Triterpenoids from *Phyllanthus acidus* (L.) Skeels

Duong Thuc Huy[1], Nguyen Huu Hung[2], Nguyen Thi Anh Tuyet[1], Bui Xuan Hao[1]

***Abstract*—The genus *Phyllanthus* (Phyllanthaceae) includes more than 900 plant species found in tropical and subtropical regions. Many of these species are widely used in folk medicine. The leaves, roots, and stem bark of *Phyllanthus acidus* (L.) Skeels have been used in Vietnamese folk medicine as an antibacterial, antiviral, analgesic, anti-inflammatory, neuroprotective, hepatoprotective, antifibrotic. From the ethanol extract of the roots of *Phyllanthus acidus* (L.) Skeels growing in Binh Thuan province, six compounds phyllanthol (1), glochidone (2), lupeol (3), glochidonol (4), α-lupene (5), and spruceanol (6) were isolated. Their structures were established by extensive spectroscopic analysis as well as comparison with NMR data in the literatures. This is the first time that compounds 4-6 were found in *Phyllanthus acidus* (L.) Skeels.**

***Keywords*—*Phyllanthus acidus* (L.) Skeels, lupane, phyllanthol, triterpene**

1 INTRODUCTION

Previous studies on chemical constituents of *Phyllanthus acidus* (L.) Skeels resulted in the discovery of various natural products such as triterpenes, phytosterols, phenolic compounds, and norbisabolane-type sesquiterpenes [1-3]. Among them, norbisabolane serquiterpenoids displayed strong anti-viral (hepatitis B) effect [3]. Our previous study on the stem bark of *Phyllanthus acidus* (L.) Skeels led to the isolation of three compounds [4].

This paper reports details of the isolation of six compounds from the roots of *Phyllanthus acidus* (L.) Skeels, including phyllanthol (**1**), glochidone (**2**), lupeol (**3**), glochidonol (**4**), *α*-lupene A (**5**), and spruceanol (**6**). Their structure were elucidated on the basis of NMR analysis.

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Duong Thuc Huy, Nguyen Thi Anh Tuyet, Bui Xuan Hao* – *Ho Chi Minh City University of Pedagogy.*
Nguyen Huu Hung – *Nguyen Tat Thanh University*
*Email: buixuanhaodhsp@gmail.com

2 MATERIALS AND METHODS

General experimental procedures

The NMR spectra were measured on a Bruker Avance III (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) spectrometer with TMS as internal standard. Proton chemical shifts were referenced to the solvent residual signal of $CDCl_3$ at δ_H 7.26. The ^{13}C–NMR spectra were referenced to the peak of $CDCl_3$ at δ_C 77.2. Gravity column chromatography was performed with Silica gel 60 (0.040–0.063mm, Himedia).

Plant material

Phyllanthus acidus (L.) Skeels was collected in Ham Thuan Nam district, Binh Thuan province. This plant was identified by Msc. Hoang Viet, Faculty of Biology, University of Science, VNU HCM. A voucher specimen (No UP-B01) was deposited in the herbarium of the Department of Organic Chemistry, Faculty of Chemistry, Ho Chi Minh University of Pedagogy.

Extraction and isolation

The ground root material (20.0kg) was extracted with 95% ethanol under reflux (3x10 L) and the filtrated solution was concentrated under the reduced pressure to obtain the crude extract (1kg). A half of this crude extract (500.0g) was applied to normal phase silica gel column chromatography eluted with increasing polarity of ethyl acetate/*n*-hexane ether (0–100%) to afford the fractions **H1** (2.0g), **H2** (4.0g), **H3** (2.1g), **H4** (3.4g), and **EA1** (67.0g). The remaining residue was eluted with ethyl

acetate: methanol (50:50) and (0:100) to afford the extracts **EA2** (85.0g) and **Me** (285.0g).

Fraction **H1** (2.0 g) was applied to silica gel column chromatography, eluted with *n*-hexane: ethyl acetate (9:1) to obtain five subfractions **H1.1** (125.0mg), **H1.2** (250.0 mg), **H1.3** (152.0 mg), **H1.4** (150.0mg), and **H1.5** (1.1g).

Subfraction **H1.2** was chromatographed, eluted with *n*-hexane: methanol (100:0.2) to obtain three subfractions **H1.2.1** (60.0mg), **H1.2.2** (55.0mg), and **H1.2.3** (75.0mg). Subfraction **H1.2.1** was rechromatographed, eluted with *n*-hexane: methanol (100:0.2) to afford three compounds **1** (6mg), **2** (30mg), and **5** (5mg). Purifying the subfraction **H1.2.3** by column chromatography, eluted with *n*-hexane: methanol (100:0.2) resulted in two compounds, **3** (22.0 mg) and **4** (8.0mg). Subfraction **H1.5** was washed many times by ethyl acetate to afford compound **1** (800mg). Fraction **EA2** was suspended in H_2O (0.5L) and partitioned with EtOAc (3x0.5L) to obtain the EtOAc-soluble subfraction **E0** (7.0g) and remaining aqueous fraction (70.0g). The subfraction **E0** was concentrated then applied to silica gel column chromatography, eluted with chloroform: methanol: water (4:0.9:0.1) to obtain five subfractions **E0.1 – E0.5**. Subfraction **E0.1** (1.16g) was chromatographed, eluted with petroleum ether: ethyl acetate: acetic acid (5:1:0.2) to obtain nineteen subfractions **E0.1.1 – E0.1.19**. Purifying the subfraction **E0.1.14** (46.0mg) by column chromatography, eluted with petroleum ether: chloroform: methanol (1:8:0.2) resulted in compound **6** (8.8mg).

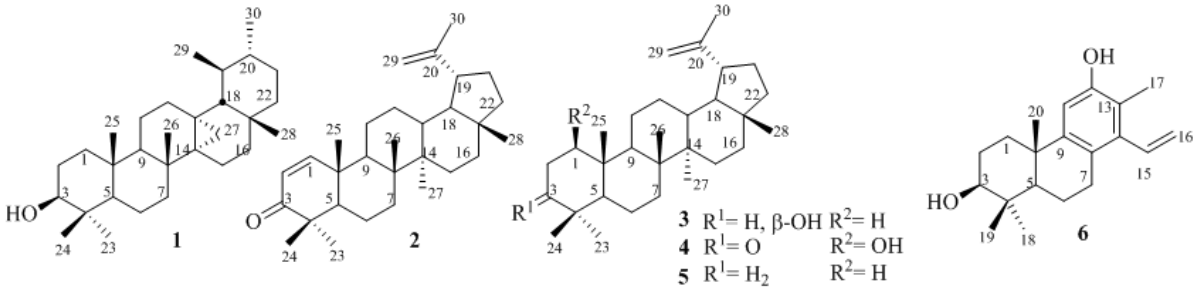

Fig. 1. Chemical structures of 1–6

Table 1. ^{13}C-NMR data of 1–6 ($CDCl_3$)

No	1	2	3	4	5	6	No	1	2	3	4	5	6
1	38.5	160.1	38.2	79.6	40.1	37.4	**16**	27.9	35.0	35.8	35.5	35.7	119.7
2	29.4	124.5	25.3	45.1	19.4	28.3	**17**	31.1	42.6	43.2	43.0	43.1	13.0
3	79.1	203.9	79.3	215.6	42.2	78.9	**18**	54.0	47.7	48.5	48.3	48.4	28.2
4	38.8	42.9	38.9	47.1	33.3	38.8	**19**	40.8	47.3	48.1	47.9	48.1	15.3
5	55.7	52.8	55.5	51.4	55.1	49.3	**20**	37.3	150.1	151.1	150.7	151.0	24.8
6	18.1	18.9	18.5	19.6	19.4	19.2	**21**	29.7	29.2	30.0	29.8	30.0	
7	38.4	33.2	34.5	35.5	34.3	29.8	**22**	42.0	39.8	40.2	40.0	39.8	
8	37.0	41.2	41.0	40.0	40.9	125.3	**23**	27.3	27.4	28.2	27.9	33.7	
9	50.1	43.9	50.6	50.7	49.9	147.9	**24**	15.3	21.2	15.6	19.9	21.6	
10	37.3	39.0	37.3	43.0	37.8	38.8	**25**	16.0	18.5	16.3	11.8	15.9	
11	17.6	20.5	21.1	23.1	21.2	109.5	**26**	17.9	16.1	16.2	16.0	16.1	
12	35.2	24.6	27.5	25.2	25.3	151.9	**27**	13.3	14.1	14.7	14.5	14.6	
13	26.6	37.5	39.0	38.0	38.3	119.0	**28**	28.2	17.8	18.2	18.0	18.2	
14	32.2	42.6	43.0	43.0	43.0	139.2	**29**	18.0	109.7	109.5	109.4	109.5	
15	21.3	26.9	27.6	27.5	27.6	135.5	**30**	20.7	18.9	19.5	19.3	19.8	

Phyllanthol (1): White amorphous powder. The ^{1}H-NMR data (δ in ppm, $CDCl_3$): 3.19 (1H, *dd*, 11.0, 5.0 Hz, H-3), 0.96 (3H, *s*, H-23), 0.77 (3H, *s*, H-24), 0.86 (3H, *s*, H-25), 1.14 (3H, *s*, H-26), 0.01 (1H, *d*, 5.5 Hz, H-27a), 0.66 (1H, *d*, 5.5 Hz, H-27b), 0.90 (3H, *s*, H-28), 0.94 (3H, *d*, 6.0 Hz, H-29), 0.87 (3H, *d*, 6.0 Hz, H-30). The ^{13}C-NMR data ($CDCl_3$): see Table 1. These spectroscopic data were suitable with those reported in the literature [5].

Glochidone (2): Colorless oil. The ^{1}H-NMR data (δ in ppm, $CDCl_3$): 7.10 (1H, *d*, 10.0 Hz, H-1), 5.79 (1H, *d*, 10.0 Hz, H-2), 2.40 (1H, *td*, 11.0, 6.0 Hz, H-19), 1.06 (3H, *s*, H-23), 0.95 (3H, *s*, H-24), 1.08 (3H, *s*, H-25), 1.12 (3H, *s*, H-26), 1.11 (3H, *s*, H-27), 0.80 (3H, *s*, H-28), 4.70 (1H, *d,* 2.0 Hz, H-29a), 4.59 (1H, *d,* 2.0 Hz, H-29b), 1.69 (3H, *s*, H-30). The ^{13}C-NMR data ($CDCl_3$): see Table 1. These spectroscopic data were suitable with those reported in the literature [6].

Lupeol (3): White amorphous powder. The ^{1}H-NMR data (δ in ppm, $CDCl_3$): 3.16 (1H, *dd*, 11.0, 4.8 Hz, H-3), 2.36 (1H, *td*, 11.0, 5.5 Hz, H-19), 0.95 (3H, *s*, H-23), 0.75 (3H, *s*, H-24), 0.82 (3H, *s*, H-25) 1.02 (3H, *s*, H-26), 0.93 (3H, *s*, H-27), 0.78 (3H, *s*, H-28), 4.68 (1H, *d*, 2.0 Hz, H-29a), 4.56 (1H, *dd*, 2.5, 1.5 Hz, H-29b), 1.67 (3H, *s*, H-30). The ^{13}C-NMR data ($CDCl_3$): see Table 1. These spectroscopic data were suitable with those reported in the literature [8].

Glochidonol (4): White amorphous powder. The ^{1}H-NMR data (δ in ppm, $CDCl_3$): 3.90 (1H, *dd*, 8.0, 3.5 Hz, H-1), 3.00 (1H, *dd,*14.5, 8.5 Hz, H-2a), 2.23 (1H, *dd*, 14.5, 3.5 Hz, H-2e), 2.37 (1H, *td*, 11.5, 5.5 Hz, H-19), 1.03 (3H, *s*, H-23), 0.97 (3H, *s*, H-24), 0.83 (3H, *s*, H-25), 1.06 (3H, *s*, H-26), 1.06 (3H, *s*, H-27), 0.80 (3H, *s*, H-28), 4.68 (1H, *d*, 2.0 Hz, H-29a), 4.56 (1H, *d*, 2.0 Hz, H-29b), 1.68 (3H, *s*, H-30). The ^{13}C-NMR data ($CDCl_3$): see Table 1. These spectroscopic data were suitable with those reported in the literature [6].

***α*-Lupene (5):** White amorphous powder. The ^{1}H-NMR data (δ in ppm, $CDCl_3$): 1.03 (3H, *s*, H-23), 0.80 (3H, *s*, H-24), 0.96 (3H, *s*, H-25), 1.07 (3H, *s*, H-26), 0.93 (3H, *s*, H-27), 0.87 (3H, *s*, H-28), 4.69 (1 H, *d*, 2.5 Hz, H-29a), 4.57 (1 H, *d*, 2.5 Hz, H-29b), 1.68 (3H, *s*, H-30). The ^{13}C-NMR data ($CDCl_3$): see Table 1. These spectroscopic data were suitable with those reported in the literature [7, 8].

Spruceanol (**6**): White amorphous powder. The ^{1}H-NMR data (δ in ppm, $CDCl_3$): 2.23 (1H, *m*, H-1e), 1.75 (1H, *m*, H-1a), 1.80 (2H, *m,* H-2), 3.29 (1H, *dd*, 11.5, 4.5 Hz, H-3), 1.29 (1H, *dd*, 2.0, 2.0 Hz, H-5), 1.89 (1H *ddd*, 13.5, 7.5, 1.0 Hz, H-6e), 1.67 (1H *ddd,* 13.5, 11.5, 6.0 Hz, H-6a), 2.78 (1H *ddd*, 17.5, 6.0, 1.0 Hz, H-7e), 2.57 (1H, *ddd*, 17.5, 11.5, 7.5 Hz, H-7a), 6.67 (1H, *s*, H-11), 6.57 (1H, *dd*, 17.5, 11.0 Hz, H-15), 5.53 (1H, *dd*, 11.0, 2.5 Hz, H-16a), 5.16 (1H, *dd*, 17.5, 2.0 Hz, H-16b), 2.18 (3H, *s,* H-17), 1.06 (3H, *s,* H-18), 0.88 (3H, *s,* H-19), 1.20 (3H, *s,* H-20). The ^{13}C-NMR data ($CDCl_3$): see Table 1. These spectroscopic data were suitable with those reported in the literature [9].

3 RESULTS AND DISCUSSION

Phyllanthol (**1**) was isolated from *P. acidus* in the first time by Sengupta and Mukhopadhyay (1966) [10] and its NMR data was revised later by Ndlebe (2008) [5]. It was found in some *Phyllanthus* species such as *P. engleri, P. sellowianus* [1], and *Phyllanthus polyanthus* [5].

Lupane-type triterpenes as glochidone (**2**), lupeol (**3**), glochidonol (**4**), and *α*-lupene (**5**) were found in many *Phyllanthus* plants [1]. Such compounds, for examples lupeol and glochidone showed good inhibition to enzyme acetylcholine esterase [11]. Nevertheless, glochidonol (**4**) and *α*-lupene (**5**) have not been isolated from *P. acidus*. Glochidonol (**4**) exerted good inhibitory effect on Epstein-Barr virus early antigen (EBV-EA) induced by TPA [12].

Compound **6** was isolated as a white amorphous powder. The ^{13}C-NMR spectrum (Table 1) displayed signals corresponding to twenty carbons, including five quaternary carbons, two quaternary carbons, one oxygenated methine, one aromatic methine, two olefinic methines, four methylenes, one methine, and four methyls. The ^{1}H-NMR spectrum displayed signals corresponding to one aromatic proton H-11 [δ_H 6.67 (1H, *s*)] and three olefinic protons H-15 [δ_H 6.57 (1H, *dd*, 17.5, 11.0 Hz)], H-16a [δ_H 5.53 (1H, *dd*, 11.0, 2.5 Hz)], and H-16b [δ_H 5.16 (1H, *dd*, 17.5, 2.5 Hz)], which were representative for one vinyl group (CH_2=CH-). Moreover, the ^{1}H-NMR spectrum revealed four *singlet* methyl H-17 (δ_H 2.18), H-18 (δ_H 1.06), H-19 (δ_H 0.83), and H-20 (1.20), one oxygenated methine H-3 at δ_H 3.29 (*dd*, 11.5, 4.5Hz). The axial position of H-3 (δ = 3.29, *J* = 11.5, 4.5Hz) in the A-ring was

determined on the basis of coupling constants. The HMBC spectrum confirmed the correlations between H-3 and the C-4, C-18, H-18 and H-19 to C-3 and C-4, indicating their vicinal positions in A-ring. Additionally, HMBC spectrum showed cross peaks of H-17 and H-16 to C-14, of H-17 and H-11 to C-12 indicating positions of H-11, 12-OH and H-17 in the C ring. Further analysis of HMBC spectrum confirmed the structure of **6**, according to comparison of the NMR data of **6** to those of spruceanol in the literature [9]. So, the structrure of compound **6** was concluded as spruceanol. This is the first time the diterpenoid skeleton was reported in *P. acidus*.

4 CONCLUSION

Six known compounds were isolated from the ethanol extract of the roots of *Phyllanthus acidus* growing in Binh Thuan province. Phyllanthol (**1**) was isolated as a major compound of the *n*-hexane extract. Glochidonol (**4**), α-lupene (**5**), and spruceanol (**6**) are reported in the plant *Phyllanthus acidus.* Further studies on this plant are in progress.

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Thành phần hóa học rễ cây chùm ruột mọc ở tỉnh Bình Thuận

Dương Thúc Huy[1], Nguyễn Hữu Hùng[2], Nguyễn Thị Ánh Tuyết[1], Bùi Xuân Hào[1,*]

[1]Trường Đại học Sư phạm TP. HCM; [2]Trường Đại học Nguyễn Tất Thành
*Tác giả liên hệ: buixuanhaodhsp@gmail.com

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***Tóm tắt*—Chi *Phyllanthus* (Phyllanthaceae) bao gồm hơn 900 loài thực vật, được tìm thấy ở vùng nhiệt đới và cận nhiệt đới. Nhiều loài trong chi này được sử dụng rộng rãi trong y học dân gian. Trong y học cổ truyền Việt Nam, lá, rễ và vỏ thân của loài *Phyllanthus acidus* (L.) Skeels đã được sử dụng để kháng khuẩn, kháng vi-rút, giảm đau, chống viêm, bảo vệ thần kinh, chống viêm gan. Từ dịch chiết ethanol của rễ cây chùm ruột mọc ở tỉnh Bình Thuận, đã phân lập được sáu hợp chất là phyllanthol (1), glochidone (2), lupeol (3), glochidonol (4), α-lupene (5), spruceanol (6). Cấu trúc của các hợp chất này được làm sáng tỏ bằng các phương pháp phổ cộng hưởng từ hạt nhân, cũng như so sánh với các tài liệu tham khảo. Đây là lần đầu tiên các hợp chất 4, 5, 6 được phát hiện trong cây chùm ruột.**

***Từ khóa*—*Phyllanthus acidus (L.)* Skeels, lupane, phyllanthol, diterpene**