Taraxastane and Lupane Triterpenoids from the Bark of *Manilkara zapota*

Flavien Aristide Alfred Toze[1][*], Marie Fomani[1], Achille Bissoue Nouga[1], Jean Rodolphe Chouna[2], Kouam[1], Alain François Kamdem Waffo[1] and Jean Duplex Wansi[1]

[1]*Department of Chemistry, University of Douala, P.O. Box 24157 Douala, Cameroon.*
[2]*Department of Chemistry, University of Dschang, P.O. Box 371, Dschang, Cameroon.*

Authors' contributions

This whole work was carried out in collaboration between all authors. Authors FAAT and K collected the plant and made the extraction. Authors FAAT, MF and ABN wrote the protocol and performed the phytochemical analysis. Authors AFKW and JDW performed the biological tests in collaboration and the spectroscopic analysis. Authors FAAT, AFKW and JDW wrote the first draft of the manuscript. Author JRC contributed in the literature searches. Author JDW managed the supervision of the study. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: Discovering new lead compounds against cancer and bacterial infections is a crucial step to ensuring a sustainable global pipeline for new effective drugs. This study focus on the isolation of secondary metabolites of methanol extract from the bark of *Manilkara zapota* (Sapotaceae) a Cameroonian medicinal plant.
Study Design: According to the literature, plants of the genus *Manilkara* are potential sources of antibacterial and anticancer secondary metabolites.
Methodology: The air-dried and powdered bark (4.0 kg) of *M. zapota* was extracted at room temperature for 72 h with a methanol. The extract was concentrated to dryness under vacuum and the residue was subjected to repeated column chromatographic separation. The structures of the

*Corresponding author: E-mail: toflavien@yahoo.fr;

isolates were established by means of spectroscopic methods. These compounds were screened *in vitro* for their activity against bacterial and human Caucasian prostate adenocarcinoma cell line PC-3.

Results: Two new pentacyclic triterpenoids, 3-acetyltaraxer-14-en-12-one (**1**) and 3-hydroxy-7-oxolup-20(29)-en-28-oic acid (**4**), together with eleven known compounds were isolated from the methanol extract from the bark of *Manilkara zapota*. The structures of all compounds were determined by comprehensive analyses of their 1D and 2D NMR, mass spectral (EI and ESI) data and comparison with previously known analogs. The agar diffusion test delivered low to missing antimicrobial activities, corresponding with MICs > 1mg/ml. In addition, compounds 1, 4-7, 11 and 12 displayed moderate cytotoxic activity against the human Caucasian prostate adenocarcinoma cell line PC-3 with IC_{50} value ranging from 14.1-30.6 μg/ml, while compounds 2 and 3 showed weak cytotoxic activity with an IC_{50} value of 61.2-62.5 μg/ml, compared to the standard doxorubicin (IC_{50} = 0.9 μg/ml).

Conclusion: Two new triterpenoids were isolated, some isolated compounds displayedmissing or low activities against bacterial, plant pathogen oomycetes and moderate cytotoxic activity against the human Caucasian prostate adenocarcinoma cell line PC-3.

1. INTRODUCTION

Manilkara zapota (Sapotaceae), known commonly as "Sapodilla", is an evergreen tree up to 10 m high. Native to Mexico and Central America, it is cultivated in tropical areas including Cameroon [1]. The fruit has a rusty brown skin and a yellowish-brown or orange pulp with a sweet pleasant flavour and a mild aroma when ripe. Sapodilla fruit is often eaten fresh, but the pulp is also incorporated into sherbets, milkshakes and ice cream [2].

The seeds of *M. zapota* are applied traditionally as aperients, diuretic tonic and febrifuge, while the stem bark is used as astringent and febrifuge. In addition, leaves and bark are taken against cough, cold, dysentery and diarrhoea [3]. Previous phytochemical investigations revealed triterpenoids, saponins, polyphenols and flavonoids, some of these compounds exhibiting potent α-amylase and α-glucosidase inhibition, as well as antimicrobial, antioxidant and cytotoxic activities [4,5]. Widespread traditional medicinal use and significant biological activities of compounds investigated so far justified continued investigation of *M. zapota*. This paper reports the isolation and structure elucidation of two new pentacyclic triterpenoids (**1** and **4**), together with antimicrobial and cytotoxic activities of isolated compounds.

2. MATERIALS AND METHODS

2.1 General

The melting points were recorded on a Buchi 535 melting point apparatus and are uncorrected. Optical rotations were measured in $CHCl_3$ on a JASCO DIP-360 digital polarimeter using a 10 cm cell. CD (Circular Dichroism) spectra were measured on a JASCO J-810 spectro polarimeter. Infrared spectra were recorded on a JASCO 302-A spectrophotometer. ESI-HR (*Electrospray Ionisation High Resolution)* mass spectra were recorded on a Bruker FTICR 4.7 T mass spectrometer. EI-MS (*Electron Impact – Mass Spectrometry)* were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluorokerosene as reference substance for EI-HR-MS (*Electron Impact- High Resolution-Mass Spectrometry)*. The ^{1}H- and ^{13}C-NMR (*Nuclear Magnetic Resonance)* spectra were recorded at 500 MHz and 125 MHz, respectively, on Bruker DRX 500 NMR spectrometers. Methyl, methylene and methine carbons were distinguished by DEPT (*Distorsionless Enhancement by Polarisation Transfer)* experiments. Homonuclear ^{1}H connectivities were determined by using the COSY (*Correlation Spectroscopy)* experiment. One-bond ^{1}H-^{13}C connectivities were determined with HMQC (*Heteronuclear Multiple Quantum Coherence*) gradient pulse factor selection. Two- and three-bond ^{1}H-^{13}C connectivities were determined by HMBC (*Heteronuclear Multiple Bond Correlation*) experiments. Chemical shifts are reported in δ (ppm) using TMS (*Tetramethylsilane*) as internal standard, and coupling constants (*J*) were measured in Hz. Column Chromatography (CC) was carried out on silica gel (70-230 mesh, Merck). TLC (*Thin Layer Chromatography)* was performed on Merck precoated silica gel 60 F_{254} aluminium foil, and spots were detected using ceric sulfate spray reagent. Phenolic compounds were detected using $FeCl_3$ reagent. The purity of the compounds was investigated by means of

^{1}H-NMR and ESI-MS experiment. The degree of purity of the tested compounds was > 95%, and of the positive control (Doxorubicin) 99.9%. All other substances, if otherwise not specified, were purchased from Sigma-Aldrich (Germany). All reagents used were of analytical grade.

2.2 Plant Material

The bark of *M. zapota* was collected at the Dibombari locality in the Littoral region of Cameroon in May 2013 and identified by Mr. Nana Victor, National Herbarium, Yaounde, Cameroon. A voucher specimen (ref. 00006 FSUD/CAM) has been deposited at the Faculty of Science.

2.3 Extraction and Isolation

The air-dried and powdered bark (4.0 kg) of *M. zapota* was macerated in methanol at room temperature for 72 h, then filtrated and evaporated under reduced pressure to obtain 102.3 g of the crude extract. A portion of 52.0 g was purified by column chromatography over silica gel 60 (230-400 mesh) and preparative TLC using a gradient system of hexane, ethyl acetate and MeOH. 125 sub-fractions (*ca.* 250 mL each) were collected and pooled on the basis of TLC analysis leading to four main fractions (A – D).

Fraction A (1.5 g, combined from sub-fractions 1-27) was chromatographed over a silica gel 60C column with a hexane-EtOAc gradient. A total of 26 fractions of *ca.* 100 mL each was collected and combined on the basis of TLC. Fractions 5-10 were further chromatographed over a silica gel 60H column with a mixture of hexane-EtOAc (5:1) for elution to yield lupeol (**7**) (31.3 mg), lupeone (**9**) (6.4 mg) and 3-acetyllupeol (**8**) (15.6 mg). Fractions 11-27 were further chromatographed over a silica gel 60H column with a mixture of hexane-EtOAc (4:1) to yield 3-acetyltaraxerol (**2**) (11.0 mg) and taraxerone (**3**) (8.0 mg). Fraction B (16.5 g, combined from sub-fractions 28-49) was chromatographed over a silica gel 60C column with a hexane- EtOAc gradient. A total of 45 fractions of *ca.* 100 mL each was collected and combined on the basis of TLC. Fractions 1-7 were purified and yielded 3-acetyltaraxer-14-en-12-one (**1**) (15.5 mg), *β*-amyrin (**10**) (7.2 mg) and *α*-amyrin (**13**) (13.0 mg). Fraction C (15.3 g, combined from sub-fractions 50-92) was chromatographed over a silica gel 60C column with a hexane-EtOAc gradient. A total of 25 fractions of *ca.* 100 mL

each was collected and combined on the basis of TLC. Fractions 1-13 were further chromatographed over a silica gel 60H column with a mixture of hexane-EtOAc (1:3) to yield oleanolic acid (**11**) (7.5 mg), 3-acetyloleanolic acid (**12**) (6.8 mg) and 7-oxobetulinic acid (**4**) (9.8 mg). Similarly, fraction D (15.5 g, combined from sub-fractions 93-150) was chromatographed over a silica gel 60C column with a hexane-EtOAc (2:5) and EtOAc gradient. The resulting 28 fractions of *ca.* 100 mL each were collected and combined on the basis of TLC. Fractions 1-13 were further chromatographed over a silica gel 60H column with a mixture of hexane-EtOAc (1:5) to yield 3-oxolup-20(29)-en-28-oic acid (**6**) (14.5 mg) and betulinic acid (**5**) (25.5 mg).

3-acetyltaraxer-14-en-12-one (1): White crystals (CHCl$_3$); mp 210-213°C; R_f = 0.79, silica gel 60 F$_{254}$, hexane-EtOAc (5:1); $[\alpha]^{25}_D$ + 12.5° (*c* = 0.50, CHCl$_3$).CD [CDCl$_3$, nm (Δ*ε*)] 310 (- 0,81), 290 (-1,00), 220 (+ 1,00), 210 (+1,40); IR (CH$_2$Cl$_2$): υ$_{max}$= 3300, 3050, 1750, 1700, 1640, 1445 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR data see Table 1; EI-MS (%) *m/z* = 482 (5) [M][+], 439 (70), 316 (100), 300 (20), 290 (55), 238 (50), 219 (60), 126 (15); HR-EI-MS [M][+]*m/z* = 482.3752 (calcd. for C$_{32}$H$_{52}$O$_3$, 482.3760).

3-hydroxytaraxer-14-en-12-one (1a): White crystals (CHCl$_3$); mp 255-259°C; R_f = 0.57, silica gel 60 F$_{254}$, hexane-EtOAc (5:1); $[\alpha]^{25}_D$ + 15.5° (*c* = 0.45, CHCl$_3$); IR (CH$_2$Cl$_2$): υ$_{max}$= 1730, 1710, 1650, 1455 cm^{-1}; ^{1}H-NMR (500 MHz, CDCl$_3$): δ 0.83 (s, Me-28), 0.88 (s, Me-23), 0.91 (s, Me-29), 0.93 (s, Me-24), 0.94 (s, Me-25), 0.98 (s, Me-30), 1.13 (s, Me-26), 1.15 (s, Me-27), 2.64 (dd, *J* = 11.7, 7.3 Hz, 1H, H-11a), 2.37 (dd, *J* = 11.7, 5.6 Hz, 1H, H-11b), 3.21 (dd, *J* = 11.0, 6.0 Hz, 1H, H-3), 5.54 (dd, *J* = 8.1, 3.1 Hz, 1H, H-15); EI-MS (%) *m/z* = 440 (14) [M][+], 423 (25), 316 (100), 299 (70), 290 (60), 238 (53), 219 (66), 124 (55); HR-EI-MS [M][+]*m/z* = 440.3649 (calcd. for C$_{30}$H$_{48}$O$_2$, 440.3654).

3-hydroxy-7-oxolup-20(29)-en-28-oic acid (4): White crystals (CHCl$_3$-MeOH); mp 215-217 °C; R_f = 0.50, silica gel 60 F$_{254}$, hexane-EtOAc (3/2); $[\alpha]^{25}_D$ + 18.5° (*c* = 0.50, CHCl$_3$); CD [CDCl$_3$, nm (Δ*ε*)] 290 (-0,68), 248 (-2.50), 220 (-0.10); IR (CHCl$_3$-MeOH): υ$_{max}$ = 3350, 3000, 2930, 1750, 1640, 1470 cm^{-1}; ^{1}H-NMR and ^{13}C-NMR data see Table 1; EI-MS (%) *m/z* = 470 (9) [M][+], 454 (28), 425 (19), 332 (18), 285 (20), 280 (18), 251 (58), 223 (40), 222 (40), 203 (57), 189 (45), 177 (70), 153 (100), 142 (57), 126 (65); HR-EI-MS

$[M]^+ m/z = 470.3391$ (calcd. for $C_{30}H_{46}O_4$, 470.3396).

2.3.1 Chemical derivatives

Transesterification: Compound **1** (10.0 mg) was refluxed at 70°C in dry MeOH (5.0 ml) with NaOMe (5.0 ml) for 2 h. The reaction mixture was extracted successively with H_2O and $CHCl_3$. The chloroform phase was dried over Na_2SO_4 and evaporated to give CH_3COOCH_3. Addition of HCl (1%) to the water phase followed by extraction with $CHCl_3$ afforded compound **1a** (5.2 mg).

2.4 Biological activities

2.4.1 Antimicrobial assays

Agar diffusion test plates with the bacteria *Bacillus subtilis* and *Escherichia coli* (on peptone agar), *Staphylococcus aureus* (Bacto nutrient agar) and the fungi *Mucor miehei* and *Candida albicans* (Sabouraud agar) as test strains were performed as previously described [6]. For the plant pathogen oomycetes *Aphanomyces cochlioides*, *Pythium ultimum* and *Rhizoctonia solani*, squares of 0.5 x 0.5 cm were cut with a microbiological hook from the growth margins of mycelial mats grown on PDA plates, inversely placed into the centers of fresh plates and cultivated for 24 h at 28°C to initiate growth.
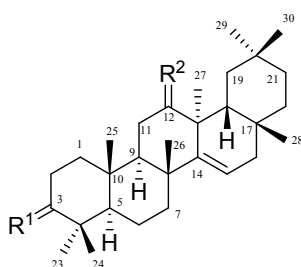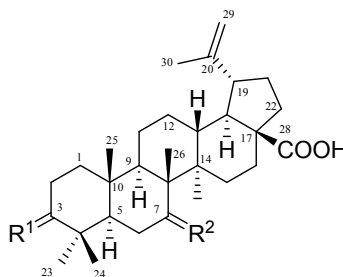

1. R^1 = H, OAc; R^2 = O

1a. R^1 = H, OH; R^2 = O
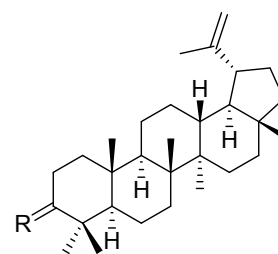
2. R^1 = H, OAc; R^2 = H, H

3. R^1 = O; R^2 = H, H

4. R^1 = H, OH; R^2 = O

5. R^1 = H, OH; R^2 = H, H

6. R^1 = O; R^2 = H, H

7. R = H, OH

8. R = H, OAc

9. R = O
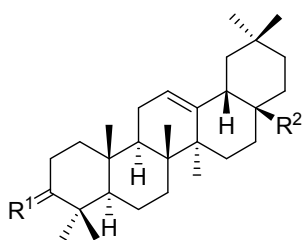
10. R^1 = H, OH; R^2 = CH_3

11. R^1 = H, OH; R^2 = COOH

12. R^1 = O, OAc; R^2 = COOH
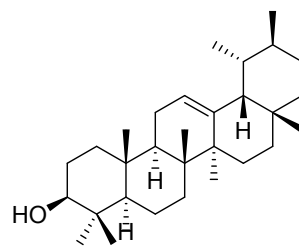
13

Fig. 1. Structures of some of the isolated compounds

Compounds were dissolved in CH_2Cl_2/MeOH (9:1), and paper disks (Ø 9 mm) impregnated with 40.0 μg, dried for 1 h under sterile conditions and arranged evenly on the pre-made agar test plates containing bacterial or fungal test strains, whereas for oomycete plates, disks were placed around the central mycelial squares at a distance of 30 mm. Bacteria and fungi plates were kept in an incubator at 37°C for 15 h, oomycetes at 28°C for 48 h. The diameter of inhibition zones (in mm) was measured directly or calculated from the radius. Nystatin (Maneesh Pharmaceutic PVT. Ltd., Govandi, Mumbai, 400 043 India) was used as positive control for fungi and gentamycin (Jinling Pharmaceutic (Group) corp., Zhejiang Tieng Feng Pharmaceutic Factory, No. 11 Chezhan Road, Huzhou city, Zhejiang, China) for bacteria.

2.4.2 Cytotoxicity assay

Cytotoxic activities of the compounds were evaluated against the human Caucasian prostate adenocarcinoma cell line PC3 by the MTT method according to a reported protocol [7]. Freshly trypsinized cell suspensions were seeded into 96-well microtiter plates at densities of 1×10^4 cells per well, and the test compounds were added from DMSO-diluted stock. After 3 days, the attached cells were incubated with MTT and subsequently solubilized in DMSO. The absorbance at 550 nm was measured by using a microplate reader. The IC_{50} is the concentration of agent that reduced cell growth under experimental conditions by 50%, with Doxorubicin as positive control (IC_{50} = 0.9 μg/ml). S.E.M = Standard error of the mean, 4 number of replicates.

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white powder. Its specific rotation was found to be $[\alpha]^{25}_D$ + 12.5° ($CHCl_3$). The molecular composition was found to be $C_{32}H_{50}O_3$ by EI-MS and HR-EI-MS ($[M]^+$ at *m/z* 482.3752, calcd. 482.3760). These data confirmed by ^{13}C NMR and DEPT indicated a triterpenoid skeleton. The IR spectrum showed the presence of carbonyls (1750-1700 cm^{-1}).

The ^{13}C-NMR spectrum (Table 1) of compound (**1**) revealed 32 carbon signals which were sorted by DEPT as nine methyls, nine methylenes, three methines, six quarternary carbons, one hydroxymethine (δ 81.0) one ester (δ 170.2), one ketone (δ 217.5) and two olefinic carbons (δ158.0 and 116.9). The typical ^{13}C-NMR

resonances at δ158.0 and 116.9 suggested the compound **1** to be a taraxer-14-ene derivative [8].

The ^{1}H-NMR data (Table 1) of compound **1**, exhibited the characteristic signals of taraxer-14-ene at δ0.84 (s), 0.88 (s), 0.90 (s), 0.93 (s), 0.93 (s), 0.98 (s), 1.12 (s) and 1.12 (s) assigned to eight methyls and, at δ 5.56 (dd, *J* = 8.2; 3.2 Hz) assigned to the olefinic proton at H-15 [9]. In addition, the ^{1}H-NMR spectrum revealed the presence of an ABX system of three protons neighbouring the carbonyl group at δ 2.60 (dd, *J* = 11.8; 7.1 Hz), 2.33 (dd, *J* = 11.8; 5.1 Hz) and 1.34 (m), the acetyl methyl at δ 2.08 (s) and the hydroxymethine at δ 4.48 (dd, *J* = 10.4; 5.8 Hz, H-3). Transesterification of compound **1** yielded the methylacetate (CH_3COOCH_3) and compound **1a**. The ^{1}H-NMR spectrum **1a** exhibited the signal of H-3 at δ3.21 (dd, *J* = 11.0; 6.0 Hz), the variation of the chemical shift of H-3 of **1** and **1a** confirmed therefore the location of the acetyl group at C-3 position, in agreement with the reported data [9].

The positions of the carbonyl function were determined by the HMBC and EI-MS spectra. In the HMBC spectrum, correlations between H-27 (δ1.12) and C-12 (δ 217.5), C-14 (δ158.0), C-18 (δ 48.8) and C-13 (δ 49.8), and between H-8 (δ 1.34) and C-12 (δ 217.5), C-5 (δ 55.7 ppm), C-1 (δ 38.4) and C-7 (δ 40.6) indicated the position of the carbonyl group at C-12. This assignment was in agreement with the EIMS, which showed two prominent fragment ion peaks at *m/z* 316 ($C_{21}H_{32}O_2$) resulting from the RDA, indicating that the carbonyl functions is located on the rings A, B or C. The fragment ion peak at *m/z* 219 ($C_{15}H_{24}O$) arising from the cleavage of bonds C-11/C-12 and C-8/C-14 confirmed the position of the carbonyl to be located at C-12 [10]. A negative CD cotton effect at 310 and 290 nm (carbonyl n → π ∗) and the positive one at 210 and 220 nm (carbonyl π → π ∗) observed for compound **1** compared with the value of serratene, which have the same rings [11] confirm that the absolute stereochemistry of compound **1** is 3*S*,5*R*,8*R*,9*R*,10*S*,13*S*,17*S*,18*R*. From the above spectroscopic studies, the structure of compound **1** was determined as (3*S*,5*R*,8*R*,9*R*,10*S*,13*S*,17*S*,18*R*) 3-acetyltaraxer-14-en-12-one (Fig. 1).

Compound **4** was obtained as a white powder. Its specific rotation was found to be $[\alpha]^{25}_D$ + 18.5° ($CHCl_3$). The molecular composition was assigned as $C_{30}H_{46}O_4$ by EI-MS and HR-EI-MS

([M]$^+$ at *m/z* 470.3391, calcd. 470.3396). The IR spectrum of **4** showed a hydroxyl group (υ_{max} 3350 cm^{-1}), a ketone (υ_{max} 1750 cm^{-1}) and a carboxyl group (υ_{max} 3000, 2930 and 1640 cm^{-1}).

The ^{1}H-NMR spectrum (Table 1) of compound **4** exhibited signals for six methyls at δ 0.76 (s), 0.77 (s), 0.86 (s), 0.87 (s), 0.92 (s) and 1.64 (s), an ABX system of three protons neighboring the carbonyl group at δ 2.66 (dd, *J* = 11.1; 7.5 Hz), 2.37 (dd, *J* = 11.1; 5.5 Hz) and 1.28 (m), a hydroxymethine at δ 2.99 (dd, *J* = 11.5; 4.5 Hz, H-3) and an exocyclic methylene group at δ 4.56 (d, *J* = 1.8 Hz) and 4.68 (dd, *J* = 1.8 Hz). These detailed analyses of the ^{1}H NMR confirmed the presence of the $\Delta^{20,29}$ lupene skeleton bearing one ketone group [12].

The above $\Delta^{20,29}$ lupene skeleton was further confirmed by the ^{13}C-NMR spectrum (table 1) which revealed the signals of 30 carbons, sorted by DEPT as six methyls, nine methylenes, five methines, five quarternary carbons, one hydroxymethine (δ 76.7),one carboxyl group (δ177.2), one ketone (δ 206.5) and two olefinic carbons (δ150.3 and 109.6). The ^{13}C-NMR resonances at δ 206.5, 177.2, 150.3 and 109.6 suggested the compound **4** to be a betulinic acid bearing one ketone group [13].

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR assignments for 1 (CDCl$_3$) and 4 (CDCl$_3$+MeOD)

| Position | 1 | | 4 | |
	^{13}C	^{1}H [m, *J* (Hz)]	^{13}C	^{1}H [m, *J* (Hz)]
1	38.4	-	38.3	-
2	23.5	-	27.2	-
3	81.0	4.48 (dd, 10.4, 5.8)	76.7	2.99 (dd, 11.5, 4.5)
4	37.4	-	38.2	-
5	55.7	-	54.8	1.28 (m)
6	18.7	-	33.9	2.66 (dd, 11.1, 7.5)
				2.37 (dd, 11.1, 5.5)
7	40.6	-	206.5	-
8	39.0	-	54.8	-
9	49.2	1.34 (m)	49.9	-
10	37.1	-	37.5	-
11	37.4	2.60 (dd, 11.8, 7.1)	20.4	-
		2.33 (dd, 11.8, 5.1)		
12	217.5	-	25.0	-
13	49.8	-	37.5	-
14	158.0	-	41.9	-
15	116.9	5.56 (dd, 8.2, 3.2)	30.0	-
16	36.7	-	31.7	-
17	35.1	-	55.4	-
18	48.8	-	46.6	-
19	42.2	-	48.6	-
20	33.7	-	150.3	-
21	33.4	-	29.2	-
22	35.1	-	36.3	-
23	28.0	0.88 (s)	28.0	0.92 (s)
24	16.5	0.93 (s)	15.9	0.86 (s)
25	14.7	0.94 (s)	15.8	0.86 (s)
26	26.5	1.12 (s)	15.7	0.76 (s)
27	15.3	1.12 (s)	14.4	0.77 (s)
28	25.9	0.84 (s)	177.2	-
29	33.4	0.98 (s)	109.6	4.68 (d, 1.8)
				4.56 (d, 1.8)
30	21.3	0.90 (s)	18.9	1.64 (s)
-COOR	170.2	-		-
-CH$_3$	21.5	2.08 (s)		-

Assignments were based on HMQC, HMBC and NOESY experiments

The complete assignment of compound 4 was based on COSY, NOESY, HMQC, and HMBC experiments. In order to determine the position of the ketone group, a HMBC analysis was done. The spectrum revealed correlations from the proton H-5 (δ1.28) to carbons C-7 (δ206.5), C-3 (δ76.7), C-4 (δ38.2) and C-1 (δ38.3) and from the methyl proton H-26 (δ0.76) to carbons C-7 (δ206.5) and C-8 (δ54.8), suggesting that the ketone group is located at C-7 position.

To establish the absolute stereochemistry, we also measured the CD spectrum, which revealed a negative Cotton effect at 290 and 248 nm (carbonyl $n \to \pi*$) in the CD spectra. These values compared with those of Hancokinol [14] confirm that the absolute stereochemistry of compound **4** is 3*S*,5*R*,8*R*,9*S*,10*R*,13*S*,14*S*,17*R*,18*S*,19*S*. From the above spectroscopic data, the structure of compound **4** was deduced as (3*S*,5*R*,8*R*,9*S*,10*R*,13*S*,14*S*,17*R*,18*S*,19*S*) 3-hydroxy-7-oxolup-20(29)-en-28-oic acid (Fig 1).

Tests of pure compounds by paper disk diffusion assay against the bacteria *Bacillus subtilis, Staphylococcus aureus, Escherichia coli*, the fungi *Mucor miehei* and *Candida albicans*, and the plant pathogen oomycetes *Aphanomyces cochlioides*, *Pythium ultimum* and *Rhizoctonia solani* resulted in missing or low activities, the latter corresponding with MIC values > 1 mg/mL.

However, compounds (**1**, **4**-**7**, **11** and **12**) displayed moderate cytotoxic activity against the human Caucasian prostate adenocarcinoma cell line PC-3 with IC_{50} values ranging from 14.1-30.6 *μ*g/ml, while compounds **2** and **3** showed weak cytotoxic activity with an IC_{50} value of 61.2 respectively 62.5 *μ*g/ml, compared to the standard Doxorubicin displaying IC_{50} = 0.9 *μ*g/ml (Table 2).

Table 2. Cytotoxicity against human prostate adenocarcinoma cell line PC-3

Compounds	IC_{50} ±S.E.M.(μg/ml)
1	27.8±0.5
2	61.2±0.7
3	62.5±0.6
4	14.1±0.5
5	19.8±0.6
6	24.8±0.8
7	30.6±0.9
11	15.5±0.5
12	16.2±0.7
Doxorubicin[a]	0.9±0.1

[a]*Standard used in the assay*

4. CONCLUSION

Two new pentacyclic triterpenoids, 3-acetyltaraxer-14-en-12-one (**1**) and 3-hydroxy-7-oxolup-20(29)-en-28-oic acid (**4**), together with eleven known compounds were isolated from the methanol extract from the bark of *Manilkara zapota*. The agar diffusion test delivered low to missing antimicrobial activities; in addition, compounds **1**, **4**-**7**, **11** and **12** displayed moderate cytotoxic activity against the human Caucasian prostate adenocarcinoma cell line PC-3.

ACKNOWLEDGEMENTS

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Aubreville A. Flore du Cameroun, Muséum national d'histoire naturelle, Laboratoire de phanérogamie Paris. 1963;1(2):157.
2. Ma J, Luo X-D, Protiva P, Yang H, Ma C, Basile MJ, Weinstein IB, Kennelly EJ. Bioactive novel polyphenol from the fruits of *Manilkara zapota* (Sapodilla). J. Nat. Prod. 2003;66:983-986.
3. Kirtikar KR, Basu BD. Indian medicinal plants. International book distributors, publication, New Delhi. 2006;2:1486.
4. Awasare S, Bhujbal S, Nanda R. In vitro cytotoxic of novel oleanane type of triterpenoid saponin from stem bark of *Manilkara zapota* Linn. Asian J. Pharm. Clin. Res. 2012;5(Suppl.)4:183-188.
5. Wang H, Liu T, Song L, Huang D. Profiles and α-amylase inhibition activity of proanthocyanidins in unripe *Manikara zapota* (Chiku). J. Agri. Food Chem. 2012; 60:3098-3104.
6. Maskey RP, Asolkar RN, Kapaun E, Wagner-Döbler I, Laatsch H. Phytotoxic arylethylamides from limnic bacteria using a screening with microalgae. J. Antibiot. 2002;55:643-649.
7. Zhao Q, Chen Q, Hao XJ, Han J, Zuo YG, Zuo C, Xu GL. Cytotoxicity of laddane-type diterpenoids from *Hedychium forrestii*. Chem. Pharm. Bull. 2008;56:210-212.

8. Huang MY, Zhong LJ, Xie JM, Wang F, Zhang YH. A new triterpenes from *Vitextrifolia* var. *simplicifolia*. Helv. Chim. Acta. 2013;96:2040-2044.

9. Wandji J, Tillequin F, Mulholland DA, Wansi JD, Fomum ZT, Fuendjiep V, Libot F, Tsabang N. Fatty acid esters of triterpenoids and steroid glycosides from *Gambeya africana*. Planta Med. 2002; 68:822-826.

10. Sakurai N, Yaguchi Y, Inoue T. Triterpenoids from *Myricarubra*. Phytochemistry. 1986;26:217-219.

11. Drake AF, Salvaldori P, Marsili A, Morelli I. Optical activity in chiral olefins: UV and CD spectra of some triterpenoid hydrocarbons. Tretrahedron. 1977;33:199-204.

12. Fukuda Y, Yamada T, Wada S-I, Sakai K, Matsunaga S, Tanaka R. Lupane and Oleanane Triterpenoids from the Cones of *Liquidamber styraciflua*. J. Nat. Prod. 2006;69:142-144.

13. Mahato SB, Kundu AP. ^{13}C NMR spectra of pentacyclic triterpenoids. A compilation and some salient fearures. Phytochemistry. 1994;37:1517-1575

14. Lou H, Li X, Onda M, Konda Y, Urano M, Harigaya Y, Takayanagi H, Ogura H. Stereochemistry of novel triterpenes from *Cynanchum hancokianum*. Chem. Pharm. Bull. 1991;39:2271-2276.