

## FLAVONOIDS FROM FLOWERS OF *AMESIODENDRON CHINENSE*

Ho Van Ban<sup>1,2,3</sup>, Trinh Thi Thanh Van<sup>1,2</sup>, Vu Van Chien<sup>1</sup>, Nguyen Thi Hue<sup>1</sup>,  
Pham Thi Hang<sup>1</sup>, Pham Van Cuong<sup>1,2</sup>, Nguyen Le Tuan<sup>3\*</sup>, Nguyen Quoc Vuong<sup>1,2,\*</sup>

<sup>1</sup>*Institute of Marine Biochemistry, Vietnam Academy of Science and Technology (VAST),  
18 Hoang Quoc Viet Street, Cau Giay, Ha Noi, Viet Nam*

<sup>2</sup>*Graduate University of Science and Technology, VAST, 18 Hoang Quoc Viet Street,  
Cau Giay, Ha Noi, Viet Nam*

<sup>3</sup>*Department of Natural Sciences, Quy Nhon University, 170 An Duong Vuong Street,  
Quy Nhon City, Viet Nam*

\*Email: nguyenvuong@imbc.vast.vn

Received: 10 June 2020; Accepted for publication: 30 September 2020

**Abstract.** From the flowers of *Amesiodendron chinense* (Merr.) Hu, six known flavonoids, (–)-catechin (**1**), (–)-*epi*-catechin (**2**), chrysoeriol (**3**), kaempferide 3-*O*- $\beta$ -D-glucopyranoside (**4**), astragalol (**5**), quercetin 3-*O*- $\beta$ -D-glucopyranoside (**6**) were isolated. Their chemical structures were elucidated by analysis of the physicochemical parameters, the NMR and mass spectral data, and comparison with those reported in the literatures.

*Keyword:* flavonoids, flowers, *Amesiodendron chinense*.

*Classification numbers:* 1.1.1, 1.1.6.

### 1. INTRODUCTION

Flavonoids are small molecular weight phenolic compounds found in all plant parts such as flowers, branches, leaves, seeds, bark, and fruit. They form a class of specific substances in plants and are divided into subheadings such as flavones, flavonols, flavanones, flavanonols, flavanols or catechins, anthocyanins, and chalcones [1]. Due to possessing antioxidant, anti-inflammatory, anti-mutagenic and anti-cancer activities, flavonoids were used for the improvement of human's health and they are indispensable ingredients in the productions of pellets, cosmetics and medicines. Hence, the search and discovery of natural source flavonoids are received great interest today [1 - 3]. In our project, *Amesiodendron chinense* (Merr.) Hu, also is called "Truong sang" in Viet Nam [4, 5], was studied on its chemical constituents. Herein, we present the isolation and chemical structural elucidation of six known flavonoids from the flowers of *A. chinense*, including two flavan-3-ols, (–)-catechin (**1**) and (–)-*epi*-catechin (**2**); one flavone, chrysoeriol (**3**); and three flavonols, kaempferide 3-*O*- $\beta$ -D-glucopyranoside (**4**), astragalol (**5**) and quercetin 3-*O*- $\beta$ -D-glucopyranoside (**6**).

## 2. MATERIALS AND METHODS

### 2.1. General experimental procedures

The  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$  and 2D-NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer. Optical rotations were recorded on a JASCO P-2000 Polarimeter. The ESI-MS were measured on an Agilent 1100 Series LC/MSD Trap SL. Column chromatography (CC) was performed using a silica gel 60 (230 - 400 mesh, Merck) or RP-18 resins (30 - 50  $\mu\text{m}$ , Fuji Silysia Chemical Ltd, Aichi, Japan). Thin layer chromatography (TLC) used percolated silica gel 60 F254 (Merck) and RP-18 F254S plates (Merck).

### 2.2. Plant material

The flowers of *Amesiodendron chinense* (Merr.) Hu (Sapindaceae) species were collected in May 2019 from Son Tra District, Da Nang City, and the scientific name was identified by Dr. Do Van Hai, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. A voucher specimen (PTH15032018) was deposited at the Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology.

### 2.3. Extraction and isolation

The dried flowers of the plant (2.3 kg) were pulverized then extracted with 85% MeOH (10L  $\times$  4) by sonification at 50  $^\circ\text{C}$  (each time 2 h). The extracts were collected and solvent were removed in reduced pressure to give a crude MeOH extract (1 L). The MeOH extract was suspended with water (1 L) and successively partitioned with *n*-hexane and ethyl acetate (EtOAc) to give *n*-hexane (AFH, 20.0 g) and ethyl acetate (AFE, 70.0 g) residues and a water layer (AFW, 1.5 L).

The AFE residue was applied on a silica gel CC eluting with EtOAc to give five fractions (fr. AFE1–AFE5).

The fraction AFE1 was chromatographed on a silica gel column eluting with *n*-hexane/EtOAc (1/1) to give five sub-fractions (AFE1.1–AFE1.5). The sub-fraction AFE1.2 was subjected to a silica gel CC eluting with  $\text{CH}_2\text{Cl}_2$ /acetone (10/1) to afford four fractions (AFE1.2.1–AFE1.2.4). The fraction AFE1.2.3 was further purified by sephadex LH-20 CC eluting with MeOH to give compound **1** (80 mg). The fraction AFE1.3 was separated by RP-18 CC eluting with MeOH/ $\text{H}_2\text{O}$  (1/2) to yield compounds **2** (50 mg) and **3** (70 mg).

The fraction AFE4 was chromatographed on a silica gel column eluting with  $\text{CH}_2\text{Cl}_2$ /EtOAc/MeOH (4/2/1) to give six sub-fractions (AFE4.1–AFE4.6). The fraction AFE4.2 was subjected to a silica gel CC eluting with  $\text{CH}_2\text{Cl}_2$ /MeOH (8/1) to afford five fractions (AFE4.2.1–AFE4.2.5). The fraction AFE4.2.3 was chromatographed on a silica gel RP-18 column eluting with MeOH/ $\text{H}_2\text{O}$  (1/1) to give compound **4** (30 mg). The fraction AFE4.4 (2.5 g) was separated by a silica gel RP-18 CC eluting with MeOH/ $\text{H}_2\text{O}$  (1/1) to yield four fractions (AFE4.4.1–AFE4.4.4). The fraction AFE4.4.1 was purified further by a Sephadex LH-20 CC eluting with MeOH to give compound **5** (25 mg). The fraction AFE4.4.3 was subjected to a silica gel RP-18 CC eluting with MeOH/ $\text{H}_2\text{O}$  (1/1) to give three fractions (AFE4.4.3.1–AFE4.4.3.3). The fraction AFE4.4.3.3 was purified further by a Sephadex LH-20 CC eluting with MeOH to give compound **6** (15 mg).

(-)-Catechin (**1**): yellow solid,  $[\alpha]_D^{25} -54$  (*c* 0.1, MeOH). Negative ESI-MS:  $m/z$  290  $[M]^-$ .  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}$ , 500 MHz) and  $^{13}\text{C-NMR}$  ( $\text{CD}_3\text{OD}$ , 125 MHz) see Table 1.

(-)-*Epi*-catechin (**2**): yellow solid,  $[\alpha]_D^{25} -19$  (*c* 0.1, MeOH). Negative ESI-MS:  $m/z$  290  $[M]^-$ .  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}$ , 500 MHz) and  $^{13}\text{C-NMR}$  ( $\text{CD}_3\text{OD}$ , 125 MHz) see Table 1.

Chrysoeriol (**3**): yellow solid,  $[\alpha]_D^{25} -23$  (*c* 0.1, MeOH). Positive ESI-MS:  $m/z$  301  $[M+H]^+$ ;  $^1\text{H-NMR}$  (acetone- $d_6$ , 500 MHz) and  $^{13}\text{C-NMR}$  (acetone- $d_6$ , 125MHz) see Table 1.

Kaempferide 3-*O*- $\beta$ -D-glucopyranoside (**4**): yellow solid,  $[\alpha]_D^{25} -16$  (*c* 0.1, MeOH). Negative ESI-MS:  $m/z$  461  $[M-H]^-$ .  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}$ , 500 MHz) and  $^{13}\text{C-NMR}$  ( $\text{CD}_3\text{OD}$ , 125 MHz) see Table 2.

Astragalin (**5**): yellow solid,  $[\alpha]_D^{25} -28$  (*c* 0.1, MeOH). Negative ESI-MS:  $m/z$  448  $[M]^-$ .  $^1\text{H-NMR}$  ( $\text{CD}_3\text{OD}$ , 500 MHz) and  $^{13}\text{C-NMR}$  ( $\text{CD}_3\text{OD}$ , 125 MHz) see Table 2.

Quercetin 3-*O*- $\beta$ -D-glucopyranoside (**6**), yellow solid,  $[\alpha]_D^{25} -10$  (*c* 0.1, MeOH). Negative ESI-MS:  $m/z$  463  $[M-H]^-$ .  $^1\text{H-NMR}$  (DMSO- $d_6$ , 500 MHz):  $^{13}\text{C-NMR}$  (DMSO- $d_6$ , 125 MHz) see Table 2.

### 3. RESULTS AND DISCUSSION

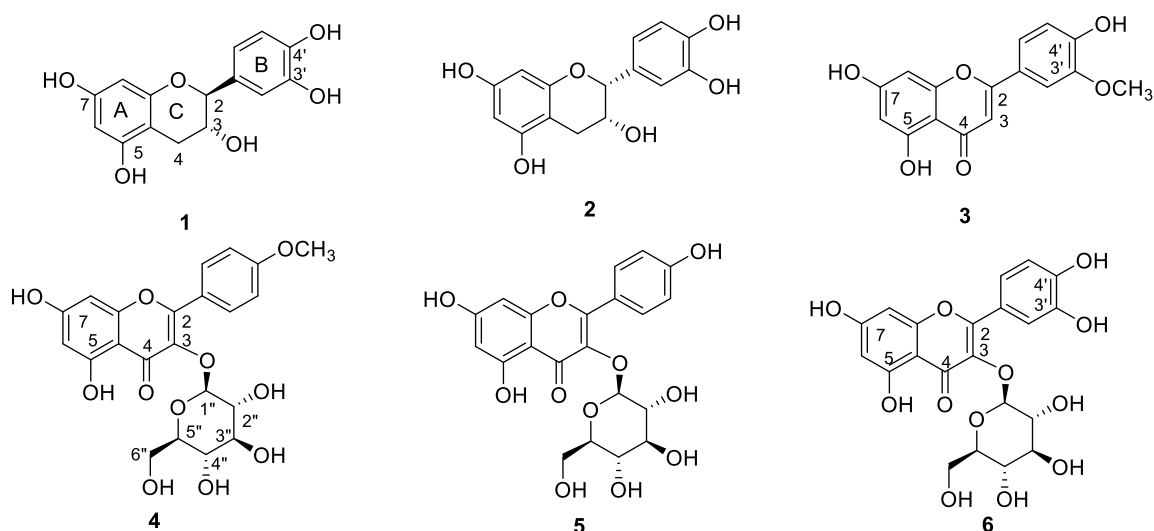


Figure 1. The chemical structure of compounds 1–6.

Compound **1** was isolated as a yellow amorphous powder. The ESI-MS spectrum gave a negative *quasi*-molecular ion peak at  $m/z$  290  $[M]^-$  ( $\text{C}_{15}\text{H}_{14}\text{O}_6^-$ ) and  $^{13}\text{C-NMR}$  spectrum of **1** indicated a molecular formula of  $\text{C}_{15}\text{H}_{14}\text{O}_6$  ( $M = 290$ ). 1D and 2D NMR spectra of **1** revealed recognizable signals to a flavanol with 3 rings A, B and C ( $\text{C}_6\text{-C}_3\text{-C}_6$ ). The  $^1\text{H-NMR}$  spectrum of **1** (Table 1) displayed signals for ABX system aromatic protons of ring B at  $\delta_{\text{H}}$  6.86 (d,  $J = 2.0$  Hz, H-2'), 6.79 (d,  $J = 8.0$  Hz, H-5'), and 6.74 (dd,  $J = 8.0, 2.0$  Hz, H-6'); two meta coupling aromatic protons of the ring A at  $\delta_{\text{H}}$  5.96 (d,  $J = 2.0$  Hz, H-6) and  $\delta_{\text{H}}$  5.89 (d,  $J = 2.0$  Hz, H-8); and signals of C ring including two protons of two oxymethine groups at  $\delta_{\text{H}}$  4.59 (d,  $J = 7.5$  Hz, H-2) and 4.00 (ddd,  $J = 8.0, 7.5, 5.5$  Hz, H-3), and two protons of a methylene group at  $\delta_{\text{H}}$  2.87 (dd,  $J = 16.0, 5.5$  Hz, H-4eq) and 2.54 (dd,  $J = 16.0, 8.0$  Hz, H-4ax). The  $^{13}\text{C-NMR}$  (Table 1) and

DEPT spectra showed corresponding signals to fifteen carbons including seven non-protonated carbons at  $\delta_C$  157.5 (C-5), 157.8 (C-7), 156.9 (C-9), 146.2 (C-3', C-4'), 100.9 (C-10) and 132.2 (C-1'); five methines at  $\delta_C$  96.3 (C-6), 95.4 (C-8), 115.3 (C-2'), 116.1 (C-5') and 120.0 (C-6'); two oxygenated aliphatic methines at  $\delta_C$  82.8 (C-2) and 68.8 (C-3); and one aliphatic methylene at  $\delta_C$  28.4 (C-4). The HMBC correlations between H-2 and C-4/C-3/C-1'/C-9/C-2'/C-6'; H-3 and C-1'/C-10; H-4ax and C-3/C-2/C-10/C-9/C-5, H-4eq and C-3/C-2/C-10 confirmed that these protons located at C-2, C-3, and C-4, respectively. Moreover, the HMBC correlations between H-6 and C-5/C-8, H-8 and C-6/C-9/C-10, indicated that two protons located at C-6 and C-8, respectively. Above 1D, 2D NMR spectra analysis established compound **1** to be catechin having the 2,3-*trans* configuration by the large coupling constant ( $J = 7.5$  Hz) of H-2 ( $\delta_H$  4.59) with H-3 and the resonance position of C-2 at  $\delta_C$  82.8 ppm. The optical rotation of **1** was determined to be  $[\alpha]_D^{25} -54$  ( $c$  0.1, MeOH), establishing **1** to be (-)-catechin [6, 7].

Compound **2** was isolated as a yellow amorphous powder. Its ESI-MS spectrum gave a negative *quasi*-molecular ion peak at  $m/z$  290  $[M]^-$  ( $C_{15}H_{14}O_6^-$ ) and  $^{13}C$ -NMR spectrum of **2** indicated a molecular formula of  $C_{15}H_{14}O_6$  ( $M = 290$ ). The NMR spectra of **2** (Table 1) is almost identical with those of **1** except for the following signals: C-2 resonated at  $\delta_C$  79.9 and H-2 at  $\delta_H$  4.83 (overlap by signal of HOD) identified base on analysis of COSY and HSQC, HMBC spectra. The chemical shift H-2 and C-2 suggested that **2** possesses the *cis*-2,3 stereochemistry, this was supported by the small value for the coupling constant ( $J < 1$  Hz) between the H-2 and H-3 protons, which appeared as a broad singlet at H-3 ( $\delta_H$  4.20) [6, 7]. Thus, **2** was assigned to be *epi*-catechin, its  $[\alpha]_D^{25}$  was determined to be  $-19$  ( $c$  0.1, MeOH), establishing **2** as (-)-*epi*-catechin.

Compound **3** was isolated as a yellow amorphous powder,  $[\alpha]_D^{25} -23$  ( $c$  0.1, MeOH). The ESI-MS gave a positive *quasi*-molecular ion peak at  $m/z$  301  $[M+H]^+$  ( $C_{16}H_{13}O_6^+$ ) and  $^{13}C$ -NMR spectrum of **3** indicated a molecular formula of  $C_{16}H_{12}O_6$  ( $M = 300$ ).  $^1H$ -NMR spectrum of compound **3** revealed recognizable signals to a flavon one, its molecule includes three ABX coupling protons of B ring at  $\delta_H$  7.62 (d,  $J = 2.0$  Hz, H-2'), 7.59 (dd,  $J = 8.0, 2.0$  Hz, H-6') and 7.00 (d,  $J = 8.0$  Hz, H-5'); two meta coupling protons of A ring at  $\delta_H$  6.54 (d,  $J = 2.0$  Hz, H-8) and  $\delta_H$  6.25 (d,  $J = 2.0$  Hz, H-6); and one methine proton of C ring at  $\delta_H$  6.69 (s, H-3).  $^{13}C$ -NMR and DEPT spectra showed corresponding carbons to fifteen carbons including one carbonyl at  $\delta_C$  183.1 (C-4); eight non-protonated carbons at 163.3 (C-5), 165.1 (C-7), 158.8 (C-9), 148.9 (C-3'), 151.5 (C-4'), 165.0 (C-2), 105.3 (C-10) and 123.5 (C-1'); and six methines at  $\delta_H$  99.7 (C-6), 94.8 (C-8), 110.6 (C-2'), 116.4 (C-5'), 121.3 (C-6') and 104.4 (C-3). In addition, the HMBC correlations between H-3 and C-1'/C-2/C-4/C-10 and between H-8 and C-6/C-7/C-9/C-10 confirmed two protons at C-3 and C-8, respectively; between H-6' and C-2'/C-4'/C-2 and between protons of  $OCH_3$  and C-3' ( $\delta_C$  148.9) revealed the methoxy group attaching on position C-3' of B ring. The analysis of NMR spectra indicated the structure of **3** was similar to those of chrysoeriol, which were reported in literature (Table 1) [8, 9]. Thus, **3** was confirmed as chrysoeriol (3'-methoxy-4',5,7-trihydroxyflavone).

Compound **5** was also isolated as a yellow amorphous powder. The ESI-MS spectrum gave a negative *quasi*-molecular ion peak at  $m/z$  448  $[M]^-$  ( $C_{21}H_{21}O_{11}^-$ ) and  $^{13}C$ -NMR spectrum of **5** indicated a molecular formula of  $C_{21}H_{21}O_{11}$  ( $M = 448$ ). The  $^1H$ ,  $^{13}C$ -NMR spectrum of **5** revealed the signals to a flavonol glucoside. The  $^1H$ -NMR spectrum showed four aromatic protons of B ring at  $\delta_H$  8.07 (d,  $J = 9.0$  Hz, H-2' and H-6') and 6.91 (d,  $J = 9.0$ , H-3' and H-5'), two meta coupling proton of A ring at  $\delta_H$  6.40 (d,  $J = 2.0$  Hz, H-8) and 6.21 (br s, H-6), assigned to flavonol aglycone; otherwhile, one anomeric proton at  $\delta_H$  5.25 (d,  $J = 7.5$  Hz, H-1'"); four methine protons at  $\delta_H$  3.47 (dd,  $J = 9.0, 7.5$  Hz, H-2''), 3.45 (t,  $J = 9.0$  Hz, H-3''), 3.33 (overlapped,

H-4''), and at  $\delta_{\text{H}}$  3.23 (m, H-5''); and two protons of methylene at  $\delta_{\text{H}}$  3.71 (dd,  $J = 12.0, 2.5$  Hz, Ha-6'') and 3.55 (dd,  $J = 12.0, 5.5$  Hz, Hb-6''); assigned to *O*-glucosyl moiety.

Table 1. The NMR spectroscopic data for compounds 1–3.

No.	1			2			3		
	$\delta_{\text{C}}^{\#}$	$\delta_{\text{C}}^{a,b}$	$\delta_{\text{H}}^{a,c}$ (mult., $J$ in Hz)	$\delta_{\text{C}}^{\#\#}$	$\delta_{\text{C}}^{a,b}$	$\delta_{\text{H}}^{a,c}$ (mult., $J$ in Hz)	$\delta_{\text{C}}^{\#\#\#}$	$\delta_{\text{C}}^{a,b}$	$\delta_{\text{H}}^{a,c}$ (mult., $J$ in Hz)
2	82.8	82.8	4.59 (d, 7.5)	79.5	79.9	4.83 (ovl.)	164.9	165.0	
3	68.3	68.8	4.00 (ddd, 8.0, 7.5, 5.5)	67.0	67.5	4.20 (brs)	104.5	104.4	6.69 (s)
4	28.8	28.4	2.87 Heq (dd, 16.0, 5.5)	29.0	29.3	2.88 Heq (dd, 16.5, 5.0)	182.9	183.1	-
			2.54 Hax (dd, 16.0, 8.0)			2.76 Hax (dd, 16.5, 3.0)			-
5	157.2	157.5	-	157.6	157.4	-	163.3	163.3	-
6	96.1	96.3	5.96 (d, 2.0)	96.2	96.4	5.96 (d, 2.0)	99.7	99.7	6.25 (d, 2.0)
7	157.7	157.8	-	157.6	158.0	-	165.0	165.1	-
8	95.3	95.4	5.89 (d, 2.0)	95.7	95.9	5.94 (d, 2.0)	94.7	94.8	6.54 (d, 2.0)
9	156.9	156.9	-	157.2	157.7	-	158.7	158.8	-
10	100.6	100.9	-	100.0	100.1	-	105.2	105.3	-
1'	131.8	132.2	-	132.3	132.3	-	123.6	123.5	-
2'	115.2	115.3	6.86 (d, 2.0)	115.3	115.4	7.00 (d, 2.0)	110.7	110.6	7.62 (d, 2.0)
3'	146.1	146.2	-	145.4	146.0	-	148.8	148.9	-
4'	146.0	146.2	-	145.3	145.8	-	151.3	151.5	-
5'	115.7	116.1	6.79 (d, 8.0)	115.5	115.9	6.78 (d, 8.0)	116.4	116.4	7.00 (d, 8.0)
6'	118.8	120.0	6.74 (dd, 8.0, 2.0)	119.4	119.4	6.82 (dd, 8.0, 2.0)	121.4	121.3	7.59 (dd, 8.0, 2.0)
3'-OCH <sub>3</sub> -	-	-	-	-	-	-	56.6	56.6	4.00 (s)

<sup>a</sup>recorded in CD<sub>3</sub>OD, <sup>b</sup>125 MHz, <sup>c</sup>500 MHz, <sup>#</sup> $\delta_{\text{C}}$  of (-)-catechin and <sup>\#\#</sup> $\delta_{\text{C}}$  of (-)-*epi*-catechin (in acetone-*d*<sub>6</sub> at 125 MHz) [6], <sup>\#\#\#</sup> $\delta_{\text{C}}$  of chrysoeriol (in acetone-*d*<sub>6</sub> at 150 MHz) [8], ovl. means overlapped.

The <sup>13</sup>C-NMR and DEPT spectra showed signals of corresponding carbons including one carbonyl at  $\delta_{\text{C}}$  179.5; eight non-protonated carbons at  $\delta_{\text{C}}$  158.5 (C-2), 135.5 (C-3), 163.1 (C-5), 166.4 (C-7), 159.1 (C-9), 161.6 (C-4'), 105.6 (C-10) and 122.8 (C-1'); and six methines at  $\delta_{\text{C}}$  100.1 (C-6), 94.9 (C-8), 132.3 (C-2' and C-6') and 116.1 (C-3' and C-5'); revealed the flavonol aglycone as kaempferol. The glucose moiety includes one anomeric carbon at  $\delta_{\text{C}}$  104.2 (C-1''); four oxygenated methines at  $\delta_{\text{C}}$  75.7 (C-2''), 78.4 (C-3''), 71.4 (C-4'') and 78.1 (C-5''); and one oxygenated methylene at  $\delta_{\text{C}}$  62.7 (C-6''). The HMBC correlation between H-1'' and C-3, and between H-2'' and C-1'', showed sugar moiety linked to aglycone at C-3. The large coupling constant between H-1'' and H-2'', H-2'' and H-3'', H-3'' and H-4'', confirmed their *axial* orientation and the sugar moiety was identified as a glucose and connected to kaempferol

through  $\beta$ -linkage. The analysis of NMR spectra indicated the structure of **5** was similar to those of kaempferol 3-*O*- $\beta$ -D-glucopyranoside reported in literature (Table 2) [11]. Thus, the compound **5** was confirmed as kaempferol 3-*O*- $\beta$ -D-glucopyranoside or astragalin.

Table 2. The NMR spectroscopic data for compounds **4–6**.

No	<b>4</b>			<b>5</b>			<b>6</b>		
	$\delta_C^{\#}$	$\delta_C^{a,b}$	$\delta_H^{a,c}$ (mult., <i>J</i> in Hz)	$\delta_C^{\#\#}$	$\delta_C^{d,b}$	$\delta_H^{d,c}$ (mult., <i>J</i> in Hz)	$\delta_C^{\#\#\#}$	$\delta_C^{d,b}$	$\delta_H^{d,c}$ (mult., <i>J</i> in Hz)
2	156.9	156.4		158.0	158.5		158.6	158.4	
3	133.5	133.5		135.3	135.5		135.7	135.6	
4	177.6	177.4		179.2	179.5		179.5	179.4	
5	161.4	161.2		162.7	163.1		163.0	162.9	
6	98.8	98.8	6.20 (d, 2.0)	99.8	100.1	6.21 (brs)	100.2	99.9	6.20 (d, 2.0)
7	164.6	164.6		165.7	166.4		166.7	166.1	
8	94.2	93.7	6.43 (d, 2.0)	94.8	94.9	6.40 (d, 2.0)	94.9	94.8	6.39 (d, 2.0)
9	156.7	155.7		158.8	159.1		159.0	159.0	
10	103.9	104.1		105.6	105.6		105.5	105.6	
1'	121.5	122.5		122.5	122.8		123.1	123.1	
2'	131.4	130.7	8.13 (d, 9.0)	132.1	132.3	8.07 (d, 9.0)	116.0	116.0	7.73 (d, 2.0)
3'	116.7	113.7	7.07 (d, 9.0)	115.9	116.1	6.91 (d, 9.0)	146.0	145.8	
4'	157.5	158.8		161.2	161.6		149.9	149.8	
5'	116.7	113.7	6.91 (d, 9.0)	115.9	116.1	6.91 (d, 9.0)	117.6	117.6	6.89 (d, 8.5)
6'	131.4	130.7	8.13 (d, 9.0)	132.1	132.3	8.07 (d, 9.0)	123.2	123.2	7.59 (dd, 8.5, 2.0)
1''	101.5	100.8	5.47 (d, 7.5)	104.0	104.2	5.25 (d, 7.5)	104.5	104.4	5.25 (d, 7.5)
2''	74.3	74.2	3.2-3.6 (ovl.)	75.6	75.7	3.47 (dd, 9.0, 7.5)	75.8	75.7	3.51 (dd, 9.0, 7.5)
3''	76.5	76.4	3.2-3.6 (ovl.)	78.2	78.4	3.45 (t, 9.0)	78.4	78.1	3.46 (t, 9.0)
4''	70.3	69.9	3.2-3.6 (ovl.)	71.2	71.4	3.33 ovl.	71.3	71.2	3.38 (dd, 9.5, 9.0)
5''	76.0	77.5	3.2-3.6 (ovl.)	78.0	78.1	3.23 (m)	78.2	78.3	3.55 (ddd 9.5, 5.5, 2.5)
6''	62.2	60.8	3.2-3.6 (ovl.)	62.4	62.7	3.71 Ha (dd, 12.0, 2.5) 3.55 Hb (dd, 12.0, 5.5)	62.6	62.6	3.74 (dd 12.0, 2.5) 3.60 (dd 12.0, 5.5)
3'- OCH <sub>3</sub>	56.7	55.4	3.85 (s)	-	-		-	-	

<sup>a</sup>recorded in DMSO-*d*<sub>6</sub>, <sup>b</sup>125 MHz, <sup>c</sup>500 MHz, <sup>d</sup> recorded in CD<sub>3</sub>OD, <sup>#</sup> $\delta_C$  of kaempferide 3-*O*- $\beta$ -D-glucopyranoside (recorded in DMSO-*d*<sub>6</sub> at 125 MHz) [10] and <sup>\#\#</sup> $\delta_C$  of astragalin (recorded in CD<sub>3</sub>OD at 100 MHz) [11], <sup>\#\#\#</sup> $\delta_C$  of isoquercetin (in CD<sub>3</sub>OD at 75.5 MHz) [12], ovl. means overlapped.

Compound **4** was also isolated as a yellow amorphous powder. The ESI-MS gave a negative *quasi*-molecular ion peak at *m/z* 461[M-H]<sup>-</sup> (C<sub>22</sub>H<sub>21</sub>O<sub>11</sub><sup>-</sup>) and <sup>13</sup>C-NMR spectrum of **4** indicated a molecular formula of C<sub>22</sub>H<sub>22</sub>O<sub>11</sub> (M = 462). The <sup>1</sup>H- and <sup>13</sup>C-NMR spectral data of **4** resembled closely those of **5** (astragalin), including a flavonol aglycone as kaempferol bearing a

glucose, except for an additional methoxy group. The large coupling constant of anomeric proton between H-1'' and H-2'' ( $J = 7.5$  Hz) confirmed the sugar moiety as a  $\beta$ -linkage. The HMBC correlation between H-1'' ( $\delta_{\text{H}} 5.47$ ) and C-3 ( $\delta_{\text{C}} 133.5$ ) showed sugar moiety linked to aglycone at C-3, and the HMBC correlation from the methoxy group ( $\delta_{\text{H}} 3.85$ ) to C-4' ( $\delta_{\text{C}} 158.8$ ) suggested the position of methoxy group at C-4'. In addition, the  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectral data of **4** is very closed with those of kaempferide 3-*O*- $\beta$ -D-glucopyranoside, which was also recorded in DMSO- $d_6$  (Table 2) [10], identified the compound **4** as kaempferide 3-*O*- $\beta$ -D-glucopyranoside.

Compound **6** was isolated as a yellow amorphous powder,  $[\alpha]_{\text{D}}^{25} = -10^{\circ}$  (c 0.1, MeOH). The ESI-MS gave a pseudomolecular negative ion peak at  $m/z$  463[M-H] $^{-}$  ( $\text{C}_{21}\text{H}_{19}\text{O}_{12}^{-}$ ) and  $^{13}\text{C}$ -NMR spectrum of **6** indicated a molecular formula of  $\text{C}_{21}\text{H}_{20}\text{O}_{12}$  ( $M = 464$ ).  $^1\text{H}$ ,  $^{13}\text{C}$ -NMR spectra of compound **6** revealed recognizable signals of a flavonol bearing a sugar moiety. The signals of the flavonol aglycone includes three ABX coupling protons of B ring at  $\delta_{\text{H}} 7.73$  (d,  $J = 2.0$  Hz, H-2'), 7.59 (dd,  $J = 8.5, 2.0$  Hz, H-6') and 6.89 (d,  $J = 8.5$  Hz, H-5'); two meta coupling protons of A ring at  $\delta_{\text{H}} 6.39$  (d,  $J = 2.0$  Hz, H-8) and  $\delta_{\text{H}} 6.20$  (d,  $J = 2.0$  Hz, H-6). The corresponding carbons to the flavonol moiety include one carbonyl carbon at  $\delta_{\text{C}} 179.4$  (C-4); nine non-protonated carbons at  $\delta_{\text{C}} 158.4$  (C-2), 135.6 (C-3), 162.9 (C-5), 166.1 (C-7), 159.0 (C-9), 145.8 (C-3'), 149.8 (C-4'), 105.6 (C-10) and 123.1 (C-1'); five methine carbons at  $\delta_{\text{C}} 99.9$  (C-6), 94.8 (C-8), 116.0 (C-2'), 117.6 (C-5') and 123.2 (C-6'). The signals of glucosyl moiety include one anomeric proton at  $\delta_{\text{H}} 5.25$  (d,  $J = 7.5$  Hz); four oxygenated methine protons at  $\delta_{\text{H}} 3.51$  (dd,  $J = 9.0, 7.5$  Hz, H-2''), 3.46 (t,  $J = 9.0$  Hz, H-3''), 3.38 (dd,  $J = 9.5, 9.0$  Hz, H-4'') and 3.55 (ddd,  $J = 9.5, 5.5, 2.5$  Hz, H-5''); and two methylene protons at  $\delta_{\text{H}} 3.74$  (dd,  $J = 12.0, 2.5$  Hz, Ha-6'') and 3.60 (dd,  $J = 12.0, 5.5$  Hz, Hb-6''). The HMBC correlation between H-1'' ( $\delta_{\text{H}} 5.25$ ) and C-3 ( $\delta_{\text{C}} 135.6$ ) showed sugar moiety linked to aglycone at C-3, the large coupling constant between H-1'' and H-2'' ( $J = 7.5$  Hz) confirmed the presence of  $\beta$ -glycosidic linkage. The NMR spectroscopic analysis of **6** to those of quercetin 3-*O*- $\beta$ -D-glucopyranoside reported in literature (Table 2) [12], confirmed compound **6** as quercetin 3-*O*- $\beta$ -D-glucopyranoside or isoquercetin.

The six obtained compounds (**1–6**), as known, possess high antioxidant activity used for the prevention and treatment of a number of chronic diseases as cardiovascular, diabetic and cancers (catechins, astragalins) [13, 14, 15], osteoporosis (chrysoeriol) [9, 16]; On the other hand, compounds **3–5** exhibited potent antimicrobial and anti-inflammatory activities [10, 17, 18].

#### 4. CONCLUSION

From the flowers of *Amesiodendron chinense*, six known flavonoids were isolated as (-)-catechin (**1**), (-)-*epi*-catechin (**2**), chrysoeriol (**3**), kaempferide 3-*O*- $\beta$ -D-glucopyranoside (**4**), astragalins (**5**), and quercetin 3-*O*- $\beta$ -D-glucopyranoside (**6**). Their structures were elucidated by spectroscopic analysis including MS, 1D, 2D-NMR spectra, physical properties as well as by the comparison with reported data in literatures. This is the first time the study on the chemical constituents of the flowers of *Amesiodendron chinense* (Merr.) Hu was performed. Biological activities and medicinal properties of the obtained compounds suggested the flowers of *Amesiodendron chinense* as a potential candidate for preparing a functional food to prevent and treat cardiovascular, diabetes, and cancer diseases.

**Acknowledgement.** This work was supported by the Vietnam Academy of Science and Technology (VAST) under grant no. VAST 04.04/18-19.

## REFERENCES

1. Panche A. N., Diwan A. D. and Chandra S. R. - Flavonoids: an overview, *Nutritional Science* **5**(47) (2010) 1-15.
2. Nagula R. L., Wairka S. - Recent advances in topical delivery of flavonoids, *Journal of controlled release* **296** (2019) 190-201.
3. Rengasamy K. R. R., Khan H., Gowrishankar S., Lagoa R. J. L., Mahoomodally F. M., Khan Z., Suroowan S., Tewari D., Zengin G., Hassan S. T. S., Pandian S. K. - The role of flavonoids in autoimmune disease: therapeutic updates, *Pharmacology & Therapeutics* **194** (2019) 107-131.
4. Ban N. T. - List of Vietnamese plant species, Agriculture Publishing House, Hanoi, 2003, Vol. **II**, p. 1016 (in Vietnamese).
5. Ho P. H. - An illustrated flora of Vietnam, Young Publisher, Ho Chi Minh city, 2003, Vol. **II**, p. 326 (in Vietnamese).
6. Razek M. H. A. - NMR Assignments of Four Catechin Epimers, *Asian Journal of Chemistry* **19** (6) (2007) 4867-4872.
7. Martin T. S., Kikuzaki H., Hisamoto M., and Nakatani N., - Constituents of amomum tsao-ko and their radical scavenging and antioxidant activities, *Journal of the American Oil Chemists' Society* **77**(6) (2000) 667-673.
8. Silva L. A. L., Faqueti L. G., Reginatto F. H., Santos A. D. C., Barison A., Biavatti M. W. - Phytochemical analysis of *Vernonanthura tweedieana* and a validated UPLC-PDA method for the quantification of eriodictyol, *Revista Brasileira de Farmacognosia* **25** (2015) 375-381.
9. Tai B. H., Cuong N. M., Huong T. T., Choi E. M, Kim J. A., and Kim Y. H. - Chrysoeriol isolated from *Eurya cillata* leaves stimulates proliferation and differentiation of osteoblastic MC3T3-E1 cells, *Journal of Asian Natural Products Research* **11**(9) (2009) 817-823.
10. Al-Musayeib N., Perveen S., Fatima I., Nasir M. and Hussain A. - Antioxidant, anti-glycation and anti-inflammatory activities of phenolic constituents from *Cordia sinensis*, *Molecules* **16** (2011) 10214-10226
11. Seo K. H., Jung J. W., Nhan N. T., Lee Y. H., and Baek N. I. - Flavonoid glycosides from the flowers of *Pulsatilla koreana* Nakai, *Natural Product Sciences* **22**(1) (2016) 41-45.
12. Gutzeit D., Wray V., Winterhalter P., Jerz G. - Preparative isolation and purification of flavonoids and protocatechuic acid from sea buckthorn juice concentrate (*Hippophaë rhamnoides* L. ssp. *rhamnoides*) by High-speed counter-current chromatography, *Chromatographia* **65** (2007) 1-7.
13. Jeong W. S. & Kong A. N. T. - Biological properties of monomeric and polymeric catechins: Green tea catechins and procyanidins, *Pharmaceutical Biology* **42** (2004) 84-93.
14. Isemura M., Catechin in human health and disease, *Molecules* **24** (2019) 528-533.
15. Riaz A., Rasul A., Hussain G., Zahoor M. K., Jabeen F, Subhani Z., Younis T., Ali M., Sarfraz I., and Selamoglu Z. - Astragalins: A bioactive phytochemical with potential therapeutic activities, *Advances in Pharmacological Sciences* Volume **2018**, article 9794625, 15 pages.



16. Khanh P. N., Ha V. T., Huong T. T., Tai N. V., Thao D. T., Cuc D. T., Cuong N., M. - Chrysoeriol preventing osteoporosis induced by prednisolone in BALB/c model, Vietnam Journal of Science and Technology **62**(5A) (2014) 350-357.
17. Bashyal B., Parajuli P., Pandey R. P., and Sohng J. K. - Microbial biosynthesis of antibacterial chrysoeriolin recombinant *Escherichia coli* and bioactivity assessment, Catalysts **9** (2019) 112-127.
18. Choi S. J., Tai B. H., Cuong N.M., Kim Y. H., Jang H. D. - Antioxidative and anti-inflammatory effect of Quercetin and its glycosides isolated from Mampat (*Cratoxylum formosum*), Food Science and biotechnology **21**(2) (2012) 587-595.