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Triterpenoids Firstly Isolated from the Stem Bark of *Glochidion littorale* Blume (Phyllanthaceae)

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ABSTRACT

Glochidion littorale Blume belongs to the family Phyllanthaceae**,** is a tropical shrub that grows wild throughout Vietnam and is found in the mangroves of Southeast Asia. In traditional medicine, this species has been applied to treat stomach ache and dysentery. However, there is not much information about chemical composition and bioactivity of this species. This study focuses on the isolation and identification of compounds from *G. littorale.* The methanol extract of the *G. littorale* stem bark was separated by liquid-liquid extraction, followed by different chromatographic techniques to purify the chemical composition. The result showed that seven terpenoids including lupeol (1), 3-epi-lupeol (2), betulin (3), lup-20(29)-ene-1β,3β-diol (4), lupenone (5), glochidonol (6) and glochidone (7) were isolated from the methanol extract. The chemical structures of these terpenoids were elucidated based on NMR and MS spectral analysis, and by comparison with previous references. These compounds have not been previously isolated from ***G. littorale.***

***Keywords*:** *Glochidion littorale*, Triterpenoid, Lupeol, Betulin, Lupenone, Glochidone, glochidonol

Introduction

Glochidion littorale Blume (synonym *Phyllanthus littoralis* (Blume) Müll.Arg.) is a species in the genus *Glochidion.* In the past *Glochidion* belonged to the family Euphorbiaceae, but a phylogenetic study by P. Hoffmann in 2006 showed that *Phyllanthus* was paraphyletic to *Glochidion*. Then, the genus *Glochidion* has been subsumed into *Phyllanthus* which belongs to the family Phyllanthaceae.[1]

Glochidion littorale Blume is a tropical shrub that can grow up to 6 m tall. The leaves of this species are oval-ovate or almost round, leathery, shiny and simple. The plant flowers all year round, and the fruit is red and smooth, measuring about 1 to 1.5 cm (Figure 1). This species is found along the coast or in the mangroves from South Asia through Southeast Asia such as in Cambodia, Malaysia, Philippines, Vietnam, Thailand, Singapore and Indonesia.[2] In traditional medicine, the leaves were used to treat stomach ache, blood in the stools, dysentery and tonsillitis.[2,3]

Study on chemical constituents of the genus *Glochidion* showed that they contained many types of metabolites including triterpenoid,[4,5,6] steroids,[7] flavonoid,[8] lignans.[9] … However, there is limited publication about the phytochemical study of *G. littorale*.

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The research of Bagoudou in 2021 showed that the leaf extract of this plant exhibited high antioxidant activity by DPPH radical-scavenging assay and also displayed neuroprotective effects against neurodegeneration *in vivo* test on *Caenorhabditis elegans* via the activation of DAF-16.[10] This species was rich in flavonoids and phenolic compounds in which coumestrin, myricetin, hesperidin, and chlorogenic acid were the major metabolites by LC-HRMS analysis.[10] A recent study in 2023 by Wannachai Chantan confirmed the high concentrations of flavonoids and phenolic derivatives in *G. littorale* leaves and displayed high antioxidant activity with an IC_{50} value of 67.18 µg/ml by DPPH assay.[11] Furthermore, the free radical scavenging efficiency of ethanol extract was slightly higher than that of ethyl acetate extract from *G. littorale* fruits in Hassan's report in 2023.[12]

With the purpose of providing more information about the chemical constituents of *G. littorale*, this paper describes the isolation and structure elucidation of triterpenoid compounds from the methanol extract of *G. littorale* stem bark including lupeol (1), 3-*epi*-lupeol (2), betulin (3), lup-20(29)-ene-1*β*,3*β*-diol (4), lupenone (5), glochidonol (6) and glochidone (7). These metabolites have not been previously isolated from the *G. littorale*.

Materials and Methods

Plant materials

The bark of *Glochidion littorale* Blume was collected in Ben Tre, Viet Nam with approximate coordinates at Latitude: 9°53'10.3"N and Longitude: 106°37'39.2"E in December 2021 and its scientific name was determined by Assoc. Prof. Dang Minh Quan, Department of Biology Education, School of Education, Can Tho University. A voucher specimen, designated as Gloch122021-TVVN, was deposited at the Botanical Laboratory, Department of Biology Education, School of education, Can Tho University.

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General experimental procedures

Nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS) were used to determine the chemical structures of the isolated compounds. NMR spectra were measured using a Bruker Avance NEO 600 MHz (Bruker BioSpin, Switzerland), mass spectra were performed on an Agilent 1100 series LC/MSD Trap SL (Agilent, USA) at the Institute of Chemistry, Vietnam Academy of Science and Technology. The melting point (m.p.) was recorded on a non-adjustable melting apparatus (Electrothermal IA 9100, UK). All solvents used for extraction and purification were of analytical grade by Xilong Scientific Co., Ltd (China) or Chemsol (Vietnam), including acetone (99.5%), dichloromethane (99.5%), chloroform (99.0%), ethyl acetate (99.5%), *n*-hexane (96%), methanol (99.5%) and distilled water. Chromatographic techniques were performed to purify and isolate compounds. Vacuum liquid chromatography (VLC) and column chromatography (CC) was realized on silica gel 60 (0.040 – 0.063 mm, Merck, Germany). Thin layer chromatography (TLC) was conducted on pre-coated silica gel 60 F_{254} (Merck, Germany). Visualization of TLC plates was carried out under UV light (254 and 365 nm), and then the plates were dipped in a 5% vanillin/H_2SO_4 or 10% H_2SO_4 solution and heated at 120°C for 5 min.

Extraction and isolation

The dried stem bark of *G. littorale* (4.60 kg) was ground and extracted with methanol (3 x 20L) at room temperature for 24 hours. The combined solution was evaporated under reduced pressure at 45°C to obtain crude methanol extract (650 g). The crude extract was suspended in 4L of water and then partitioned sequentially with *n*-hexane and ethyl acetate to obtain the corresponding fractions: *n*-hexane (31.8 g, HF), EtOAc (40.0 g, EF), and water layer (4L, WF). The *n*-hexane fraction (31.8 g) was subjected to a silica gel VLC (10 cm x 20 cm) eluted step by step with a gradient of *n*-hexane – acetone (100:0 to 0:100, v/v) to afford 13 sub-fractions (HF1 to HF13).

Sub-fraction HF3 (5.66 g) was separated by silica gel CC (*n*-hexane – dichloromethane, 100:0 to 0:100, v/v) to yield compound 5 (645 mg) and compound 7 (45 mg). Sub-fraction HF5 (1.32 g) was purified by another silica gel CC (*n*-hexane – dichloromethane, 100:0 to 0:100, v/v) to give eleven sub-fractions HF5.1 to HF5.11. Sub-fraction HF5.7 (140 mg) was subjected on silica gel CC eluted with an isocratic solvent of *n*-hexane – acetone (8:2, v/v) to obtain compound 1 (55 mg). Sub-fraction HF5.4 (244 mg) was separated by silica gel CC (*n*-hexane – acetone, 100:0 to 0:100 v/v) to yield nine sub-fractions HF5.4.1 to HF5.4.9. Sub-fraction HF5.4.3 (117 mg) was purified on silica gel CC (*n*-hexane – dichloromethane, 9:1-8:2, v/v) to afford compound 2 (85 mg). Sub-fraction HF8 (1.30 g) was subjected on silica gel CC eluted step by step with a gradient of *n*-hexane – acetone (100:0 – 0:100, v/v) to yield twelve sub-fractions HF8.1 to HF8.12. Sub-fraction HF8.9 (72 mg) was recrystallized in *n*-hexane-acetone mixture (2:1, v/v) to give compound 3 (35 mg). Sub-fraction HF8.7 (107 mg) was purified by silica gel CC eluted with an isocratic solvent of *n*-hexane – acetone (8:2, v/v) to give compound 6 (100 mg). Purification of sub-fraction HF9 (3.20 g) was repeated by silica gel CC (*n*-hexane – acetone, 100:0 - 0:100, v/v) to yield twelve sub-fractions HF9.1 to HF9.12. Sub-fraction HF9.6 (200 mg) was purified by another silica gel CC eluted with an isocratic solvent of 100% CH_2Cl_2 to yield compound 4 (20 mg).

Validations of novel contribution

The isolated compounds were compared with library data on Reaxys® and Scifinder® to confirm the novel contribution of this study.

Results and Discussion

The *n*-hexane fraction from the stem bark of *G. littorale* was separated and purified by silica gel VLC and CC many times to afford seven compounds. The chemical structures of these compounds (Figure 2) were elucidated by analysis of spectroscopic data including 1D-and 2D-NMR, ESI-MS, as well as comparison with previous publications. All of these metabolites were identified as triterpenoid derivatives possessed a skeleton of lup-20(29)-ene by the characteristic signals of an isopropylene group at about δ_C 151, 109 and 19 ppm in the ^{13}C-NMR spectrum, combined with the observation of two olefin protons at about δ_H 4.7 and 4.6 ppm in the ^{1}H-NMR spectrum.

Lupeol (1): white powder, m.p. 210°C. TLC: Rf values 0.15 (*n*-hexane : $CHCl_3$ = 70:30), 0.36 (*n*-hexane : Ethyl acetate = 95:5), and 0.60 (*n*-hexane : acetone = 85:15) (Figure S1). ESI-MS *m/z* = 427.1 $[M+H]^+$ (molecular formula $C_{30}H_{50}O$) (Figure S2). ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm): see Table 1 (Figure S3). ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C (ppm): see Table 3 (Figure S4).

3-*epi*-lupeol (2): white powder, m.p. 199-200°C. TLC: Rf values 0.27 (*n*-hexane : $CHCl_3$ = 80:20), 0.39 (*n*-hexane : acetone = 75:25), and 0.7 (*n*-hexane : Ethyl acetate = 95:5) ((Figure S5). ESI-MS *m/z* = 427.2 $[M+H]^+$ (molecular formula $C_{30}H_{50}O$) (Figure S6). ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm): see Table 1 (Figure S7). ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C (ppm): see Table 3 (Figure S8). HSQC and HMBC spectra (Figure S9-S10)

Table 1: The important ^{1}H-NMR data (δ_{ppm}) for **1** to **3** ($CDCl_3$, 600 MHz)

Nº	1	2	3
3	3.19. dd. 11.4. 4.8	3.38. t. 2.4	3.19. dd. 11.4. 4.8
19	2.39. dt. 6.0. 10.8	2.38. dt. 6.0. 10.8	2.38. dt. 6.0. 10.8
23	0.96. s	0.83. s	0.97. s
24	0.76. s	0.94. s	0.76. s
25	0.83. s	0.84. s	0.83. s
26	1.03. s	1.03. s	0.98. s
27	0.95. s	0.96. s	1.03. s
28	0.79. s	0.79. s	3.80. d. 10.8 3.34. d. 10.8
29	4.68. d. 2.4 4.58. dd. 2.4. 1.2	4.68. d. 2.4 4.58. m	4.68. d. 2.4 4.58. dq. 2.4. 1.2
30	1.68. s	1.68. s	1.68. s

Betulin (3): white powder, m.p. 256-257°C. TLC: Rf values 0.14 (*n*-hexane : Ethyl acetate = 90:10), 0.36 (*n*-hexane : acetone = 85:15), and 0.74 ($CHCl_3$: Ethyl acetate = 95:5) (Figure S11). ESI-MS *m/z* = 443.3 $[M+H]^+$ (molecular formula $C_{30}H_{50}O_2$) (Figure S12). ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm): see Table 1 (Figure S13). ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C (ppm): see Table 3 (Figure S14).

Lup-20(29)-ene-1*β*,3*β*-diol (4): white powder, m.p. 213-214°C. TLC: Rf values 0.14 ($CHCl_3$: Ethyl acetate = 95:5), 0.5 ($CHCl_3$: acetone = 90:10), and 0.81 ($CHCl_3$: MeOH = 90:10) (Figure S15). ESI-MS: *m/z* = 443.3 $[M+H]^+$ (molecular formula $C_{30}H_{50}O_2$) (Figure S16). ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm): see Table 2 (Figure S17). ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C (ppm): see Table 3 (Figure S18).

Lupenone (5): white powder, m.p. 169-170°C. TLC: Rf values 0.2 (*n*-hexane : $CHCl_3$ = 90:10), 0.6 (*n*-hexane : ethyl acetate = 95:5), and 0.75 (*n*-hexane : acetone = 95:5) (Figure S19). ESI-MS: *m/z* = 425.2 $[M+H]^+$ (molecular formula $C_{30}H_{48}O$). ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm):

see Table 2 (Figure S20). ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C (ppm): see Table 3 (Figure S21).
Glochidonol (6): white powder, m.p. 228-230°C. TLC: Rf values 0.23 (*n*-hexane : ethyl acetate = 90:10), 0.49 (*n*-hexane : acetone = 85:15), and 0.77 ($CHCl_3$: acetone = 95:5) (Figure S22). ESI-MS: *m/z* = 441.3 $[M+H]^+$ (molecular formula $C_{30}H_{48}O_2$). ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm): see Table 2 (Figure S23). ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C (ppm): see Table 3 (Figure S24).
Glochidone (7): white powder, m.p. 163-164°C. TLC: Rf values 0.13 (*n*-hexane : $CHCl_3$ = 90:10), 0.54 (*n*-hexane : ethyl acetate = 95:5), and 0.77 (*n*-hexane : acetone = 95:5) (Figure S25). ESI-MS: *m/z* = 423.1 $[M+H]^+$ (molecular formula $C_{30}H_{46}O$) (Figure S26). ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H (ppm): see Table 2 (Figure S27). ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C (ppm): see Table 3 (Figure S28).

Figure 1: *Glochidion littorale* Blume

Compound **1** was obtained as white powder, and its molecular formula was identified as $C_{30}H_{50}O$ by the ESI-MS pseudo-molecular ion peak at *m/z* 427.1 $[M+H]^+$. The ^{1}H-NMR spectrum of **1** (Table 1) exhibited the signals of two olefin protons at δ_H 4.68 (1H, *d*, *J* = 2.4 Hz) and δ_H 4.58 (1H, *m*) together with a singlet of methyl group at δ 1.68 (3H, *s*) indicating the existence of a terminal isopropylene group. Besides, the presence of six other methyl groups was observed by the six singlets (three protons for each signal) at δ_H 1.03, 0.96, 0.95, 0.83, 0.79 and 0.76. These signals were characteristics of a triterpenoid compound with a skeleton of lup-20(29)-ene. Moreover, the ^{1}H-NMR spectrum also exhibited a 3*β*-hydroxyl group identified by the doublet of doublets at δ_H 3.19 (1H, *dd*, *J* = 11.4, 4.8 Hz) which was at axial orientation. The ^{13}C-NMR spectrum showed 30 carbons for the triterpenoid of lup-20(29)-ene skeleton which was characteristic by the observation of two olefinic carbons of isopropylene functional group at δ_C 151.0 and 109.3, and seven methyl carbons at δ_C 28.0, 15.4, 16.1, 16.0, 14.6, 18.0, 19.3 (Table 3). The presence of one hydroxyl group at position 3 was determined by an oxygenated carbon signal at δ_C 79.0. From this evidence combined with comparison of the spectral data of a previous publication of lupeol isolated from *Bombax costatum*,[13] compound **1** was identified as lupeol (or 3*β*-hydroxylup-20(29)-ene).
Compound **2** exposed a pseudo-molecular ion peak at *m/z* 427.2 $[M+H]^+$ in the ESI-MS spectrum corresponding to the molecular formula of $C_{30}H_{50}O$. The ^{1}H-NMR and ^{13}C-NMR spectra data of **2** was very similar to those of **1** indicating that these two compounds were isomers (Table 1 and 3). The difference between two metabolites was the signal of oxygenated methine proton at δ_H 3.38 (1H, *t*, *J* = 2.4 Hz**,** H-3) which had a small coupling constant of ^3J = 2.4 Hz proved that this proton was at equatorial orientation. Based on the spectroscopic evidence and comparison with the reported publication of 3-*epi*-lupeol,[4] compound **2** was determined as 3-*epi*-lupeol.
Compound **3** displayed a pseudo-molecular ion peak at *m/z* = 443.3 $[M+H]^+$ in the ESI-MS spectrum suggesting a molecular formula of $C_{30}H_{50}O_2$. 1D-NMR spectra data of **3** was similar to the NMR data of **1**. However, the ^{1}H-NMR spectrum of **3** (Table 1**)** showed the observation of two protons of methylene at δ_H 3.80 (1H, *d*, *J* = 10.8 Hz, H-28a), and 3.34 (1H, *d*, *J* = 10.8 Hz, H-28b) instead of the presence of a singlet of methyl group at δ_H 0.79 ppm in compound **1** indicating the existence of -CH_2OH group in compound **3**. Similarly, the ^{13}C-NMR spectrum (Table 3**)** confirmed the presence of this methylene carbon at δ_C 60.6 ppm. The absence of a methyl carbon at δ_C 18.0 (C-28) indicated that this carbon was replaced by a -CH_2OH group in compound **3**. By comparing the NMR data between compound **3** and betulin in previously reported literature,[14] compound **3** was assigned as betulin.
The NMR data of **4** was also similar to those of **1** with the characteristic signals of a triterpenoid of lup-20(29)-ene skeleton. The difference between the two compounds was the presence of more than one hydroxyl group in the ^{1}H-NMR spectrum of **4** revealed by the doublet of doublets signal at δ_H 3.42 (1H, *dd*, *J* = 11.4, 4.8 Hz, H-1) (Table 2**)**. The ^{13}C-NMR data of **4** confirmed this analysis by the observation of an oxygenated methine carbon at δ_C 79.0 ppm (Table 3**)**. The MS spectrum of **4** was in agreement with NMR spectra data, in which molecular formula of **4** was identified to be $C_{30}H_{50}O_2$ by the observation of a pseudo-molecular ion peak at m/z 443.3 $[M+H]^+$ in the positive ESI-MS spectrum. This corresponded to an additional 16 amu unit (more than one oxygen atom) when compared to compound **1** ($C_{30}H_{50}O$). Based on the above analysis and comparison of NMR spectral data with lup-20(29)-ene-1*β*,3*β*-diol previously isolated from *Glochidion eriocarpum*, [5] the structure of **4** was identified as lup-20(29)-ene-1*β*,3*β*-diol (or 3-epiglochidiol).
The comparison of the NMR data of **5** and **1** showed that they were similar. However, the absence of an oxygenated methine proton signal at δ_H 3.19 in the ^{1}H-NMR spectrum of **5** (Table 2**)** indicated that this compound did not have a hydroxyl group at position 3. In addition, the observation of carbonyl carbon at δ_C 218.2 ppm instead of the oxygenated methine carbon at δ_C 79.0 ppm in the ^{13}C-NMR spectrum of **5** (Table 3**)** revealed that the 3*β*-hydroxyl group of **1** has been supplanted by a ketone group at position 3 in compound **5**. The MS spectrum also confirmed the above analysis. The ESI-MS spectrum of **5** exhibited a pseudo-molecular ion peak at *m/z* = 425.2 $[M+H]^+$ suggesting a molecular formula of $C_{30}H_{48}O$, its molecular was less than 2 hydrogens compared to the molecular formula of **1** as $C_{30}H_{50}O$. From this evidence and in comparison with the NMR data of lupenone,[5] the structure of **5** was identified as lupenone.
Compound **6**, a white powder, indicated a pseudo-molecular ion peak at *m/z* 441.3 $[M+H]^+$ in the ESI-MS spectrum, consistent with the molecular formula of $C_{30}H_{48}O_2$, this compound had more than one oxygen atom compared to those of **5**. The NMR data of **6** was similar to those of **5** as a ketone triterpenoid compound (Table 2**)**. However, the observation of a multiple signal of oxygenated methine proton at δ_H 3.90 (1H, *m*, H-1) in the ^{1}H-NMR spectrum of **6,** corresponding to the presence of a deshielded methine carbon at δ_C 79.6 ppm in the ^{13}C-NMR spectrum (Table 3**)**, indicated the existence of a hydroxyl group in its structure. This evidence was in agreement with the above analysis of MS data. From the results of NMR and MS spectral data analysis, and comparison with the NMR data of glochidonol isolated from *Glochidion eriocarpum*,[5] the chemical structure of compound **6** was determined as glochidonol.
The ESI-MS spectral data of 7 displayed a pseudo-molecular ion peak at *m/z* = 423.1 $[M+H]^+$ (18 amu units less than that of 6, corresponding to a water molecule), determining a molecular formula of $C_{30}H_{46}O$. This analysis was agreement with the NMR data, the presence of two olefin protons at δ_H 7.10 (1H, *d*, *J* = 10.2 Hz, H-1) and 5.79 (1H, *d*, *J* = 10.2 Hz, H-2) in the ^{1}H-NMR spectrum of 7 (Table 2) instead of the signal of methine proton of CHOH group at δ_H 3.90 (1H, *m*, H-1) in compound 6. In addition, the olefin group was also observed in the ^{13}C-NMR spectrum of 7 (Table 3) by the signals at δ_C 159.8 (C-1) and 125.2 (C-2), combined with the absence of the signal of hydroxyl carbon at δ_C 79.6 ppm as present in compound 6, demonstrating that structure of 7 was formed by the reduction of a water molecule from compound 6. The NMR spectral analysis of compound 7 showed high compatibility with glochidone from a previous publication,[5] the structure of 7 was elucidated as glochidone.
In summary, seven triterpenoids were isolated from the *n*-hexane fraction of *G. littorale* comprising lupeol (1), 3-*epi*-lupeol (2), betulin (3), lup-20(29)-ene-1*β*,3*β*-diol (4), lupenone (5), glochidonol (6) and glochidone (7). Comparison with library data of *G. littorale* on Reaxys® and Scifinder® showed that these metabolites have never been isolated from this species before. These triterpenoids possess a wide range of biological activities. Previous studies have shown that lupeol has antibacterial,[15] anti-inflammatory,[16] and anti-cancer activities.[16,17]

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Notably, lupeol was non-toxic to normal cells and tissues at therapeutically effective doses, making it a potential candidate for drug development in the treatment of inflammation and cancer.[17] Besides, betulin also exhibited numerous significant activities such as antibacterial, antiviral and anticancer effects.[18] Another triterpenoid, lupenone, was found in many species of the families Acanthaceae, Betulaceae, Compositae, Euphorbiaceae, Fabaceae, Rosaceae, Zingiberaceae… This compound also displayed many biological activities including antidiabetic, antiviral, anti-inflammatory, anticancer.[19] In addition, the research of Chen in 2020 showed that glochidone and 3-epiglochidiol exhibited anti-lung cancer effects, however, the activity of these compound was less than that of glochidiol (an isomer of 3-epiglochidiol).[20] This study also concluded that glochidiol could be a promising compound for the treatment of lung cancer.[20] Moreover, glochidone also possessed antidiabetic effects.[21] These important data may orientate for further studies on the biological activity and phytochemical of *G. littorale*.

Table 2: The important ^{1}H-NMR data (δ_{ppm}) for **4** to **7** ($CDCl_3$, 600 MHz)

Nº	4	5	6	7
1	3.42. dd. 11.4. 4.8		3.90. m	7.10. d. 10.2
2		2.48, ddd. 7.8, 9.6, 15.6	3.00. dd. 14.4. 7.8	5.79. d. 10.2
		2.41, ddd. 4.2, 7.8, 15.6	2.22. dd. 14.4. 3.6	
3	3.24. brd. 12.0			
19	2.37. dt. 6.0. 10.8	2.38, dt. 6.0. 10.8	2.38 dt. 6.0. 10.8	2.39. dt. 6.0. 10.8
23	0.75. s	1.03. s	1.06. s	1.08. s
24	0.95. s	1.07. s	1.06. s	1.13. s
25	0.90. s	0.93. s	0.84. s	1.07. s
26	1.04. s	1.07. s	1.04. s	1.11. s
27	0.95. s	0.96. s	0.98. s	0.96. s
28	0.79. s	0.80. s	0.80. s	0.81. s
29	4.68. d. 2.4	4.69. d. 2.4	4.68. d. 2.4	4.71. d. 2.4
	4.55. dq. 2.4. 1.2	4.57. m	4.57. m	4.59. dq. 2.4. 1.2
30	1.67. s	1.68. s	1.68. s	1.69. s

Table 3: ^{13}C-NMR data (δ_{ppm}) for **1** to **7** ($CDCl_3$, 150 MHz)

Nº	1	2	3	4	5	6	7
1	38.7	33.3	38.9	79.0	34.2	79.6	159.8
2	27.4	25.4	27.5	38.1	39.6	45.1	125.2
3	79.0	76.3	79.0	75.8	218.2	215.8	205.5
4	38.9	37.5	38.9	38.9	47.3	47.1	44.7
5	55.3	49.1	55.3	53.2	55.0	51.4	53.5
6	18.3	18.3	18.3	18.0	19.7	19.6	19.0
7	34.3	34.2	34.3	34.1	33.6	33.0	33.8
8	40.9	41.1	41.0	42.9	40.8	43.0	41.8
9	50.5	50.2	50.4	51.5	49.8	50.7	44.5
10	37.2	37.3	37.4	43.6	36.9	42.9	39.6
11	21.0	20.8	20.9	23.9	21.5	23.1	21.3
12	25.2	25.2	25.3	25.1	25.2	25.2	25.1
13	38.1	38.1	37.2	37.6	38.2	38.0	38.3
14	42.9	43.0	42.7	41.4	43.0	41.2	43.0
15	27.5	27.4	27.1	27.5	27.5	27.5	27.4
16	35.6	35.6	29.2	35.6	35.5	35.5	35.5
17	43.0	42.9	47.8	42.9	42.9	43.0	43.1
18	48.3	48.3	47.8	48.4	48.3	48.3	48.2
19	48.0	48.0	48.8	48.0	48.0	47.9	47.9
20	151.0	151.0	150.5	150.8	150.9	150.7	150.8
21	29.9	29.9	29.8	29.8	29.9	29.8	29.8
22	40.0	40.0	34.0	40.0	40.0	40.0	40.0
23	28.0	28.3	28.0	27.9	26.7	27.9	27.8
24	15.4	22.1	15.4	14.9	21.0	19.9	21.4
25	16.1	16.0	16.1	11.9	16.0	11.8	19.3
26	16.0	15.9	16.0	16.2	15.8	16.0	16.5
27	14.6	14.7	14.8	14.5	14.5	14.5	14.4
28	18.0	18.0	60.6	18.0	18.0	18.0	18.1
29	109.3	109.3	109.7	109.4	109.4	109.5	109.5
30	19.3	19.3	19.1	19.2	19.3	19.3	19.2

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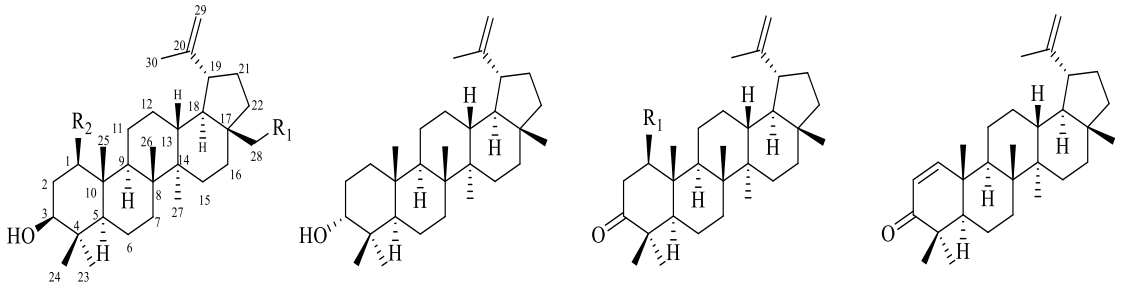

1. R_1 = H, R_2=H
3. R_1 = OH, R_2 = H
4. R_1 = H, R_2 = OH

2

5. R_1 = H
6. R_1 = OH

7

Figure 2: Structure of the isolated compounds from *G. littorale*

Conclusion

Seven terpenoids were isolated from the *n*-hexane fraction of *G. littorale* stem bark including lupeol (1), 3-*epi*-lupeol (2), betulin (3), lup-20(29)-ene-1*β*,3*β*-diol (4), lupenone (5), glochidonol (6) and glochidone (7). The chemical structures of these triterpenoids were elucidated based on NMR and MS spectral analysis. These terpenoids have not been previously reported from *G. littorale* collected in Vietnam. In agreement with previous publications on the chemical constituents of other species in the genus *Glochidion*, the isolated triterpenoids compounds from *G. littorale* could be seen as characteristic metabolites of genus *Glochidion*. Further studies on the biological activities and chemical composition of *G. littorale* are being conducted to provide scientific information on its use in traditional medicine.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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