



## Research Article

# TERPENOID COMPOUNDS FROM THE EXTRACTED ETHYL ACETATE OF THE BARK STEM OF *PHYLLANTHUS ACIDUS*

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## ABSTRACT

Thin layer chromatography and column chromatography were performed from the ethyl acetate extraction of the bark stem of *Phyllanthus acidus*, and six terpenoids were isolated, including four diterpenes and two triterpenes. Their names were phyllanthol (1), 19-acetoxy-16 $\alpha$ , 17-dihydroxy-ent-kaurane (2), 16 $\alpha$ , 17-dihydroxy-ent-kaurane (3), 16 $\alpha$ ,17-acetonide-3 $\alpha$ -hydroxykauran (4), maslinic acid (5) and phyllane A (6). The structures of these compounds were elucidated by nuclear magnetic resonance spectroscopy. This is the first time that compounds 2, 3, 4, and 5 were found in the genus of *Phyllanthus*.

**Keywords:** kaurene; *Phyllanthus acidus*; terpenoid

## 1. Introduction

*Phyllanthus acidus* has a Vietnamese name, Chum ruot. The other names of *Phyllanthus acidus* are *Phyllanthus distichus*, *Cicca disticha*, and *Cicca acida*. *P. acidus* grows in tropical regions such as Asia and Africa. In traditional medicine, *P. acidus* is used to treat many diseases. Fruits have the effect of improving liver function, supporting the treatment of cirrhosis very effectively. The leaves and roots have a hemolytic, expectorant, antiseptic, antivenom, and snake antivenom effect. The leaves help with boils and cure sore throats and stomatitis. The stem can reduce fever quickly; the bark can destroy toxic lymph nodes, boils, and expectorants. Ethanol extract from the bark of the stem can cure purulent ear rot, cure scabies, ulcers, bleeding skin wounds, lozenges, toothache, and sore throat (Do, 2006).

In this report, we announce the isolation and structural elucidation of six compounds from the extracted ethyl acetate of the bark stem of *P. acidus*. These compounds were phyllanthol (1), 19-acetoxy-16 $\alpha$ , 17-dihydroxy-ent-kaurane (2), 16 $\alpha$ , 17-dihydroxy-ent-kaurane (3), 16 $\alpha$ ,17-acetonide-3 $\alpha$ -hydroxykauran (4), maslinic acid (5) and phyllane A (6).

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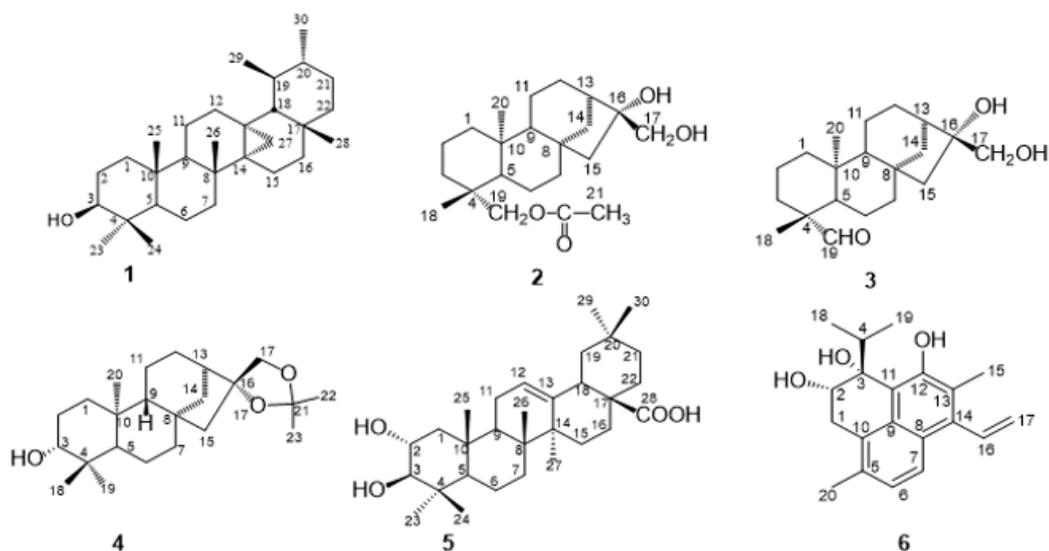


Figure 1. Chemical structures of isolated compounds 1-6

## 2. Experiment

### 2.1. Experimental methods

Thin-layer chromatography was implemented on a 60 F<sub>254</sub> silica gel plate (Merck), and spots were detected by ultraviolet (UV) illumination and by spraying with 20% sulfuric acid reagent followed by heating. Column chromatography was performed on silica gel (70-230 mesh, Merck) in the normal phase and C18-reversed phase silica gel at atmospheric pressure. The NMR spectroscopic data were recorded on an Avance III HD spectrometer (500 MHz for <sup>1</sup>H NMR, 125 MHz for <sup>13</sup>C NMR) in deuterated solvents using tetramethylsilane or residual non-deuterated solvent peak as an internal standard. Chemical shifts are shown in units of  $\delta$  (ppm), and the coupling constants are expressed in Hertz.

### 2.2. Plant materials

The bark stem of *P. acidus* was collected in Ham Thuan Bac district, Binh Thuan province, in 2018 and identified by traditional medicine physician Nguyen Thien Chung, chairman of the Oriental Medicine Association of Tinh Bien district, An Giang province.

### 2.3. Extraction and isolation

The bark stem of *P. acidus* (19 kg) was macerated in methanol (MeOH) for three days at room temperature (repeated five times), and the extract was concentrated in vacuo to obtain a methanol residue (995 g). This residue was dissolved sequentially in *n*-hexane and ethyl acetate to gain the extracted liquid, respectively, followed by concentration in vacuo, yielding the respective residues *n*-hexane (300 g) and ethyl acetate (105 g).

The ethyl acetate residue (100 g) was chromatographed on a silica gel column, eluted with a stepwise gradient of *n*-hexane/EtOAc (30:1, 20:1, 15 :1, 9:1, 4:1, 2:1, 1:1, 0:1) and EtOAc/MeOH (9:1, 8:2) to yield ten main fractions (EA1-EA10). Fraction EA1 (7.1 g) was separated by silica gel column chromatography, eluting with *n*-hexane/EtOAc (40:1, 30:1, 20:1, 15 :1, 9:1, 4:1) to give six subfractions (EA1.1-EA1.6). Subfraction EA1.2 (120 mg)

was chromatographed repeatedly on silica gel column chromatography, eluting with *n*-hexane/EtOAc (20:1) to obtain compound **1** (15.8 mg).

Fraction EA3 (4.5 g) was separated by silica gel column chromatography, eluting with *n*-hexane/EtOAc (20:1, 15:1, 9:1, 4:1) to give four subfractions (EA3.1-EA3.4). Subfraction EA3.2 (620 mg) was chromatographed repeatedly on silica gel column chromatography, eluting with *n*-hexane/EtOAc (20:1, 15:1, 9:1) to give three subfractions (EA3.2.1-EA3.2.3). Conduct chromatography using the C18-reversed phase column on subfraction EA3.2.1, eluting with H<sub>2</sub>O/THF (4:1, 3:1, 2:1, 1:1) to obtain compound **2** (6.4 mg) and **4** (4.5 mg). Carry out C18-reversed phase column chromatography on subfraction EA3.2.2, eluting with H<sub>2</sub>O/THF (4:1, 3:1, 2:1, 1:1) to obtain compound **3** (5.5 mg).

Fraction EA4 (4.5 g) was separated by silica gel column chromatography, eluting with *n*-hexane/EtOAc (20:1, 15:1, 9:1, 4:1) to give four subfractions (EA4.1-EA4.4). Subfraction EA4.3 (420 mg) was chromatographed repeatedly on silica gel column chromatography, eluting with *n*-hexane/EtOAc (15:1, 9:1, 4:1) to give three subfractions (EA4.3.1-EA4.3.3). Conduct chromatography using the C18-reversed phase column on subfraction EA4.3.2, eluting with H<sub>2</sub>O/THF (4:1, 3:1, 2:1, 1:1) to obtain compounds **5** (10.5 mg) and **6** (3.5 mg).

#### 2.4. Spectroscopic data

• **Phyllanthol (1)**. White amorphous powder; <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) δ<sub>H</sub> 3.19 (dd, 1H, *J* = 11.5, 5.5 Hz, H-3), 3.20 (dd, *J* = 12.0, 4.8 Hz), 0.94 (s, 3H, H-23), 0.77 (s, 3H, H-24), 0.86 (s, 3H, H-25), 1.14 (s, 3H, H-26), 0.66 (1H, d, *J* = 5.5 Hz, H-27a), 0.00 (1H, d, *J* = 5.5 Hz, H-27b), 0.96 (s, 3H, H-28), 0.93 (3H, d, *J* = 6.0 Hz, H-29), 0.86 (3H, d, *J* = 5.4 Hz, H-30); <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>) δ<sub>C</sub> 79.1 (C-3), 55.7 (C-5), 54.0 (C-9), 50.1 (C-18), 42.1 (C-22), 40.8 (C-19), 38.9 (C-4), 38.5 (C-1), 38.4 (C-7), 38.4 (C-20), 37.3 (C-10), 37.0 (C-8), 35.2 (C-12), 32.3 (C-14), 31.9 (C-17), 31.1 (C-21), 28.2 (C-28), 27.9 (C-23), 27.3 (C-16), 27.3 (C-2), 26.6 (C-13), 21.3 (C-15), 20.7 (C-30), 18.1 (C-11), 18.1 (C-6), 18.0 (C-26), 17.9 (C-29), 16.0 (C-25), 15.3 (C-24), 13.3 (C-27).

• **19-Acetoxy-16 $\alpha$ , 17-dihydroxy-ent-kaurane (2)**. White amorphous powder; <sup>1</sup>H-NMR (acetone-d<sub>6</sub>, 500 MHz) δ<sub>H</sub> 4.29 (d, *J* = 10.8 Hz, H-19a), 3.85 (d, *J* = 10.8 Hz, H-19b), 3.71 (d, *J* = 10.8 Hz, H-17a), 3.55 (d, *J* = 10.8 Hz, H-17b), 1.09 (s, H-20), 2.01 (s, H-21), 0.96 (s, H-18); <sup>13</sup>C-NMR (acetone-d<sub>6</sub>, 125 MHz) δ<sub>C</sub> 171.1 (COO), 81.6 (C-16), 67.1 (C-19), 66.5 (C-17), 57.9 (C-9), 57.5 (C-5), 54.0 (C-15), 46.2 (C-13), 45.2 (C-8), 43.3 (C-7), 41.0 (C-1), 40.0 (C-10), 37.9 (C-14), 37.8 (C-3), 37.0 (C-4), 27.8 (C-18), 26.9 (C-12), 21.3 (C-21), 20.7 (C-6), 19.1 (C-2), 18.9 (C-11), 18.6 (C-20).

• **16 $\alpha$ , 17-Dihydroxy-ent-kaurane (3)**. White amorphous powder; <sup>1</sup>H-NMR (acetone-d<sub>6</sub>, 500 MHz) δ<sub>H</sub> 9.70 (1H, CHO), 3.70 (1H, d, *J* = 10.8 Hz, H-17a), 3.55 (1H, d, *J* = 10.8 Hz, H-17b), 0.97 (s, H-18), 0.90 (s, H-20); <sup>13</sup>C-NMR (acetone-d<sub>6</sub>, 125 MHz) δ<sub>C</sub> 205.7 (C-19), 81.7 (C-16), 66.5 (C-17), 57.2 (C-9), 56.3 (C-5), 53.9 (C-15), 48.9 (C-4), 46.1 (C-13), 45.1

(C-8), 42.9 (C-7), 40.3 (C-1), 40.2 (C-10), 38.1 (C-14), 34.9 (C-3), 26.9 (C-12), 24.4 (C-18), 20.8 (C-2), 19.1 (C-6), 19.1 (C-11), 16.9 (C-20).

**16 $\alpha$ ,17-Acetonide-3 $\alpha$ -hydroxykauran (4).** White amorphous powder;  $^1\text{H-NMR}$  (500 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{H}}$  3.18 (1H, dd,  $J = 11.4, 4.8$  Hz, H-3), 2.12 (1H, m, H-13), 4.05 (1H, d,  $J = 9.0$  Hz, H-17a), 3.90 (1H, d,  $J = 8.4$  Hz, H-17b), 0.97 (3H, s, H-18), 0.77 (3H, s, H-19), 1.01 (3H, s, H-20), 1.38 (3H, s, H-22), 1.35 (3H, s, H-23);  $^{13}\text{C-NMR}$  (125 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{C}}$  108.4 (C-21), 70.0 (C-17), 89.1 (C-16), 56.5 (C-15), 56.1 (C-9), 45.7 (C-13), 39.0 (C-14), 38.4 (C-10), 28.4 (C-18), 27.1 (C-12), 26.9 (C-23), 26.8 (C-22), 20.1 (C-11), 17.8 (C-20), 15.5 (C-19).

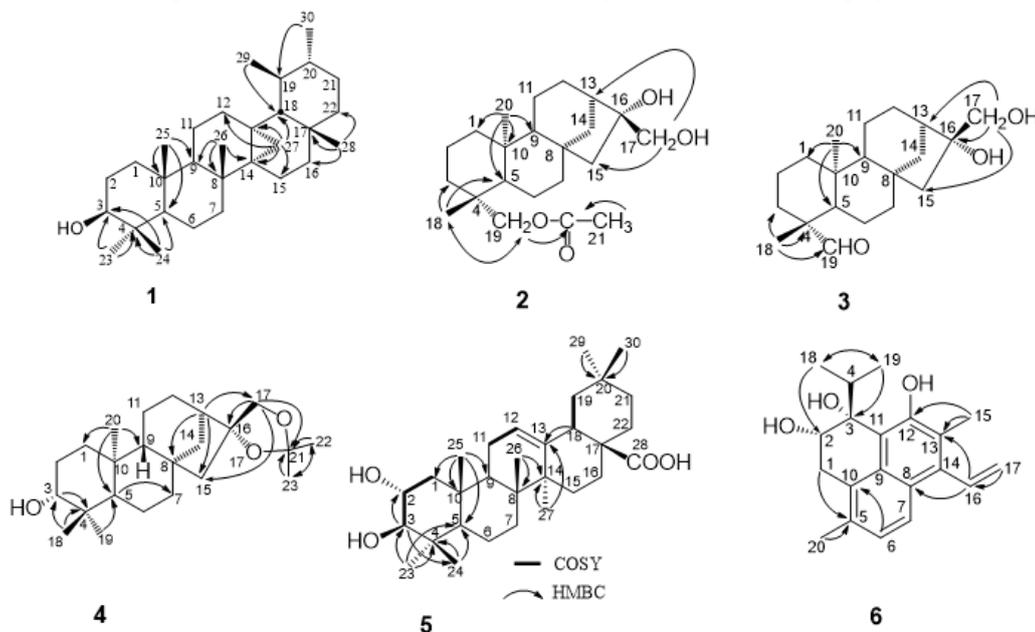
• **Maslinic acid (5).** White amorphous powder;  $^1\text{H-NMR}$  (600 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{H}}$  3.61 (1H, m, H-2), 2.90 (1H, d,  $J = 9.5$  Hz, H-3), 5.25 (1H, t,  $J = 3.5$  Hz, H-12), 2.88 (1H, m, H-18), 1.02 (3H, s, H-23), 0.81 (3H, s, H-24), 1.00 (3H, s, H-25), 0.80 (3H, s, H-26), 1.18 (3H, s, H-27), 0.94 (3H, s, H-29), 0.96 (3H, s, H-30);  $^{13}\text{C-NMR}$  (150 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{C}}$  178.8 (C-28), 145.0 (C-13), 122.9 (C-12), 84.0 (C-3), 68.9 (C-2), 56.2 (C-5), 48.5 (C-8), 48.5 (C-9), 47.6 (C-1), 46.8 (C-19), 46.7 (C-17), 42.5 (C-14), 42.2 (C-18), 39.8 (C-4), 39.8 (C-10), 34.5 (C-7), 34.2 (C-21), 34.1 (C-22), 33.4 (C-29), 31.3 (C-20), 29.2 (C-23), 28.4 (C-15), 26.3 (C-27), 23.9 (C-11), 23.9 (C-16), 23.7 (C-30), 19.1 (C-6), 17.6 (C-26), 17.4 (C-24), 17.0 (C-25).

• **Phyllane A (6).** White amorphous powder;  $^1\text{H-NMR}$  (500 MHz, acetone- $d_6$ )  $\delta_{\text{H}}$  3.25 (1H, dd,  $J = 18.0, 3.5$  Hz, H-1a), 3.16 (1H, dd,  $J = 18.0, 2.4$  Hz, H-1b), 4.38 (1H, m, H-2), 1.85 (1H, sept,  $J = 7.2$  Hz, H-4), 7.09 (1H, d,  $J = 8.4$  Hz, H-6), 7.74 (1H, d,  $J = 8.4$  Hz, H-7), 2.30 (3H, s, H-15), 7.04 (1H, dd,  $J = 18.0, 11.4$  Hz, H-16), 5.74 (1H, dd,  $J = 11.4, 1.8$  Hz, H-17a), 5.32 (1H, dd,  $J = 17.7, 1.8$  Hz, H-17b), 1.05 (1H, d,  $J = 7.2$  Hz, H-18), 0.96 (1H, d,  $J = 7.2$  Hz, H-19), 2.34 (3H, s, H-20), 10.55 (1H, s, 12-OH);  $^{13}\text{C-NMR}$  (125 MHz, acetone- $d_6$ )  $\delta_{\text{C}}$  156.0 (C-12), 136.1 (C-14), 135.8 (C-16), 133.3 (C-5), 129.5 (C-9), 126.6 (C-10), 126.2 (C-6), 126.0 (C-8), 124.8 (C-13), 124.0 (C-7), 121.1 (C-17), 114.7 (C-11), 82.2 (C-3), 69.8 (C-2), 36.4 (C-4), 33.4 (C-1), 19.6 (C-20), 17.6 (C-19), 16.6 (C-18), 14.0 (C-15).

### 3. Results and discussion

The  $^1\text{H-NMR}$  spectrum of compound **1** displayed the presence of seven methyl groups, including five singlets at  $\delta_{\text{H}}$  0.94, 0.77, 0.86, 1.14, and 0.96, two doublets at  $\delta_{\text{H}}$  0.93 ( $J = 6.0$  Hz), 0.86 ( $J = 5.4$  Hz), one proton carbinol at  $\delta_{\text{H}}$  3.19 (dd,  $J = 11.5, 5.5$  Hz). In addition, there are two protons of the cyclopropyl ring at  $\delta_{\text{H}}$  0.01 (d,  $J = 5.5$  Hz) and  $\delta_{\text{H}}$  0.66 (d,  $J = 5.5$  Hz). The  $^{13}\text{C}$  and HSQC NMR spectra showed 30 carbon signals, of which seven are methyl, five methine signals, 11 methylene, and one oxymethine at  $\delta_{\text{C}}$  79.1. Two proton signals in upfield at  $\delta_{\text{H}}$  0.00 (d,  $J = 5.5$  Hz) and  $\delta_{\text{H}}$  0.66 (d,  $J = 5.5$  Hz) and carbon in HSQC spectrum at  $\delta_{\text{H}}$  13.3 (C-27) showing the cyclopropane ring in compound **1** (V. J. Ndebe et al., 2007). The HMBC spectrum of **1** (Figure 2) shows the cross-peaks of methylene protons above-mentioned at  $\delta_{\text{H}}$  0.00 (d,  $J = 5.5$  Hz) and  $\delta_{\text{H}}$  0.66 (d,  $J = 5.5$  Hz) to carbon at  $\delta_{\text{C}}$  35.2 (C-12), 26.6 (C-13), 32.3 (C-14), 21.3 (C-15), 50.1 (C-18), confirmed that three-membered ring

located between C-14 and C-15. The HMBC spectrum is also displaying proton at  $\delta_H$  0.96 (H-23) and 0.77 (H-24) having cross-peak to  $\delta_C$  79.1 (C-3), 38.9 (C-4), and 55.7 (C-5) confirmed to locate the -OH group attached to C-3. The other HMBC correlations are shown in Figure 2. The spectroscopic data of compound **1** is compatible with the published data (V. J. Ndlebe et al., 2007). So, compound **1** was confirmed as 13,27-cycloursan-3 $\beta$ -ol or phyllanthol.



**Figure 2.** Some key HMBC and COSY correlations of 1-6

The  $^1\text{H-NMR}$  spectrum of compound **2** shows the presence of two singlet methyl groups at  $\delta_H$  0.96 and 1.09, one group  $\text{COOCH}_3$  at  $\delta_H$  2.01, two doublet oxymethylene groups at 3.71, 3.55 (each 2H,  $J = 10.8$ ), and 4.29, 3.85 (each 2H,  $J = 10.8$ ), nine methylene groups between 1.0 and 2.0 ppm. The  $^{13}\text{C}$  NMR spectrum of compound **2** shows the presence of 22 carbons, including two methyl carbons, one methyl in  $\text{COOCH}_3$ , four quaternary carbons, two oxymethylene, three methine, one carboxyl, and nine methylene. The HMBC spectrum of compound **2** shows that two protons oxymethylene at  $\delta_H$  3.71, 3.55 (d,  $J = 10.8$ , H-20) display the cross-peaks to carbon at  $\delta_C$  46.2 (C-13), 54.0 (C-15), confirmed that position of oxymethylene C-17. Protons methyl at  $\delta_H$  0.96 (H-18) correlate with carbons at  $\delta_C$  67.1 (C-19) show position of oxymethylene (C-19) attached at C-4, the cross-peaks of protons at  $\delta_H$  2.01 (H-21) to carbon at  $\delta_C$  171.1 (carbon carboxyl) show presence of  $\text{COOCH}_3$ . The other HMBC significant correlation of compound **2** are showed in Figure 2. The data spectrum of **2** is similar to the published data, so it is suggested that the structure of compound **2** is 19-acetoxy-16 $\alpha$ , 17-dihydroxy-ent-kaurane (Rumbero et al., 2000). The stereochemistry of compound **2** at C-16 was determined based on previously published data, confirming  $\alpha$  the orientation of hydroxyl at C-16 (Yang et al., 2002).

The  $^1\text{H}$  and  $^{13}\text{C}$ -NMR spectrum of compound **3** show like compound **2**, exhibits the presence of two singlet methyl groups at  $\delta_{\text{H}}$  0.97 and 0.90, one doublet oxymethylene group at 3.70, 3.55 (each 2H,  $J = 10.8$ ), nine methylene groups between 1.0 and 2.0 ppm. The  $^{13}\text{C}$  NMR spectrum of compound **3** shows 20 carbon signals, including two methyl carbons, one carbon carbonyl at  $\delta_{\text{C}}$  205.7 (C-19), four quaternary carbons, one oxymethylene, three methine, and nine methylene. Similar to compound **2**, the HMBC spectrum of compound **3** reveals that two protons oxymethylene at  $\delta_{\text{H}}$  3.70, 3.55 (d,  $J = 10.8$ , H-17) present the cross-peaks to carbon at  $\delta_{\text{C}}$  46.1 (C-13), 53.9 (C-15), 81.7 (C-16). Protons methyl at  $\delta_{\text{H}}$  0.97 (H-18) correlate with carbons at  $\delta_{\text{C}}$  205.7 (C-19), confirming carbonyl (C-19) attached at C-4. The other HMBC expressive correlation of compound **3** is shown in Figure 2. The data spectrum of **3** is similar to the published data (Piacente et al., 1994), so it is suggested that the structure of compound **3** is  $16\alpha$ , 17-dihydroxy-ent-kaurane. The stereochemistry of hydroxyl at C-16 was confirmed to be  $16\alpha$  compared to previously published data (Yang et al., 2002).

The  $^1\text{H}$  and  $^{13}\text{C}$ -NMR spectra of compound **4** are similar to compounds **2** and **3**. Compound **4** also belongs to the diterpene skeleton, showing five singlet methyl groups at  $\delta_{\text{H}}$  0.97, 0.77, 1.01, 1.38, and 1.35, one doublet oxymethylene group at 3.90, 4.5 (each 2H,  $J = 9.0$ ), nine methylene groups, one doublet proton carbinol at  $\delta_{\text{H}}$  3.18 ( $J = 11.4$ , 4.8 Hz). The  $^{13}\text{C}$  NMR spectrum of compound **4** showed 23 carbon signals, including five methyl carbons, one carbon carbinol at  $\delta_{\text{C}}$  79.0, one oxymethylene at 70.0, one isopropylidenedioxygen group at 108.5, three methines, and nine methylene. The HMBC of compound **4** is similar to compounds **2** and **3** (Figure 2). The difference is that there are cross-peaks of protons at  $\delta_{\text{H}}$  1.38 (H-22) and 1.35 (H-25) to quaternary carbon at  $\delta_{\text{C}}$  108.4, confirming the presence of isopropylidenedioxygen, and position of this group are verified by the cross-peaks of protons at  $\delta_{\text{H}}$  3.90, 4.5 (H-17) to carbon at  $\delta_{\text{C}}$  108.4 (C-21). In addition, the cross-peaks of doublet proton at  $\delta_{\text{H}}$  3.18 ( $J = 11.4$ , 4.8 Hz, H-3) to carbon at  $\delta_{\text{C}}$  38.6 (C-4) and the correlation of proton at  $\delta_{\text{H}}$  0.97 (H-18) to carbon at  $\delta_{\text{C}}$  79.0 (C-3) exhibit hydroxyl group attached at C-3. The data spectrum of **4** is similar to the published data, so it is suggested that the structure of compound **4** is 16,17-acetonide- $3\alpha$ -hydroxykauran (Turak et al., 2018). Based on a comparison with previously published data (Yang et al., 2002), the hydroxyl group at C-16 was concluded to be  $16\alpha$ .

The  $^1\text{H}$ -NMR spectrum of compound **5** shows the appearance of seven methyl singlets, one triplet olefinic proton at  $\delta_{\text{H}}$  5.25 ( $J = 3.5$  Hz), two proton carbinol, including one doublet proton at  $\delta_{\text{H}}$  2.90 ( $J = 9.5$  Hz) and one multiplet proton at  $\delta_{\text{H}}$  3.61. The  $^{13}\text{C}$  and HSQC NMR spectrum showed 30 carbon signals, containing seven methyl, two olefinic carbon at  $\delta_{\text{C}}$  122.9 and 145.0, one carbon carboxyl at  $\delta_{\text{C}}$  178.8, six methine carbons in which two carbon carbinol at  $\delta_{\text{C}}$  84.0 and 68.9, five quaternary carbons, nine methylene. Two proton carbinol at  $\delta_{\text{H}}$  2.90 ( $J = 9.5$  Hz) and 3.61 (m) are attached at C-3 and C-2, respectively, by correlations

in the HMBC and COSY spectrum (Figure 2). The HMBC spectrum of **2** (Figure 2) shows the cross-peaks of proton carbinol at  $\delta_{\text{H}}$  2.90 ( $J = 9.5$  Hz) to carbon at  $\delta_{\text{C}}$  68.9 (C-2) and 17.4 (C-24), proving this proton attached at C-3. The spin-spin coupling constant of H-3 exhibits the  $\beta$  orientation of 3-OH. Besides, the HMBC spectrum is also displaying proton at  $\delta_{\text{H}}$  1.02 (H-23) having cross-peak to  $\delta_{\text{C}}$  84.0 (C-3), 39.8 (C-4), and 56.2 (C-5), proton at  $\delta_{\text{H}}$  0.81 (H-24) correlate to  $\delta_{\text{C}}$  39.8 (C-4), and 56.2 (C-5). The other HMBC correlations are shown in Figure 2. The spectroscopic data of compound **5** is adaptable to the published data (Quach et al., 2019; Woo et al., 2014; Ikuta et al., 1995). So, compound **5** was confirmed as maslinic acid.

The  $^1\text{H-NMR}$  spectrum of compound **6** shows one phenolic hydroxy group at  $\delta_{\text{H}}$  10.55, two *ortho*-coupled doublets of aromatic protons at  $\delta_{\text{H}}$  7.74 ( $J = 8.4$  Hz) and 7.09 ( $J = 8.4$ ), one vinyl group manifest doublet of doublets at  $\delta_{\text{H}}$  7.04 ( $J = 18.0, 11.4$  Hz), one methylene appear doublet of doublets at  $\delta_{\text{H}}$  3.25 ( $J = 18.0, 3.5$  Hz) and 3.16 ( $J = 18.0, 2.4$  Hz), one oxymethine at  $\delta_{\text{H}}$  4.38, one isopropyl is split into a septet at 1.85 ( $J = 7.2$  Hz), two methyl at  $\delta_{\text{H}}$  2.34 and 2.30. The  $^{13}\text{C-NMR}$  displays 20 carbons, including two aromatic carbons at  $\delta_{\text{C}}$  126.2 and 124.0, one isopropyl group contains three carbons at  $\delta_{\text{C}}$  36.5, 17.7, and 16.6, one oxymethine carbon at  $\delta_{\text{C}}$  69.8, one methylene at 33.4, nine aromatic carbons appear from 110.0 to 116.0. The HMBC spectrum of compound **6** indicated correlations between proton of methyl at  $\delta_{\text{H}}$  2.3 (H-15) to carbon at  $\delta_{\text{C}}$  156.0 (C-12) and 124.8 (C-13) show position of this methyl, the cross-peaks of proton vinyl at  $\delta_{\text{H}}$  7.04 ( $J = 18, 11.4$  Hz) to carbon at  $\delta_{\text{C}}$  126.0 (C-8) and 136.1 (C-14), proving this vinyl group attached at C-13. Furthermore, the correlations of proton methyl at  $\delta_{\text{H}}$  0.96 ( $J = 7.2$  Hz, H-19) to  $\delta_{\text{C}}$  82.2 (C-3), from 1.05 ( $J = 7.2$  Hz, H-18) to  $\delta_{\text{C}}$  69.8 (C-2) showing isopropyl group connected at C-3. The significant HMBC correlations are shown in Figure 2. The spectroscopic data of compound **6** is unanimous to the published data (Duong et al., 2018). So, compound **6** was confirmed as phyllane A.

#### 4. Conclusions

From the extracted ethyl acetate of the bark stem of *Phyllanthus acidus*, six terpenoid compounds (**1-6**) were isolated, and their chemical structures were elucidated. To the best of our knowledge, 19-acetoxy-16, 17-dihydroxy-ent-kaurane, 16, 17-dihydroxy-ent-kaurane, 16,17-acetonide-3 $\alpha$ -hydroxykauran, and maslinic acid were the compounds that are the first time were known from this genus.

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**CÁC HỢP CHẤT TERPENOID TỪ DỊCH CHIẾT ETHYL ACETATE  
CỦA THÂN VỎ CÂY PHYLLANTHUS ACIDUS**

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**TÓM TẮT**

Thực hiện sắc kí lớp mỏng và sắc kí cột từ dịch chiết ethyl acetate của vỏ thân cây *Phyllanthus acidus*, sáu hợp chất terpenoid đã phân lập, bao gồm bốn diterpene và hai triterpene. Tên của chúng là phyllanthol (1), 19-acetoxy-16 $\alpha$ , 17-dihydroxy-ent-kaurane (2), 16 $\alpha$ , 17-dihydroxy-ent-kaurane (3), 16 $\alpha$ ,17-acetonide-3 $\alpha$ -hydroxykauran (4), manilic acid (5) và phyllane A (6). Cấu trúc của các hợp chất này được làm sáng tỏ bằng phổ cộng hưởng từ hạt nhân. Đây là lần đầu tiên các hợp chất 2, 3, 4 và 5 được tìm thấy trong chi *Phyllanthus*.

**Từ khóa:** kaurene; *Phyllanthus acidus*; terpenoid