1, France
[e] Laboratoire de Chimie Analytique, UFR de Médecine Pharmacie Rouen, 22-Bld Gambetta, 76183 Rouen Cedex 1, France

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ABSTRACT

The air-dried stems and ripe fruit of *Drypetes inaequalis* Hutch. (Euphorbiaceae) were studied. Four triterpene derivatives, characterized as lup-20(29)-en-3β,6α-diol, 3β-acetoxylup-20(29)-en-6α-ol, 3β-caffeoyloxylup-20(29)-en-6α-ol and 28-β D-glucopyranosyl-30-methyl 3β-hydroxyolean-12-en-28,30-dioate along with 10 known compounds were isolated from the whole stems. One triterpene, characterized as 3α-hydroxyfriedelan-25-al along with six known compounds were isolated from the ripe fruit. Their structures were established on the basis of spectroscopic analysis and chemical evidence. The triterpenes were tested for antimicrobial activity against some Gram-positive and Gram-negative bacteria, and two of them appeared to be modestly active.

1. Introduction

Drypetes inaequalis Hutch. (Euphorbiaceae) is a forest shrub growing in the Centre and East provinces of Cameroon. Therapeutic applications of the *Drypetes* plants in West and Central Africa concern the treatment of sinusitis, swellings, boils, gonorrhoea and dysentery (Dalziel, 1937; Irvine, 1961; Bouquet and Debray, 1974; Walker et al., 1961). In our previous study on the *Drypetes* genus, we have reported on the anti-inflammatory and analgesic actions of the crude extract and compounds isolated from *D. molunduana* (Wandji et al., 2000; Chungag-Anye et al., 2001, 2002), the phenolic constituents from *D. armoracia* (Wandji et al., 2003) and the antileishmanial furanosesquiterpenes and tritrepenoids from *D. chevalieri* (Wansi et al., 2007). As a continuation of our search for compounds with biological activities from the *Drypetes* species, we studied the whole stems and the ripe fruit of *D. inaequalis*. From the whole stems, we isolated four new and 10 known compounds, and from the ripe fruit we obtained one new and six known compounds. The known compounds from both parts of the plant were identified as serjanic acid (**5**) (Javasinghe et al., 1993), oleanolic acid (**6**) (Mahato and Kundu, 1994), hederagenin (**7**) (Mahato and Kundu, 1994), queretaroic acid (**8**) (Agrawal and Jain, 1992), serragenic acid (**9**) (Agrawal and Jain, 1992), 28-β-D-glucopyranosyl 3β-hydroxyolean-12-en-28-oate (**10**) (Srivastava and Jain, 1989), friedelin (**12**) (Li et al., 2006), 3,7-dioxofriedelane (**13**) (Mahato and Kundu, 1994), 3α-friedelanol (**14**) (Salazar et al., 2000), 3-oxofriedelan-25-al (**15**) (Anjaneyulu and Narayana, 1980), stigmasterol (**16**) (Wandji et al., 2000), 3β-D-glucopyranosylstigmasterol (**17**) (Wandji et al., 2000), sitosterol (**18**) (Wandji et al., 2003) and 3β-D-glucopyranosylsitosterol (**19**) (Wandji et al., 2003). The structures of the five new compounds have been determined as lup-20(29)-en-3β,6α-diol (**1**), 3β-acetoxylup-20(29)-en-6α-ol (**2**), 3β-caffeoyloxylup-20(29)-en-6α-ol (**3**), 28-β-D-glucopyranosyl-30-methyl 3β-hydroxyolean-12-en-28,30-dioate (**4**) and 3α-hydroxyfriedelan-25-al (**11**), on the basis of spectroscopic analysis and chemical evidence. In the present paper, the isolation, structural determination and antimicrobial activity of the new compounds will be described.

2. Results and discussion

The whole stems and the ripe fruit of *D. inaequalis* were sun-dried, ground into a powder form, macerated with a mixture of solvents and chromatographed on silica gel to afford 19 compounds

* Corresponding author. Tel.: +237 77 39 02 87, +237 22 31 09 57; fax: +237 22 23 44 96.
E-mail address: jeanwandji@yahoo.fr (J. Wandji).

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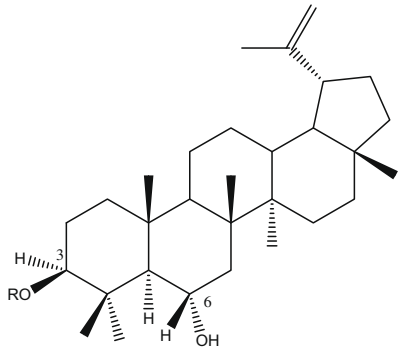
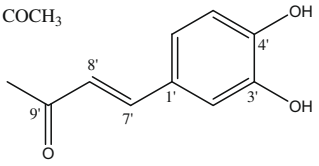
1–19. Compounds **5–10** and **12–19** were identified as known compounds by comparison of their ^{13}C NMR data and other physical properties with reported values. Compounds **1–4** and **11** were characterized as five new triterpene derivatives.

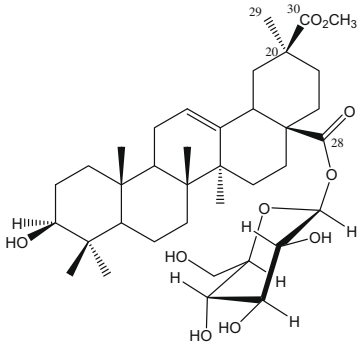


1 R = H

2 R = $COCH_3$

3 R =





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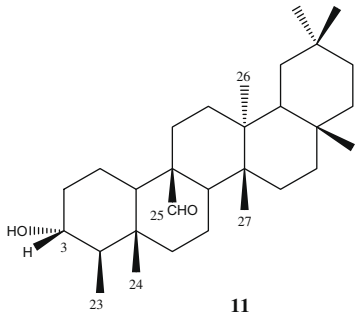

11

Compound **1** was obtained as a colourless amorphous solid. The pseudo molecular ion peaks at *m/z* 443 $[M+H]^+$ and 460 $[M+NH_4]^+$ in its CI/NH_3 MS, and the HR TOF MS ES^+ at *m/z* 442.3825 suggested its molecular formula to be $C_{30}H_{50}O_2$. The IR spectrum indicated the presence of hydroxyl (3400 cm^{-1}) and olefinic (1660 cm^{-1}) groups. The ^{13}C NMR and DEPT spectra of **1** showed 30 carbon signals including seven methyls, nine methylenes, seven methines (two of which are oxygenated (δ_H 3.20 and 4.10)) and six quaternary carbons (Table 1). The signals exhibited at δ_C 109.4 and 150.8 confirmed **1** to be a lupane-type triterpene bearing two hydroxyl

Table 1
^{13}C NMR data for compounds **1**[a], **2**[a], **3**[b], **4**[c], **11**[c] and **15**[c] (100 MHz)[d].

N° C	1	2	3	4	11	15
1	38.5	38.1	38.3	39.2	21.0	22.4
2	27.0	23.3	23.3	28.1	37.0	40.9
3	78.7	80.8	81.2	78.8	71.2	211.7
4	39.1	38.0	38.2	39.5	53.3	59.0
5	60.6	60.6	60.5	55.9	38.3	41.6
6	68.8	68.5	67.6	I9.0	41.9	40.2
7	46.7	46.7	45.8	33.4	18.0	17.2
8	42.1	42.0	41.9	39.8	52.2	52.5
9	49.9	49.8	49.9	48.2	51.5	51.4
10	39.3	39.1	39.1	37.6	58.2	57.0
11	20.8	20.8	20.5	24.4	28.2	28.8
12	25.0	24.9	25.2	123.1	31.2	31.6
13	37.6	37.5	37.9	144.3	39.2	39.4
14	43.0	43.0	42.9	41.4	38.2	38.4
15	27.4	27.4	27.5	28.3	31.4	31.7
16	35.5	35.5	35.4	24.0	35.7	35.1
17	42.9	42.8	42.8	46.4	30.0	30.1
18	48.3	48.2	48.2	43.1	42.8	42.7
19	47.9	47.9	47.9	42.2	35.5	34.9
20	150.8	150.7	150.6	44.2	28.1	28.1
21	29.8	29.7	29.6	30.7	32.8	31.9
22	39.9	39.9	39.8	33.8	38.9	39.0
23	30.9	30.6	30.1	29.3	10.6	7.2
24	15.5	16.5	16.5	17.1	14.7	15.9
25	17.1	17.2	16.0	16.2	204.7	204.9
26	17.5	17.5	16.8	17.7	19.6	19.7
27	14.5	14.5	13.8	26.5	18.5	18.4
28	18.0	18.0	17.2	175.8	31.9	31.7
29	109.4	109.4	109.0	28.8	35.1	35.1
30	19.3	19.2	18.3	177.3	31.6	31.6
		Ac	Caffeoyl	Glc		
1'			126.5	95.1		
2'			113.9	73.4		
3'			145.6	77.7		
4'			148.4	70.6		
5'			115.3	77.4		
6'			121.7	61.7		
7'			145.5			
8'			114.4			
9'			168.1			
3-OCOCH$_3$		21.2				
		171.0				
30-CO–OC*H*$_3$				52.7		

[a] $CDCl_3$.
[b] CD_3OD.
[c] C_5D_5N.
[d] Assignments were made on the basis of DEPT, ^1H–^1H COSY, NOESY, HMQC and HMBC experiments.

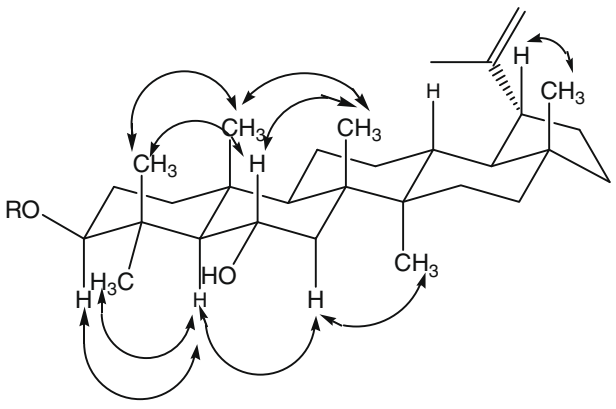

Fig. 1. Important NOESY correlations in compounds **1**; **2** and **3**.

groups. The EIMS of **1** exhibited key peaks at *m/z* 218, 205, 203 and 189, indicating that the two hydroxyl groups are located on rings A and B. The determination of the positions of both hydroxyl groups is based on the HMBC and NOESY experiments. The HMBC spectrum of **1** showed correlations between the oxymethine proton signal at δ_H 3.20 and the carbon signals at δ_C 27.0 (C-2), 39.1 (C-4), 60.6 (C-5), 30.9 (C-23), and 15.5 (C-24), confirming the location of one hydroxyl group at C-3. Interactions were also exhibited between the second oxymethine proton signal at δ_H 4.10 and the carbon signals at δ_C 39.1 (C-4), 60.6 (C-5), 46.7 (C-7), 42.1 (C-8), and 39.3 (C-10) suggesting that the second hydroxyl group was connected to C-6. The NOESY (Fig. 1) cross-peaks exhibited between both protons H-3α and H-5 (δ 0.78), in addition to the coupling constant of H-3α (J = 10.7, 5.6 Hz) confirmed the β-orientation of the 3-hydroxyl group; the NOESY spectrum also showed correlations between the proton signal at δ_H 4.10 (H-6β) and the methyl proton signals at δ_H 0.98 (CH_3-24), 0.85 (CH_3-25) and 1.08 (CH_3-26), confirming the α-equatorial orientation of the 6-hydroxyl group. The configuration of C-6 was also supported by the coupling constant of H-6β (J = 9.6, 4.3 Hz) and the ^{13}C NMR spectrum of **1** which exhibited a key and characteristic signal at δ_C 60.6 for carbon C-5 as deduced from the HMQC spectrum; this value was 4 ppm higher than that of some reported data for C-5 (56.6 ppm) in similar lupane-type triterpenes with a 6β-OH group (Núnez et al., 2005). Therefore, the structure of compound **1** was established as, lup-20(29)-ene-3β,6α-diol. The same 3β,6α-structure was postulated before for a metabolite isolated from *Periploca aphylla* (Ghulam et al., 2000). However, the configuration of C-6 subsequently corresponded to lup-20(29)-en-3β,6β-diol (Núnez et al., 2005).

Compound **2** was obtained as a colourless amorphous solid, and was deduced to have the molecular formula $C_{32}H_{52}O_3$ on the basis of CI/NH_3 MS (*m/z* 485 $[M+H]^+$, 502 $[M + NH_4]^+$, and the HR TOF MS ES^+ (*m/z* 484.3934). The IR spectrum of **2** showed signals assigned to hydroxyl (3400 cm^{-1}), ester (1740 cm^{-1}) and olefinic (1668 cm^{-1}) groups. On comparison, the ^{1}H NMR spectra of compounds **2** and **1** were almost identical, apart from the change of chemical shift of H-3 from δ_H 3.20 in **1** to δ_H 4.42 in **2**, and the presence of one additional acetyl proton singlet at δ_H 2.04 in **2**. The second oxymethine proton at δ_H 4.10 in **1** did not change in **2** (δ_H 4.03). In the ^{13}C NMR (Table 1) the carbon C-3 signal, δ_C 78.7 in **1**, changed to δ_C 80.8 in **2**. Thus, compound **2** was deduced to be the 3-monoacetylated derivative of **1**. On the basis of the ^{1}H–^{1}H COSY and NOESY (Fig. 1) spectra, the stereochemistry of the two oxymethine carbons C-3 and C-6 in both compounds **2** and **1** were confirmed to be the same. Accordingly, the structure of **2** was established as 3β-acetoxylup-20(29)-en-6α-ol.

Compound **3** was obtained as colourless crystals. The pseudo molecular ion peaks at *m/z* 605 $[M+H]^+$ and 622 $[M + NH_4]^+$ in its CI/NH_3 MS, and the HR TOF MS ES^+ at *m/z* 604.4105 suggested its molecular formula to be $C_{39}H_{56}O_5$. The IR spectrum showed absorption bands assigned to hydroxyl (3500, 3310 cm^{-1}), ester (1680 cm^{-1}), and aromatic (1600 cm^{-1}) groups. The ^{1}H NMR spectral data of **3** showed two sets of signals: the first set of signals were analysed as an (*E*)-caffeoyl moiety [δ_H 6.22 (H-7′), 7.50 (H-8′), and three aromatic protons at δ_H 6.75 (H-5′), 7.01 (H-2′) and 6.92 (H-6′)]; the second set of signals in **3** were almost similar to those of **1,** except few modifications observed on the oxymethine protons, δ_H 3.20 in **1** and δ_H 4.50 in **3**. The second oxymethine proton at δ_H 4.10 in **1** did not change in **3** (δ_H 4.00). The ^{13}C NMR (Table 1) confirmed the presence of an (*E*)-caffeoyl moiety in **3** (δ_C 113.9, 114.4, 115.5, 121.7, 126.5, 145.5, 145.6, 148.4 and 168.1). The carbon C-3 signal at δ_C 78.7 in **1** changed to δ_C 81.2 in **3**. Thus, compound **3** was deduced to be the 3-(*E*)-caffeoyl derivative of **1**. On the basis of the ^{1}H–^{1}H COSY and NOESY (Fig. 1) spectra, the stereochemistry of the two oxymethine carbons (C-3 and C-6) in both compounds **3** and **1** were confirmed to be the same. Therefore, the structure of **3** was established as 3β-caffeoyloxylup-20(29)-en-6α-ol.

The molecular formula of compound **4** was deduced as $C_{37}H_{58}O_{10}$ from the FAB MS and ^{13}C NMR data. The positive FAB MS of **4** revealed a quasi-molecular ion at *m/z* 669.5 $[M + Li]^+$. The ^{1}H NMR of **4** showed the presence of six tertiary methyl singlets at δ_H 0.80–1.19 (each, 3H, s), a doublet of doublets at δ_H 2.73 (1H, J = 3.65, 14.35 Hz, H-18) and a triplet at δ_H 5.36 (1H, J = 3.42 Hz, H-12). These signals and the ^{13}C NMR signals (Table 1) at δ_C 123.1 and 144.3 were in agreement with reported data of olean-12-ene type triterpenes. The ^{13}C NMR and DEPT spectra of **4** showed an oxymethine carbon signal at δ_C 78.8 and two C=O groups at δ_C 175.8 and 177.3. The ^{1}H and ^{13}C NMR signals at δ_H 3.73 (3H, *s*) and δ_C 52.7 indicated that one of them was present as a carbomethoxyl group. Moreover, the ^{13}C NMR resonances of the carbons C-12, C-13, C-14, C-17 and C-20 were identical with spectral values of compounds having carboxyl functions at C-17 and C-20 (Hassanean and Mohamed, 1998). The HMBC spectrum showed key correlations between the proton H-18 (δ_H 2.73) and carbons C-28 (δ_C 177.3), C-20 (δ_C 44.2), and between the proton H-1′ (δ_H 5.37) and carbon C-28 (δ_C 177.3), confirming the position of connectivity of the β-glucopyranosyl ester to be to the 28-carboxyl group. Consequently, the carbomethoxyl was deduced to be connected at C-20, and its position was established to be C-30, as deduced from the ^{13}C NMR spectrum of **4** which exhibited resonance for C-29 methyl at δ_C 28.8 in agreement literature (Hassanean and Mohamed, 1998). In addition, alkaline saponification of **4** gave glucose and the corresponding sapogenin which was identical to compound **5**, isolated from the same plant and identified as serjanic acid (**5**) (Javasinghe et al., 1993). Accordingly, compound **4** was elucidated as, 28-β-D-glucopyranosyl-30-methyl 3β-hydroxyolean-12-en-28,30-dioate.

Compound **11** was obtained as colourless amorphous powder. Its molecular formula $C_{30}H_{50}O_2$ was established on the basis of the HR TOF MS ES^+, *m/z* 442.3821, the CI/NH_3 MS, *m/z* 443 $[M+H]^+$, 460 $[M+NH_4]^+$ and NMR data. The ^{13}C NMR (Table 1), ^{1}H NMR, HMQC, HMBC and DEPT spectra suggested a friedelin-type triterpene skeleton containing one oxymethine (δ_C 71.2 and δ_H 3.49) and one aldehyde function (δ_C 204.4 and δ_H 10.19). From the GC-SM, the fragment ion at *m/z* 205 suggested the absence of oxygen function on rings D and E. Also, the fragments at *m/z* 125 and 315 resulting from the cleavage of ring B suggested the location of one oxygen function on ring A. The HMBC spectrum showed correlations between the proton signal (δ_H 3.49) and carbons C-4 (δ_C 53.3), C-5 (δ_C 38.3) and C-23 (δ_C 10.6), and between the aldehyde proton signal at δ_H 10.19 and the carbons C-8 (δ_C 52.2), C-9 (δ_C 51.5), C-10 (δ_C 58.2).and C-11 (δ_C 28.2). These data confirmed the position of the hydroxyl group at C-3 and the aldehyde function (C-25) at C-9. The NOESY spectrum of **11** (Fig. 2) showed interactions between the aldehyde proton (δ_H 10.19) and the methyl groups CH_3-24 (δ_H 0.65) and CH_3-26 (δ_H 0.95). The NOESY cross-peaks from H-3 (δ_H 3.49) to CH_3-23 (δ_H 1.01) and CH_3-24 (δ_H 0.65), in addition to the coupling constant of H-3 (J = 10.0,

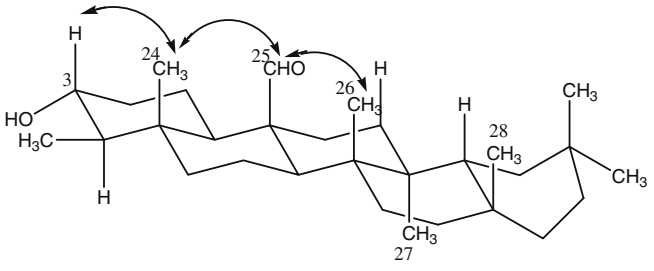

Fig. 2. Important NOESY correlations in compound **11**.

Table 2
Antimicrobial activities of compounds **1**, **2**, **3**, **4** (each conc. 200 mg/l in DMSO).

Micro-organisms used	Inhibition zone diameter (mm)				
	1	**2**	**3**	**4**	Gentamicin (control)
Staphylococcus aureus Gram-(+)	15			11	34
Escherichia coli Gram-(−)				14	35
Salmonella typhi Gram-(−)				13	42
Shigella dysenteriae Gram-(−)					30
Klebsiella pneumoniae Gram-(−)					40
Pseudomonas aeruginosa Gram-(−)					43

4.0 Hz) confirmed the β-axial orientation of H-3 and consequently the α-equatorial orientation of the hydroxyl group in agreement with reported data (Salazar et al., 2000). Therefore, the structure of **11** was established as 3α-hydroxyfriedelan-25-al.

From the antimicrobial test results (Table 2), it appears that compound **1** exhibits antimicrobial activity against *Staphylococcus aureus*. Compounds **2** and **3** which are the 3-acylated derivatives of compound **1** showed no inhibitory activity on the six bacterial strains. Compound **4** reveals antimicrobial activity against *S. aureus*, *Escherichia coli* and *Salmonella typhi*. The activities of both compounds **1** and **4** were lower in comparison to that of gentamicin which was used as control.

3. Experimental

3.1. General

MPs were determined using a Kofler microhot stage apparatus. IR spectroscopy was performed on a Perkin–Elmer 257 spectrometer. Specific rotations were measured on a Perkin–Elmer 241 polarimeter. MS were registered on a Micromass Q-Tof instrument, on a Nermag R10-10C spectrometer and a HP-5973 Mass Selective Detector. NMR experiments were performed on a Varian Gemini 400 MHz instrument and a Bruker AC 400 spectrometer, the residual solvent signal was taken as reference in each case ($CDCl_3$, CD_3OD and C_5D_5N). Si gel 60 (240–400 mesh) was used for CC at normal pressure while Si gel 60 H (5–40 μm) and Si gel 60 C (20–40 μm) were used for CC under compressed air (300 mbar). Precoated Si gel 60 F_{254} aluminium plates were used for TLC.

3.2. Plant material

The whole stems and the ripe mature fruit of *D. inaequalis* Hutch. (Euphorbiaceae) were collected from Eloundem (Centre province of Cameroon) in August 2004. The herbarium specimen documenting the collection has been deposited in the National Herbarium, Yaoundé, Cameroon (Ref 4981/SRFK).

3.3. Extraction and isolation

The whole stems and the ripe fruit of *D. inaequalis* were sun-dried and ground separately into a powder form. The ground stems (10.0 kg) were macerated at room temperature with a mixture of CH_2Cl_2–MeOH (1:1) (3 × 25 l) for 9 days. The solvents were evaporated under reduced pressure to yield the total crude extract (390.0 g). Part of the extract (250.0 g) was subjected to CC over Si gel [60 (240–400 mesh), 800 g]. A total of 75 fractions (400 ml each) were eluted with hexane, CH_2Cl_2 and MeOH in increasing polarity. TLC permitted the combination the resulting fractions into 8 groups of fractions coded A, B, C, D, E, F, G and H, obtained as follow: A (5.0 g) [Fr. 1–5 (hexane–CH_2Cl_2 100:0 to 75:25)]; B (30 g) [Fr. 6–12 (hexane–CH_2Cl_2 70:30 to 50:50)]; C (35 g) [Fr. 13–20 (hexane–CH_2Cl_2 45:55 to 25:75)]; D (27 g) [Fr. 21–27 (hexane–CH_2Cl_2 20:80 to 0:100)]; E (25 g) [Fr. 28–35 (CH_2Cl_2–MeOH 100:0 to 95:5)]; F (30 g) [Fr. 36–45 (CH_2Cl_2–MeOH 93:7 to 90:10)]; G (36 g) [Fr. 46–57 (CH_2Cl_2–MeOH 88:12 to 80:20)] and H (50 g) [Fr. 58–75 (CH_2Cl_2–MeOH 75:25 to 50:50)]. Further CC over Si gel 60 C (20–40 μm) of group C fractions using hexane – CH_2Cl_2 (50:50) yielded compounds **2** (30 mg), **12** (40 mg), **13** (30 mg) and **16** (50 mg). Further CC over Si gel 60 C (20–40 μm) of group D fractions using hexane–CH_2Cl_2 (75:25) afforded compounds **1** (22 mg), **5** (15 mg) and **6** (70 mg). Further CC over Si gel 60 H (5–40 μm) of group E fractions by using CH_2Cl_2 (100%) yielded compounds **7** (20 mg), **8** (12 mg) and **9** (10 mg). Further CC over Si gel 60 H (5–40 μm) of group F fractions using CH_2Cl_2–MeOH (95:5) yielded compounds **3** (20 mg), **4** (45 mg), **10** (15 mg) and **17** (20 mg). The ground fruit (2.0 kg) was macerated at room temperature with a mixture of EtOAc–MeOH (3:1), (3 × 4 l) for 6 days. The solvents were evaporated under reduced pressure to yield the total crude extract (157.0 g) which was subjected to CC over Si gel [60 (230–400 mesh), 500 g]. A total of 144 fractions (200 ml each) were eluted with hexane, EtOAc and MeOH in increasing polarity. TLC permitted the combination the resulting fractions into five series of fractions coded A, B, C, D and E, obtained as follows: series A (10.0 g) [Fr. 1–10 (hexane–EtOAc, 100:0 to 75:25)]; series B (15 g) [Fr. 11–19 (hexane–EtOAc 70:30 to 50:50)]; series C (10 g) [Fr. 20–50 (hexane–EtOAc 45:55 to 25:75)]; series D (50 g) [Fr. 51–102 (hexane–EtOAc 20:80 to 0:100)]; series E (55 g) [Fr. 103–144 (EtOAc–MeOH 100:0 to 50:50)]. Further CC over Si gel 60 C (20–40 μm) of series A using hexane–EtOAc in increasing polarity yielded compounds **12** (50 mg) and **14** (17 mg). Repeated CC of series B over Si gel 60 C (20–40 μm) using hexane–EtOAc in increasing polarity afforded compounds **11** (7 mg) and **15** (18 mg). Further CC of series C over Si gel 60 C (20–40 μm) using hexane–EtOAc in increasing polarity yielded compound **18** (60 mg). Repeated CC over Si gel 60 C (20 40 μm) of series D using EtOAc–MeOH in increasing polarity afforded compounds **10** (65 mg) and **19** (200 mg).

3.3.1. lup-20(29)-ene-3β,6α-diol (**1**)

Colourless amorphous solid; $[\alpha]_D^{20}$ = +24.5° ($CHCl_3$, c 0.60); IR (KBr) ν_{max} cm^{-1}: 3400, 3030, 1660, 1260, 1180, 890; ^1H NMR spectral data (400 MHz, $CDCl_3$): δ 3.20 (1H, dd, *J* = 5.6, 10.7 Hz, H-3), 4.10 (1H, dt, *J* = 4.3, 9.6 Hz, H-6), 4.58 (1H, d, *J* = 2.3 Hz, H-29), 4.68 (1H, d, *J* = 2.3 Hz, H-29), 2.35 (1H, m, H-19), 1.68 (3H, s, CH_3-30), 1.32 (3H, s, CH_3-23), 1.08 (3H, s, CH_3-26), 0.98 (3H, s, CH_3-24), 0.96 (3H, s, CH_3-27), 0.85 (3H, s, CH_3-25), 0.78 (m, H-5), 0.75 (3H, s, CH_3-28), 1.40 and 1.67 (m, H-7); ^{13}C NMR spectral data (100 MHz, $CDCl_3$), see Table 1; CI/NH_3 MS, *m/z*: 443 $[M+H]^+$, 460 $[M+NH_4]^+$; EIMS (70 eV) *m/z* 442 $[M]^+$ (10), 424 $[M-H_2O]^+$ (20), 409 $[M-H_2O-CH_3]^+$ (5), 406 $[M-2H_2O]^+$ (3), 236 (6), 218 (20), 205 (40), 203 (12), 189 (50); HR TOF MS ES^+ (calcd. for $C_{30}H_{50}O_2$ 442.3811, found 442.3825).

3.3.2. 3β-acetoxylup-20(29)-en-6α-ol (**2**)

Colourless amorphous solid; $[\alpha]_D^{20}$ = +18.5° ($CHCl_3$, c 0.80); IR (KBr) ν_{max} cm^{-1}: 3400, 3000, 1740, 1668, 1255, 880; ^1H NMR spectral data (400 MHz, $CDCl_3$): δ 4.42 (1H, dd, *J* = 5.5, 10.5 Hz, H-3), 4.03 (1H, dt, *J* = 4.2, 9.5 Hz, H-6), 4.56 (1H, d, *J* = 2.3 Hz, H-29), 4.68 (1H, d, *J* = 2.3 Hz, H-29), 2.35 (1H, m, H-19), 1.68 (3H, s, CH_3-30), 1.16 (3H, s, CH_3-23), 1.10 (3H, s, CH_3-26), 1.04 (3H, s, CH_3-24), 0.95 (3H, s, CH_3-27), 0.91 (3H, s, CH_3-25), 0.78 (3H, s, CH_3-28), 2.04 (3H, s, $COCH_3$-3); ^{13}C NMR spectral data (100 MHz, $CDCl_3$), see Table 1; CI/NH_3 MS, *m/z* 485 $[M+H]^+$, 502 $[M+NH_4]^+$; EIMS (70 eV) *m/z* 484 $[M]^+$ (7), 466 (8), 426 (10), 408 (5), 383 (5), 218 (100), 205 (40), 203 (12), 189 (12); HR TOF MS ES^+ (calcd. for $C_{32}H_{52}O_3$ 484.3916, found 484.3934).

*3.3.3. 3β-caffeoyloxylup-20(29)-en-6α-ol (**3**)*

Colourless crystals; mp 272.2–273.7 °C; $[\alpha]_D^{20}$ = +105.4° ($CHCl_3$, c 0.08); IR (KBr) ν_{max} cm^{-1}: 3500, 3310, 2930, 2860, 1680, 1600, 1255, 1180, 880; UV (CH_3OH) λ_{max} nm log(ε) 303.6 (0.504), 327.6 (0.636); ^1H NMR spectral data (400 MHz, CD_3OD): *triterpene moiety*: δ 4.50 (1H, dd, *J* = 5.70, 10.50 Hz, H-3), 4.00 (1H, (1H, dt, *J* = 4.10, 9.50 Hz, H-6), 4.55 (1H, d, *J* = 2.30 Hz, H-29), 4.68 (1H, d, *J* = 2.30 Hz, H-29), 2.39 (1H, m, H-19), 1.68 (3H, s, CH_3-30), 1.17 (3H, s, CH_3-23), 1.14 (3H, s, CH_3-26), 0.96 (3H, s, CH_3-24), 1.02 (3H, s, CH_3-27), 1.12 (3H, s, CH_3-25), 0.82 (3H, s, CH_3-28); *caffeoyl moiety*: δ 7.01 (1H, d, *J* = 1.83 Hz, H-2′), 6.75 (1H, d, *J* = 8.06 Hz, H-5′), 6.92 (1H, dd, *J* = 2.00, 8.24 Hz, H-6′), 7.50 (1H, d, *J* = 15.74 Hz, H-7′), 6.22 (1H, d, *J* = 15.74 Hz, H-8′); ^{13}C NMR spectral data (100 MHz, CD_3OD), see Table 1; CI/NH_3 MS, *m/z*: 605 $[M+H]^+$, 622 $[M+NH_4]^+$; EIMS (70 eV) *m/z* 604 $[M]^+$ (25), 239(10), 221 (15), 203 (65), 163 (100); HR TOF MS ES$^+$ (calcd. for $C_{39}H_{56}O_5$ 604.4128, found 604.4105).

*3.3.4. 28-β-D-glucopyranosyl-30-methyl 3β-hydroxyolean-12-en-28,30-dioate (**4**)*

White crystals from CH_2Cl_2; mp 230-232 °C; $[\alpha]_D^{20}$ = +45.5° (MeOH, c 0.75); IR (KBr) ν_{max} cm^{-1}: 3450, 1720 (COOR), 1710 ($COOCH_3$); ^1H NMR spectral data (400 MHz, C_5D_5N) δ: 5.37 (1H, d, *J* = 8.00 Hz, H-1′), 5.36 (1H, t, *J* = 3.42 Hz, H-12), 3. 73 (3H, s, $COOCH_3$), 3.19 (1H, t, *J* = 8.00 Hz, H-3), 2.73 (1H, dd, *J* = 3.65, 14.35 Hz, H-18), 1.19 (3H, s, CH_3), 1.17 (3H, s, CH_3), 1.00 (3H, s, CH_3), 0.98 (3H, s, CH_3), 0.82 (3H, s, CH_3), O.80 (3H, s, CH_3); ^{13}C NMR spectral data (100 MHz, C_5D_5N) data, see Table 1. CI/NH_3 MS, *m/z* 663 $[M+H]^+$, 680 $[M+NH_4]^+$; FAB/NBA + Li: ion mode FAB+, *m/z*: 669.5 $[M+Li]^+$,630, 625, 581.6, 580.6, 460.3, 397.5, 307.0, 292.1, 291.1, 290.0, 154.1. Alkaline hydrolysis of compound (**4**): The glycoside **4** (15 mg) was refluxed with 5% KOH for about 5 h. After completion, the reaction mixture was neutralized with diluted H_2SO_4 and extracted with *n*-BuOH. Work up of the *n*-BuOH soluble portion yielded glucose identified by ^1H and ^{13}C NMR to the available authentic compound. The purification of the aqueous fraction afforded the aglycone, identical to serjanic acid (**5**), also isolated from the same plant.

*3.3.5. Serjanic acid (3β-hydroxyolean-12-en-28,30-dioic acid 30-methyl ester) (**5**)*

White crystals from CH_2Cl_2; mp 280–281 °C; CI/NH_3 MS, *m/z*: 501 $[M+H]^+$, 518 $[M+NH_4]^+$; EI MS (probe) 70 eV, *m/z*: 500 $[M]^+$ (1.5), 454 (15.4), 292 (64.6), 247 (41.5), 246 (100.0), 233 (15.4), 232 (17.7), 207 (33.8), 187 (93.8), 186 (46.2), 173 (23.1), 159 (21.5).

*3.3.6. 3α-hydroxyfriedelan-25-al (**11**)*

Colourless amorphous powder; $[\alpha]_D^{20}$ = +14.5° ($CHCl_3$, c 0.60); IR (KBr) ν_{max}: 3400, 3030, 1720, 1260, 1180, 890 cm^{-1}; ^1H NMR spectral data (400 MHz, $CDCl_3$): δ 10.19 (1H, s, H-25), 3.49 (1H, dt, *J* = 10.0, 4.0 Hz, H-3ax), 2.16 (qd, *J* = 13.0, 3.0, H-2eq), 1.19 (3H, s, CH_3-28); 1.07 (3H, s, CH_3-30); 1.01 (3H, d, *J* = 6.0 Hz, CH_3-23); 0.95 (3H, s, CH_3-26); 0.94 (3H, s, CH_3-29); 0.93 (3H, s, CH_3-27); 0.65 (3H, s, CH_3-24); ^{13}C NMR spectral data (100 MHz, $CDCl_3$), see Table 1. GC–SM *m/z*: $[M]^+$ 442, 315, 205, 125. CI/NH_3 MS, *m/z*: 443 $[M+H]^+$, 460 $[M+NH_4]^+$.

3.4. Antimicrobial activity

3.4.1. Microbial strains

A total of six micro-organisms belonging to one Gram-(+) bacterial species (*S. aureus*) and five Gram-(−) bacteria (*E. coli*, *S. typhi*, *Shigella dysenteriae*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) were clinically isolated from patients in the "Centre Pasteur de Yaoundé" Cameroon. They were maintained on agar slants at 4 °C in the Laboratory of the Applied Microbiology and Molecular Pharmacology (Faculty of Science, Yaounde).

3.4.2. Antimicrobial assays

Antimicrobial activity was evaluated using the agar diffusion method, according to the NCCLS (2002) protocol with slight modifications. Briefly, sterile cylinders of 6 mm were used to make wells inside Mueller-Hinton agar plates. The plates were inoculated with 2×10^{-4} l of the test micro-organisms equivalent to 5×10^5 CFU/ml. All the compounds were dissolved in DMSO or in heated sterilized distilled water at a concentration 200 mg/l. Wells were filled with 15×10^{-5} l of solution of each test compound, the positive control drug (gentamicin) and the negative control DMSO, and allowed to diffuse for 45 min at 4 °C. The plates were incubated at 37 °C for 24 h. The sensitivity was recorded by measuring the clear zone of growth inhibition around the wells (mm diameter). Each set was tested in triplicate.

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