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ISOLATION OF SOME FLAVONOIDS FROM *Vitex trifolia* IN BACHMA NATIONAL PARK, THUA THIEN HUE, VIETNAM

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Abstract. Using combined chromatographic methods, three flavonoids, (*2S*)-7,4'-dihydroxy-5-methoxyflavanone (**1**), luteolin (**2**), and 2″-*O*-rhamnosylvitexin (**3**) were isolated from the methanol extract of the leaves of *Vitex trifolia.* Their structures were established on the basis of spectroscopic evidence and comparison with those reported in the literature.

Keywords: *Vitex* genus, *V. trifolia*, flavonoid.

1. Introduction

From ancient years, the traditional medical plants were used for treatment of various diseases based on knowledge and experience. Many of these plants failed to draw attention to their useful medicinal properties and their active pharmacological contents remain to be undiscovered. In recent years, many traditional plants from various genus, species and families have been evaluated scientifically. Many active phytoconstituents were isolated and evaluated for their role in prevention and treatment of many diseased conditions.

The genus *Vitex* is one of the largest genus in the *Verbenaceae* family with approximately 250 species [1]. The plants are mostly shrubs or trees, and mainly found in the tropical areas with a few in subtropical regions [1]. Traditionally, some of its species are being used for rheumatic pains, sprains, anti-fungal, and anti-cancer activities [2]. Phytochemical investigation of *Vitex* genus led to the isolation of numerous terpenoids, flavonoids, ecdysteroids, lignans, and other compounds. Pharmacological studies had shown that the extractions and isolated compounds possess antitumor, anti-inflammatory, antibacterial, antioxidant activities, and so on. However, there are few researches on the chemical components and biological activities of *Vitex* species growing in Vietnam [3-7].

According to Dictionary of Vietnamese medicinal plants, *Vitex trifolia* was used for treating many diseases, such as: cough, colds, headache, fever, skin diseases [8].

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Phytochemical study of *Vitex trifolia* has led to the isolation of diterpenoids, triterpenoids, flavonoids [9-12], and many others. In Vietnam, there has been only one study on fruits of this species [7]. This paper reported the isolation and structure elucidation of three flavonoids from the methanol extract of the leaves of *Vitex trifolia* growing in Bachma National Park, Thua Thien Hue, Vietnam. This is the first announcement in Vietnam about the chemical composition of leaves species *Vitex trifolia.*

2. Content

2.1. Material and methods

**** Plant materials***

The leaves of *Vitex trifolia* L. were collected in Bachma National Park, Thua Thien Hue, Vietnam in September, 2015, and identified by Prof. Dr. Ninh Khac Ban. A voucher specimen was deposited at the Herbarium Institute of Marine Biochemistry, VAST.

**** General experimental procedures***

Optical rotations were determined on a Jasco DIP-370 automatic Polari meter. The NMR spectra were recorded using a Bruker DRX 500 spectrometer (^{1}H, 500 MHz; ^{13}C, 125 MHz). Column chromatography was performed using silica-gel (Kieselgel 60, 70 - 230 mesh and 230 - 400 mesh, Merck) or RP-18 resins (30 - 50 μm, Fujisilisa Chemical Ltd.), and thin layer chromatography (TLC) was performed using a precoated silica gel 60 F_{254} (0.25 mm, Merck) and RP-18 F_{254S} plates (0.25 mm, Merck).

**** Extraction and isolation***

The dried leaves of *V. trifolia* (2.2 kg) were extracted with hot MeOH three times (3 × 5 L) using sonicator for 3 h to yield 130 g extract after evaporation of the solvent. This extract was suspended in H_2O and successively partitioned with CH_2Cl_2, EtOAc to obtain the CH_2Cl_2 (VIT1, 51.0 g), EtOAc (VIT2, 27.0 g), and H_2O (VIT3, 52.0 g) extracts after removal of the solvents *in vacuo.*

The VIT2 fraction was chromatographed on a silica gel column eluting with a gradient of *n*-hexane:acetone (100:0 → 0:1) to give four fractions, VIT2A–VIT2D. VIT2C was chromatographed on an RP-18 column eluting with MeOH:water (1.2:1, v/v) to yield compounds **1** (10.0 mg) and **2** (9.0 mg).

VIT3 was chromatographed on a Diaion HP-20P column, using H_2O to remove sugar and then eluting with the increasing MeOH in water (25, 50, 75, and 100 %) to obtain four sub-fractions, VIT3A-VIT3D. VIT3C was chromatographed on an RP-18 column eluting with MeOH:water (1:1.5, v/v) to yield compounds **3** (15.0 mg).

***(2S)*-7,4'-Dihydroxy-5-methoxyflavanone** (**1**): yellowish powder; $C_{16}H_{14}O_5$; optical rotation $[\alpha]_D^{25}$: -20.5 (*c* 0.1, MeOH); ^{1}H- and ^{13}C-NMR (DMSO-d_6): see Table 1.

Luteolin (2): yellowish powder; $C_{15}H_{10}O_6$; ^{1}H- and ^{13}C-NMR (DMSO-d_6): see Table 1.

2″-*O*-Rhamnosylvitexin (**3**): yellowish powder; $C_{27}H_{30}O_{14}$; optical rotation $[\alpha]_D^{25}$: +30.0 (*c* 0.1, MeOH); ^{1}H- and ^{13}C-NMR (CD_3OD): see Table 1.

Table 1. The ^{1}H- and ^{13}C-NMR data for compounds 1-3

		1					**3**	
C	**δ_C[#]**	**δ_C[a,c]**	**δ_H[a,d] (*J* in Hz)**		**C**	**δ_C[$]**	**δ_C[b,c]**	**δ_H[b,d] (*J* in Hz)**
2	78.0	78.1	5.32 (dd, 2.5, 13.0)		2	166.5	166.7	-
3	44.7	44.8	2.52 (dd, 2.5, 16.5) 2.98 (dd, 13.0, 16.5)		3	103.5	103.6	6.62 (s)
4	187.7	187.8	-		4	184.0	184.1	-
5	162.1	162.2	-		5	162.6	162.7	-
6	93.1	93.2	6.05 (d, 2.0)		6	99.8	100.1	6.26 (s)
7	164.2	164.2	-		7	164.0	164.9	-
8	95.5	95.6	5.95 (d, 2.0)		8	105.5	105.8	-
9	164.2	164.3	-		9	157.8	158.0	-
10	104.0	104.5	-		10	105.9	105.8	-
1′	129.3	129.4	-		1′	123.4	123.6	-
2′	128.0	128.1	7.28 (d, 8.5)		2′	130.0	130.1	8.01 (d, 8.5)
3′	115.0	115.2	6.78 (d, 8.5)		3′	117.0	117.0	6.97 (d, 8.5)
4′	157.5	157.6	-		4′	162.6	162.8	-
5′	115.0	115.2	6.78 (d, 8.5)		5′	117.0	117.0	6.97 (d, 8.5)
6′	128.0	128.1	7.28 (d, 8.5)		6′	130.0	130.1	8.01 (d, 8.5)
5-OMe	55.7	55.6	3.73 (s)		8-*C*-Glu			
		2			1″	73.6	73.8	5.06 (d, 10.0)
C	**δ_C[¥]**	**δ_C[a,c]**	**δ_H[a,d] (*J* in Hz)**		2″	78.0	78.2	4.28 (d, 8.0)
2	163.9	166.0	-		3″	81.5	81.6	3.66 (m)
3	102.9	103.9	6.55 (s)		4″	72.1	72.3	3.87 (m)
4	181.7	183.9	-		5″	82.7	82.9	3.34 (m)
5	161.5	163.2	-		6″	63.0	63.1	3.80 (dd, 5.5, 12.0) 3.97 (d, 12.0)
6	98.8	100.1	6.22 (d, 2.0)		2’’-*O*-Rha			
7	164.2	166.4	-		1‴	102.4	102.5	5.11*
8	93.8	95.0	6.45 (d, 2.0)		2‴	72.4	72.5	3.65 (m)
9	157.3	159.4	-		3‴	71.8	72.0	3.42 (m)
10	103.7	105.3	-		4‴	73.4	73.6	3.13 (m)
1′	121.5	123.7	-		5‴	69.8	70.0	2.50 (t, 6.0)
2′	113.4	114.2	7.39*		6‴	18.0	18.0	0.67 (d, 6.0)
3′	145.8	147.0	-					
4′	149.7	151.0	-					
5′	116.0	116.8	6.92 (d, 8.5)					
6′	119.0	120.3	7.39*					

*[a]recorded in DMSO-d_6, [b] CD_3OD, [c]125MHz, [d]500MHz, [#]δ_C of (2S)-7,4'-dihydroxy-5-methoxyflavanone [13], [¥]δ_C of luteolin [14], [$]$\delta_C$ of 2″-O-rhamnosylvitexin [15]; *)overlapped*

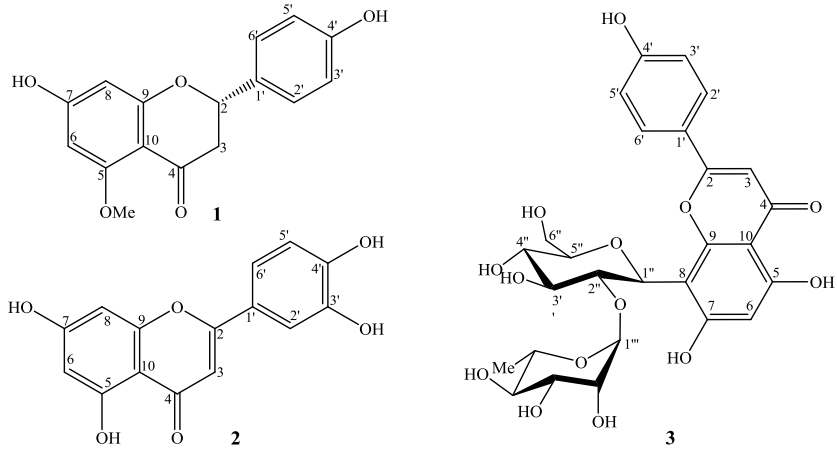

Figure 1. Chemical structures of compounds 1 - 3

2.2. Results and discussion

Compound **1** was obtained as a yellowish powder. The ^{1}H-NMR spectrum of **1** showed the following proton signals: six aromatic protons in two groups with ABX system at δ_H 5.95 (1H, d, *J* = 2.0 Hz), 6.05 (1H, d, *J* = 2.0 Hz), and 6.78 (2H, d, *J* = 8.5 Hz), 7.28 (2H, d, *J* = 8.5 Hz); one oxymethine proton at δ_H 5.32 (dd, *J* = 2.5, 13.0 Hz); one methoxy group at δ_H 3.73 (s); and two methylen protons at 2.52 (dd, *J* = 2.5, 13.0 Hz), 2.98 (dd, *J* = 13.0, 16.5 Hz), suggested the presence of a flavone. The ^{13}C-NMR and HSQC spectra showed the signals of 16 carbons, including seven non-protonated carbons at δ_C 104.5, 129.4, 157.6, 162.2, 164.2, 164.3, and 187.8; seven methines at δ_C 78.1, 93.2, 95.6, 115.2 × 2, and 128.1 ×2; one methylen at δ_C 44.8; and one methoxy carbon at δ_C 55.6. This also confirmed the presence of the flavone structure with one methoxy group. The HMBC correlations between methyl proton (δ_H 3.73) and C-5 (δ_C 162.2) indicated methoxy group at C-5. The HMBC correlations between H-6 (δ_H 6.05)/H-8 (δ_C 5.95) and C-7 (δ_C 164.3) indicated hydroxyl group at C-7. The hydroxyl group at C-4′ of B ring was confirmed by HMBC correlations between H-2′ (δ_H 7.28)/H-3′ (δ_H 6.78) and C-4′ (δ_C 157.6). All NMR assignments of **1** were confirmed by detailed analyses of HSQC and HMBC spectra, which are in good agreement with those reported in the literature [13]. Furthermore, compound **1** had optical rotation $[\alpha]_D^{25}$: -20.5 (*c* 0.1, MeOH), which is similar to (2*S*)-7,4'-dihydroxy-5-methoxyflavanone. Thus, compound **1** was identified as (2*S*)-7,4'-dihydroxy-5-methoxyflavanone.

Compound **2** was also obtained as a yellowish powder. The ^{1}H-NMR spectrum of **2** showed the signals of six aromatic protons at δ_H 6.22 (d, *J* = 2.0 Hz), 6.45 (d, *J* = 2.0 Hz), 6.55 (s), 6.92 (d, *J* = 8.5 Hz), and 7.39 (overlapped) × 2. The ^{13}C-NMR and HSQC spectra showed the signals of 15 carbons, including nine non-protonated carbons at δ_C 105.3, 123.7, 147.0, 151.0, 159.4, 163.2, 166.0, 166.4 and 183.9; six methines at δ_C 95.0, 100.1, 103.9, 114.2, 116.8 and 120.3. The ^{1}H-NMR and ^{13}C-NMR data of **2** suggested the presence of a flavone, and they were also similar to those of luteolin [14]. Thus, compound **2** was identified as luteolin.

Compound **3** was obtained as a yellowish powder. The ^{1}H-NMR spectrum of **3** showed the signals of six protons at δ_H 6.26 (1H, s), 6.62 (1H, s), 6.97 (2H, d, J = 8.5 Hz), 8.01 (2H, d, J = 8.5 Hz), and two anomeric proton at δ_H 5.06 (d, J = 10.0 Hz) and 5.11 (overlapped), suggested the presence of a flavone with two sugar units. The ^{13}C-NMR spectrum of **3** showed the signals of 27 carbons, including nine non-protonated carbons, sixteen methines, one methylene, and one methyl proton. Of which, 15 carbons were assigned to flavone moiety at δ_C 100.1, 103.6, 105.8 × 2, 117.0 × 2, 123.6, 130.1 × 2, 158.0, 162.7, 162.8, 164.9, 166.7 and 184.1, six carbons to a sugar unit at δ_C 63.1 (CH_2), 72.2 (CH), 73.8 (CH), 78.2 (CH), 81.6 (CH) and 82.9 (CH), and six carbons to the remaining sugar unit at δ_C 18.0 (CH_2), 70.0 (CH), 72.0 (CH), 72.5 (CH), 73.6 (CH) and 102.5 (CH). The ^{1}H- and ^{13}C-NMR data of 3 were similar to those of 2″-*O*-rhamnosylvitexin [15]. All NMR assignments of **3** were confirmed by detailed analyses of HSQC and HMBC spectra.

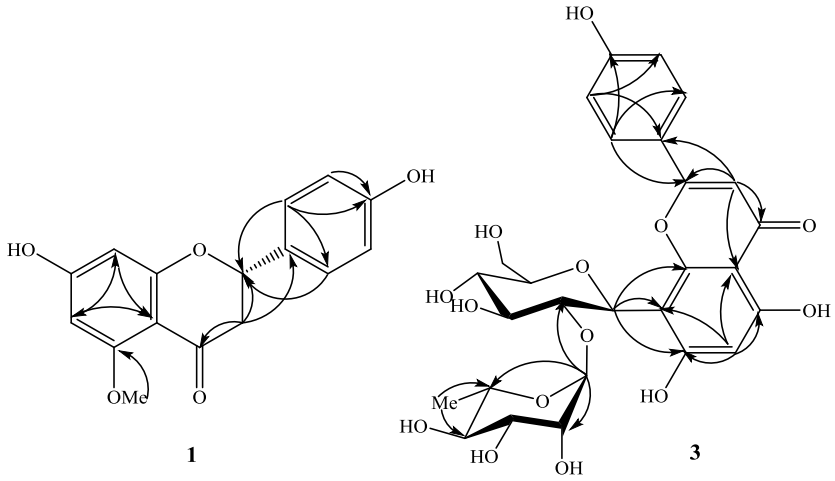

Figure 2. The key HMBC correlations of compounds 1 and 3

The HMBC correlations between H-3 (δ_H 6.62) and C-2 (δ_C 166.7)/C-4 (δ_C 184.1)/C-10 (δ_C 105.8) suggested the position of ketone groups at C-4. The two hydroxyl groups at C-5 and C-7 were confirmed by HMBC correlations between H-6 (δ_H 6.29) and C-5 (δ_C 162.7)/C-7 (δ_C 164.9)/C-8 (δ_C 105.8)/C-10 (δ_C 105.8). The HMBC correlations between H-2′ (δ_H 8.01) and C-2 (δ_C 166.7)/C-4′ (δ_C 162.8)/C-6′ (δ_C 130.1); and between H-3′(δ_H 6.97) and C-1′ (δ_C 123.6)/C-4′ (δ_C 162.8)/C-5′ (δ_C 117.0) suggested the position of hydroxyl group at C-4′.

In addition, the ^{13}C-NMR of the first sugar unit and the coupling constants of H-1″ and H-2″, J = 10 Hz (H-1″, δ_H 5.06, d, J = 10.0 Hz), confirmed the presence of *C-β*-glucopyranose. The HMBC correlations between glc H-1″ (δ_H 5.06) and C-7 (δ_C 164.9)/C-8 (δ_C 105.8)/C-9 (δ_C 158.0) suggested the position of *C*-glucopyranose at C-8 of flavone moiety. The HMBC correlations between anomeric proton H-1‴ (δ_H 5.11) and C-2″ (δ_C 78.1)/C-2‴ (δ_C 72.5)/C-5‴ (δ_C 70.0) suggested the position of the second sugar unit at C-2″ of glucopyranose moiety. Base on the above evidence and literature [15], compound **3** was determined to be 2″-*O*-rhamnosylvitexin.

3. Conclusions

From the leaves of *Vitex trifolia*, three flavonoids, (*2S*)-7,4'-dihydroxy-5-methoxyflavanone (**1**), luteolin (**2**), and 2′′-*O*-rhamnosylvitexin (**3**) were isolated. Their chemical structures were elucidated the by using one/two-dimension nuclear magnetic resonance (NMR) spectra and comparison with those reported in the literature. This result has contributed to the category of natural compounds in Vietnam and it is foundation for further studies on biological activities of compounds from *Vitex trifolia*, such as cytotoxic, anti-inflammatory, antibacterial activities.

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