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ORIGINAL ARTICLE

New triterpene isovanniloyl and antibacterial activity of constituents from the roots of *Paullinia pinnata* Linn (Sapindaceae)

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Abstract In addition to lupeyl steryl ether (**1**) and 3-oxo-11α-hydroxyl-20 (29) lupen (**2**), a new lupeol-3-isovanniloyl ester (**3**) was isolated from *Paullinia pinnata*. Using detailed 1D and 2D NMR spectra and comparison with the published data, the new compound was characterised as 3-*O*-isovanilloyl-3*R*,5*R*,8*R*,9*R*,10*R*,13*R*,14*S*,17*S*,18*R*,19*R*-lup-20(29)-en, for which the trivial name Paullinoyl was proposed. Compound **3** demonstrated significant antibacterial activity on the tested strains (MIC 15.2–30.20 μg/ml). The antibacterial activities obtained in this study confirmed the use of *P. pinnata* in traditional medicine for treating bacterial infections.

1. Introduction

Paullinia pinnata Linn (Sapindaceae) is an African endemic woody vine that inhabits the tropical rainforest zones of West Africa, stretching from Nigeria to Cameroon. Sapindaceae consists of about 136 genera and nearly 2000 species (Ferruci, 2000; Prance, 2001). *Paullinia* consists of 180 species, located in Neotropical zones, except *P. pinnata* which is found also in the African tropics (Hennmann, 1982). Its morphology is documented (Fransworth and Morris, 1976). Aqueous decoctions and powdered root from *P. pinnata* are used in Nigeria, Togo and Ghana traditional medicine for treating sores, wounds, snake bites and other diseases such as erectile dysfunction, malaria, dysentery, menstrual pain and coughs (Irvine, 1991; Taylor et al., 1995; Zanble et al., 2006; Jimoh et al., 2007). *P. pinnata* is called *ogbe-okuje* by the Yoruba natives of South-Western Nigeria, meaning instant wound healer (oral communication). Previous phytochemical studies on *P. pinnata* resulted in the isolation of alkaloid (Martins, 1877), triterpene saponins (Bowden, 1962), flavone glycoside (Abouraseed et al., 1999), triterpenes and steroids (Miemanang et al., 2006). Recently, 5α-portiferastane-3β,6α-diol and 2-(4-hydroxyl-3,5-dimethoxyl

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phenyl)-3-hydroxymethyl-2,3-dihydro-1,4,5-trioxaphenanthrene-6-one were isolated from the leaf EtOAc extract of *P. pinnata* (Dongo et al., 2009). Pharmacological study on aqueous extracts from *P. pinnata* was conducted by a Togolese group (Annanil et al., 2000). The strong antibacterial activity exhibited by *P. pinnata*, in addition to the search for antibacterial phytochemicals from natural sources, justified further attempts to identify and isolate active compounds from *P. pinnata*. Details of isolation, structural and antibacterial evaluation of isolated compounds are described. To our knowledge, compounds **1**, **2** and **3** are reported for the first time from *Paullinia*.

2. Experimental

2.1. General

Melting points, uncorrected were determined on the Gallenkamp (Phillip Harris, England) apparatus. Unless otherwise specified, UV spectra were taken in EtOH, recorded with a Perkin–Elmer Lambda 2 UV/VIS spectrophotometer. IR spectra were recorded on a Perkin Elmer FT-IR spectrophotometer using KBr pellets. The GC–MS was performed with a Hewlett Packard model 5890 Gas chromatography coupled to an EI (70 eV) mass spectrometer, Hewlett–Packard model 5971. Initial and final temps were 70 °C and 310 °C, respectively (rate of heating 60 °C/min) with He carrier gas. ^{1}H and ^{13}C NMR spectral data were recorded on a Jeol spectrometer (200 MHz for ^{1}H and 100 MHz for ^{13}C NMR in $CDCl_3$) using TMS as internal reference. Column chromatography was performed on silica gel [60, 70–230 mesh, ASTM (Merck, Darmstadt, Germany)]. TLC was performed on precoated aluminium sheets [silica gel 60 $PF_{254+360}$, 0.25 mm (Merck)], detected under UV light (254 and 360 nm).

2.2. Plant material

The roots of *P. pinnata*, Linn (Sapindaceae) were collected at the premises of Forest Research Institute of Nigeria (FRIN), Jericho, Ibadan, Oyo State, Nigeria. The plant material was collected and authenticated in July, 2008 by Mr. Felix Inyang – a forester in the herbarium section of FRIN. A voucher specimen was compared with authentic sample in FRIN, labelled FHI 10572.

2.3. Extraction and isolation

Air-dried roots (1.3 kg) from *P. pinnata* were extensively and exhaustively extracted with MeOH (10 L) to give a gummy red solid (400 g) after the evaporation of the solvent. The gummy red solid was suspended in water, extracted with *n*-hexane and $CHCl_3$ in succession. $CHCl_3$ extract was evaporated in vacuo, at reduced pressure to yield brown gummy solid (8 g). Five grams of $CHCl_3$ extract was chromatographed on a column of silica gel (350 g), eluting with increasing percentages of *n*-hexane (66–68 °C) in diethyl ether (Et_2O). Fifty five fractions (Frs) collected were grouped into four according to their TLC profile: Frs 1–15 (hexane), Frs 16–29 (hexane–Et_2O, 8:1), Frs 30–45 (hexane–Et_2O, 1:1) and Frs 46–55 (hexane–Et_2O, 1:3). Frs 16–29 afforded compound **1** (200 mg), Frs 30–44 furnished compound **2** (180 mg), Frs 45–50 yielded compound **3** (120 mg).

2.3.1. Lupeyl steryl ether (**1**)

Elution of the column with *n*-hexane–Et_2O (8:1) yielded a white gum, mp 220–222 °C; TLC: R_f = 0.50 (hexane:EtOAc, 3:1 vol/vol); ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 4.80 (1H, d, J = 2 Hz, H-29b), 4.65 (1H, dd, J = 8, 2 Hz, H-29a), 4.15 (2H, dd, J = 5 Hz, 10.8 Hz, H-1′a,b), 3.61 (2H, t, J = 2 Hz, H-1a,b), 3.18 (1H, dd, J = 11, 5.5 Hz, H-3), 2.36 (m, H-19), 1.67 (br, s, H-30), 1.25 (br, s, –$(CH_2)_n$–), 1.02, 0.96, 0.94, 0.82, 0.78, 0.75 (each 3H, s, H-23, 24, 25, 26, 27, 28); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 151.2 (C-20), 109.6 (C-29), 78.6 (C-3), 63.4 (C-1′), 55.5 (C-5), 50.5 (C-9), 48.4 (C-18), 48.1 (C-19), 37.4 (C-8), 43.0 (C-17), 42.9 (C-14), 41.7 (C-8), 39.9 (C-22), 38.5 (C-4), 38.3 (C-1), 37.5 (C-13), 37.2 (C-10), 35.5 (C-16), 34.6 (C-7), 33.1 (C-2′), 32.1 (C-16′), 29.8 (C-21), 29.5–29.9 (C-3′-15′), 28.1 (C-23), 28.0 (C-15), 27.4 (C-2), 25.2 (C-12), 22.8 (C-17′), 21.0 (C-11), 19.4 (C-30), 18.3 (C-6), 18.1 (C-28), 16.5 (C-26), 16.0 (C-25), 15.4 (C-24), 14.5 (C-27), 14.5 (C-18′).

2.3.2. 3-Oxo-11α-hydroxyl-20 (29) lupen (**2**)

Elution of column with *n*-hexane–Et_2O (1:1) afforded a yellow powder, mp 245–245 °C; TLC: R_f = 0.40 (hexane:EtOAc, 3:1 vol/vol); ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 4.7 (1H, d, J = 2 Hz, H-29b), 4.5 (1H, dd, J = 8, 2 Hz, H-29a), 3.75 (1H, m, H-11), 2.30 (1H, m, H-13), 1.76 (1H, m, H-13), 1.45 (1H, d J = 3.9, H-5), 1.41(1H, t, J = 7.8, H-9), 1.34 (1H, dd, J = 10.9, 4.8) 1.67, 1.05, 1.04, 1.02, 0.98, 0.96, 0.79 (each 3H, s, H-30, 28, 26, 25, 23, 27, 24); ^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm): 212.2 (C-3), 148.3 (C-20), 109.9 (C-29), 70.4 (C-11), 54.9 (C-9), 54.6 (C-5), 47.8 (C-18), 47.7 (C-19), 47.6 (C-4), 43.0 (C-8), 42.4 (C-14), 42.3 (C-17), 42.1(C-1), 39.7 (C-22), 37.9 (C-10), 37.3 (C-15), 37.2 (C-13), 35.4 (C-7), 34.1 (C-2), 34.0 (C-16), 29.5 (C-21), 27.3 (C-12), 27.1 (C-23), 20.5 (C-24), 19.5 (C-6), 19.3 (C-30), 18.1 (C-28), 16.8 (C-26), 16.7 (C-25), 14.2 (C-27).

2.3.3. 3-O-Isovanilloyl-lup-20 (29)-en [(Paullinoyl, (**3**)]

Elution of the column with hexane–Et_2O (1:1) furnished light-yellow powder; mp 245–246 °C; IR: KBr, ν_{max} (cm^{-1})]: 3350 [O–H, broad], 3090 [vinylic (=C–H) stretch], 3050 [C–H stretch, arene], 1645 [C=O stretch], 1580 [C=C stretch], 1402 cm^{-1} [C–O–H stretch].

HR-EI-MS m/z (rel int, %): 576.102 [(M^+), 5%], 546 [($M^+ - CO_2$, 3%], 452 [($M^+ - CO_2 - C_7H_7O_2$), 10%], 248 [3%], [395, 10%], [248, 3%], [207.3, 5%], [189, 100%], [123.1, 60%], [147.3, 2%], [119, 51%], [1076, 3%], [82, 38%]. ^{1}H and ^{13}C NMR ($CDCl_3$): see Table 1.

2.3.4. Acid hydrolysis of compound **3**

Compound **3** (20 mg) was refluxed for 75 min with 4.0 M HCl in MeOH (35 ml). The acid hydrolysate was concentrated, extracted with EtOH and examined by TLC on silica gel in $CHCl_3$/EtOAC (3:1). ^{1}H NMR of the liberated triterpene moiety was compared with naturally-occurring triterpene and found to be similar. The acidic mother liquor was neutralised with Na_2CO_3, filtered, extracted with CH_2Cl_2 and evaporated to dryness for the examination of the isovanillic acid moiety; which proved to be isovanillic acid by direct comparison of

Table 1 ^{1}H, ^{13}C NMR and HMBC connectivities for compound **3**.

C	HMQC		HMBC	DEPT	^{1}H-^{1}H	
	δC	δH			COSY	NOESY
1′	114.5	–	H-5′	C		
2′	112.0	7.40 d (1.9)	H-6′	CH	6′	
3′	151.4	–	H-2′, H-5′	C		
4′	146.5	–	H-2′, H-6′OCH3	C		
5′	114.3	6.90 d (8.3)	H-6′	CH	5′	
6′	124.3	7.50 dd (8.3, 1.9)	H-2′	CH	6′	
7′	165.7	–	H-2′, H-3, H-1, H-2	C		
–OCH3	56.3	3.98 br, s	H-5′, H-6′	CH_3		
–OH	–	6.1 br, s	–	CH_3		
1	38.8	1.52 ddd (13.5, 13.5, 3.7), H-1α 1.24 d (13.5), H-1β	2H-2, H-9, 3H-25	CH_2	2H-2	
2	24.1	1.99 m, H-2α 1.86 m, H-2β	H-3	CH_2	2H-1, H-3	
3	77.9	5.36 dd (12.4, 3.9)	3H-23, 3H-24	CH	2H-2	H-5, 3H-23
4	37.9	–	2H-6, 3H-23, 3H-24	C		
5	56.1	1.39 dd (12.6, 3.9)	H-1, 3H-23 or 3H-25	CH	2H-6	
6	18.2	1.52 m, H-6α 1.27 m, H-6β	H-5	CH_2	H-5, 2H-7	
7	35.5	1.47 m, H-7β 1.75 m, H-7α	3H-26	CH_2		
8	41.7	–	2H-6, 3H-26, 3H-27	C		
9	49.2	1.44 t (7.8)	2H-11, H-12, H-22	CH	2H-11	
10	37.9	–	H-5, 2H-6, H-9, 3H-25	C		
11	22.1	1.52 m, H-11α 1.27 m, H-11β	H-9, H-12	CH_2		
12	25.3	1.24 m, H-12β 1.49 m, H-12α	H-18	CH_2	2H-11	H-18
13	37.9	2.65 m	3H-27	CH	H-18	
14	43.2	–	2H-16, 3H-27	C		
15	27.6	1.49 m, H-15β 1.24 m, H-15α	3H-27	CH_2	2H-16	
16	35.5	1.49 m, H-16β 1.29 m, H-16α	3H-28	CH_2	2H-15	3H-27
17	43.2	–	2H-16	C		
18	48.1	2.20 dd (10.94.8)	H-16, 3H-28	CH	H-13, H-19	
19	46.9	2.16 ddd (10.9, 10.9, 4.8)	2H-29, 3H-30	CH	H-18	
20	150.2	–	3H-30	C		
21	30.0	1.62 m (H-21β) 1.38 m (H-21α)	2H-22	CH_2	2H-22	
22	39.9	1.55 m (H-22β) 1.30 m (H-22α)	2H-16, 3H-28	CH_2	2H-21	
23	27.6	0.79 s		CH_3	H-1	
24	14.7	0.84 s	H-5	CH_3	H-2	
25	16.3	1.09 s	H-5 or H-9	CH_3	H-11	
26	17.8	0.86 s	H-9	CH_3	H-11	
27	14.7	1.22 s		CH_3	H-7, H-16	
28	18.1	1.25 s	2H-22	CH_3	2H-22	
29	109.8	4.81 dd (8.3, 1.8) 4.63 d (1.8)	3H-30	CH_2		
30	19.6	1.71 s	2H-29	CH_3		

^{1}H and ^{13}C NMR spectra were acquired in $CDCl_3$ at 200 MHz. Chemical shifts values are shown in the δ scale (ppm), with coupling constants (J, Hz) in parentheses, s = singlet, d = doublet, dd = doublet of doublet, m = multiplet.

its ^{13}C NMR with an authentic sample of isovanillic acid (Merck).

2.4. Antibacterial assay

The cultures of bacterial strains used were of the National Collection for Industrial bacteria (NCIB) and some locally isolated organisms (LIO). They were obtained at the University College Hospital (UCH), Ibadan, Oyo State, Nigeria. The bacterial strains consist of *Escherichia coli* (NCIB, 15520), *Bacillus subtilis* (NCIB 85650), *Staphylococcus aureus* (NCIB, 22350), *Pseudomonas aeriginosa* (NCIB, 27850), *Shigella dysenteriae* (NCIB, 2350), *Clostridium sporogenes* (LIO) and *anaerobic Clostridium tetani* (LIO).

2.4.1. Determination of minimum inhibitory concentrations (MIC)

The MIC of isolated compounds were evaluated using the broth dilution assay (Ieven et al., 1979; Kudi et al., 1999). Varying concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml, 15 mg/ml) of isolated compounds were prepared. Each concentration (0.1 ml) was added to 9 ml nutrient broth; in a 0.1 ml standardised test organism of bacterial cells. The tubes were incubated at 37 °C for 24 h. Positive controls were set up, using DMSO and test organisms without extracts. The tube with the least concentration of extract without growth after incubation was taken and recorded as the minimum inhibitory concentration.

2.4.2. Statistical analysis

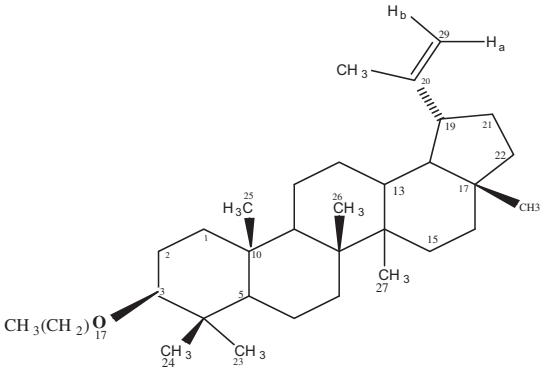
All data were expressed as mean ± SEM. Data were subjected to two-way ANOVA followed by Student's *t*-test, using Microsoft Excel* and Statistical* computer software packages. Difference in mean was considered significant when $p \leqslant 0.05$.

3. Results and discussion

The root chloroform extract from *P. pinnata* was submitted to repeated column chromatography and monitored by TLC to afford lupeol steryl ether (**1**), 3-oxo-11α-hydroxyl-20 (29) lupene (**2**), and a new lupeol-3-isovanilloyl ester (**3**) (Fig. 1). ^{1}H and ^{13}C NMR of compounds, **1** and **2** were consistent with those reported in the literature (Babady-Byla and Werner, 1996; Josinete et al., 2000).

Compound **3** was isolated as a light-yellow powder from hexane–Et_2O (1:4), recrystallised from hexane–EtOAc (7:10), mp 245–246 °C. Compound **3** tested positive to ferric chloride solution, indicating its phenolic nature. IR spectrum shows characteristic bands ($\nu_{max,}$ cm^{-1}) at 3350, attributable to phenolic hydroxyl group; 1645, suggestive of conjugated carbonyl groups; 1600, indicative of aromatic C=C double bonds; 1402, for C–O–H stretch and 1050, for C–O stretch of ether. The UV spectrum of **1** displayed two maximum bands at 293 and 273 nm.

High Resolution Electron Impact Mass Spectrum (HR-EI-MS) of **3** exhibited molecular ion [M^+] peak at m/z 576.102, corresponding to the molecular formula $C_{38}H_{56}O_4$. The ^{1}H NMR spectrum of **3** displayed signals of seven tertiary methyl groups and a doublet of doublet at δ 5.63 (J = 12.4, 3.9) characteristic of axial H-3 of a triterpene under an ester function; in this case that of an isovanniloyl (Ahmadu and Attah-ur-Rahman, 1994). A singlet at δ 3.98 (3H, br) was assigned to aromatic group on C-4′ position through HMBC and NOE difference spectroscopy. The signals at δ 4.59 [1H, d, J = 1.8 Hz, H-29a] and δ 4.71 [1H, dd, J = 8.3, 1.8 Hz, H-29b] are ascribable to the vinylic protons on the side chain



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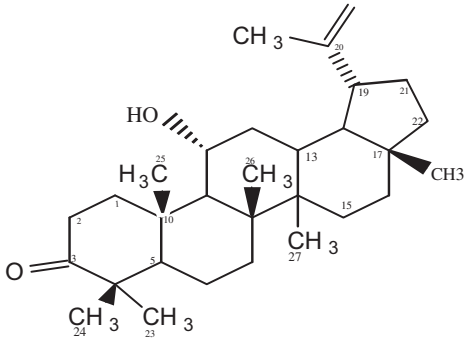

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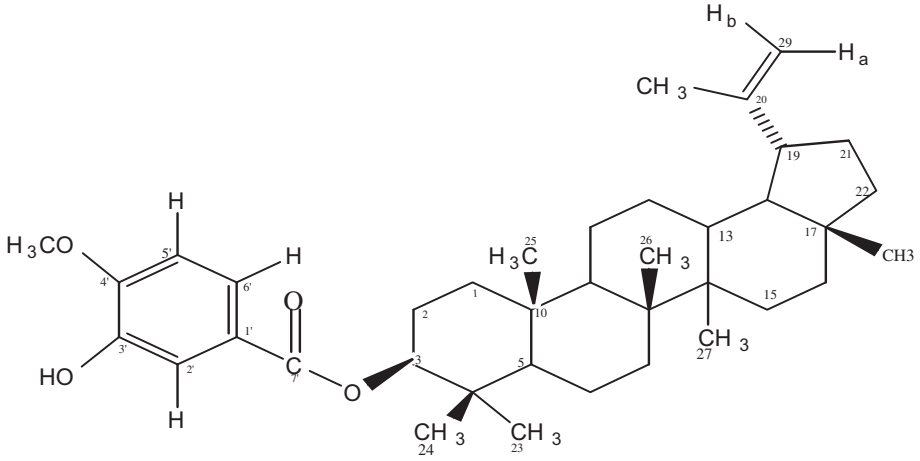

3

Figure 1 Structure of compounds **1**, **2** and **3**.

Table 2 Minimum inhibitory concentrations (MIC) of isolated compounds and reference antibacterial drug (gentamicin).

Minimum inhibitory concentration [MIC, (μg/ml)]							
Isolated compounds	*S. aureus*	*B. subtilis*	*C. sporogenes*	*E. coli*	*P. aueriginosa*	*S. dysenteriae*	*Clostridium tetani*
1	24.3 ± 2.5	22.3 ± 4.6	NA	37.2 ± 1.8	33.4 ± 1.7	15.4 ± 1.3	18.4 ± 0.6
2	28.2 ± 2.5	35.2 ± 8.2	NA	40.1 ± 5.5	37.4 ± 1.5	15.6 ± 0.8	25.2 ± 1.7
3	15.2 ± 5.5	21.1 ± 15.2	25.5 ± 1.5	30.2 ± 12.5	15.4 ± 11.3	20.0 ± 1.2	15.5 ± 5.5
Gentamicin	14.6 ± 10.3	15.1 ± 1.1	20.6 ± 2.4	27.1 ± 5.5	14.2 ± 7.2	12.1 ± 7.5	20.7 ± 1.5
DMSO	NA	NA	NA	NA	NA	NA	NA

Key: NA = no activity.

of the triterpene in **3**. In addition, three aromatic hydrogens on an AMX spin system at δ 7.50 [1H, dd, J = 8.3, 1.9 Hz, H-6′], δ 7.40 [1H, d, J = 1.9 Hz, H-2′] and δ 6.90 [1H, d, J = 8.4 Hz, H-5′] were ascribable to the presence of additional isovanniloyl moiety. ^{1}H NMR spectrum of **3** exhibited one-proton, broad singlet at δ 6.10 (which lost on deuteration), assignable to phenolic-OH. The broad band decoupled ^{13}C NMR and DEPT spectra of **3** exhibited 38 carbons, comprising 10 quaternary, 11 methylene, 9 methine, 8 methyl and a methoxyl carbon at δ 56.3 (br, s) (Table 1). A signal appearing at a conspicuously low field (δ 109.8, C-29) was assigned to vinylic carbon on the triterpene moiety (Ahmadu and Attah-ur-Rahman, 1994). The signals for three methine carbons at δ 112.0, 114.5 and 124.3 ppm were assigned to the methine carbons on the isovanniloyl moiety (Table 1). The unequivocal NMR assignments of compound **3** (Table 1) were established by analyses from COSY, HMQC, HMBC and NOESY experiments. The HMBC spectrum of **3** confirmed the correlation between methoxyl protons at δ 3.98 and carbon signal at δ 146.5 (C-4′). A correlation was observed in the COSY spectrum of **3** justifying methoxyl at C-4′ position (Fig. 1). The position of vanilloyl ester linkage at C-3 was confirmed by HMBC spectrum, where cross peaks between H-2′/C-3 were observed. Furthermore, the ^{1}H–^{1}H scalar correlation observed from the COSY analysis revealed correlations between the methylene groups at δ 1.99 and 1.86 [2H, H-2] with methine proton at δ 5.36 [1H, H-3]; which shows a long-range coupling with methine hydrogen at δ 1.39 [1H, H-5]. A pair of methylene signals (2H, H-1 and 2H, H-2) showed distinct correlations with the quaternary oxygenated carbon at δ 84.7 [C-3]. The relative stereochemistry of compound **3** at C-3 was assigned for axial orientation on the basis of J value and comparison of chemical shift pattern of carbinyl proton (C-3–H) of related lupane triterpene (Ahmadu and Attah-ur-Rahman, 1994; Kobayashi, 1997). The absolute stereochemistry of compound **3** was assigned by Optical Rotatory Dispersion (ORD) spectrum. This was established by comparing the ORD spectrum of **3** with known lupane triterpene at the same concentration (0.2 mg/ml) in MeOH (Chen et al., 1983; Li et al., 1998). On the basis of this comparison, the absolute stereochemistry of ten asymmetric centres in **3** are determined to be 3*R*,5*R*,8*R*,9*R*,10*R*,13*R*,14*S*,17*S*,18*R*,19*R*; consistent with the conventional biosynthetic pathway of lupane triterpenoids (Kobayashi, 1998). The structure of compound **3** was established as 3-*O*-isovanniloyl-3*R*,5*R*,8*R*,9*R*,10*R*,13*R*,14*S*,17*S*,18*R*,19*R*-lup-20(29)-en.

Compounds **1, 2** and **3** were evaluated for antibacterial activities against selected Gram-positive and Gram-negative bacteria, as well as anaerobic *Clostridium* spp. usually implicated for sores and wounds (Birkhauser and Basel, 1978). Compound **3** exhibited significant inhibition on the tested pathogenic strains with MIC values between 15.2 and 30.2 μg/ml especially on *C. tetani* (MIC 15.5 μg/ml). The result of MIC (Table 2) suggests that compounds **1**–**3** could possibly act as a bactericidal agent to these organisms. Gram-positive bacteria are common pathogens responsible for certain diseased conditions; this includes *S. aureus* and *E. coli* for skin infections (Damstatd and Lane, 1994; Trilla and Miro, 1995; Noble, 1998) and *C. tetani*, implicated for sores and wounds (Birkhauser and Basel, 1978; Park and Chun, 2001). From these results, a kind of structure–activity relationship can be drawn to some degree of confidence. Earlier pharmacological reports had attributed phenolic constituents as a criterion for assessing the probable biological effects of the extracts of *P. pinnata* (Zanble et al., 2006). Hydroxyl moiety is a reactive group which hydrogen bond with the active sites of enzymes. Pentacyclic triterpenes isolated from natural sources have been reported as compounds with good medicinal properties (Park and Chun, 2001, Jiri, 2003, Cássia et al., 2010). Results of antibacterial activities in this study validated the use of root decoctions from *P. pinnata* in traditional medicine for treating sores, wounds and other bacterial infections.

4. Conclusion

This research study reports a new lupeol-3-isovanilloyl ester (**3**) from *P. pinnata*. The new compound was fully characterised and it is one of the rare reported phytochemicals in *P. pinnata*. Compound **3** and other isolates from *P. pinnata* were evaluated for their antibacterial activities on selected bacteria. Compound **3** demonstrated good *in vitro* antibacterial activities on *C. tetani*, implicated for sores and wounds. Results obtained in this study supported the use of root decoctions from *P. pinnata* in traditional medicine for treating bacterial infections.

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Nigeria for the pathogenic strains provided for the antibacterial assay.

References

Abouraseed, E.A., Toyang, N.T., Choinski, J., Khan, I.A., 1999. Two new flavone glycosides from *Paullinia pinnata*. J. Nat. Prod. 62, 1179–1181.

Ahmadu, V.I.U., Attah-ur-Rahman, 1994. In: Handbook of Natural Product Data – Pentacyclic Triterpenoids, vol. 2. Elsevier, Amsterdam, pp. 1225–1227.

Annanil, K., Hudson, J.B., Dasouzal, C., Akpaguanal, K., Tower, G.H.N., Ananson, J., Gbeassori, M., 2000. Investigating medicinal plants of Togo for antiviral and antimicrobial activities. Pharm. Biol. 38 (1), 40–45.

Babady-Byla, Werner, H., 1996. Triterpenes and 1-(-hydroxylceratyl) glycerols from *Pentaclethra eetveldeana* root bark. Phytochemistry 42 (2), 501–504.

Birkhauser, Basel, H., 1978. Wound healing properties of naphthaquinone pigment from *Alkana tinctoria*. Cell. Mol. Life Sci. 34 (11), 2021–2025.

Bowden, K., 1962. Triterpene saponins from *P. pinnata*. J. Pharmacol. 18, 173–174.

Cássia, G.M., Fernanda, C.F., Denia, A.S., Gracia, D.F., Silva, L.P., Duarte, R.C., Figueiredo Sidney, A.V.F., 2010. *Maytenus salicifolia* Reissek, Celastraceae: triterpenes isolated from stems and antioxidant property of extracts from aerial parts. Braz. J. Pharmacol. 25, 1–5.

Chen, T.K., Ales, D.C., Baenziger, N.C., Wiemer, D.F., 1983. Antirepellant triterpenoids from *Cordial alliodoro*. J. Org. Chem. 48, 3525–3531.

Damstatd, G.L., Lane, A.T., 1994. Impetigo: an overview. Pediatr. Dermatol. 25, 293–303.

Dongo, E., Hussain, H., Miemanang, R.S., Tazoo, D., Schulz, B., Krohn, K., 2009. Chemical constituents of *Klainedoxa gabonensis* and *Paullinia pinnata*. Rec. Nat. Prod. 3, 165–169.

Ferruci, M.S., 2000. Cytotaxonomy of the Sapindaceae with special reference to Prance tribe, *Paullinieae*. Genet. Mol. Biol. 23, 941–946.

Fransworth, A.N., Morris, J.L., 1976. Economic aspect of medicinal plants. Phytochemistry 8, 309–311.

Hennmann, A.R., 1982. Ecological and social perspectives on an economic plant of the Central Amazon basin. J. Ethnopharmacol. 6, 311–338.

Ieven, M., Vanden, I., Berghe, D.A., Mertens, F., Vlentinck, A., Lammens, E., 1979. Screening higher plants for biological activity. Planta Med. 36, 311–321.

Irvine, E.R., 1991. In: Woody Plants of Ghana with Special Reference to their Use, vol. 446. Oxford University Press, London, pp. 547–549.

Jimoh, F.O., Sofidiya, M.O., Afolayan, A., 2007. Antioxidant properties of the methanol extracts from the leaves of *Paullinia pinnata*. J. Med. Food 15, 215–219.

Jiri, Patocka, 2003. Biologically active pentacyclic triterpenes and their current medicine signification. J. Appl. Biomed. 1, 7–12.

Josinete, S.A., Janiza, C.M., Maisa, O.F., Emidio, V., Leitao da-Cunha, , Jose, M.B., Marcelo de Silva, 2000. Complete assignments of the ^{1}H- and ^{13}C-NMR spectra of four triterpenes of the ursane, artane, lupane and friedelane groups. Magn. Reson. Chem. 38, 201–206.

Kobayashi, M., 1997. The fucofuranoside method, a new ^{1}H- and ^{13}C-nuclear magnetic resonance method to determine the absolute configuration of secondary alcohol. Tetrahedron 53, 5973–5994.

Kobayashi, M., 1998. The fucofuranoside method for determining the absolute configuration of the tertiary alcohols substituted with methyl and two methylene groups. Tetrahedron 54, 10987–10998.

Kudi, A.C., Umoh, J.U., Eduvie, L.O., Gefu, 1999. Screening of Nigerian medicinal plants for antibacterial activity. J. Ethnopharmacol. 67, 225–228.

Li, T.S., Wang, J.X., Zheng, X.J., 1998. Simple synthesis of allobetuline, 28-oxyallobetuline and related biomarkers from betulin and betulinic acid catalysed by solid acid. J. Chem. Soc., Perkin Trans. I, 3957–3965.

Martins, Stanilaus, 1877. An alkaloid timbonine from *Paullinia pinnata*. Pharm. J. Trans. VII, 1020–1023.

Miemanang, R.S., Krohn, K., Hussain, H., Dongo, E.Z., 2006. Paullinoside A: a new cerebroside and a new ceramide from leaves of *P. pinnata*. Z. Naturforsch. 61b, 1123–1127.

Noble, W.C., 1998. Skin bacteriology and role of *E. coli* infections. Br. J. Dermatol. 139 (53), 9–12.

Park, E.H., Chun, M.J., 2001. Wound healing activity of *Opuntia ficus-indica*. Fitoterapia 72, 165–167.

Prance, G.T., 2001. Discovering the plant world. Taxon 50, 345–359.

Taylor, R.L., Manandhar, N.P., Towers, G.N., 1995. Screening of selected medicinal plants of Nepal for antimicrobial activities. J. Ethnopharmacol. 546, 153–159.

Trilla, A., Miro, J.M., 1995. Identifying high risk patients for *Staphylococcus aureus*, skin and soft tissue infections. J. Chemother. 7, 37–43.

Zanble, A.M.S., Carpentier, M., Kandoussi, A., Schpaz, S., Petrault, O., Ouk, T., Hennuyer, N., Fruchert, J.C., Staeles, B., Bodret, R., Duriez, P., Baillent, F.J., 2006. *P. pinnata* extracts rich in polyphenols, promote vascular relaxation via endothelium-dependent mechanism. Cardiovasc. Pharmacol. 47 (4), 559–608.