

## Full Length Research Paper

**Terpenoids from *Phaulopsis imbricata* (Acanthaceae)****Archile Bernabe Ouambo Kengne<sup>1</sup>, Mathieu Tene<sup>1\*</sup>, Alembert Tiabou Tchinda<sup>2</sup>, Pierre Tane<sup>1</sup> and Michel Frédérick<sup>3</sup>**

<sup>1</sup>Laboratory of Natural Products Chemistry, Chemistry Department, University of Dschang, P. O. Box 67 Dschang, Cameroon.

<sup>2</sup>Laboratory of Phytochemistry, Institute of Medical Research and Medicinal Plant Studies, (IMPM), P.O. Box 6163, Yaoundé, Cameroon.

<sup>3</sup>Laboratory of Pharmacognosy, Interfaculty Centre for Research on Medicine (CIRM), Department of Pharmacy, University of Liège, B36, B-4000, Liège, Belgium.

Received 8 December, 2015; Accepted 16 February, 2016

The whole plant of *Phaulopsis imbricata* (Forssk.) Sweet (Acanthaceae) was collected at Bansa, Cameroon, shade dried and extracted by maceration in methanol. This study was carried out to isolate secondary metabolites from this plant species that has not been investigated so far. Two lupane-type triterpenoids, one  $\beta$ -type carotenoid, one eudesmane-type sesquiterpenoid, and one sterol glycoside were isolated from the dried methanol extract using solvent partitioning, column chromatography and re-crystallization. They were identified as lupeol, betulin, (*all-E*)-lutein, cryptomeridiol, and sitosterol 3-O- $\beta$ -D-glucopyranoside, respectively. The structures of the isolated compounds were elucidated on the basis of spectroscopic methods including 1D- and 2D- nuclear magnetic resonance (NMR), infrared (IR) and mass spectrometry (MS). This is the first report of these compounds from the genus *Phaulopsis*. To the best of our knowledge, *P. imbricata* is also the first species of the genus to be phytochemically studied.

**Key words:** *Phaulopsis imbricata*, chromatography, spectroscopy, terpenoids, lupeol, betulin, (*all-E*)-lutein, cryptomeridiol.

**INTRODUCTION**

The genus *Phaulopsis* (Acanthaceae) comprises about 22 species encountered in tropical Africa and Asia (Ke et al., 2011). *Phaulopsis imbricata* (Forssk.) Sweet (syn. *Aetheilema anisophyllum* R. Brown) is an erect herb of about 1 m high mainly distributed in West Africa (Kayode and Omotoyinbo, 2008; Brummet, 2005). It has been used as medicinal plant for the treatment of pain, arthritis, rheumatism, skin diseases, diarrhoea, dysentery,

stomachache, nausea and sores (Burkill, 1985). It is also used as chewing stick for oral and dental healthcare (Kayode and Omotoyinbo, 2008). Another species, *P. fascicepala*, has been reported to possess antioxidant activity (Adesegun et al., 2009).

Many workers have isolated terpenoids from some species of Acanthaceae (Berrondo et al., 2003; Tamokou et al., 2011; Sudhanshu et al., 2000). But, according to

\*Corresponding author. E-mail: mtene2001@yahoo.fr. Tel: +237- 677 808 420. Fax: +237- 243 451 735.

our knowledge, no phytochemical study has been reported from the genus *Phaulopsis*. This study approach has been to investigate the chemical constituents of *P. imbricata*. We now report, herein, the isolation and structural identification of two triterpenoids (lupeol and betulin), one carotenoid [(*all-E*)-lutein], one sesquiterpenoid (cryptomeridiol), and one sterol glycoside (sitosterol 3-*O*- $\beta$ -D-glucopyranoside) from the methanol extract of the whole plant of *P. imbricata*.

## MATERIALS AND METHODS

### General

Melting points (m.p.) were recorded with a Reichert microscope and uncorrected.  $^1\text{H}$  NMR (500 MHz) and  $^{13}\text{C}$  NMR (125 MHz) with APT program were recorded at room temperature in  $\text{CDCl}_3$ , using a Bruker DMX 500 spectrometer. The chemical shifts ( $\delta$ ) are reported in parts per million (ppm) with the solvent signals as reference relative to TMS ( $\delta = 0$ ) as internal standard, while the coupling constants ( $J$  values) are given in Hertz (Hz). COSY, NOESY, HSQC and HMBC experiments were recorded with gradient enhancements using sine shape gradient pulses. The Infra red (IR) spectra were recorded with a Shimadzu FTIR-8400S Infrared spectrophotometer and Gas chromatography-Mass spectrometry (GC-MS) data were obtained with an Agilent 6890N Network GC system/5975 Inert XL Mass Selective Detector at 70 eV and 20°C. The GC column (VARIAN, USA) was a CP-Sil 8 CB LB/MS, chromatopack capillary column (0.25 mm  $\times$  30 m, film thickness 0.25  $\mu\text{m}$ ). The initial temperature was 50°C for 1 min, and then heated at 10°C/min to 300°C. For the carrier gas, helium was used with a flow rate of 1.20 ml/min. Kovat's retention index (KI) was determined using a calibration curve of *n*-alkanes. Column chromatography was run on Merck silica gel 60 (70 to 230 mesh) (MERCK, Germany) and gel permeation on Sephadex LH-20 (SIGMA-ALDRICH, St. Louis, MO, USA) while thin layer chromatography (TLC) was carried out on silica gel GF<sub>254</sub> pre-coated plates (MERCK, Germany) with detection accomplished by spraying with 50%  $\text{H}_2\text{SO}_4$  followed by heating at 100°C, or by visualizing with a UV lamp at 254 and 365 nm.

### Plant

The whole plant of *P. imbricata* was collected from Bansa, West Region, Cameroon, in January, 2010. Authentication was done by Mr Paul Mezili, a retired Botanist of the Cameroon National Herbarium, Yaoundé, where the voucher specimen (No. 19290 / SRF / Cam) is deposited.

### Extraction and isolation of compounds

The fresh plant of *P. imbricata* was dried under shadow for two weeks. The powdered material (2 kg) was extracted three times by maceration with MeOH (10 L) at room temperature (three days for each time). Evaporation of solvent under vacuum afforded 109 g of crude extract. A portion of this extract (105 g) was successively extracted with *n*-hexane, EtOAc and *n*-butanol. TLC analysis showed that the *n*-hexane and EtOAc extracts (21 and 3 g, respectively) were qualitatively the same. They were thus combined and a portion (23 g) subjected to silica gel (70 to 230 mesh) column chromatography eluted with gradient of *n*-hexane-EtOAc (100:0, 9:1, 4:1, 7:3, 1:1 and 0:100) followed by gradient of EtOAc-MeOH

(100:0, 19:1, 9:1 and 0:100). Sixty-six fractions of 300 ml each were collected and combined on the basis of TLC profile to give six major fractions A to F (A: 1 to 9; B: 10 to 20; C: 21 to 27; D: 28 to 45; E: 46 to 53; F: 54 to 66). Fraction A (3.2 g) was purified on a silica gel column eluted with gradient of *n*-hexane-EtOAc (10:0, 9:1, 4:1 and 7:3). 25 fractions of 50 ml each were collected to afford compound **1** (31 mg) after re-crystallization of sub-fractions 11 to 19 (A2). Fraction B (5 g) was chromatographed on Sephadex LH-20 column eluted with the isocratic *n*-hexane- $\text{CH}_2\text{Cl}_2$ -MeOH (7:4:0.5). 21 fractions of 10 ml each were collected to afford compound **2** (14 mg) from sub-fraction B2 (fractions 8 to 17) and mixture of chlorophylls. Fraction C (2.5 g) was also passed through Sephadex LH-20 column chromatography eluted with the isocratic *n*-hexane- $\text{CH}_2\text{Cl}_2$ -MeOH (7:4:0.5). 14 fractions of 10 ml each were collected to give compound **3** (11 mg) after re-crystallization of sub-fraction C1 (fractions 1 to 9) in *n*-hexane. Fraction D was also chromatographed on Sephadex LH-20 column eluted with the isocratic *n*-hexane- $\text{CH}_2\text{Cl}_2$ -MeOH (7:4:0.5). 20 fractions of 10 ml each were collected yielding three sub-fractions. Sub-fraction D2 (9 to 14) was purified through silica gel column chromatography eluted with the gradient  $\text{CH}_2\text{Cl}_2$ -MeOH (100:0, 49:1, 19:1, 9:1, 7:3 and 1:1) giving 10 mg of compound **4**.

The *n*-butanol extract (20 g) was also subjected to silica gel (70 to 230 mesh) column chromatography eluted with the gradient EtOAc-MeOH (100:0, 19:1, 9:1, 4:1 and 0:100). Seventeen fractions of 300 ml each were collected and combined on the basis of TLC profile to give three major fractions G to I (G: 1 to 8; H: 9 to 11; I: 12 to 17). Fraction G (3.7 g) crystallized to afford compound **5** (101 mg). An attempt to purify fractions E, F, H, I and filtrate of G failed, affording only complex mixtures in each case.

## RESULTS

Compound **1** was obtained as white pellets from MeOH. It had a melting point (m.p.) of 216°C and showed the following spectral characteristics:

IR (KBr)  $V_{\text{max}}$   $\text{cm}^{-1}$ : 3300, 3080, 2920, 1640, 1470, 1420, 1390, 1045, 885. EIMS:  $m/z$  (rel. int.) = 426 $[\text{M}]^+$  (22), 207 (51), 189 (84), 161 (48), 135 (91), 121 (98), 95 (100), 81 (86), 55 (65), 41 (38).

Compound **2** was obtained as white crystals from MeOH. m.p. 237-238 °C. It showed the following spectral characteristics:

IR (NaCl)  $V_{\text{max}}$   $\text{cm}^{-1}$ : 3456, 3080, 2925, 1639, 1480, 1460, 1345, 1060, 885. EIMS:  $m/z$  (rel. int.) = 442 $[\text{M}]^+$  (4), 207 (52), 189 (81), 135 (82), 121 (72), 95 (100), 81 (93), 55 (80). HR-EI-MS:  $m/z$  = 442.3786  $[\text{M}]^+$  (calcd. 442.3811 for  $\text{C}_{30}\text{H}_{50}\text{O}_2$ ).  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectral data (Table 1).

Compound **3** was obtained as reddish crystals from *n*-hexane. m.p. 190-191°C. It showed the following spectral data:

IR (NaCl)  $V_{\text{max}}$   $\text{cm}^{-1}$ : 3500, 2962, 2921, 1662, 1390. EIMS:  $m/z$  (rel. int.) = 568  $[\text{M}]^+$  (1), 550 (12), 479 (3), 442 (5), 313 (3), 135 (80), 121 (75), 95 (100), 81 (90), 55 (83). HR-EI-MS:  $m/z$  = 568.4276  $[\text{M}]^+$  (calcd. 568.4280 for  $\text{C}_{40}\text{H}_{56}\text{O}_2$ ).  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectral data (Table 2).

**Table 1.**  $^1\text{H}$ - and  $^{13}\text{C}$ /APT- NMR spectral data of betulin (**2**) ( $\text{CDCl}_3$ ,  $\delta$  in ppm), and, selected HMBC data for **2**.

No	$\delta_{\text{C}}$	APT	$\delta_{\text{H}}$ , mult.(J in Hz)	HMBC (H→C)
1	38.7	CH <sub>2</sub>	0.72, m; 1.62, m	2, 10, 25
2	27.4	CH <sub>2</sub>	1.42, m; 1.58, m	1, 3, 4, 10
3	79.0	CH	3.18, dd (15.0, 5.0)	1, 4, 5, 23, 24
4	38.9	C	-	-
5	55.3	CH	0.67, m	3, 4, 6, 10, 23, 25
6	18.3	CH <sub>2</sub>	1.38, m; 1.52, m	4, 5, 7, 8, 10
7	34.0	CH <sub>2</sub>	1.39, m; 1.39, m	5, 6, 8, 9, 14, 26
8	40.5	C	-	-
9	50.4	CH	1.27, m	7, 8, 10, 11, 12
10	37.2	C	-	-
11	20.8	CH <sub>2</sub>	1.21, m; 1.42, m	8, 9, 10, 12, 13
12	25.0	CH <sub>2</sub>	1.02, m; 1.63, m	9, 11, 13, 14, 18
13	37.5	CH	1.60, m	8, 12, 14, 17, 18, 19, 27
14	42.5	C	-	-
15	27.0	CH <sub>2</sub>	1.05, m; 1.67, m	13, 14, 16, 17, 27
16	29.7	CH <sub>2</sub>	1.21, m; 1.90, m	14, 15, 17, 18, 22, 28
17	47.8	C	-	-
18	48.9	CH	1.57, m	12, 13, 14, 16, 17, 19, 20
19	47.8	CH	2.38, m	13, 17, 18, 20, 21, 29, 30
20	150.5	C	-	-
21	29.7	CH <sub>2</sub>	1.42, m; 1.95, m	17, 18, 19, 20, 22
22	34.6	CH <sub>2</sub>	1.02, m; 1.83, m	16, 17, 18, 21, 28
23	27.4	CH <sub>3</sub>	0.93, s	3, 4, 5, 24
24	15.9	CH <sub>3</sub>	0.76, s	3, 4, 5, 23
25	16.3	CH <sub>3</sub>	0.82, s	1, 5, 9, 10
26	16.1	CH <sub>3</sub>	0.98, s	7, 8, 9, 14
27	15.4	CH <sub>3</sub>	0.90, s	8, 13, 14, 15
28	60.6	CH <sub>2</sub>	3.33, d (10.0); 3.80, d (10.0)	16, 17, 18, 22
29	109.7	CH <sub>2</sub>	4.58, br s; 4.66, br s	19, 20, 30
30	19.1	CH <sub>3</sub>	1.68, s	19, 20, 29

Compound **4** was obtained as white crystals from MeOH. m.p. 141 to 143°C. It showed the following spectral characteristics:

IR (NaCl)  $V_{\text{max}}$   $\text{cm}^{-1}$ : 3600-3200, 2920, 2848, 1456, 1377, 1238, 1166, 1049. EIMS:  $m/z$  (rel. int.) = 240 [ $\text{M}$ ]<sup>+</sup> (1), 222 (1), 204 (17), 189 (33), 149 (100), 123 (39), 109 (52), 95 (45), 43 (65). HR-EI-MS:  $m/z$  = 240.2094 [ $\text{M}$ ]<sup>+</sup> (calcd. 240.2089 for  $\text{C}_{15}\text{H}_{28}\text{O}_2$ ).  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectral data (Table 3).

Compound **5** was obtained as white powder. m.p. 252-254 °C. IR (KBr)  $V_{\text{max}}$   $\text{cm}^{-1}$ : 3500-3300, 2953, 2866, 1108.

## DISCUSSION

Compound **1** was considered to be a triterpenoid due to a positive (violet coloration) Liebermann Burchard test. Its

electron impact mass spectrum (EIMS) showed molecular ion peak at  $m/z$  426 (GC-MS). Analysis of the IR spectrum suggested that it contained a hydroxyl group ( $3300\text{ cm}^{-1}$ ) and a terminal double bond ( $3080, 1640, 885\text{ cm}^{-1}$ ). On the basis of TLC, m.p. and IR analyses, compound **1** was suggested to be lupeol, a lupane-type triterpenoid previously isolated in our laboratory (Tene et al., 2009). The identity of compound **1** was confirmed by comparison of its mass spectrum with that of lupeol in the NIST GC-MS library (NIST, USA).

Compound **2** showed a positive (violet coloration) Liebermann Burchard test and was suggested to be a triterpenoid. Its EIMS indicated a molecular ion peak at  $m/z$  442 and a molecular formula  $\text{C}_{30}\text{H}_{50}\text{O}_2$  on the basis of its HR-EIMS (442.3793 [ $\text{M}$ ]<sup>+</sup>). The IR spectrum showed vibration bands corresponding to hydroxyl group ( $3456\text{ cm}^{-1}$ ) and a terminal double bond ( $3080, 1639, 885\text{ cm}^{-1}$ ). Its  $^1\text{H}$  NMR spectrum showed five methyl singlets at  $\delta_{\text{H}}$

**Table 2.**  $^1\text{H}$ - and  $^{13}\text{C}$ /APT- NMR spectral data of (all-*E*)-lutein (**3**) ( $\text{CDCl}_3$ ,  $\delta$  in ppm), and, selected HMBC data for **3**.

No	$\delta_{\text{C}}$	APT	$\delta_{\text{H}}$ , mult.(J in Hz)	HMBC (H→C)
1	37.2	C	-	-
2	48.4	CH <sub>2</sub>	1.48, t (10.7) 1.84, dd (6.1, 13.5)	- -
3	65.1	CH	4.02, m	-
4	42.6	CH <sub>2</sub>	2.03, dd (10.7, 15.0) 2.37, m	3, 5, 6 2, 3, 5, 6
5	126.2	C	-	
6	137.7	C	-	
7	125.6	CH	6.12, m	
8	138.5	CH	6.11, m	
9	135.7	C	-	
10	131.3	CH	6.15, m	
11	125.0	CH	6.63, m	
12	137.6	CH	6.36, d (15.6)	
13	136.5	C	-	
14	132.6	CH	6.26, m	
15	130.1	CH	6.63, m	
16	29.5	CH <sub>3</sub>	1.09, s	1, 2, 6
17	30.3	CH <sub>3</sub>	1.09, s	1, 2, 6
18	21.7	CH <sub>3</sub>	1.74, s	4, 5, 6
19	12.9	CH <sub>3</sub>	1.99, s	8, 9, 10
20	12.9	CH <sub>3</sub>	1.99, s	12, 13, 14
1'	34.1	C	-	-
2'	44.7	CH <sub>2</sub>	1.37, dd (7.1, 13.5) 1.84, dd (6.1, 13.5)	1', 3', 16', 17' 1', 3', 16', 17'
3'	65.9	CH	4.27, br s	-
4'	124.5	CH	5.55, br s	2', 3', 5', 6', 18'
5'	138.0	C	-	-
6'	55.0	CH	2.39, m	1', 2', 4', 5', 7', 16', 18'
7'	128.8	CH	5.43, dd (10.9, 15.6)	1', 5', 6', 8', 9'
8'	137.7	CH	6.15, m	6', 7', 9'
9'	135.1	C	-	-
10'	130.8	CH	6.15, m	-
11'	124.8	CH	6.63, m	-
12'	137.6	CH	6.36, d (15.6)	-
13'	136.4	C	-	-
14'	132.6	CH	6.26, m	-
15'	130.1	CH	6.63, m	-
16'	24.3	CH <sub>3</sub>	1.02, s	1', 2', 6', 17'
17'	28.8	CH <sub>3</sub>	0.87, s	1', 2', 6', 16'
18'	22.9	CH <sub>3</sub>	1.63, s	4', 5', 6'
19'	13.2	CH <sub>3</sub>	1.93, s	8', 9', 10'
20'	12.9	CH <sub>3</sub>	1.99, s	12', 13', 14'

**Table 3.**  $^1\text{H}$ - and  $^{13}\text{C}$ /APT- NMR spectral data of cryptomeridiol (**4**) ( $\text{CDCl}_3$ ,  $\delta$  in ppm), and, selected HMBC data for **4**.

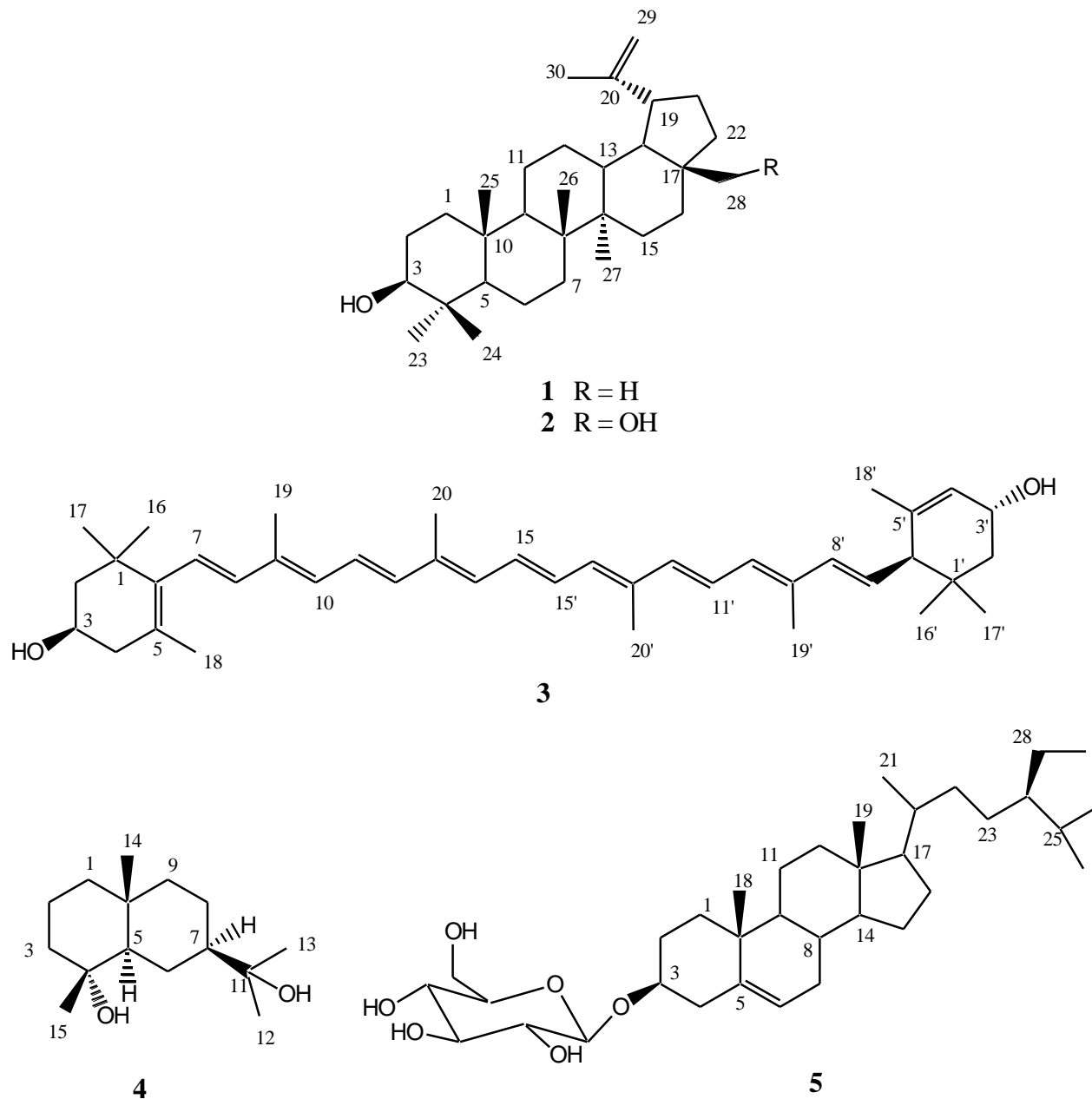
No	$\delta_{\text{C}}$	APT	$\delta_{\text{H}}$ , mult.(J in Hz)	HMBC (H→C)
1	41.0	CH <sub>2</sub>	1.09, m; 1.42, m	9, 14
2	20.2	CH <sub>2</sub>	1.57, m	3
3	43.5	CH <sub>2</sub>	1.38, m; 1.80, d (12.5)	1, 2, 4, 5, 15
4	72.4	C	-	-
5	54.8	CH	1.22, m	6, 14, 15
6	21.5	CH <sub>2</sub>	1.05, m; 1.92, d (12.2)	4, 5, 7, 8, 10, 11
7	49.9	CH	1.38, m	8, 11
8	22.5	CH <sub>2</sub>	1.30, m; 1.59, m	7, 9, 11
9	44.6	CH <sub>2</sub>	1.15, m; 1.47, m	5, 7
10	34.5	C	-	-
11	73.0	C	-	-
12	27.1	CH <sub>3</sub>	1.23, s	7, 11, 13
13	27.3	CH <sub>3</sub>	1.23, s	7, 11, 12
14	18.7	CH <sub>3</sub>	0.88, s	1, 5, 9, 10
15	22.6	CH <sub>3</sub>	1.13, s	3, 4, 5

0.76, 0.82, 0.90, 0.93 and 0.98, a vinyl methyl group at  $\delta_{\text{H}}$  1.68 (3H-30, s), a double doublet of one oxygenated methine proton at  $\delta_{\text{H}}$  3.18 (H-3, dd, 5.0, 15.0), two doublets of one oxygenated methylene protons at  $\delta_{\text{H}}$  3.33 (H-28a, d, 10.0) and 3.80 (H-28b, d, 10.0) and two broad singlets of a terminal double bond, one proton each at  $\delta_{\text{H}}$  4.58 (H-29a) and 4.66 (H-29b) (Table 1). The  $^{13}\text{C}$  NMR/APT revealed 30 carbon signals including 6 methyl groups, 12 methylene groups, 6 methine carbons and 6 quaternary carbons (Table 1). The oxygenated carbon signals appeared at  $\delta_{\text{C}}$  60.6 (C-28) and 79.0 (C-3) and the double bond at  $\delta_{\text{C}}$  109.7 (C-29) and 150.5 (C-20), characteristics of triterpenes of the lup-20(29)-ene type (Mahato and Kundu, 1994). In the HSQC spectrum correlations observed between H-3 ( $\delta_{\text{H}}$  3.18) and C-3 ( $\delta_{\text{C}}$  79.0), H-28a ( $\delta_{\text{H}}$  3.33) and C-28 ( $\delta_{\text{C}}$  60.6), H-28b ( $\delta_{\text{H}}$  3.80) and C-28 ( $\delta_{\text{C}}$  60.6), H-29a ( $\delta_{\text{H}}$  4.58) and C-29 ( $\delta_{\text{C}}$  109.7), H-29b ( $\delta_{\text{H}}$  4.66) and C-29 ( $\delta_{\text{C}}$  109.7), suggested compound **2** to be a 3, 28-dihydroxylup-20(29)-ene. The positions of the two hydroxyl (3-OH and 28-OH) and vinylidene (C=CH<sub>2</sub>) groups were confirmed in the HMBC spectrum by correlations between H-3 ( $\delta_{\text{H}}$  3.18) and C-1 ( $\delta_{\text{C}}$  38.7), C-4 ( $\delta_{\text{C}}$  38.9), C-5 ( $\delta_{\text{C}}$  55.3), C-23 ( $\delta_{\text{C}}$  27.4), and C-24 ( $\delta_{\text{H}}$  15.9), H-28a ( $\delta_{\text{H}}$  3.33)/H-28b ( $\delta_{\text{H}}$  3.80) and C-16 ( $\delta_{\text{C}}$  29.7), C-17 ( $\delta_{\text{C}}$  47.8), C-18 ( $\delta_{\text{C}}$  48.9) and C-22 ( $\delta_{\text{C}}$  34.6), H-29a ( $\delta_{\text{H}}$  4.58)/H-29b ( $\delta_{\text{H}}$  4.66) and C-19 ( $\delta_{\text{C}}$  47.8), C-20 ( $\delta_{\text{C}}$  150.5) and C-30 ( $\delta_{\text{C}}$  19.1). Furthermore, correlations observed in the NOESY spectrum between H-3 ( $\delta_{\text{H}}$  3.18, dd, J = 5.0, 15.0 Hz) and H-5 ( $\delta_{\text{H}}$  0.67, m), and H-3 ( $\delta_{\text{H}}$  3.18) and H-23 ( $\delta_{\text{H}}$  0.93, s) suggested the 3-OH group to be  $\beta$ -oriented.

Compound **2** was identified to 3 $\beta$ ,28-dihydroxylup-20(29)-ene (betulin), a lupane-type triterpenoid previously reported from other sources (Tinto et al., 1992; Joshi et

al., 2013).

HR-EIMS of compound **3** showed the molecular ion at  $m/z$  568.4276  $[\text{M}]^+$  in agreement with the molecular formula  $\text{C}_{40}\text{H}_{56}\text{O}_2$  (13 $^{\circ}$  of unsaturation). The IR spectrum showed bands for hydroxyl group (3500  $\text{cm}^{-1}$ ) and double bond (1662  $\text{cm}^{-1}$ ).  $^{13}\text{C}$ -NMR/APT indicated signals for forty carbon atoms including ten methyls, three methylenes, eighteen methines and nine quaternary carbons (see Table 2). Its  $^1\text{H}$  NMR spectrum showed signals for four angular methyl groups at  $\delta_{\text{H}}$  0.87 (3H-17', s), 0.99 (3H-16 and 3H-17, s) and 1.02 (3H-16', s), six vinyl methyl groups at  $\delta_{\text{H}}$  1.63 (3H-18', s), 1.74 (3H-18, s), 1.93 (3H-19', s), 1.99 (3H-19, 3H-20 and 3H-20', s) and three methylene groups at  $\delta_{\text{H}}$  1.37 (H-2' $\alpha$ , dd, J = 7.1, 13.5 Hz), 1.48 (H-2 $\alpha$ , t, J = 10.7 Hz), 1.84 (H-2 $\alpha$ , H-2' $\alpha$ , each dd, J = 6.1, 13.5 Hz), 2.03 (H-4 $\alpha$ , dd, J = 10.7, 15.0 Hz) and 2.37 (H-4 $\alpha$ , m). Also on this spectrum, three aliphatic methine protons resonated respectively at  $\delta_{\text{H}}$  2.39 (H-6', m), 4.02 (H-3, m) and 4.27 (H-3', *br* s) (Table 2). The proton sequence of the compound was deduced from the  $^1\text{H}$ - $^1\text{H}$  COSY and the gradient HMQC spectra while the HMBC spectrum permitted the construction of the skeleton of **3**.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectral data suggested the presence in the molecule of two different and six-membered aliphatic rings, each bearing one hydroxyl (C-3 / C-3'), one double bond (C-5, C-6 / C-4', C-5') and three methyl (C-16, C-17, C-18 / C-16', C-17', C-18') groups. Each cyclohexenyl moiety is attached in one end of a symmetrical polyene system. But due to the structure difference of the two rings the molecule is not symmetric (Figure. 1 and Table 2). Signals of the polyene region of the  $^1\text{H}$  NMR spectrum were identical to that of the (*all-E*)-lutein, as previously described (Aman et al., 2005). The structure was determined as (3*R*, 3'*R*, 6'*R*)-



**Figure 1.** Structures of the isolated compounds.

$\beta,\beta$ -carotene-3,3'-diol using  $^1\text{H}$ ,  $^{13}\text{C}$ /APT, HMBC, HSQC,  $^1\text{H}$ - $^1\text{H}$  COSY and NOESY spectra. Its NMR data (see Table 2) agree with those reported in the literature (Srividya and Vishnuvarthan, 2014). (*all-E*)-Lutein (**3**) was previously isolated from *Rhus leptodictya* (Songca et al., 2012).

The EIMS of compound **4** showed a molecular ion peak at  $m/z$  240 and a molecular formula  $\text{C}_{15}\text{H}_{28}\text{O}_2$  on the basis of its HR-EIMS (240.2094  $[\text{M}]^+$ ). The IR spectrum showed a vibration band at  $3400\text{ cm}^{-1}$  corresponding to hydroxyl group. Its  $^{13}\text{C}$  NMR/APT spectrum showed fifteen carbon signals including four methyls ( $\delta_{\text{C}}$  27.3, 27.1, 22.6, 18.7),

six methylenes ( $\delta_{\text{C}}$  44.6, 43.5, 42.0, 22.5, 21.5, 20.2), two methines ( $\delta_{\text{C}}$  54.8, 49.7), and three quaternary carbons ( $\delta_{\text{C}}$  73.0, 72.4, 34.5) two of which are oxygenated (Figure 1 and Table 3). Its  $^1\text{H}$  NMR spectrum showed signals for four tertiary methyl groups three of which are attached to carbons bearing oxygen atom, at  $\delta_{\text{H}}$  0.88 (3H-14, s), 1.13 (3H-15, s) and 1.23 (3H-12 and 3H-13, s). The two methine protons appeared respectively at  $\delta_{\text{H}}$  1.22 (H-5, m) and 1.38 (H-7, m). Other signals were attributed to the methylene protons of the molecule (Table 3). The proton sequence of the compound was deduced from the  $^1\text{H}$ - $^1\text{H}$  COSY and the gradient HMQC spectra while the HMBC

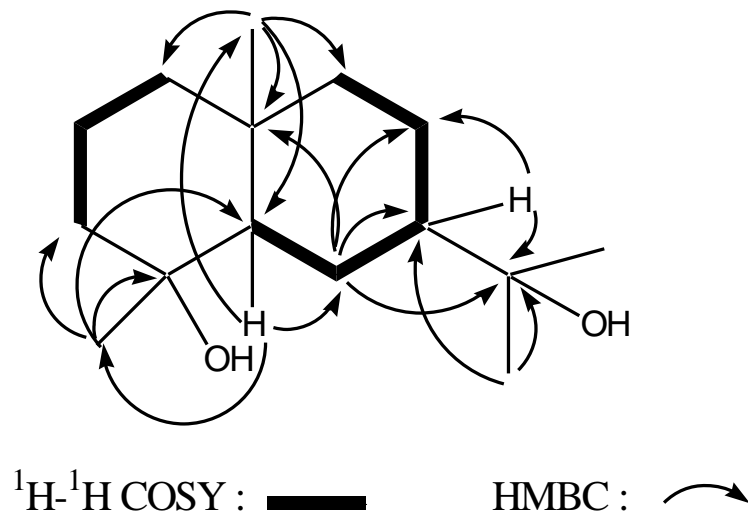


Figure 2. Selected correlations for compound 4

spectrum permitted the construction of the skeleton of 4 (Figure 2). In the NOESY spectrum, correlations observed between H-5 ( $\delta_{\text{H}}$  1.22) and H-7 ( $\delta_{\text{H}}$  1.38), and between 3H-14 ( $\delta_{\text{H}}$  0.88) and 3H-15 ( $\delta_{\text{H}}$  1.13) permitted the attribution of configurations at the chiral centers of compound 4. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data (Table 3) were in good agreement with those reported in the literature for cryptomeridiol, an eudesmane-type sesquiterpenoid previously isolated from *Artemisia pygmaea* (Irwin and Geissman, 1973) and *Blumea basalmifera* (Ruangrunsi et al., 1985).

Compound 5 was identified to sitosterol 3-O- $\beta$ -D-glucopyranoside after TLC, m.p. and IR analyses, compared to a referenced sample previously isolated in our laboratory (Tene et al., 2008). This compound is widely distributed in plant kingdom.

## Conclusion

The phytochemical study of the whole plant of *Phaulopsis imbricata* (Acanthaceae) afforded five known compounds including lupeol, betulin, (*all-E*)-lutein, cryptomeridiol and sitosterol 3-O- $\beta$ -D-glucopyranoside. To the best of our knowledge, this plant is phytochemically studied here for the first time. In addition, pure compounds are here reported for the first time from the genus *Phaulopsis*. The antimicrobial activity of the extracts and pure compounds from *P. imbricata* will be evaluated, with a view of verifying the hypothesis that this plant species is used by traditional healers for specific medicinal ends.

## Conflict of interests

The authors have not declared any conflict of interests.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge financial support from the research grant committees of both the University of Dschang and the Cameroonian Ministry of Higher Education.

## REFERENCES

- Adesegun SA, Fajana A, Orabueze CI, Coker HAB (2009). Evaluation of antioxidant properties of *Phaulopsis fascispala* C. B. Cl. (Acanthaceae). *Evid. Based Complement. Altern. Med.* 6(2):227-231.
- Aman R, Biehl J, Carle R, Conrad J, Beifuss U, Schieber A (2005). Application of HPLC coupled with DAD, APci-MS and NMR to the analysis of lutein and zeaxanthin stereoisomers in thermally processed vegetables. *Food Chem.* 92(4):753-763.
- Berrondo LF, Gabriel FT, Fernandes SBO, Menezes FS, Moreira DL (2003). Dirhamnosyl flavonoid and other constituents from *Brillantaisia palisatii*. *Quim. Nova* 26(6):922-923.
- Brummett RK (2005). *Vascular plants. Families and genera*. Royal Botanical Gardens. pp. 20-25.
- Burkill HM (1985). *The useful plants of West Tropical Africa vol. 1; Edition 2; Families A-D*. Royal Botanic Gardens, kew. 960p.
- Irwin MA, Geissman TA (1973). Sesquiterpene alcohols from *Artemisia pygmaea*. *Phytochemistry* 12(4):849-852.
- Joshi H, Saxena GK, Singh V, Arya E, Singh RP (2013). Phytochemical investigation, isolation and characterization of betulin from bark of *Betula utilis*. *J. Pharmacogn. Phytochem.* 2(1):145-151.
- Kayode J, Omotyinbo MA (2008). Cultural erosion and biodiversity: conserving chewing stick knowledge in Ekiti State, Nigeria. *Afr. Scientist* 9(1):41-51.
- Ke JC, Jiaqi H, Yunfei D, Wood JRI, Daniel TF (2011). Acanthaceae. In: Wu ZY, Raven PH, Hong DY (Eds), *Flora of China*, vol. 19; Science Press, Beijing, and Missouri Botanical Garden Press, St. Louis. pp. 429-430.
- Mahato SB, Kundu AP (1994).  $^{13}\text{C}$  NMR spectra of pentacyclic triterpenoids-a compilation and some salient features. *Phytochemistry* 37(6):1517-1575.
- Ruangrunsi N, Tantivatana P, Tappayuthpijarn P, Borris RP, Cordell GA (1985). Traditional medicinal plants of Thailand VI. Isolation of cryptomeridiol from *Blumea balsamifera*. *J. Sci. Soc. Thailand* 11(1):47-50.

- Songca SP, Sebothoma C, Samuel BB, Eloff JN (2012). A biflavonoid and a carotenoid from *Rhus leptodictya*: isolation, characterization and antibacterial properties. *Afr. J. Biochem. Res.* 6(13):172-178.
- Srividya AR, Vishnuvarthan VJ (2014). Physical, chemical and biological properties of lutein: A Review. *World J. Pharm. Res.* 3(5):105-118.
- Sudhanshu S, Dharam CJ, Madan MG, Rajendra SB, Hari OM, Ram PS (2000). High performance thin layer chromatographic analysis of hepatoprotective diterpenoids from *Andrographis paniculata*. *Phytochem. Anal.* 11(1):34-36.
- Tamokou JD, Kuate JR, Tene M, Nwemegueta TJK, Tane P (2011). The antimicrobial activities of extract and compounds isolated from *Brillantaisia lamium*. *Iran. J. Med. Sci.* 36(1):24-31.
- Tene M, Ndontsa BL, Tane P, Tamokou J-D, Kuate J-R (2009). Antimicrobial diterpenoids and triterpenoids from the stem bark of *Croton macrostachys*. *Int. J. Biol. Chem. Sci.* 3(3):538-544.
- Tene M, Tane P, Tamokou JD, Kuate JR, Connolly JD (2008). Degraded diterpenoids from the stem bark of *Neoboutonia mannii*. *Phytochem. Lett.* 1(2):120-124.
- Tinto WF, Blair LC, Alli A, Reynolds WF, McLean S (1992). Lupane triterpenoids of *Salacia cordata*. *J. Nat. Prod.* 55(3):395-398.