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Terpenoids from *Phaulopsis imbricata* (Acanthaceae)

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The whole plant of *Phaulopsis imbricata* (Forssk.) Sweet (Acanthaceae) was collected at Bansoa, 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 β-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-β-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

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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 (cryptomeridol), and one sterol glycoside (sitosterol 3-O-β-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. 1H NMR (500 MHz) and 13C NMR (125 MHz) with APT program were recorded at room temperature in CDCl3, using a Bruker DMX 500 spectrometer. The chemical shifts (δ) are reported in parts per million (ppm) with the solvent signals as reference relative to TMS (δ = 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 X (GC/MS) system. The GC column (VARIAN, USA) was a CP- Sil 8 CB LB/MS, chromapack capillary column (0.25 mm x 30 m, film thickness 0.25 μm). 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 GF254 pre-coated plates (MERCK, Germany) with detection accomplished by spraying with 20% H2SO4 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 Bansoa, West Region, Cameroon, in January, 2010. Authentication was done by Mr Paul Mezili, a retired Botanist of the Cameron 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-CH2Cl2-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-CH2Cl2-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-CH2Cl2-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 CH2Cl2-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 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) Vmax cm⁻¹: 3300, 3080, 2920, 1640, 1470, 1420, 1390, 1045, 885. EIMS: m/z (rel. int.) = 426[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) Vmax cm⁻¹: 3456, 3080, 2925, 1639, 1480, 1460, 1345, 1060, 885. EIMS: m/z (rel. int.) = 442[M⁺] (4), 207 (52), 189 (81), 135 (82), 121 (72), 95 (100), 81 (93), 55 (80). HR-ESI-MS: m/z = 442.3786 [M⁺] (calcld. 442.3811 for C30H50O2). 1H- and 13C-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) Vmax cm⁻¹: 3500, 2962, 2921, 1662, 1390. EIMS: m/z (rel. int.) = 568 [M⁺] (1), 550 (12), 479 (3), 442 (5), 313 (3), 135 (80), 121 (75), 95 (100), 81 (90), 55 (83). HR-ESI-MS: m/z = 568.4276 [M⁺] (calcld. 568.4280 for C40H50O2). 1H- and 13C-NMR spectral data (Table 2).
Compound 4 was obtained as white crystals from MeOH. m.p. 141 to 143°C. It showed the following spectral characteristics:

IR (NaCl) ν max cm⁻¹: 3600-3200, 2920, 2848, 1640, 1377, 1238, 1166, 1049. EIMS: m/z (rel. int.) = 240 [M⁺] (1), 222 (1), 204 (17), 189 (33), 149 (100), 123 (39), 109 (52), 95 (45), 43 (65). HR-El-MS: m/z = 240.2094 [M⁺] (calcld. 240.2089 for C₁₅H₂₆O₂). ¹H- and ¹³C-NMR spectral data (Table 3).

Table 1. ¹H- and ¹³C/APT- NMR spectral data of betulin (2) (CDCl₃, δ in ppm), and, selected HMBC data for 2.

| No | δC | APT | δH, mult.(J in Hz) | HMBC (H→C) |
|----|----|-----|-------------------|------------|
| 1  | 38.7 | CH₂ | 0.72, m; 1.62, m | 2, 10, 25 |
| 2  | 27.4 | CH₂ | 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 | CH₂ | 1.38, m; 1.52, m | 4, 5, 7, 8, 10 |
| 7  | 34.0 | CH₂ | 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 | CH₂ | 1.21, m; 1.42, m | 8, 9, 10, 12, 13 |
| 12 | 25.0 | CH₂ | 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 | CH₂ | 1.05, m; 1.67, m | 13, 14, 16, 17, 27 |
| 16 | 29.7 | CH₂ | 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 | CH₂ | 1.42, m; 1.95, m | 17, 18, 19, 20, 22 |
| 22 | 34.6 | CH₂ | 1.02, m; 1.83, m | 16, 17, 18, 21, 28 |
| 23 | 27.4 | CH₃ | 0.93, s          | 3, 4, 5, 24 |
| 24 | 15.9 | CH₃ | 0.76, s          | 3, 4, 5, 23 |
| 25 | 16.3 | CH₃ | 0.82, s          | 1, 5, 9, 10 |
| 26 | 16.1 | CH₃ | 0.98, s          | 7, 8, 9, 14 |
| 27 | 15.4 | CH₃ | 0.90, s          | 8, 13, 14, 15 |
| 28 | 60.6 | CH₂ | 3.33, d (10.0); 3.80, d (10.0) | 16, 17, 18, 22 |
| 29 | 109.7 | CH₂ | 4.58, br s; 4.66, br s | 19, 20, 30 |
| 30 | 19.1 | CH₃ | 1.68, s          | 19, 20, 29 |

Compound 5 was obtained as white powder. m.p. 252-254 °C. IR (KBr) ν max 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 cm⁻¹) and a terminal double bond (3080, 1640, 885 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 C₅₀H₇₀O₂ on the basis of its HR-EIMS (442.3793 [M⁺]). The IR spectrum showed vibration bands corresponding to hydroxyl group (3456 cm⁻¹) and a terminal double bond (3080, 1639, 885 cm⁻¹). Its ¹H NMR spectrum showed five methyl singlets at δH
Table 2. $^1$H- and $^{13}$C/APT-NMR spectral data of (all-E)-lutein (3) (CDCl$_3$, δ in ppm), and, selected HMBC data for 3.

| No | δC  | APT | δH, mult.(J in Hz) | HMBC (H→C) |
|----|-----|-----|-------------------|-------------|
| 1  | 37.2| C   | -                 | -           |
| 2  | 48.4| CH$_2$ | 1.48, t (10.7)  | -           |
|    |     |      | 1.84, dd (6.1, 13.5) | -           |
| 3  | 65.1| CH  | 4.02, m           | -           |
| 4  | 42.6| CH$_2$ | 2.03, dd (10.7, 15.0)  | 3, 5, 6 |
|    |     |      | 2.37, m           | 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$_3$| 1.09, s          | 1, 2, 6 |
| 17 | 30.3 | CH$_3$| 1.09, s          | 1, 2, 6 |
| 18 | 21.7 | CH$_3$| 1.74, s          | 4, 5, 6 |
| 19 | 12.9 | CH$_3$| 1.99, s          | 8, 9, 10 |
| 20 | 12.9 | CH$_3$| 1.99, s          | 12, 13, 14 |
| 1' | 34.1| C   | -                 | -           |
| 2' | 44.7| CH$_2$| 1.37, dd (7.1, 13.5)  | 1', 3', 16', 17' |
|    |     |      | 1.84, dd (6.1, 13.5)  | 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$_3$| 1.02, s          | 1', 2', 6', 17' |
|17' | 28.8 | CH$_3$| 0.87, s          | 1', 2', 6', 16' |
|18' | 22.9 | CH$_3$| 1.63, s          | 4', 5', 6' |
|19' | 13.2 | CH$_3$| 1.93, s          | 8', 9', 10' |
|20' | 12.9 | CH$_3$| 1.99, s          | 12', 13', 14' |
Table 3. 1H- and 13C/APT- NMR spectral data of cryptomeridiol (4) (CDCl3, δ in ppm), and, selected HMBC data for 4.

| No | δ C | APT | δ H, mult.(J in Hz) | HMBC (H→C) |
|----|-----|-----|--------------------|------------|
| 1  | 41.0| CH2 | 1.09, m; 1.42, m   | 3, 14      |
| 2  | 20.2| CH2 | 1.57, m            | 3          |
| 3  | 43.5| CH2 | 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| CH2 | 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| CH2 | 1.30, m; 1.59, m   | 7, 9, 11   |
| 9  | 44.6| CH2 | 1.15, m; 1.47, m   | 5, 7       |
| 10 | 34.5| C   | -                  |            |
| 11 | 73.0| C   | -                  |            |
| 12 | 27.1| CH3 | 1.23, s            | 7, 11, 13  |
| 13 | 27.3| CH3 | 1.23, s            | 7, 11, 12  |
| 14 | 18.7| CH3 | 0.88, s            | 1, 5, 9, 10|
| 15 | 22.6| CH3 | 1.13, s            | 3, 4, 5    |

0.76, 0.82, 0.90, 0.93 and 0.98, a vinyl methyl group at δH 1.68 (3H-30, s), a double doublet of one oxygenated methine proton at δH 3.18 (H-3, dd, 5.0, 15.0), two doublets of one oxygenated methylene protons at δH 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 δH 4.58 (H-29a) and 4.66 (H-29b) (Table 1). The 13C 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 δC 60.6 (C-28) and 79.0 (C-3) and the double bond at δC 109.7 (C-29) and 150.5 (C-20), characteristics of triterpenes of the lup-29(e)-ene type (Mahato and Kundu, 1994). In the HSQC spectrum correlations observed between H-3 (δH 3.18) and C-3 (δC 79.0), H-28a (δH 3.33) and C-28 (δC 60.6), H-28b (δH 3.80) and C-28 (δC 60.6), H-29a (δH 4.58) and C-29 (δC 109.7), H-29b (δH 4.66) and C-29 (δC 109.7), suggested compound 2 to be a 3, 28-dihydroxy-lup-20(29)-ene. The positions of the two hydroxy (3-OH and 28-OH) and vinylidene (C=CH2) groups were confirmed in the HMBC spectrum by correlations between H-3 (δH 3.18) and C-1 (δC 38.7), C-4 (δC 38.9), C-5 (δC 55.3), C-23 (δC 27.4), and C-24 (δH 15.9), H-28a (δH 3.33)/H-28b (δH 3.80) and C-16 (δC 29.7), C-17 (δC 47.8), C-18 (δC 48.9) and C-22 (δC 34.6), H-29a (δH 4.58)/H-29b (δH 4.66) and C-19 (δC 47.8), C-20 (δC 150.5) and C-30 (δC 19.1). Furthermore, correlations observed in the NOESY spectrum between H-3 (δH 3.18, dd, J = 5.0, 15.0 Hz) and H-5 (δH 0.67, m), and H-3 (δH 3.18) and H-23 (δH 0.93, s) suggested the 3-OH group to be β-oriented.

Compound 2 was identified as 3β,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 [M]+ in agreement with the molecular formula C40H58O2 (130 of unsaturation). The IR spectrum showed bands for hydroxyl group (3500 cm⁻¹) and double bond (1662 cm⁻¹). 13C-NMR/APT indicated signals for forty carbon atoms including ten methyls, three methylene, eighteen methines and nine quaternary carbons (Table 2). Its 1H NMR spectrum showed signals for four angular methyl groups at δH 0.87 (3H-17’, s), 0.99 (3H-16 and 3H-17, s) and 1.02 (3H-16e, s), six vinyl methyl groups at δH 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 δH 1.37 (H-2’α, dd, J = 7.1, 13.5 Hz), 1.48 (H-2’α, t, J = 10.7 Hz), 1.84 (H-2’α, H-2’α, each dd, J = 6.1, 13.5 Hz), 2.03 (H-4’α, dd, J = 10.7, 15.0 Hz) and 2.37 (H-4’α, m). Also on this spectrum, three aliphatic methine protons resonated respectively at δH 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 1H-1H COSY and the gradient HMOC spectra while the HMBC spectrum permitted the construction of the skeleton of 3. The 1H- and 13C-NMR spectral data suggested the presence in the molecule of two different and six-membered aliphatic rings, each bearing one hydroxy (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 polylene system. But due to the structure difference of the two rings the molecule is not symmetric (Figure. 1 and Table 2). Signals of the polylene region of the 1H NMR spectrum were identical to that of the (all-E)-lutein, as previously described (Aman et al., 2005). The structure was determined as (3R, 3′R, 6′R)-
Figure 1. Structures of the isolated compounds.

β,β-carotene-3,3'-diol using $^1$H, $^{13}$C/APT, HMBC, HSQC, $^1$H-$^1$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 C$_{15}$H$_{28}$O$_2$ on the basis of its HR-EIMS (240.2094 [M$^+$]). The IR spectrum showed a vibration band at 3400 cm$^{-1}$ corresponding to hydroxyl group. Its $^{13}$C NMR/APT spectrum showed fifteen carbon signals including four methyls ($\delta_C$ 27.3, 27.1, 22.6, 18.7), six methylenes ($\delta_C$ 44.6, 43.5, 42.0, 22.5, 21.5, 20.2), two methines ($\delta_C$ 54.8, 49.7), and three quaternary carbons ($\delta_C$ 73.0, 72.4, 34.5) two of which are oxygenated (Figure 1 and Table 3). Its $^1$H NMR spectrum showed signals for four tertiary methyl groups three of which are attached to carbons bearing oxygen atom, at $\delta_H$ 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 $\delta_H$ 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 $^1$H-$^1$H COSY and the gradient HMBC spectra while the HMBC
The authors have not declared any conflict of interests.

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Figure 2. Selected correlations for compound 4

$^{1}H-^{1}H$ COSY:

HMBC:

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

Compound 5 was identified to sitosterol 3-O-β-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-α)-lutein, cryptomeridol and sitosterol 3-O-β-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.
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