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Kaempferol and kaempferol glycosides from *Phyllanthus acidus* leaves

Nguyen Cong Thuy Tram[1], Ninh The Son[2], Do Thi Thao[3], Nguyen Manh Cuong[2*]

[1]*Faculty of Biology and Environmental Science, University of Education, University of Danang*

[2]*Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology*

[3]*Institute of Biotechnology, Vietnam Academy of Science and Technology*

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Abstract

In the search for hepatoprotective plants in Vietnam, the leaves of *Phyllanthus acidus* (L.) Skeels were selected for chemical investigation. Phytochemical analysis of the ethyl acetate fraction of the methanol extract led to the isolation of four compounds, including kaempferol (**1**), and its glycosides, kaempferol-3-*O*-*β*-D-glucoside (**2**), kaempferol-3-*O*-*α*-L-rhamnopyranoside (**3**), and kaempferol-3-*O*-*α*-L-rhamnopyranosyl-(1→2)- *α*-L-arabinopyranoside (**4**). The structures of the natural compounds were determined from the spectroscopic evidences, including 1D, 2D-NMR. Here is the first report of the isolation of compound **4** from the genus *Phyllanthus*, family Phyllanthaceae.

Keywords. *Phyllanthus acidus*, leaves, kaempferol, glycosides.

1. INTRODUCTION

The genus *Phyllanthus* (Phyllanthaceae family) in Vietnam comprises about 53 species, several of which are very common and widely distributed throughout the country such as *P. chamaepeuce* (Cau troi), *P. urinaria* (Cho de), *P. virgatus* (Vay oc) [1]. Previous investigations from various plant parts of *Phyllanthus* species led to the isolation of triterpenes [2], tannins [3], alkaloids, steroids, saponins [4], flavonoids and flavonoid glycosides [5], and acids [6]. The species *Phyllanthus acidus* (L.) Skeels distributed in tropical and subtropical countries, with local name "Chum ruot" in Vietnam or Grosella in Puerto Rico, Jimbilin in Jamaica, and Karamay in Northern Philippines [1, 7]. This herb was commonly found and has been employed as folk medicines for the treatment of several diseases such as asthma, hepatic disease, diabetes, and gonorrhoea [7].

In current paper, we describe the isolation and structural elucidation of four compounds, including kaempferol (**1**), and its glycosides, kaempferol-3-*O*-*β*-D-glucoside (**2**), kaempferol-3-*O*-*α*-L-rhamnopyranoside (**3**), and kaempferol-3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabino-pyranoside (**4**). The structures of the natural compounds were identified by comparison of the physicochemical and spectroscopic data 1D, 2D-NMR and with those reported in the literature.

2. EXPERIMENTAL

2.1. General experimental procedures

^{1}H-NMR (500 MHz), ^{13}C NMR (125 MHz) spectra were measured on a Bruker AVANCE 500 spectrometer. Column chromatography (CC) was carried out on silica gel (Si 60 F_{254}, 230-400 mesh, Merck). All solvents were distilled before use. Precoated plates of silica gel 60 F_{254} were used for analytical purposes. Compounds were visualized under UV radiation (254, 365 nm) and by spraying plates with 10% H_2SO_4 followed by heating with a heat gun.

2.2. Plant material

The leaves of *Phyllanthus acidus* (L.) Skeels were collected in Lien Chieu, Da Nang, Vietnam and identified by ethnobotanist Nguyen Thi Dao (Faculty of Biology and Environmental Science). A voucher specimen (PA-1) was deposited in the Faculty of Biology and Environmental Science, University of Education.

2.3. Extraction and isolation

Dried powdered leaves of *P. acidus* (6.0 kg, PA) were extracted with MeOH for 3 days (3 x10L) at room temperature and concentrated under reduced

pressure to yield a black crude MeOH extract (300.5 g).

The crude MeOH extract was then suspended in MeOH:H_2O (1:1, v/v) and successively partitioned with *n*-hexane, dichloromethane, and ethyl acetate. The resulting fractions were concentrated under decreased pressure to give the corresponding *n*-hexane (PA-H, 55.1 g), dichloromethane (PA-D, 45.2 g), ethyl acetate (PA-E, 25.2 g), and water residue, respectively.

The PA-E fraction residue (25.2 g) was chromatographed on a silica gel column, using gradient of CH_2Cl_2:MeOH (50:1, v/v) to afford 5 sub-fractions (PA-E1 to PA-E5). The sub-fraction PA-E1 (1.0 g) was rechromatographed on a RP-18 column, eluting with MeOH:H_2O (3:2, v/v) to yield compound **1** (20.0 mg). In the same manner, the sub-fraction PA-E3 (0.8 g) was separated on a RP-18 column, eluting with MeOH:H_2O (3:2, v/v) to give 4 sub-fractions (PA-E3A to PA-E3D). The sub-fraction PA-E3A (0.28 g) was purified by a Sephadex LH-20 column with MeOH:H_2O (2:3, v/v) to afford compound **2** (12.0 mg). Meantime, compound **3** (11.0 mg) was derived from the sub-fraction PA-E3B (0.31 g), using a Sephadex LH-20 column with MeOH:H_2O (2:3, v/v). In a similar way, the sub-fraction PA-E3D (0.35 g) was subjected to chromatography on a Sephadex LH-20 column, eluting with MeOH:H_2O (2:3, v/v) to afford compound **4** (6.0 mg).

2.4. Spectral and physical data

Kaempferol (**1**): Yellow powder; ESI-MS (*m/z*): 285 $[M-H]^-$, $C_{15}H_{10}O_6$; ^{1}H-NMR (500 MHz, CD_3OD, δ ppm): 8.08 (2H, d, 8.5 Hz, H-2′, H-6′), 6.92 (2H, d, 8.5 Hz, H-3′, H-5′), 6.40 (1H, s, H-8), 6.20 (1H, s, H-6); ^{13}C-NMR (125 MHz, CD_3OD, δ ppm): 177.3 (s, C-4), 165.5 (s, C-7), 162.4 (s, C-5), 160.5 (s, C-4′), 158.2 (s, C-9), 148.1 (s, C-2), 137.1 (s, C-3), 130.7 (d, C-2′, C-6′), 123.7 (s, C-1′), 116.3 (d, C-3′, C-5′), 104.5 (s, C-10), 99.3 (d, C-6), 94.5 (d, C-8).

Kaempferol-3-*O*-*β*-D-glucoside (**2**): Yellow powder; ESI-MS (*m/z*): 447 $[M-H]^-$, $C_{21}H_{20}O_{11}$; ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) (CD_3OD, δ ppm), see table 1.

Kaempferol-3-*O*-*α*-L-rhamnopyranoside (**3**): Yellow powder; ESI-MS (*m/z*): 431 $[M-H]^-$, $C_{21}H_{20}O_{10}$; ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) (CD_3OD, δ ppm), see table 1.

Kaempferol-3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranoside (**4**): Yellowish powder; ESI-MS (*m/z*): 563 $[M-H]^-$, $C_{26}H_{28}O_{14}$; ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) (CD_3OD, δ ppm), see table 1.

3. RESULTS AND DISCUSSION

Repeated column chromatography of the ethyl acetate fraction of *P. acidus* leaves resulted in the isolation of four known compounds (**1**–**4**), including kaempferol (**1**), and its glycosides, to be kaempferol 3-*O*-*β*-D-glucoside (**2**), kaempferol-3-*O*-*α*-L-rhamnopyranoside (**3**), and kaempferol-3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranoside (**4**).

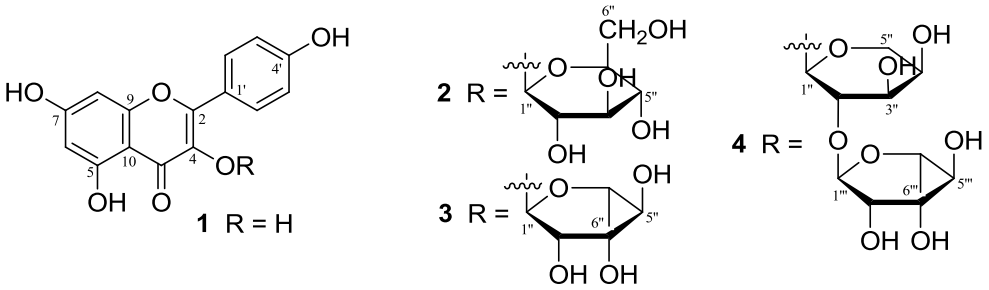

Figure 1: Structures of isolated compounds (**1**-**4**) from the leaves of *Phyllanthus acidus* (L.) Skeels

Compound **1** was isolated as yellow powder ($C_{15}H_{10}O_6$, M = 286). The ^{1}H NMR spectrum of compound **1** showed signals characteristic for a flavonol (table 1). A tetrasubstituted phenyl ring system (ring A) in **1** was presented by two singlet signals at δ_H 6.22 (1H, s, H-6) and 6.42 (1H, s, H-8). An AA′BB′ spin system of a symmetrically disubstituted phenyl ring (ring B) was observed at δ_H 6.91 (2H, d, *J* = 8.5 Hz, H-3', H-5'), 8.07 (1H, *J* = 8.5 Hz, H-2', H-6'). The ^{13}C NMR spectrum of compound **1** contained 15 carbon signals in the downfield, including 7 quaternary aromatic carbons at δ_C 104.5-165.7 ppm and a carbonyl group at δ_C 177.3 (C-4), 2 methine aromatic carbons at δ_C 99.3 (C-6) and δ_C 94.5 (C-8) of ring A, 4 methine carbons of ring B at δ_C 130.7 (d, C-2′, C-6′), and 116.3 (d,

C-3′, C-5′) (table 1). Based on the above analysis and a comparison with literature data, the structure of compound **1** was elucidated as a flavonol, namely kaempferol [8]. Kaempferol was isolated from several species in the genus *Phyllanthus*, for example *P. emblica* [9].

Table 1: ^{1}H- and ^{13}C-NMR data (CD_3OD) of compounds (**2**-**4**)

Position	**2**		**3**		**4**	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
Aglycone moiety						
2	158.5		159.3		158.4	
3	135.5		136.2		135.1	
4	179.5		179.6		179.5	
5	163.1		163.2		163.2	
6	100.0	6.22, s	100.0	6.22, s	99.8	6.21, d, 2.0 Hz
7	166.1		166.2		165.9	
8	94.8	6.42, s	94.8	6.39, s	94.7	6.41, d, 2.0 Hz
9	159.1		158.6		158.6	
10	105.7		105.9		105.8	
1'	122.8		122.7		122.8	
2'	132.3	8.07, d, 8.5 Hz	131.9	7.78, d, 8.5 Hz	132.2	8.05, d, 8.5 Hz
3'	116.1	6.91, d, 8.5 Hz	116.6	6.95, d, 8.5 Hz	116.4	6.93, d, 8.5 Hz
4'	161.6		161.6		161.5	
5'	116.1	6.91, d, 8.5 Hz	116.6	6.95, d, 8.5 Hz	116.4	6.93, d, 8.5 Hz
6'	132.3	8.07, d, 8.5 Hz	131.9	7.78, d, 8.5 Hz	132.2	8.05, d, 8.5 Hz
Glycone moieties						
1"	104.2	5.26, d, 7.0 Hz	103.5	5.40, brs	101.0	5.54, d, 4.5 Hz
2"	75.8	3.33, overlap	72.2	4.24, brs	77.2	4.12, dd, 4.5, 6.5 Hz
3"	78.1	3.45, m	72.0	3.73, brd, 5.0	72.8	3.84, m
4"	71.4	3.45, m	73.2	3.36, m	68.4	3.81, m
5"	78.4	3.23, m	71.9	3.34, m	65.2	3.35, dd, 2.0, 11.5 Hz 3.77, t, 11.5 Hz
6"	62.7	3.71, brd, 12.0 Hz 3.55, dd, 5.0, 12.0 Hz	17.7	0.94, d, 4.5 Hz		
1"'					102.2	5.10, brs
2"'					72.5	3.93, brs
3"'					72.3	3.73, dd, 3.0, 9.0 Hz
4"'					74.0	3.39, m
5"'					70.2	3.90, dd, 6.0, 9.5 Hz
6"'					17.7	1.11, d, 6.0 Hz

Compound **2** was isolated as yellow powder, ($C_{21}H_{20}O_{11}$, M = 448). The ^{1}H- and ^{13}C-NMR of the compound is similar to those of **1** except for 3-OH group of **1** was replaced by a *β*-D-glucopyranosyl group, with remarkable anomeric signals at δ_H 5.26 (d, 7.0 Hz, H-1") and δ_C 104.2 (C-1") (table 1). Based on the above analysis and literature data, the structure of compound **2** was elucidated as flavonol glycoside, named kaempferol-3-*O*-*β*-D-glucoside, with trivial name astragalin [10]. This compound was also isolated from many species in genus *Phyllanthus*, for instance *P. muellerianus* [11].

Compound **3** was isolated as yellow powder ($C_{21}H_{20}O_{10}$, M = 432). Analysis of the ^{1}H and ^{13}C NMR spectra of **3** revealed the presence of an aglycone skeleton similar to those of **1** and **2**, excepting for glycone moiety. In compound **3**, the sugar group located at carbon C-3, to be identified as *α*-L-rhamnopyranosyl moiety, with significant signals of anomeric group at δ_H 5.40 (brs, H-1"), δ_C 103.5 (C-1") and methyl group δ_H 0.94 (d, 4.5 Hz, H-6"), δ_C 17.7 (C-6") (Table 1). Based on the above analysis, the structure of compound **3** was elucidated as kaempferol-3-*O*-*α*-L-rhamnopyranoside, given trivial name kaempferin or afzelin [12].

Compound **4** was obtained as yellowish powder ($C_{26}H_{28}O_{14}$, M = 564). Same as in the case of compounds **2** and **3**, the ^{1}H and ^{13}C NMR spectra of

4 showed patterns of a flavonol glycoside, with the same aglycone skeleton of compounds **1-3**. Regarding to the glycone moiety, sugar in compound **4** was identified as α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside, with characteristic signals of two anomeric protons at δ_H 5.54 (d, 4.5 Hz, H-1"), δ_H 5.10 (brs, H-1'"), and methyl group at 1.11 (d, 6.0 Hz, H-6'"), together with 11 carbon signals at δ_C 101.1 (C-1")/102.2 (C-1'"), 77.2 (C-2")/72.5 (C-2'"), 77.8 (C-3")/72.3 (C-3'"), 68.4 (C-4")/74.0 (C-4'"), 65.2 (C-5")/70.2 (C-5'"), and 17.7 (C-6'") (table 1). In the HMBC spectrum, correlation between H-1'" and C-2" exhibited the (1→2) glycosyl linkage, whereas connectivity between sugar moiety and aglycone kaemferol was confirmed by correlation of H-1" and C-3. The 1D, 2D-NMR spectroscopic data and also literature data led to identify the structure of compound **4** as a flavonol glycoside, named kaempferol-3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (trivial name drabanemoroside) [13]. Herein, this compound was firstly found from the genus *Phyllanthus*.

4. CONCLUSION

Phytochemical analysis of the ethyl acetate fraction of the methanol extract of *Phyllanthus acidus* (L.) Skeels leaves led to the isolation of four compounds including flavonol kaempferol (**1**), and its glycosides as kaempferol-3-*O*-β-D-glucoside (**2**), kaempferol-3-*O*-α-L-rhamnopyranoside (**3**), and kaempferol-3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (**4**). The structures of the natural compounds were identified by comparison of the physicochemical and 1D, 2D-NMR spectroscopic data and with those reported in the literature. In addition, compound **4** has been isolated for the first time from the genus *Phyllanthus*, family Phyllanthaceae.

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Corresponding author: **Nguyen Manh Cuong**
Institute of Natural Products Chemistry, VAST
1H Buiding, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam
E mail: nmcuong@inpc.vast.vn.