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Research Article

A STUDY ON THE CHEMICAL CONSTITUENT OF EXTRACTED ETHYL ACETATE OF THE LEAVES OF *PHYLLANTHUS ACIDUS*

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ABSTRACT

Leaves of Phyllanthus acidus were collected in Binh Thuan province and extracted with methanol at room temperature to obtain a methanol extract. Carry out complete dispersion of methanol residue into the water and extracts with increased polarization solvents: n-hexane, chloroform, ethyl acetate, and n-butanol solvents, respectively, to obtain the respective residue fractions. The ethyl acetate extraction was performed with column chromatography several times and isolated eight pure compounds to be ***1, 2, 3, 4, 5, 6, 7, and 8****. Their chemical structures were determined based on analysis of 1D, 2D-NMR nuclear magnetic resonance spectroscopy data, combined with reference comparison. This is the first time eight compounds have been reported on the isolation and determination of their structure from ethyl acetate extraction from the leaves of Phyllanthus acidus collected in Binh Thuan province.*

Keywords: Euphorbiaceae; kaempferol; *Phyllanthus acidus*; quercetin

1. Introduction

Chum ruot has the scientific name *Phyllanthus acidus*, which belongs to the genus *Phyllanthus*, family Euphorbiaceae. The plant was introduced to Asia and Africa from Madagascar and is becoming increasingly popular. In Vietnam, Chum ruot is commonly grown in the southern provinces and some houses in the North for shade, ornamental purposes, or fruit (Do, 2004). In addition, it grows wild and is grown in Laos, and the tree is also widely distributed in tropical Asia (Malaysia, India, Indonesia, Philippines, and Mangat Island). According to traditional medicine in many countries, the leaves are used for cooking water for bathing to help treat itching, urticaria, and skin diseases. The bark of the stem has the effect of detoxification, boils, and phlegm. The bark soaked in alcohol is used to treat ear rot and pus, scabies, sores, and skin wounds to treat toothache and sore throat. The roots are toxic, hemolytic, detoxifying, and antiseptic effects, often used by Malaysians to treat

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headaches and coughs and Javars for asthma. The fruit has a sour taste and is used to eat raw or cook soup to clear heat, detoxify, cure headaches, and improve liver function in patients with liver disease (Do, 2006).

In this article, we describe the isolation and determination of the chemical structure of the eight compounds from the extract of ethyl acetate of *P. acidus* leaves. These compounds were elucidated as phyllanthol (**1**), olean-12-en-3β,15α-diol (**2**), kumatakenin (**3**), kaempferol (**4**), quercetin (**5**), 4-hydroxybenzoic acid (**6**), protocatechuic acid (**7**), and quercetin 3-*O*-*β*-D-glucopyranoside (**8**).

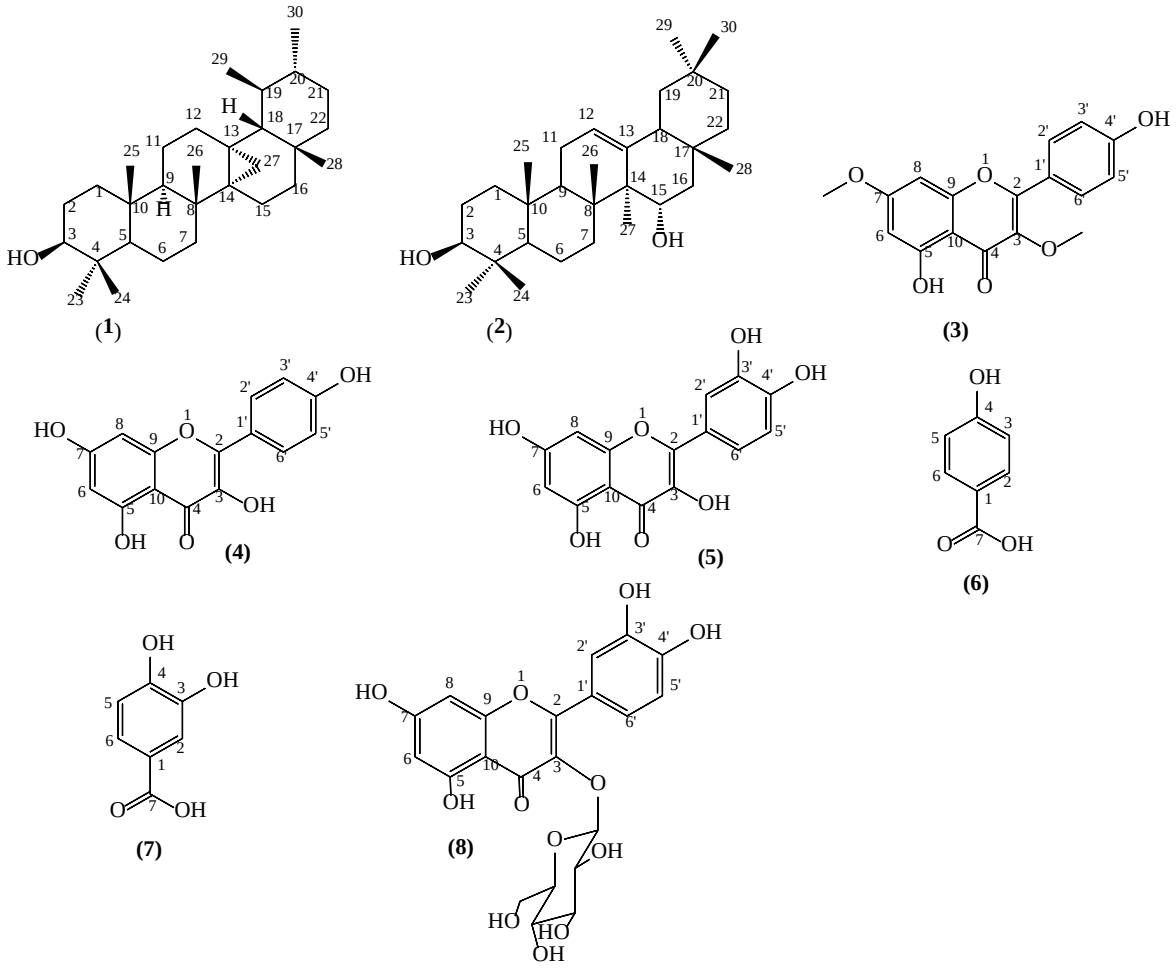

Figure 1. *Chemical structures of isolated compounds* ***1-8***

2. Experimental

2.1. General experimental methods

Column chromatography was performed on silica gel (70-230 mesh, Merck) in the normal phase and at atmospheric pressure. Thin-layer chromatography was performed on a 60 F_{254} silica gel plate (Merck), and spots were detected by ultraviolet (UV) illumination and by spraying with 10% sulfuric acid reagent followed by heating. The NMR spectroscopic data were recorded on an Avance Neo (600 MHz for ^{1}H NMR, 150 MHz for ^{13}C NMR) and

an Avance III HD spectrometer (500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR) in CD_3OD, $CDCl_3$, DMSO-d_6, acetone-d_6 using tetramethylsilane or residual non-deuterated solvent peak as an internal standard. Chemical shifts are shown in units of δ (ppm), and the coupling constants are expressed in hertz.

2.2. Plant material

Leaves of *Phyllanthus acidus* (L.) Skeels were collected in Ham Thuan Bac district, Binh Thuan province, in 2018. A voucher specimen was deposited in the Natural Product Lab, Faculty of Chemistry, Ho Chi Minh City University of Education.

2.3. Extraction and isolation

The dried leaves powder of *P. acidus* (25 kg) was macerated in methanol (MeOH) for three days at room temperature (repeated four times). The extract was concentrated in vacuo to obtain a methanol residue (991 g). This residue was suspended in H_2O and then partitioned successively with *n*-hexane, chloroform ($CHCl_3$), ethyl acetate (EtOAc), *n*-butanol, respectively, followed by concentration in vacuo, yielding the respective residues *n*-hexane (250 g), chloroform (300 g), ethyl acetate (25 g), and *n*-butanol (81.5 g). The ethyl acetate residue (25 g) was chromatographed on a silica gel column, eluted with a step-wise gradient of *n*-hexane/EtOAc (20:1, 10 :1, 8:1, 2:1, 1:1, 0:1) and EtOAc/MeOH (9:1, 8:2) to yield eight main fractions (EA1-EA8). Fraction EA1 (1.102 g) was separated by silica gel column chromatography, eluting with *n*-hexane/EtOAc (6:1) to give five subfractions (EA1.1-EA1.5). Subfraction EA1.2 (182 mg) was chromatographed repeatedly on silica gel column chromatography, eluting with *n*-hexane/EtOAc (5:1) to obtain compound **1** (4.8 mg). Compound **2** (5.0 mg) was isolated by silica gel column chromatography many times on subfraction EA1.4 (200 mg), eluting with *n*-hexane/EtOAc (5:1). Fraction EA2 (1.254 g) was subjected to a silica gel column with a gradient elution of *n*-hexane/EtOAc (3:1) to provide seven subfractions (EA2.1-EA2.7). Subfraction EA2.3 (160 mg) was separated by column chromatography over silica gel, eluting with *n*-hexane/$CHCl_3$/EtOAc/AcOH (3:3:2:1) to obtain compound **3** (3.5 mg). Subfraction EA2.4 (191 mg) was separated successively by passage over silica gel column chromatography, eluting with *n*-hexane/$CHCl_3$/AcOH (1:5:1) to afford **4** (6.0 mg). Subfraction EA2.5 (155 mg) was chromatographed over silica gel, eluting with *n*-hexane/$CHCl_3$/AcOH (5:1:2) to obtain compound **5** (7.5 mg).

Chromatography of fraction EA3 (8.831 g) on a silica gel column eluted with *n*-hexane/EtOAc (5:4) produced six subfractions (EA3.1-EA3.6). Subfraction EA3.2 (197 mg) underwent chromatography on a silica gel column and was eluted with *n*-hexane/EtOAc/AcOH (5:1:3) to provide compounds **6** (4.5 mg) and **7** (4.2 mg). Fraction EA7 (3.412 g) was separated on a silica column and eluted with $CHCl_3$/MeOH (1:0 and 8:2) to give four subfractions (EA7.1-EA7.4). Subfraction EA7.2 (120 mg) was further purified

by silica gel column chromatography and eluted with $CHCl_3$/MeOH/H_2O (9:1:0.1) to afford **8** (4.2 mg).

2.4. Spectroscopic data

- **Phyllanthol (1)**. White amorphous powder; ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 3.19 (dd, 1H, *J* = 11.5, 5.5 Hz, H-3), 0.96 (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), 0.01 (1H, d, *J* = 5.5 Hz, H-27b), 0.90 (s, 3H, H-28), 0.94 (3H, d, *J* = 6.0 Hz, H-29), 0.88 (3H, d, *J* = 6.0 Hz, H-30); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C 79.1 (C-3), 55.7 (C-5), 54.0 (C-18), 50.1 (C-9), 42.0 (C-22), 40.8 (C-19), 38.9 (C-4), 38.5 (C-1), 38.4 (C-7), 37.3 (C-20), 37.3 (C-10), 37.0 (C-8), 35.2 (C-12), 32.2 (C-14), 31.9 (C-17), 29.4 (C-21), 29.4 (C-2), 28.2 (C-28), 27.9 (C-16), 27.3 (C-23), 26.6 (C-13), 21.3 (C-15), 20.7 (C-30), 18.1 (C-6), 18.0 (C-29), 17.9 (C-26), 17.6 (C-11), 16.0 (C-25), 15.3 (C-24), 13.3 (C-27).
- **Olean-12-en-3β,15α-diol (2).** White amorphous powder; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H 3.22 (1H, dd, *J* = 11.0, 4.5 Hz, H-3), 5.28 (1H, t, *J* = 3.0 Hz, H-12), 4.23 (1H, dd, *J* = 11.5, 5.0 Hz, H-15), 0.97 (3H, *s*, H-23), 0.79 (3H, *s*, H-24), 0.86 (3H, *s*, H-25), 1.16 (3H, *s*, H-26), 0.94 (3H, *s*, H-27), 1.03 (3H, *s*, H-28), 0.87 (3H, *s*, H-29), 0.87 (3H, *s*, H-30); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ_C 146.2 (C-13), 123.4 (C-12), 79.1 (C-3), 68.5 (C-15), 54.9 (C-5), 47.8 (C-9), 47.7 (C-18), 47.6 (C-14), 46.4 (C-19), 41.2 (C-8), 38.8 (C-1), 38.7 (C-4), 37.6 (C-22), 36.9 (C-7), 36.2 (C-16), 34.7 (C-21), 33.4 (C-29), 33.1 (C-17), 31.1 (C-20), 29.0 (C-28), 28.2 (C-23), 27.3 (C-2), 23.8 (C-30), 23.8 (C-11), 20.3 (C-27), 18.7 (C-6), 17.6 (C-26), 15.7 (C-25), 15.6 (C-24), 7.1 (C-10).
- **Kumatakenin (3).** Yellow powder; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ_H 12.66 (1H, s, 5-OH), 6.37 (1H, d, *J* = 2.0 Hz, H-6), 6.74 (1H, d, *J* = 2.5 Hz, H-8), 7.98 (1H, d, *J* = 9.0 Hz, H-2′), 6.95 (1H, d, *J* = 9.0 Hz, H-3′), 6.95 (1H, d, *J* = 9.0 Hz, H-5′), 7.98 (1H, d, *J* = 9.0 Hz, H-6′), 3.80 (3H, s, OCH_3), 3.86 (3H, s, OCH_3); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ_C 178.1 (C-4), 165.1 (C-7), 161.0 (C-2), 160.3 (C-4′), 156.4 (C-5), 156.0 (C-9), 137.9 (C-3), 130.2 (C-6′), 130.2 (C-2′), 120.5 (C-1′), 115.7 (C-5′), 115.7 (C-3′), 105.2 (C-10), 97.8 (C-6), 92.4 (C-8), 59.7 (OCH_3), 56.1 (OCH_3).
- **Kaempferol (4).** Yellow powder; ^{1}H-NMR (600 MHz, acetone-d_6) δ_H 12.15 (1H, br, 5-OH), 6.26 (1H, d, *J* = 1.2 Hz, H-6), 6.53 (1H, s, H-8), 8.14 (1H, d, *J* = 9.0 Hz, H-2′), 7.01 (1H, d, *J* = 9.0 Hz, H-3′), 7.01 (1H, d, *J* = 9.0 Hz, H-5′), 8.14 (1H, d, *J* = 9.0 Hz, H-6′); ^{13}C-NMR (150 MHz, acetone-d_6) δ_C 176.6 (C-4), 165.4 (C-7), 162.3 (C-4′), 160.2 (C-5), 157.8 (C-9), 147.0 (C-2), 136.6 (C-3), 130.4 (C-2′), 130.4 (C-6′), 123.3 (C-1′), 116.3 (C-3′), 116.3 (C-5′), 104.0 (C-10), 99.3 (C-6), 94.5 (C-8).
- **Quercetin (5).** Yellow powder; ^{1}H-NMR (600 MHz, acetone-d_6) δ_H 6.13 (1H, d, *J* = 2.0 Hz, H-6), 6.39 (1H, d, *J* = 2.0 Hz, H-8), 7.68 (1H, d, *J* = 8.5 Hz, H-2′), 6.86 (1H, d, *J* = 8.5 Hz, H-5′), 7.56 (1H, dd, *J* = 8.5, 2.9 Hz, H-6′); ^{13}C-NMR (150 MHz, acetone-d_6) δ_C

176.5 (C-4), 165.2 (C-7), 162.3 (C-5), 157.8 (C-9), 148.4 (C-4′), 146.9 (C-2), 145.9 (C-3′), 136.7 (C-3), 123.7 (C-1′), 121.4 (C-6′), 116.2 (C-2′), 115.7 (C-5′), 104.0 (C-10), 99.2 (C-6), 94.5 (C-8).

- **4-Hydroxybenzoic acid (6).** White amorphous powder; ^{1}H-NMR (500 MHz, acetone-d_6) δ_H 7.90 (1H, d, J = 8.0 Hz, H-2), 6.89 (1H, d, J = 8.0 Hz, H-3), 6.89 (1H, d, J = 8.0 Hz, H-5), 7.90 (1H, d, J = 8.0 Hz, H-3); ^{13}C-NMR (125 MHz, acetone-d_6) δ_C 168.6 (C-7), 162.3 (C-4), 132.4 (C-2), 132.4 (C-6), 122.7 (C-1), 115.6 (C-3), 115.6 (C-5).
- **Protocatechuric acid (7).** White amorphous powder; ^{1}H-NMR (500 MHz, acetone-d_6) δ_H 7.51 (1H, d, J = 2.0 Hz, H-2), 7.45 (1H, dd, J = 8.0, 2.5 Hz, H-6), 6.89 (1H, d, J = 8.0 Hz, H-5); ^{13}C-NMR (125 MHz, acetone-d_6) δ_C 167.6 (C-7), 50.7 (C-4), 145.4 (C-3), 123.4 (C-1), 123.0 (C-6), 117.4 (C-2), 115.5 (C-5).
- **Quercetin 3-*O*-*β*-D-glucopyranoside (8).** Yelow amorphous powder; ^{1}H-NMR (500 MHz, CD_3OD) δ_H 6.09 (1H, d, J = 2.0 Hz, H-6), 6.24 (1H, d, J = 2.0 Hz, H-8), 7.73 (1H, d, J = 2.0 Hz, H-2′), 6.87 (1H, d, J = 8.5 Hz, H-5′), 7.59 (1H, dd, J=8.5, 2.0, H-6′), 5.11 (1H, d, J = 8.0 Hz, H-1″), 3.50 (1H, dd, J=9.0, 8.0, H-2″), 3.44 (1H, t, J = 9.0 Hz, H-3″), 3.38 (1H, t, J = 9.0 Hz, H-4″), 3.23 (1H, ddd, J = 9.0, 5.5, 2.5 Hz, H-5″), 3.60 (1H, dd, J = 12.0, 5.5 Hz, H_a-6″), 3.72 (1H, dd, J = 12.0, 2.5 Hz, H_b-6″); ^{13}C-NMR (500 MHz, CD_3OD) δ_C 158.2 (C-2), 135.4 (C-3), 178.6 (C-4), 162.6 (C-5), 102.3 (C-6), 96.4 (C-8), 159.0 (C-9), 103.6 (C-10), 123.1 (C-1′), 117.3 (C-2′), 150.1 (C-3′), 146.1 (C-4′), 116.7 (C-5′), 122.9 (C-6′), 105.2 (C-1″), 75.7 (C-2″), 78.2 (C-3″), 71.2 (C-4″), 78.3 (C-5″), 62.6 (C-6″).

3. Results and discussion

The ^{1}H-NMR spectrum of compound **1** showed the presence of seven methyl groups [δ_H 0.96 (*s*, H-23), 0.77 (*s*, H-24), 0.86 (*s*, H-25), 1.14 (*s*, H-26), 0.90 (*s*, H-28), 0.94 (*d*, J = 6.0 Hz, H-29), 0.88 (*d*, J = 6.0 Hz, H-30)]. In addition, the ^{1}H-NMR spectrum indicated signals due to one oxygenated methine proton [δ_H 3.19 (*dd*, J = 11.5, 5.5 Hz, H-3)]. Proton H-3 has the coupling constant of J = 11.5, 5.5 Hz, proves that this proton is in an axial position, so the hydroxyl group is equatorial; the 2H-27 cyclopropyl ring proton resonances at δ_H 0.01 (d, J = 5.5 Hz) and δ_H 0.66 (d, J = 5.5 Hz). The ^{13}C-NMR spectrum showed that compound **1** has thirty carbon signals, of which seven are of the methyl group [at δ_C 15.3 (C-24), 16.0 (C-25), 17.9 (C-26), 18.0 (C-29), 20.7 (C-30), 27.3 (C-23), and 28.2 (C-28); three methine group signals [δ_C 50.1 (C-9), 54.0 (C-18), and 55.7 (C-5); a oxygenated methine group at δ_C 79.1 (C-3); a methylene group at δ_C 13.3 (C-27), and other carbon signals. In the HMBC spectrum of **1** (*Figure 2*), the cross-peaks from H-23 (δ_H 0.96, s) and H-24 (δ_H 0.77, s) to C-3 (δ_C 79.1), C-4 (δ_C 38.8), and C-5 (δ_C 55.7) confirmed to locate the -OH group attached to C-3. The HMBC correlations from H-25 (δ_H 0.86, s) to C-5 (δ_C 55.7), C-9 (δ_C 50.1) and C-10 (δ_C 37.3); from H-26 (δ_H 1.14, s) to C-8 (δ_C 37.0), C-9 (δ_C 50.1) and C-14 (δ_C 32.2); H-28 (δ_H 0.90, s) to C-16 (δ_C 27.9), C-17 (δ_C 31.1), C-18 (δ_C 54.0) and C-22 (δ_C 42.0); and H-29 (δ_H 0.94, d, J = 6 Hz) to C-18 (δ_C 54.0) confirmed that the

position of the methyl groups of the compound **1**. In addition, the ^{1}H-NMR spectrum demonstrated two proton signals of the non-equivalent methylene group H-27 [δ_H 0.01 (d, *J* = 5.5 Hz), 0.66 (d, *J* = 5.5 Hz)]. The HSQC spectrum showed that these two protons are attached to the same C-27 at δ_C 13.3. The HMBC correlations from H-27 to C-12 (δ_C 35.2), C-13 (δ_C 26.6), C-14 (δ_C 32.2), C-15 (δ_C 21.3), and C-18 (δ_C 54.0) confirmed that the location of this methylene group. By analyzing ^{1}H-NMR and ^{13}C-NMR spectra as above and comparing the spectral data of compound **1** with those from other studies (Duong et al., 2018; Vuyelwa et al., 2008), compound **1** is confirmed with a structure of phyllanthol (or 3,27-cycloursan-3β-ol).

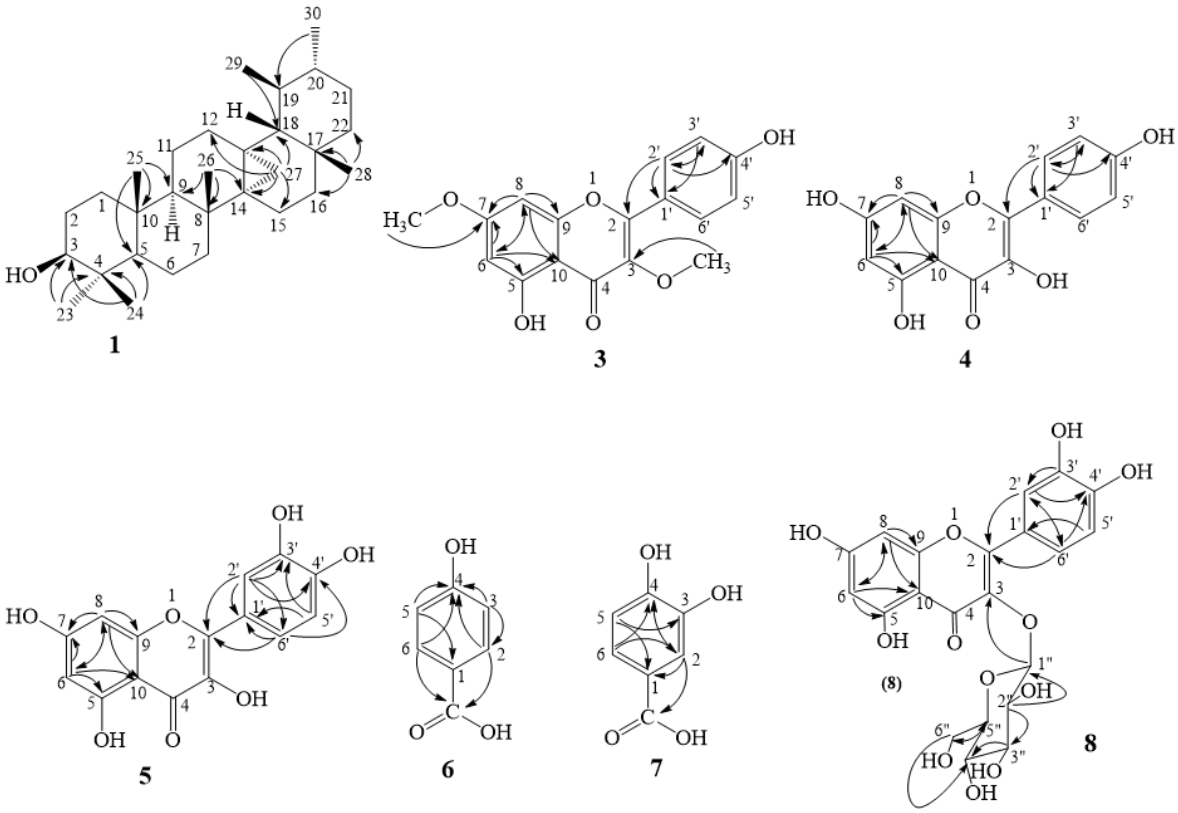

Figure 2. *Some key HMBC correlations of* ***1, 3, 4, 5, 6,*** *and* ***8***

The ^{1}H-NMR spectrum of compound **2** showed the presence of one methine olefin proton [δ_H 5.28 (t, *J* = 3.0 Hz, H-12)], two oxymethine protons [δ_H 4.23 (dd, *J* =11.5, 5.0 Hz, H-15), and 3.22 (dd, *J* = 11.0, 4.5 Hz, H-3)], eight methyl protons [δ_H 1.16 (s, H-26), 1.03 (s, H-28), 0.97 (s, H-23), 0.94 (s, H-27), 0.87 (s, H-29), 0.87 (s, H-30), 0.86 (s, H-25), 0.79 (s, H-24). The ^{13}C-NMR spectrum of compound **2** displayed for the signal of 30 carbons, which includes a quaternary olefin carbon [δ_C 146.2 (C-13)], a methine olefin carbon [δ_C 123.4 (C-12)], two oxymethine carbon [δ_C 79.1 (C-3)], 68.5 (C-15)], and signals of eight methyl groups [δ_C 33.4 (C-29), 29.0 (C-28), 28.2 (C-23), 23.8 (C-30), 20.3 (C-27), 17.6

(C-26), 15.7 (C-25), 15.6 (C-24)]. The similarities were found from the analysis of the above spectral data, combined with the NMR data of the olean-12-en-3β,15α-diol compound (Reiko Tanaka et al., 1988). Therefore, the structure of compound **2** is suggested to be olean-12-en-3*β*,15*α*-diol.

The ^{1}H-NMR spectrum of compound **3** showed two *meta*-coupled doublets for A-ring [δ_H 6.44 (d, *J* = 2.5 Hz, H-8), 6.37 (d, *J* = 2 Hz, H-6)] and two *ortho*-coupled doublets, for B-ring [δ_H 7.98 (d, *J*=8.5 Hz, H-2′/6′) and 6.96 (d, *J*=8.5 Hz, H-3′/5′)]. Also, two singlets were assigned to methoxyl groups [δ_H 3.80 (s, 3-OCH_3) and [δ_H 3.86 (s, 7-OCH_3)], and one signal for a chelated hydroxyl (OH-5) was observed at δ_H 12.66. The ^{13}C-NMR spectrum displays the resonance signals of two methoxyl groups at δ_C 59.7 (3-OCH_3) and 56.1 (7-OCH_3). Furthermore, the spectrum displayed the other carbon resonances, including aromatic carbons [δ_C 156.4 (C-5), 97.8 (C-6), 165.1 (C-7), 92.4 (C-8), 156.0 (C-9), 105.2 (C-10), 120.5 (C-1′), 130.2 (C-2′), 115.7 (C-3′), 160.3 (C-4′), 115.7 (C-5′), 130.2 (C-6′)], and two oxygenated olefin carbons [δ_C 111.1 (C-2), 137.9 (C-3)], the signal at δ_C 178.1 was attributed to a carbonyl carbon placed at C-4. The HMBC spectrum of compound **3** indicated clear correlations between the hydrogens of 3-OCH_3 (δ_H 3.80) and C-3 (δ_C 137.9), 7-OCH_3 (δ_H 3.86), and C-7 (δ_C 165.1) confirming the location of the two methoxyl groups at C-3 and C-7 for compound **3**. Moreover, the HMBC spectrum of compound **3** exhibited the proton signal at δ_H 6.37 (H-6) has correlations with the carbons at δ_C 156.4 (C-5), 165.1 (C-7), 92.4 (C-8), and 105.2 (C-10), thereby showing this proton connect at position 6. Similarly, correlations in the HMBC spectrum of H-8 (δ_H 6.74) with the carbons at δ_C 97.8 (C-6), 165.1 (C-7), 156.0 (C-9), and 105.2 (C-10) prove that this proton attaches at the position 8.

Some other critical HMBC correlations of **3** are shown in *Figure 2*. By analyzing the spectrum of ^{1}H-NMR and ^{13}C-NMR as above and comparing the spectral data of compound **3** with that of kumatakenin (Calved et al., 1979; Castillo et al., 2015), there are similarities, so it is suggested that the structure of compound **3** is kumatakenin (5,4'-dihydroxy-3,7-dimethoxyflavone).

The ^{1}H-NMR spectrum of compound **4** showed two *meta*-coupled doublets for A-ring [δ_H 6.26 (d, *J* = 1.2 Hz, H-6), 6.52 (H-8)] and two *ortho*-coupled doublets, for B-ring [δ_H 8.14 (d, *J*=9.0 Hz, H-2′/6′) and 7.01 (d, *J*=9.0 Hz, H-3′/5′)]. Also, one signal for a chelated hydroxyl (OH-5) was observed at δ_H 12.15. The ^{13}C-NMR spectrum displayed the fifteen carbon resonances, including aromatic carbons [δ_C 160.2 (C-5), 99.3 (C-6), 165.4 (C-7), 94.5 (C-8), 157.8 (C-9), 104.0 (C-10), 123.3 (C-1′), 130.4 (C-2′/6′), 116.3 (C-3′/5′), 162.3 (C-4′)], and two oxygenated olefin carbons [δ_C 147.0 (C-2), 136.6 (C-3)], carbonyl carbon is identified as C-4 with chemical shift δ_C 176.6. The HMBC spectrum of compound **4** shows the resonance proton at δ_H 6.26 (H-6) has correlations with the carbons at δ_C 160.2 (C-5), 165.4 (C-7), 94.5 (C-8), and 104.0 (C-10), it is allowed to conclude that this hydrogen attaches at position 6. Similarly, correlations in the HMBC spectrum of H-8 (δ_H 6.53) with

the carbons at δ_C 99.3 (C-6), 165.4 (C-7), 157.8 (C-9), and 104.0 (C-10) demonstrate that this proton is in position 8. Some other critical HMBC correlations of **4** are shown in *Figure 2*. The above analysis of ^{1}H-NMR and ^{13}C-NMR spectra, compared with the spectral data of compound 4 with kaempferol of reference (Xiao et al., 2006), showed similarities; hence compound **4** was suggested structure is kaempferol (or 3, 4 ′, 5.7-tetrahydroxyflavone).

Similar to compound **4**, the ^{1}H-NMR spectrum of compound **5** showed two *meta*-coupled doublets for A-ring [δ_H 6.13 (d, J = 2.0 Hz, H-6)] and δ_H 6.39 (d, J = 2.0 Hz, H-8). The B-ring demonstrated three aromatic protons [δ_H 7.68 (d, J = 8.5 Hz, H-2′), 6.84 (d, J = 8.5 Hz, H-5′) and 7.56 (dd, J=8.5, 2.0 Hz, H-6′)]. In addition, one signal for a chelated hydroxyl (OH-5) was observed at δ_H 12.02. The resonance signals on the ^{13}C-NMR spectrum indicated fifteen carbon resonances, including aromatic carbon [δ_C 162.3 (C-5), 99.2 (C-6), 165.2 (C-7), 94.5 (C-8), 157.8 (C-9), 104.0 (C-10), 123.7 (C-1′), 116.2 (C-2′), 145.9 (C-3′), 148.4 (C-4′), 115.7 (C-5′), 121.4 (C-6′)], and two oxygenated olefin carbons [δ_C 146.9 (C-2), 136.7 (C-3)]. Carbonyl carbon is assigned at C-4 because its chemical shift is δ_C 176.5. The HMBC spectrum of compound **5** indicated that the proton signal resonating at δ_H 6.13 was correlated with the carbon signals at δ_C 162.3 (C-5), 165.2, (C-7), 94.5, (C -8), 104.0, (C-10), suggesting the presence of the hydrogen at position 6, correlations with H-8 (δ_H 6.39) with the carbons at δ_C 99.2 (C-6), 165.2 (C-7), 157.8 (C-9), and 104.0 (C-10) confirming the position of the hydrogen at position 8. Some other critical HMBC correlations are shown in *Figure 2*. The compound **5** was identified as quercetin (or 3,3′,4′,5,7-pentahydroxyflavone) confirmed by comparing the spectroscopic data with values reported in the literature (Abdullah et al., 2016; Castillo et al., 2015).

The ^{1}H-NMR spectrum of compound **6** demonstrated the phenolic compound. In the ^{1}H- and ^{13}C-NMR spectra of compound **6**, two doublets of aromatic proton signals [δ_H 7.91 (d, J = 8.5 Hz) and 6.91 (d, J = 9.0 Hz)], six aromatic carbon signals [δ_C 115.6 (C-3)/(C-5), 131.8 (C-2)/(C-6), 122.7 (C-1), 162.3 (C-4)], and one carboxyl carbon signal [δ_C 168.6] were observed. The HMBC spectrum of compound **6** exhibited correlations between the proton signal at δ_H 7.91 (H-6/H-2) and the carboxyl carbon at δ_C 168.6 (1-COOH) and 162.3 (C-4), and correlations between the proton signal at δ_H 6.91(H-3/H-5) and carbon at δ_C 122.7 (C-1), 162.3 (C-4). Some other important HMBC correlations are shown in *Figure 2*. Therefore, compound **6** has the structure of 4-hydroxybenzoic acid compared to NMR spectrum data found by Liu et al. (2010).

The ^{1}H-NMR spectrum of compound **7** showed phenolic compound signals similar the compound **6**. The ^{1}H-NMR spectrum of compound **7** showed two doublets of aromatic proton signals at δ_H 7.51 (d, J = 8.5 Hz, H-2) and 6.89 (d, J = 8.0 Hz, H-5), one double doublet at δ_H 7.45 (dd, J = 8.0, 2.5 Hz, H-6). The ^{13}C- NMR spectrum indicated six aromatic carbon signals [δ_C 123.4 (C-1), 117.4 (C-2), 145.4 (C-3), 150.7 (C-4), 115.5 (C-5), 123.0 (C-6)], and one carboxyl carbon signal [δ_C 167.6]. HMBC spectrum showed correlations of H-

2 to δ_C 150.7 (C-4), 123.4 (C-1), and carboxyl carbon at 167.6; H-6 to δ_C 150.7 (C-4), 117.4 (C-2); H-5 to 123.4 (C-1), 15.5 (C-5), leading to the position of the hydrogen at positions 2, 5, 6. Accordingly, compound **7** has a chemical structure as protocatechuic acid compared to the spectral data found by Lee et al. (2013).

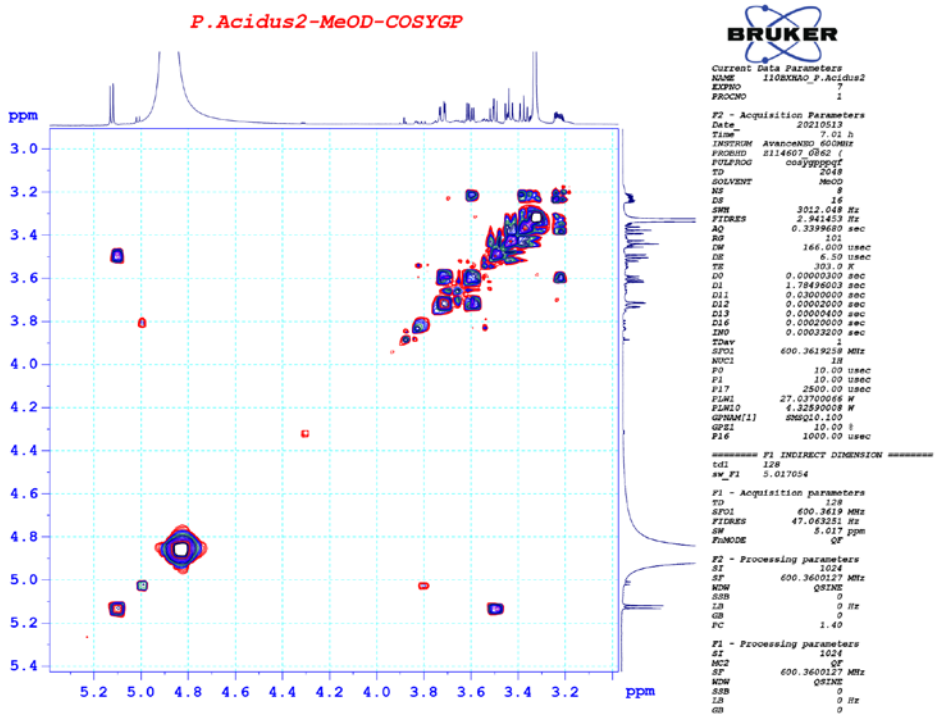

Figure 3. The** COSY spectrum of compound **8

The ^{1}H-NMR spectrum (*Figure 6*) of compound **8** denoted two *meta*-coupled doublets for A-ring [δ_H 6.09 (d, J = 2.0 Hz, H-6)] and δ_H 6.24 (d, J = 2.0 Hz, H-8). The B-ring indicated three aromatic protons [δ_H 7.73 (d, J = 8.5 Hz, H-2′), 6.87 (d, J = 8.5 Hz, H-5′) and 7.59 (dd, J=8.5, 2.0 Hz, H-6′)]. In addition, resonance signals were observed at δ_H 5.11 (d, J = 8.0 Hz, H-1″), 3.50 (dd, J=9.0, 8.0, H-2″), 3.44 (t, J = 9.0 Hz, H-3″), 3.38 (t, J = 9.0 Hz, H-4″), 3.23 (ddd, J = 9.0, 5.5, 2.5 Hz, H-5″), 3.60 (dd, J = 12.0, 5.5 Hz, H_a-6″), 3.72 (1H, dd, J = 12.0, 2.5 Hz, H_b-6″) which supported the presence of an glucopyranose moiety. The coupling constant of the anomer proton signal at δ_H 5.11 is 8.0 Hz in the ^{1}H-NMR spectrum to prove configuration β of the sugar unit. Based on the correlations in the COSY spectrum (*Figure 4*), the chemical shift of each proton in the glucose unit is determined precisely.The ^{13}C-NMR spectrum indicated carbon resonances, including aromatic carbon [δ_C 162.6 (C-5), 102.3 (C-6), 94.5 (C-8), 157.8 (C-9), 104.0 (C-10), 123.7 (C-1′), 116.2 (C-2′), 145.9 (C-3′), 148.4 (C-4′), 115.7 (C-5′), 121.4 (C-6′)], two oxygenated olefin carbons [δ_C 146.9 (C-2), 136.7 (C-3)], the signal at δ_C 178.6 was attributed to a carbonyl carbon placed at C-4. In addition, six carbons of one β-D-glucopyranose [105.2 (C-1″), 75.7 (C-2″), 78.2 (C-3″), 71.2 (C-4″), 78.3 (C-5″), 62.6 (C-6″)] were observed. The HMBC spectrum (Figure 5) showed

that the anomer proton signal [δ_H 5.11 (d, J = 8.0 Hz, H-1″)] correlates to carbon oxygenate at δ_C 135.4 (C-3), confirming that the sugar unit attached at C-3. In addition, the HMBC indicated correlations of δ_H 3,50 (H-2″) to 105.2 (C-1″) and 78.2 (C-3″), 3.44 (H-3″) to 71.2 (C-4″), 3.38 (H-4″) to 75.7 (C-2″) and 78.3 (C-5″), 3.23 (H-5″) to 62.6 (C-6″), 3.72 (H_b-6″) to 71.2 (C-4″). Some other critical HMBC correlations of **8** are shown in *Figure 2*. Based on the above spectroscopic evidence and a detailed comparison of NMR data of **8** to that 3-*O*-*β*-D-glucopyranoside (Liu et al., 2010) showed very similarity, thus compound **8** was identified as a quercetin-3-*O*-*β*-D-glucopyranoside.

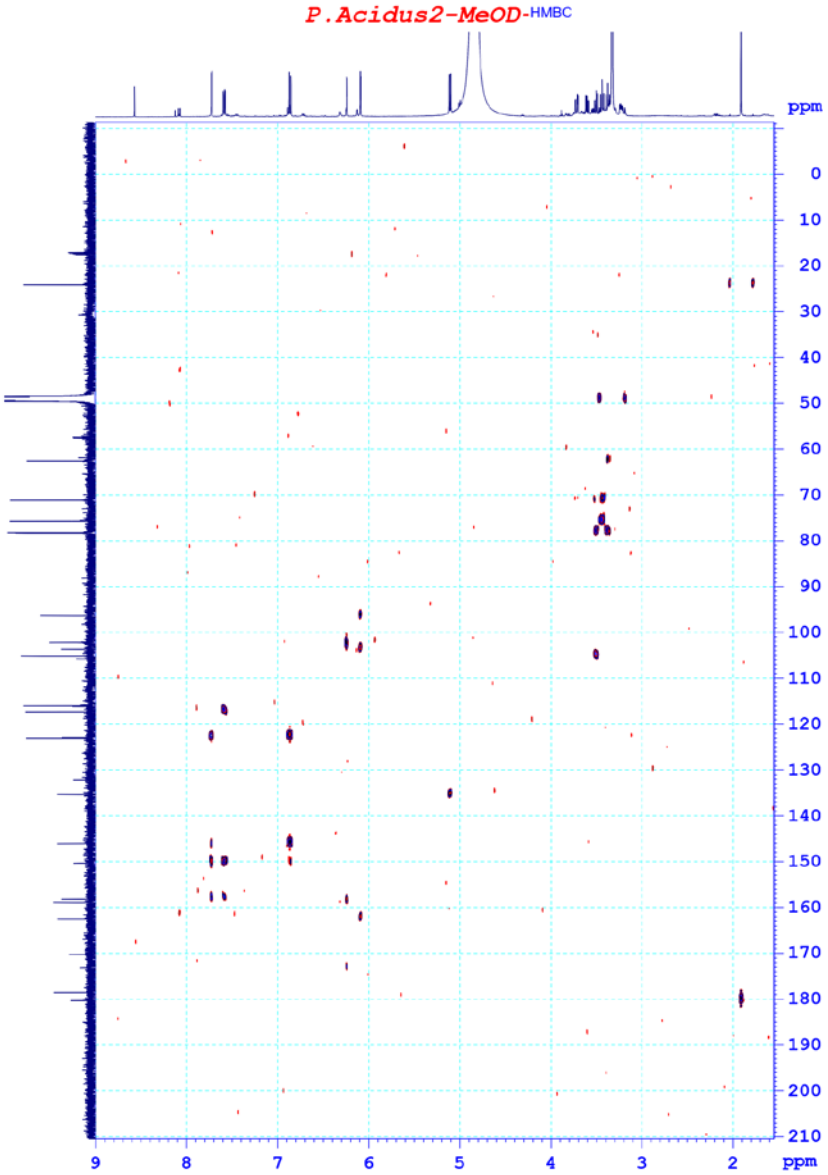

Figure 4. *HMBC spectrum of compound* ***8***

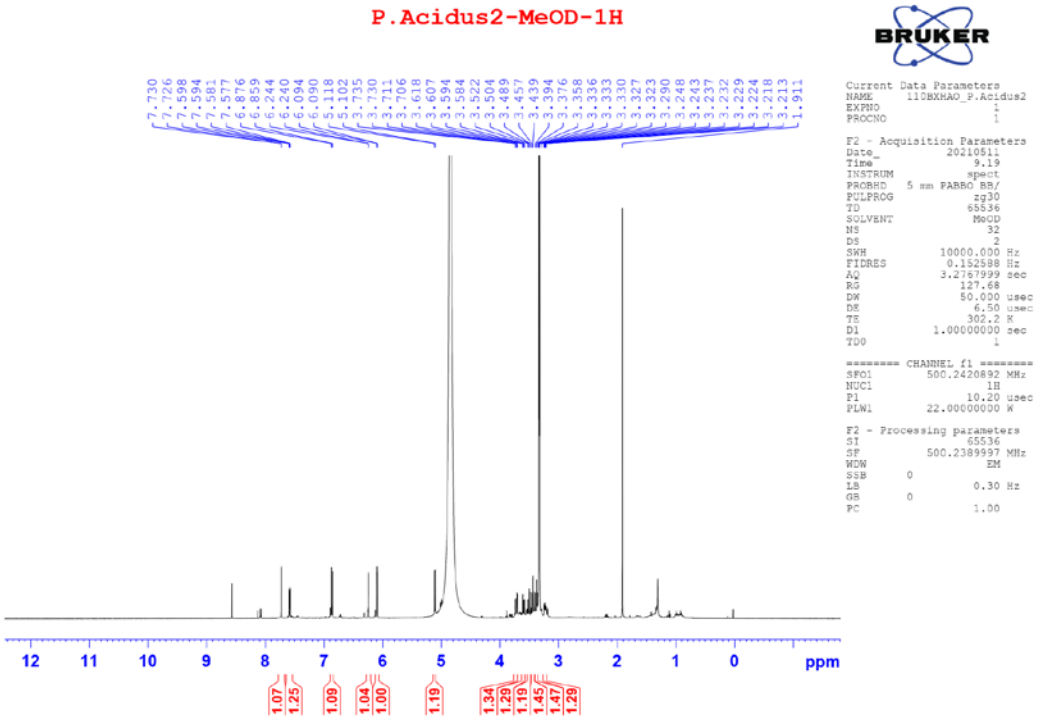

Figure 5. *^{1}H-NMR spectrum of compound* ***8***

4. Conclusions

From the extracted ethyl acetate of the leaves of *Phyllanthus acidus*, eight compounds (**1**-**8**) were isolated and chemicals were elucidated. Our study has shown compound **8** was isolated from this species for the first time.

❖ ***Conflict of Interest:*** *Authors have no conflict of interest to declare.*

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NGHIÊN CỨU THÀNH PHẦN HÓA HỌC DỊCH CHIẾT ETHYL ACETATE CỦA LÁ CÂY CHÙM RUỘT

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TÓM TẮT

*Lá của cây Chùm ruột thu hái ở tỉnh Bình Thuận và được chiết bằng methanol ở nhiệt độ phòng, thu được dịch chiết methanol. Tiến hành phân tán hoàn toàn cao metanol vào nước và chiết lỏng-lỏng bằng dung môi n-hexane, chloroform, ethyl acetate và n-butanol, thu được các cao tương ứng. Phân đoạn ethyl acetate được thực hiện sắc kí cột nhiều lần, đã phân lập được tám hợp chất tinh khiết là **1, 2, 3, 4, 5, 6, 7, 8**. Cấu trúc hóa học của chúng được xác định dựa trên phân tích dữ liệu phổ cộng hưởng từ hạt nhân 1D, 2D-NMR, kết hợp với so sánh tài liệu tham khảo. Đây là lần đầu tiên sự phân lập và xác định cấu trúc của các hợp chất từ dịch chiết ethyl acetate từ lá cây Chùm ruột được báo cáo.*

Từ khóa: Euphorbiaceae; kaempferol; *Phyllanthus acidus*; quercetin