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FLAVONOL GLYCOSIDES FROM THE LEAVES OF *FISSISTIGMA PALLENS* (FIN. & GAGN.) MERR.

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Abstract. Using combined chromatographic methods, five flavonol glycosides including: kaempferol 3-rutinoside (**1**), rutin (**2**), kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-galactopyranoside (**3**), isorhamnetin 3-robinobioside (**4**), and kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside (**5**) were isolated from the methanol extract of the leaves of *Fissistigma pallens* (Fin. & Gagn.) Merr. Their chemical structures were determined using NMR spectra as well as in comparison with the reported data. All compounds were reported from *Fissistigma* genus for the first time.

Keywords: Fissistigma pallens, Annonaceae, flavonol glycoside.

Classification numbers: 1.1.1, 1.1.6.

1. INTRODUCTION

Fissistigma pallens (Annonaceae) is a plant widely distributed in Viet Nam. The leaves of *F. pallens* have been used in traditional medicine for the treatment of abdominal pain, stomach pain, and diarrhea [1]. The chemical studies of *Fissistigma* genus indicated the presence of alcaloids, sesquiterpens, phenolics and flavonoids [2-5]. These compounds have shown the potential significant biological effects as cytotoxic and antimicrobial [5]. However, a few phytochemical investigations of *Fissistigma pallens* have been studied [6, 7]. Previously, we reported flavonol glycosides and sesquiterpene glycosides from this plant [8, 9]. In this continuous study, we report the structural elucidation of five flavonol glycosides from the leaves of *F. pallens* (Fig. 1).

2. MATERIALS AND METHODS

2.1. Plant materials

The leaves of *Fissistigma pallens* (Fin. & Gagn.) Merr. were collected in Nghe An, Viet Nam in April 2016, and identified by Dr. Nguyen The Cuong, Institute of Ecology and

Biological Resources. A voucher specimen (NCCT-P14) was deposited at the Institute of Marine Biochemistry, VAST.

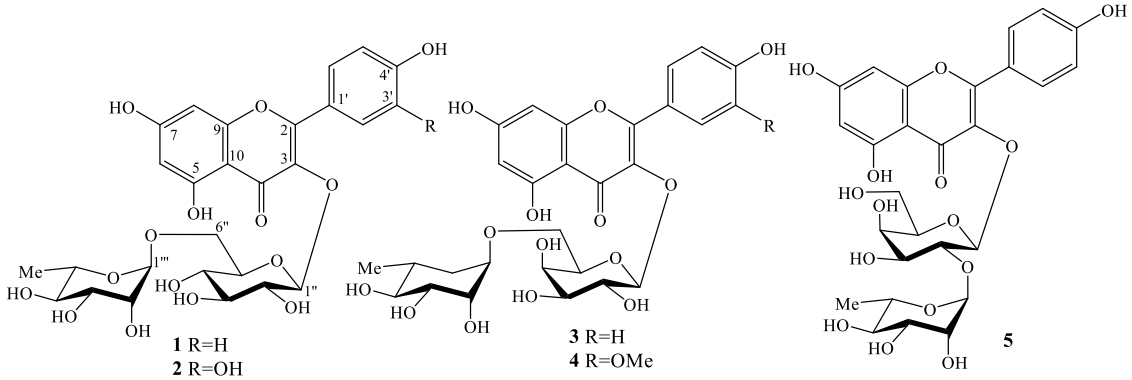

Figure 1. Chemical structures of compounds **1**-**5** from *F. pallens.*

2.2. General experimental procedures

All NMR spectra were recorded on a Varian AM400 spectrometer (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR). Column chromatography was performed using a silica gel (Kieselgel 60, 70-230 mesh and 230 - 400 mesh, Merck) or RP-18 resins (150 µm, Fuji Silysia Chemical Ltd.), thin layer chromatography (TLC) using a pre-coated silica-gel 60 F_{254} (0.25 mm, Merck) and RP-18 F_{254S} plates (0.25 mm, Merck).

2.3. Extraction and isolation

The dried powder of the *F. pallens* leaves (5.0 kg) was sonicated with hot methanol to give the methanol extract (400 g) after concentrating under reduced pressure. The extract was suspended in water and successively partitioned with *n*-hexane, dichloromethane, and ethyl acetate giving *n*-hexane (FP1 14.0 g), dichloromethane (FP2 72.0 g), ethyl acetate extracts (FP3 11.0 g) and water layer (FP4). The water layer was loaded on a Diaion HP-20 column eluting with water then increase the concentration of MeOH in water (25, 50, 75, and 100 %) to obtain four fractions, FP4A - FP4D. The FP4C fraction was chromatographed on a silica gel column eluting with gradient solvents of CH_2Cl_2: MeOH (50/1, 25/1, 10/1, 5/1, v/v) to give 4 sub-fractions, FP4C1-FP4C4. FP4C3 was chromatographed on an RP-18 column eluting with MeOH/water (1/1, v/v) to give the smaller fractions, FP4C3A- FP4C3C. FP4C3A was chromatographed on an RP-18 column eluting with acetone/water (1/1.5, v/v) to give three smaller fractions, FP4C3A1-FP4C3A3. FP4C3A1 was further chromatographed on an HPLC using J'sphere ODS H-80 column (250 mm length × 20 mm I.D) eluting with ACN in H_2O (20%, v/v) yielding compounds **1** (30.0 mg), **2** (15.0 mg), and **3** (17.0 mg). FP4C3A3 chromatographed on HPLC using J'sphere ODS H-80, (250 mm length ×20 mm I.D) eluting with ACN-H_2O (20 %, v/v) to yield compounds **4** (8.0 mg) and **5** (30.0 mg).

Kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (1): Yellow powder; ESI-MS *m/z* 595 $[M+H]^+$, $C_{27}H_{30}O_{15}$; ^{1}H- and ^{13}C-NMR (CD_3OD): see Table 1.
Rutin (2): Yellow powder; ESI-MS *m/z* 611 $[M+H]^+$, $C_{27}H_{30}O_{16}$; ^{1}H- and ^{13}C-NMR (CD_3OD): see Table 1.

Kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-galactopyran oside (3): Yellow powder; ESI-MS *m/z* 595 $[M+H]^+$, $C_{27}H_{30}O_{15}$; ^{1}H- and ^{13}C-NMR (CD_3OD): see Table 1.

Isorhamnetin 3-O-α-L-rhamnopyranosyl (1→6)-β-D-galactopyranoside (4): Yellow powder; ESI-MS *m/z* 619 $[M+H]^+$, $C_{28}H_{32}O_{16}$; ^{1}H- and ^{13}C-NMR (CD_3OD): see Table 2.

Kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside (5): Yellow powder; ESI-MS *m/z* 595 $[M+H]^+$, $C_{27}H_{30}O_{15}$; ^{1}H- and ^{13}C-NMR (CD_3OD): see Table 2.

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a yellow amorphous powder. The ^{1}H-NMR spectra showed the proton signals of 6.17 and 6.36 (each 1H, s) *para* substituted at 8.03 and 6.86 (each 2H, J = 8.0 Hz) suggesting the presence of a flavonol. Two anomeric protons at δ_H 5.10 (1H, d, J = 7.2 Hz) and δ_H 4.50 (1H, br s), suggested the appearance of two sugar units. The ^{13}C-NMR and HSQC spectra of **1** showed signals of 15 carbon atoms and 12 carbon signals of two sugar units. The signals at δ_C 104.6, 75.8, 78.1, 71.4, 77.2, and 68.5 in the ^{13}C-NMR indicated a monosaccharide as glucopyranosyl. The signals at δ_C 102.4, 72.1, 72.3, 73.9, 69.7 and 17.9 in the ^{13}C-NMR confirmed a remaining monosaccharide as rhamnopyranosyl. The analysis of ^{1}H- and ^{13}C-NMR suggested the structure of **1** was kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside [10]. The positions of the functional groups were confirmed by the analysis of HSQC and HMBC spectra. The HMBC cross peaks from H-6 (δ_H 6.17) to C-5 (δ_C 162.9)/C-7 (δ_C 166.0)/C-8 (δ_C 94.9)/C-10 (δ_C 105.6); from H-8 (δ_H 6.36) to C-6 (δ_C 100.0)/C-7 (δ_C 166.0)/C-9 (δ_C 158.5)/C-10 (δ_C 105.6) confirmed the locations of two hydroxyl groups at C-5 and C-7. The HMBC correlations between H-2′ (δ_H 8.03)/H-6′ (δ_H 6.86) and C-1′ (δ_C 122.7)/C-4′ (δ_C 161.5) suggested the position of hydroxyl group at C-4′ of B ring. The sugar linkage of **1** was proved as *α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside and at C-3 of flavonol by the observation of the HMBC correlations between rham H-1‴ (δ_H 4.50) and glc C-6″ (δ_C 68.5) and between glc H-1″ (δ_H 5.10) and C-3 (δ_C 135.5). Furthermore, ESI-MS of **1** exhibited an ion at *m/z* 595 $[M+H]^+$, corresponding to the molecular formula of $C_{27}H_{30}O_{15}$. Thus, compound **1** was elucidated as kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside. Compound **1** was reported for the first time from *Hosta ventricosa* in 1990 [11] and also reported from *Clitoria ternatea* [10]. Compound **1** showed significant antioxidant activity against the ABTS radical system [12]. As our best knowledge, compound **1** was reported for the first time from *Fissistigma* genus.

The ^{1}H-NMR of compound **2** showed the following signals: three aromatic protons of ABX aromatic system in B ring at δ_H 6.85 (1H, d, J = 8.0 Hz), 7.60 (1H, d, J = 8.0 Hz), and 7.65 (s); two *meta*-protons of A ring at δ_H 6.17 (s) and 6.36 (s); two anomeric protons at δ_H 4.50 (br s) and 5.08 (d, J = 7.2 Hz) and one secondary methyl group at δ_H 1.10 (d, J = 6.4 Hz) assigned to a flavonol disaccharide. The ^{13}C-NMR and HSQC spectra revealed signals of 27 carbons, including one carbonyl at δ_C 179.4; nine non-protonated carbons at δ_C 105.6, 123.1, 135.6, 145.8, 149.8, 158.4, 159.3, 162.9, and 166.0; fifteen methines at δ_C 69.7, 71.4, 72.1, 72.2, 73.9, 75.7, 77.2, 78.1, 94.9, 99.9, 102.4, 104.7, 116.0, 117.7, and 123.5; one methylene carbon at δ_C 68.5; and one methyl carbon at δ_C 17.9. Besides, the NMR data of **2** were compared with those of rutin and found to be similar [13]. The positions of hydroxyl group at C-3′ and C-4′ were proved by HMBC correlations between H-2′ (δ_H 6.75)/H-5′ (δ_H 6.85) and C-3′ (δ_C 145.8)/C-4′ (δ_C 149.6) (Fig. 2). ESI mass spectrum of **2** exhibited an ion at *m/z* 611 $[M+H]^+$, corresponding to the molecular formula of $C_{27}H_{30}O_{16}$. Thus, the structure of **2** was elucidated to be rutin.

Table 1. NMR data for compounds **1**- **3** and reference compounds.

C	1			2			3		
	δ_C[@]	δ_C	δ_H (mult., *J*, Hz)	δ_C[#]	δ_C	δ_H (mult., *J*, Hz)	δ_C[$]	δ_C	δ_H (mult., *J*, Hz)
2	**161.5**	**159.3**	-	158.2	159.3	-	157.7	159.3	-
3	135.5	135.5	-	135.3	135.6	-	135.2	135.7	-
4	179.4	179.3	-	179.5	179.4	-	178.5	179.6	-
5	163.0	162.9	-	162.6	162.9	-	163.0	163.0	-
6	100.0	100.0	6.17 (s)	99.8	99.9	6.17 (s)	102.5	100.0	6.18 (s)
7	166.0	166.0	-	165.7	166.0	-	166.0	166.2	-
8	94.9	94.9	6.36 (s)	94.8	94.9	6.36 (s)	96.0	94.9	6.38 (s)
9	158.6	158.5	-	159.1	158.4	-	159.1	158.5	-
10	105.7	105.6	-	105.5	105.6	-	105.1	105.5	-
1′	122.8	122.7	-	123.4	123.1	-	122.6	122.6	-
2′	132.4	132.4	8.03 (d, 8.0)	115.9	116.0	7.65 (s)	132.5	132.5	8.07 (d, 8.4)
3′	116.1	116.1	6.86 (d, 8.0)	145.6	145.8	-	116.4	116.1	6.86 (d, 8.4)
4′	**159.4**	**161.5**	-	149.6	149.8	-	162.0	161.6	-
5′	116.1	116.1	6.86 (d, 8.0)	117.6	117.7	6.85 (d. 8.0)	116.4	116.1	6.86 (d, 8.4)
6′	132.4	132.4	8.03 (d, 8.0)	122.9	123.5	7.60 (d. 8.0)	132.5	132.5	8.07 (d, 8.4)
Glc				Glc			Gal		
1″	104.6	104.6	5.10 (d, 7.2)	104.7	104.7	5.08 (d. 7.2)	102.5	105.5	5.02 (d, 7.6)
2″	75.8	75.8	3.42 (m)	75.6	75.7	3.45 (m)	71.9	73.0	3.76 (m)
3″	78.2	78.1	3.41 (m)	78.0	78.1	3.41 (m)	73.2	75.0	3.51 (m)
4″	71.5	71.4	3.24 (m)	71.2	71.4	3.26 (m)	69.0	70.1	3.75 (br s)
5″	77.2	77.2	3.32 (m)	77.0	77.2	3.31 (m)	75.7	75.3	3.60 (m)
6″	68.6	68.5	3.35 (m) 3.79 (brd 10.4)	68.5	68.5	3.36 (m) 3.78 (brd. 10.4)	66.9	67.4	3.37 (m) 3.70 (m)
Rha									
1‴	102.4	102.4	4.50 (br s)	102.2	102.4	4.50 (br s)	101.7	101.9	4.50 (br s)
2‴	72.1	72.1	3.62 (br s)	72.0	72.1	3.63 (br s)	72.1	72.1	3.57 (br s)
3‴	72.3	72.3	3.50 (m)	72.1	72.2	3.52 (m)	71.9	72.3	3.48 (m)
4‴	73.9	73.9	3.26 (m)	73.8	73.9	3.27 (m)	72.3	73.9	3.26 (m)
5‴	69.7	69.7	3.43 (m)	69.6	69.7	3.42 (m)	69.0	69.7	3.50 (m)
6‴	17.9	17.9	1.10 (d, 6.4)	17.9	17.9	1.10 (d. 6.4)	17.7	18.0	1.16 (d, 6.4)

[@]δ_C of kaempferol 3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside [10], [#]δ_C of rutin [13], [$]$\delta_C$ of kaempferol 3-O-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside [14].

The analysis of the ^{1}H and ^{13}C-NMR spectra indicated that the structure of **3** was kaempferol disaccharide. The galactopyranosyl moiety was confirmed by the presence of carbon signals at δ_C 106.5, 73.1, 75.0, 70.0, 75.5, 67.4 in the ^{13}C-NMR spectrum and a broad singlet signal of H-4 (δ_H 3.75 (br s)) in the ^{1}H-NMR spectrum. Furthermore, the NMR and ESI-MS data of **3** were compared and well agreed with those of kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-galactopyranoside in the literature [14]. Thus, the compound **3** was identified as kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-galactopyranoside. Compound **3** was isolated for the first time in 1985 from *Strychnos variabilis* [17] and also from *Rumex chalepensis* [14] and *Phoebe poilanei* [18].

The NMR spectra of **4** were almost similar to the corresponding spectra of **3**, excepted for the addition of a methoxy group at C-3′. The HMBC correlations between H-2′/H-6′ and C-4′, between H-5′ and C-3′, between methoxy proton (δ_H 3.94) and C-3′ (δ_C 148.3) confirmed the locations of the methoxy and hydroxyl groups at C-3′ and at C-4′, respectively (Fig. 2). The proton and carbon chemical shifts and ESI-MS data as well as the ^{1}H-^{1}H coupling constants of **4**

matched perfectly with isorhamnetin 3-*O*-α-L-rhamnopyranosyl (1→6)-*β*-D-galactopyranoside [15].

Table 2. NMR data for compounds **4** and **5** and reference compounds.

C	4			5		
	δ_C[#]	δ_C	δ_H (mult., *J*, Hz)	δ_C[$]	δ_C	δ_H (mult., *J*, Hz)
2	156.5	158.8	-	156.4	158.4	-
3	133.1	135.5	-	132.5	134.4	-
4	177.2	179.4	-	177.1	179.5	-
5	161.2	162.9	-	161.1	163.2	-
6	99.0	100.0	6.17 (s)	99.0	99.8	6.15 (s)
7	165.3	166.1	-	165.5	166.0	-
8	93.9	94.9	6.37 (s)	93.7	94.6	6.34 (s)
9	156.2	158.4	-	155.6	158.3	-
10	103.6	105.6	-	103.3	105.8	-
1′	121.1	122.9	-	120.9	123.0	-
2′	113.5	114.6	7.99 (s)	130.7	132.2	8.05 (d, 8.0)
3′	147.0	148.3	-	115.0	116.2	6.87 (d, 8.0)
4′	149.5	150.8	-	159.8	161.3	-
5′	115.2	115.9	6.88 (d, 8.0)	115.0	116.2	6.87 (d, 8.0)
6′	122.0	123.7	7.57 (d, 8.0)	130.7	132.2	8.05 (d, 8.0)
	55.9	56.9	3.94 (s)			
Glc	Gal			Gal		
1″	101.9	105.6	5.19 (d, 7.6)	98.7	100.6	5.68 (d, 8.0)
2″	71.2	73.1	3.80 (m)	75.1	77.7	3.92 (t, 8.0)
3″	73.0	75.0	3.55 (m)	74.0	75.8	3.68 (m)
4″	68.0	70.0	3.76 (br s)	68.1	70.8	3.80 (br s)
5″	73.6	75.5	3.64 (m)	75.5	77.0	3.46 (m)
6″	65.2	67.7	3.43 (m) 3.72 (m)	60.1	62.2	3.48 (m) 3.59 (m)
Rha						
1‴	100.1	101.9	4.51 (br s)	100.5	102.6	5.19 (br s)
2‴	70.6	72.1	3.56 (br s)	70.6	72.3	3.97 (br s)
3‴	70.4	72.3	3.47 (m)	70.6	72.4	3.75 (brd, 9.6)
4‴	71.9	73.8	3.25 (m)	71.8	74.0	3.31 (m)
5‴	68.3	69.7	3.51 (m)	68.4	69.8	4.00 (m)
6‴	17.9	18.0	1.15 (d, 6.4)	17.1	17.5	0.91 (d, 6.4)

[#]δ_C of isorhamnetin 3-O-α-L-rhamnopyranosyl (1→6)-β-D-galactopyranoside [15], [$]$\delta_C$ of kaempferol 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranoside [16].

The ^{1}H- and ^{13}C-NMR data of compound **5** were similar to those of **3** (Table 2). The difference in structure between **5** and **3** is the movement of the position of *α*-L-rhamnopyranosyl moiety from gal C-6 to gal C-2. This was proved by the HMBC correlation between rha H-1‴ (δ_H 5.19) and gal C-2″ (δ_C 77.7). Thus, the structure of **5** was identified as kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside [16] (Fig. 2) which was further confirmed by the exhibition of an ion peak at *m/z* 595 $[M+H]^+$ on the ESI mass spectrum, corresponding to the molecular formula of $C_{27}H_{30}O_{15}$. Compound **5** was isolated the first time form *Blackstonia perfoliata* in 1989 [16], was also isolated *Phyllanthus acidus* [19] and *Chenopodium quinoa* [20]. Compound **5** exhibited antioxidant activity in DPPH assay with IC_{50} value of 79.32 ± 3.14 µM and IC_{50} of 86 µM DPPH [19, 20].

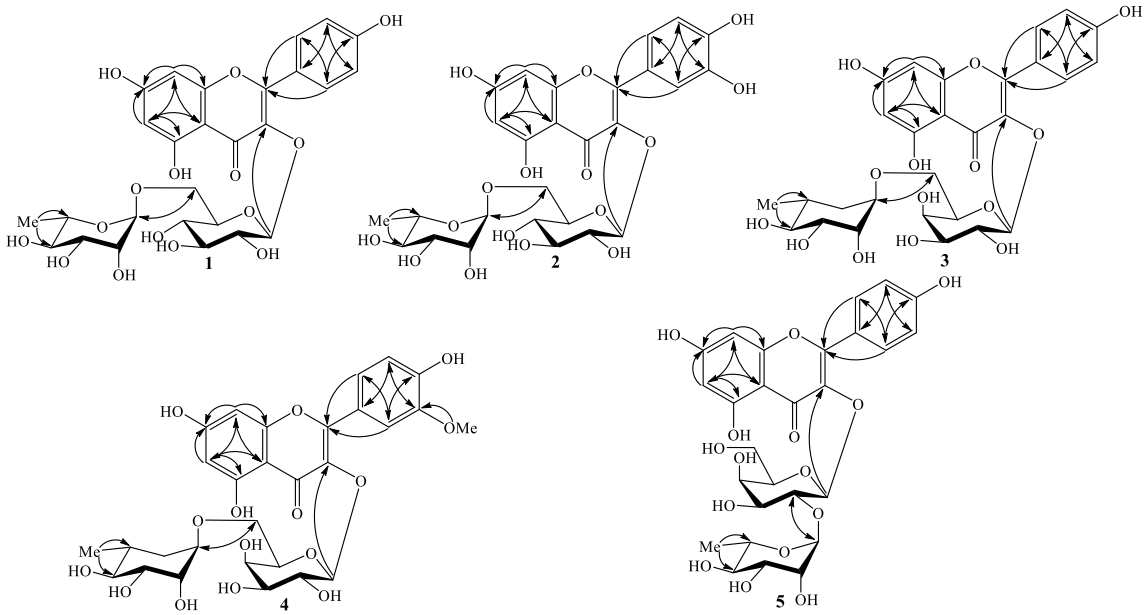

Figure 2. The key HMBC correlations of compounds **1- 5**.

4. CONCLUSION

In conclusion, five flavonol glycoside, kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (**1**), rutin (**2**), kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-galactopyranoside (**3**), isorhamnetin 3-O-α-L-rhamnopyranosyl (1→6)-β-D-galactopyranoside (**4**), and kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside (**5**) were isolated from the leaves of *Fissistigma pallens* using combined chromatographic methods.

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