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STRUCTURE AND BIOLOGICAL ACTIVITY OF FIVE FLAVONOIDS FROM *AMPELOPSIS CANTONIENSIS*

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Abstract. In this paper, phytochemical studies on ethanol extract of *Ampelopsi cantoniensis* led to obtain five flavonoid compounds: myricitrin, myricetin, quercetin, dihydromyricetin, and phloretin. Their structure was confirmed by NMR and ESI-MS methods. Antioxidant and anti-diabetic activities of the compounds were evaluated. The results showed that all five flavonoids have strong DPPH radical scavenging activity with SC_{50} values ranging from 9.42 - 35.37 μM. Four of them (myricetin, phloretin, myricitrin and quercetin) are strong α-glucosidase and α-amylase inhibitors with IC_{50} values ranging from 8.92 - 18.74 μM for α-glucosidase inhibition and from 9.64 - 136.58 μM for α-amylase inhibition.

Keywords. *Ampelopsi cantoniensis*, flavonoid, antioxidant activity, α-glucosidase and α-amylase inhibitor.

Classification numbers: 1.1.1, 1.2.1.

1. INTRODUCTION

Ampelopsis cantoniensis is a dicotyledonous plant in the Vitaceae family. This species is distributed in China, India, Japan and Viet Nam and is a wild plant rich in flavonoids. Previous chemical investigations on this plant had led to the isolation of flavonoids, stilbenes, coumarins, triterpenes, steroids, and alkaloids [1]. Those compounds are attracting more and more attention from the scientific community due to their sufficient biological activities, such as antioxidant activity, cytotoxicity, acyltransferase inhibition, anti-bacterial activity, antiviral activity, and antihypertensive effects [2, 3]. Flavonoids from *Ampelopsis cantoniensis* used to treat inflammatory diseases, such as rheumatic-arthritis, hepatitis, dermatitis, and gastritis [4].

In this paper, we report the results on isolation, structural determination, antioxidant activity, α-glucosidase and α-amylase inhibitory activity of five flavonoid compounds extracted from *Ampelopsis cantoniensis* collected in Quang Nam, Viet Nam.

2. MATERIALS AND METHODS

2.1. Materials

Ampelopsis cantoniensis were collected in Quang Nam, Viet Nam, in September 2017 and was identified by Dr. Nguyen Quoc Binh, Vietnam National Museum of Nature, VAST. The voucher specimen (CD92017) was deposited at Vietnam National Museum of Nature and University of Danang.

2.2. Methods

2.2.1. General analysis

NMR spectra were recorded on a Bruker Advance 500 III NMR spectrometer using TMS as an internal standard. ESI-MS spectra were recorded on LC-MS IonTrap, Agilent 1100 spectrometer. Column chromatography (CC) was performed on silica gel 230 - 400 mesh (0.040 - 0.063 mm, Merck) or YMC RP-18 resins (30 - 50 µm, Fujisilisa Chemical Ltd.). Thin-layer chromatography (TLC) was performed on DC-Alufolien $60F_{254}$ (Merck 1.05715) or RP_{18} F_{254}, (Merck) plates. Compounds were appeared by spraying with aqueous 10 % H_2SO_4 and heating for 5 minutes.

2.2.2. Antioxidant assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was measured following the method of Widowati *et al*. [5].

Percentage of DPPH radical scavenging activity (Sc) was calculated using equation:

$$Sc\ (\%) = (Ac - As) / Ac \times 100$$

Ac: negative control absorbance (without sample), As: sample absorbance. The results were expressed as SC_{50} values. SC_{50} values were calculated from dose-response curve by Table Curve program.

2.2.3. Inhibition assay for α-amylase activity

The α-amylase inhibition assay was performed by some modification in the method proposed by Manaharan *et al*. on 96 well plates [6]. Porcine pancreatic α-amylase (Sigma Type IV-B) was dissolved in ice-cold distiled water to give a concentration of 2 U/ml. The compounds of *A. cantoniensis* was dissolved in minimum amount of 10 % DMSO and was further dissolved in 20 mM photphate buffer at pH 6.9. The assay system, 80 µl of various dilutions of compound and 40 µl α-amylase solution was mixed and was incubated for 10 min at 37°C. Thereafter 40 µl of the starch solution (1 % w/v) was added and incubated for 10 min. The reaction was terminated by the addition of 80 µl DNSA reagent was added and solution incubated for 10 min in a water bath at 85 – 90 °C. The reaction mixture was removed from the water bath and cooled, thereafter α-amylase activity was determined by measuring the absorbance of the mixture at 540 nm using a Multiskan Sky microplate spectrophotometer. Acarbose solution at the concentrations was used as a positive control. The inhibitory activity was calculated as percentage inhibition:

$$\%Inhibition = \frac{A_{control} - A_{sample}}{A_{control}} \times 100\%$$

Each experiment was performed in triplicate. The concentration of the extract required to inhibit 50 % of α-glucosidase activity under the assay conditions was defined as the IC_{50} value.

2.2.4. Inhibition assay for α-glucosidase activity

The inhibition of α-glucosidase activity was determined by modifying the published method [7]. In a 96-well plate, reaction mixture containing 110 µl phosphate buffer (0.1 M, pH = 6. 9), 20 µl α -glucosidase (0.4 U/ml) and 20 µl of compounds or acarbose liquid at various concentrations. The mixed solution was incubated at 37 ^{o}C for 15 min. After pre-incubation, the enzymatic reaction was initiated by adding 20 µl of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) and the reaction mixture was incubated for another 15 min at 37 ^{o}C. The reaction was terminated by adding 80 µl of 0.2 M sodium carbonate solution, and the absorbance of the released ρ-NPG was measured at 405 nm. The percentage inhibition and IC_{50} value was determined as described above.

2.3. Flavonoid extraction and isolation

Ampelopsis cantoniensis (2.0 kg) was dried and grounded into powder then extracted with EtOH at room temperature (3 × 6L) and evaporated under reduced pressure to give EtOH extract (CCD, 446 g). This extract was suspended in water and then partitioned with *n*-hexane to obtain CDH (37 g), EtOAc (CDE, 180 g) and BuOH (CDB, 33 g) layers after removal of the solvents *in vacuo*. CDE layer (40 g) was chromatographed on a silicagel column and eluting with chloroform–acetone gradient (10: 3 → 10 : 5, v/v) to obtain six subfractions FCDE1 (1.5 g), FCDE2 (5.5 g), FCDE3 (5.0 g), FCDE4 (2.5 g) FCDE5 (6 g) and FCDE6 (9.0 g). FCDE2 was crystallized in chloroform-acetone (2/1, v/v) to yield **2** (100 mg). FCDE4 was chromatographed on a RP-18 column, eluting with acetone–water (6 : 1, v/v) to yield **4** (170 mg). FCDE5 was chromatographed on a silicagel column, eluting with $CHCl_3$-acetone (8:1, v/v) to give two fractions CDE5.1 (1.2 g) and CDE5.2 (1.0 g). CDE5.1 was chromatographed on a RP-18 column, eluting with methanol-water (2.5 : 1, v/v) to yield **5** (10.0 mg). FCDE6 was chromatographed on a Sephadex LH-20 column, eluting methanol to yield **1** (6.0 mg), and **3** (9.0 mg).

Myricitrin **(1):** yellow amorphous powder; ^{1}H and ^{13}C-NMR data is in Table 1; Positive ESI-MS found $[M+H]^{+}$ at *m/z* 464.9, negative ESI-MS $[M-H]^{-}$ at *m/z* 462.9. Myricetin **(2):** yellow needles; ^{1}H- and ^{13}C-NMR data is in Table 1; Positive ESI-MS found *m/z* 319 $[M+H]^{+}$, Negative ESI-MS $[M-H]^{-}$ at *m/z* 317. Quercetin **(3):** yellow needles; ^{1}H and ^{13}C-NMR data is in Table 1; ESI-MS contained $[M+H]^{+}$at *m/z* 302.9 and $[M-H]^{-}$ at *m/z* 300.8. Dihydromyricetin **(4):** white needles^{1}H and ^{13}C-NMR data is in Table 2; ESI-MS found $[M+H]^{+}$ at *m/z* 321 and $[M-H]^{-}$ ^{at} *m/z* 319. Phloretin **(5):** white amorphous powder; ^{1}H and ^{13}C-NMR data is in Table 2; ESI-MS contained $[M+H]^{+}$at *m/z* 274.9 and at *m/z* 272.8.

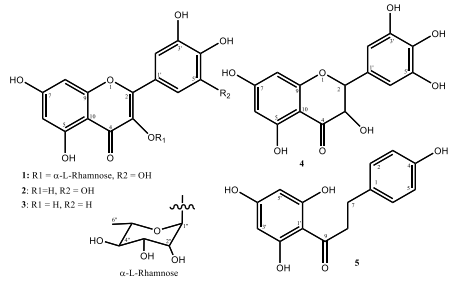

Figure 1. Structure of compounds **1** – **5**.

3. RESULTS AND DISCUSSION

3.1. Structure of isolated compounds

Compound **1** was obtained as yellow needles. Molecular formula was established as $C_{21}H_{20}O_{12}$ based on ESI-MS with the signal on positive mode $[M+H]^+$ at *m/z* 464.9, and negative mode $[M-H]^-$ at *m/z* 462.9.

Table 1. ^{1}H and ^{13}C-NMR data of compounds **1**-**3**.

		Compound 1			Compound 2			Compound 3	
	$^a\delta_C$	δ_C [b]	δ_H [b] (mult., *J* = Hz)	$^\beta\delta_C$	δ_C [d]	δ_H [d] (mult., *J* = Hz)	$^\alpha\delta_C$	δ_C [f]	δ_H [f] (mult., *J* = Hz)
	157.4	158.1		146.8	148.0		148.8	148.1	
	134.2	134.9		135.9	137.3		137.3	137.2	
	177.7	178.3		175.7	177.2		177.4	177.3	
	161.3	161.8		160.7	162.4		162.5	162.5	
	98.6	98.4	6.22 (1H, d, 2.0)	98.2	99.2	6.20 (1H, d, 2.0)	99.3	99.3	6.20 (1H, d, 2.0)
	164.2	164.5		164.1	165.5		165.6	165.6	
	93.5	93.3	6.38 (1H, d, 2.0)	93.2	94.3	6.40 (1H, d, 2.0)	94.5	94.4	6.41 (1H, d, 2.0)
	156.4	157.1		156.1	158.2		158.3	158.2	
10	104.0	104.5		102.8	104.5		104.6	104.5	
1'	119.6	120.6		120.7	123.1		124.2	124.2	
2'	107.9	108.2	6.97 (1H, s)	107.1	108.5	7.36 (1H, s)	116.0	116.0	7.75 (1H, d, 2.0)
3'	145.7	145.5		145.7	146.7		146.3	146.2	
4'	136.4	136.5		135.8	136.9		148.1	148.8	
5'	145.7	145.5		145.7	146.7		116.3	116.3	6.91 (1H, d, 8.5)
6'	107.9	108.2	6.97 (1H, s)	107.1	108.5	7.36 (1H, s)	121.7	121.7	7.65 (1H, dd, 2.5, 8.5)
1''	101.9	102.2	5.34 (1H, d, 1.5)						
2''	70.0	70.5	4.24 (1H, dd, 1.5, 2.0)						
3''	70.4	70.6	3.81 (1H, dd, 3.5, 9.5)						
4''	71.3	71.9	3.6 (1H, m)						
5''	70.6	70.7	3.53 (1H, m)						
6''	17.5	16.3	0.99 (3H, d, 6.0)						

$^a\delta_C$ – of myricitrin in DMSO – d_6 [7] , **[b]** in CD_3OD. $^\beta\delta_C$ – of myricetin in DMSO – d_6 [8], [d] in CD_3OD. $^\alpha\delta_C$ – of quercetin in CD_3OD [9], [f] in CD_3OD

^{1}H and ^{13}C-NMR (Table 1) suggested the presence of rhamnoside flavonoids. ^{1}H-NMR spectrum showed the signals: the *meta*-couple doublets at δ_H 6.22 ppm (1H; d; *J* = 2.0 Hz) and δ_H 6.38 ppm (1H; d; *J* = 2.0 Hz) were assigned to H-6 and H-8 of A ring flavonoid; singlet signal of aromatic proton has an integrate value of 2 at δ_H 7.36 (2H; s) was assigned to H-2' and H-

6' positions of B ring, respectively; one methyl proton of rhamnoside moiety at δ_H 0.99 (3H; d; *J* = 6.0 Hz); the signal of anomeric proton at δ_H 5.34 (1H; d; *J* = 1.5 Hz). ^{13}C-NMR spectrum exhibited 21 carbons: 1 carbonyl group; 6 methine; 1 methyl group; 3 oxygenated methine of sugar moiety and 10 quaternary carbons (see Table 1). HMBC correlations between H-6" (δ_H 0.99) and C-4" (δ_C 71.98), C-5" (δ_C 70.76); and between H-1" (δ_H 5.34) and C-3 (δ_C 134.93), C-3" (δ_C 70.64) indicated that the sugar linkaged at C-3 position. Based on NMR data and comparison with reported data [8], compound **1** was confirmed to myricitrin.

Compound **2** was obtained as yellow needles. ESI mass spectrum exhibited a positive ion $[M+H]^+$ at *m/z* 319.0 and negative ion $[M-H]^-$ at *m/z* 317.0, which is in agreement with molecular formula $C_{15}H_{10}O_8$. Except for the signal of the sugar moiety at C-3, ^{13}C-NMR spectrum showed fifteen carbon signals comprising one carbonyl carbon group, 4 methines, 10 quaternary carbons. ^{1}H-NMR spectrum exhibited a characteristic *meta*-coupled proton signal at δ_H 6.20 (1H, d, *J* = 2.0 Hz) and 6.40 (1H, d, *J* = 2.0 Hz) corresponding to H-6 and H-8 of flavonoid A ring. The other AX coupling system at δ 7.36 (2H, br, s) was assigned to H-2' and H-6' of B ring. Based on NMR data and comparison with literature [9], compound **2** was determined as myricetin.

Compound **3** was obtained as yellow needles. ESI-MS of **3** exhibited a positive ion $[M+H]^+$ at *m/z* 302.9 and negative $[M-H]^-$ at *m/z* 317.0, corresponding to molecular formula $C_{15}H_{10}O_7$. ^{1}H-NMR spectrum showed signals at δ_H 6.20 (1H, d, *J* = 2.0 Hz) and δ_H 6.41 (1H, d, *J* = 2.0 Hz) as doublets which are specific for the C-6 and C-8 protons of A ring. There are three proton signals of ABX system at δ_H 7.75 (1H, *d*, *J* = 2.0 Hz), δ_H 6.91 (1H, *d*, *J* = 8.5 Hz) and δ_H 7.65 (1H, dd, *J* = 2.5, 8.5 Hz). ^{13}C-NMR spectrum of compound **3** showed signals of fifteen carbons of the flavone skeleton. From NMR data and comparison with literature [8], compound **3** is quercetin.

Compound **4** was isolated as a white amorphous powder, molecular formula of **4** was established as $C_{15}H_{12}O_8$ based on ESI-MS with $[M+H]^+$ ion at *m/z* 321.0, and $[M-H]^-$ ion at *m/z* 319.0. NMR data of **4** were similar to **2** (Table 2), except for the chemical shift at C-2, C-3. ^{1}H-NMR spectrum showed methine signal δ_H 4.88 (1H, d, *J* = 11.0 Hz) and methine-oxygenated at δ_H 4.52 (1H, d, *J* = 11.0 Hz) corresponding to C-2 (δ_C 84.6) and C-3 (δ_C 72.9) in HSQC spectrum. ^{13}C-NMR spectrum of **4** contained fifteen carbon signals: 5 methine, oxygenated methine, carbonyl groups, 8 quaternary carbons. In addition, ^{1}H-NMR spectrum also showed the presence of two *meta*-coupled aromatic protons as doublets between δ_H 5.91(1H, d, 2.5 Hz) and δ_H 5.95(1H, d, 2.5 Hz), and two *meta*-coupled aromatic protons δ_H 6.59 (1H; s) corresponds to a pentahydroxyl flavanonol scaffold. All protons and carbons of compound **4** were assigned based on HSQC and HMBC spectra. Based on obtained NMR data and comparison with the reported data [10], compound **4** was determined as dihydromyricetin.

Compound **5** was isolated as a colorless powder. ESI-MS exhibited positive ion $[M+H]^+$ at *m/z* 272.9 and negative $[M-H]^-$ at *m/z* 272.8, which is in agreement with molecular formula $C_{15}H_{14}O_5$. ^{13}C-NMR spectrum showed fifteen carbon signals comprising one carbonyl carbon, 6 methines, 6 quaternary carbons, 2 methylene carbons. The ^{1}H-NMR spectrum data of **5** exhibited a characteristic four aromatic protons as doublets between δ_H 6.72 (2H, d, 8.5 Hz), and δ_H 7.07 (2H, d, *J* = 8.5 Hz), and two *meta*-coupled aromatic protons as singlet at δ_H 5.84 (2H, s). Besides, the ^{1}H-NMR spectra data also showed the presence of two methylene protons at δ_H 2.88 (2H, dd, 8.0, 7.5 Hz), and δ_H 3.33 (2H, dd, 8.0, 7.5 Hz). It suggested that there was an opening at C-2 position of flavanone skeleton. Based on NMR data and literature [11], the structure of **5** was determined as phloretin.

Table 2. ^{1}H and ^{13}C-NMR data of compounds **4**-**5**.

	$^{\mu}\delta_C$	Compound 4		C	$^{®}\delta_C$	Compound 5	
		δ_C [h]	δ_H [h] (mult., *J* = Hz)			δ_C [g]	δ_H [g] (mult., *J* = Hz)
	83.3	84.6	4.88 (1H, d, 11.0)	1	132.6	134.0	
	71.7	72.9	4.52 (1H, d, 11.0)	2	128.9	130.3	7.07 (1H, d, 8.5)
	197.7	198.0		3	114.7	116.1	6.72 (1H, d, 8.5)
	163.4	163.9		4	155.0	156.4	
	95.9	95.9	5.91 (1H, d, 2.5)	5	114.7	116.1	6.72 (1H, d, 8.5)
	166.8	168.1		6	128.9	130.3	7.07 (1H, d, 8.5)
	95.0	96.9	5.95 (1H, d, 2.5)	7	30.1	31.4	2.88 (2H, dd, 8.0, 7.5)
	162.6	164.8		8	45.9	47.2	3.33 (2H, dd, 8.0, 7.5)
10	100.5	101.3		9	205.0	206.4	
1’	127.2	128.7		1’	103.9	105.3	
2’	106.9	107.9	6.59 (1H, s)	2’	164.7	166.1	
3’	145.7	146.3		3’	94.3	95.7	5.84 (1H, s)
4’	133.5	134.3		4’	164.4	165.8	
5’	145.7	146.3		5’	94.3	95.7	5.84 (1H, s)
6’	106.9	107.9	6.59 (1H, s)	6’	164.7	166.1	

$^{\mu}\delta_C$ - of dihydromyricetin in DMSO-d6 [10], [h] in acetone-d6. $^{®}\delta_C$ – of phloretin in CD_3OD [11], [g] in CD_3OD

3.2. DPPH radical-scavenging activity

Table 3. Antioxidant activity of compounds **1**-**5**.

Compounds \ Concentration (μM)	Percentage of DPPH absorbance (%)					SC_{50} (μM/mL)
	5	10	25	50	100	
Acid ascorbic	25.66 ± 0.70	40.80 ± 1.65	50.72 ± 1.30	65.73 ± 0.68	81.20 ± 0.82	19.52 ± 0.70
Myricitrin	26.45 ± 1.40	41.27 ± 0.92	52.91 ± 1.54	65.08 ± 0.92	76.72 ± 1.06	19.64 ± 0.94
Myricetin	33.86 ± 1.40	46.03 ± 1.83	63.49 ± 0.92	74.07 ± 0.73	81.48 ± 0.53	12.50 ± 0.89
Quercetin	38.62 ± 1.16	53.44 ± 0.53	64.55 ± 0.73	74.60 ± 0.92	85.18 ± 1.60	9.42 ± 0.33
Dihydromyricetin	11.11 ± 1.59	29.63 ± 1.48	42.86 ± 0.98	56.62 ± 0.93	69.31 ± 0.53	35.37 ± 1.70
Phloretin	17.46 ± 0.82	32.27 ± 1.16	48.68 ± 0.78	56.62 ± 1.26	72.49 ± 1.06	29.56 ± 1.24

The results in Table 3 showed that the probability of radical scavenging proportional to the concentration of compound, the higher concentration, the greater likelihood of radical scavening and vice versa. Quercetin showed the highest activity of 85.18 %, while that of dihydromyricetin was 69.31 % at concentration of 100 μM. DPPH absorbance of quercetin at the concentration of 5 μM was the highest (38.62 %) and the lowest dihydromyricetin (11.11 %). The SC_{50} values were estimated from the graph (Fig. 2). The results showed that quercetin, phloretin, myricetin,

dihydromyricetin and myricitrin had significant activity in DPPH free radicals traps at the tested concentrations.

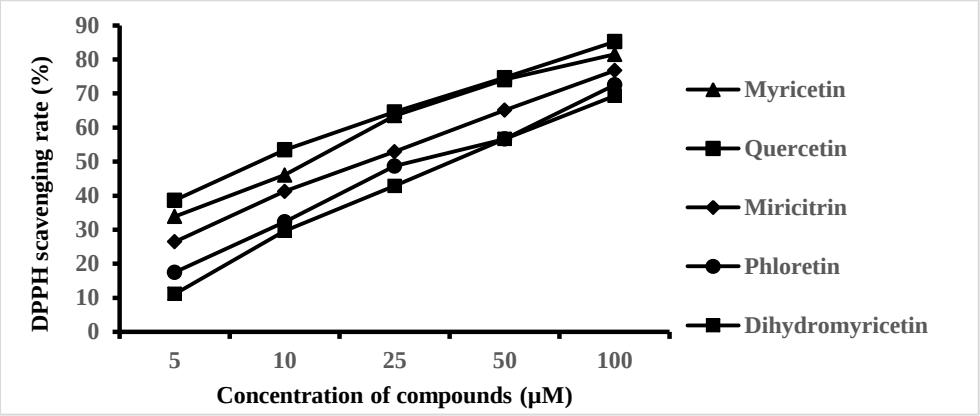

Figure 2. Effect of radical scavenging of compounds **1**-**5**.

3.3. α-glucosidase and α-amylase inhibitory activity

Among the isolate, myricitrin showed the most active α-amylase and α-glucosidase inhibitory activities with IC_{50} values of 9.6 ± 0.68 and 8.92 ± 0.35 µM, respectively. Myricetin, quercetin, phloretin showed moderate inhibitory effects against α-amylase when compared with those of a standard reference drug Acarbose (Table 4).

Table 4. IC_{50} values of isolated compounds against *α*-amylase and α-glucosidase.

Compounds	*α*-amylase (IC_{50} µM)	α-glucosidase (IC_{50} µM)
Acarbose	211.35 ± 4.305	187.41 ± 0.80
Myricetin	86.31 ± 4.91	9.20 ± 0.04
Dihydromyricetin	-	374.47 ± 2.69
Phloretin	199.11 ± 7.60	18.74 ± 0.07
Myricitrin	9.64 ± 0.68	8.92 ± 3.65
Quercetin	136.58 ± 6.77	10.64 ± 1.62

Myricetin, quercetin, and phloretin, also were inhibitors against α-glucosidase with IC_{50} of 9.20 ± 0.04, 10.64 ± 1.62 and 18.74 ± 0.07 µM, respectively. Dihydromyricetin exhibited a poor inhibitory activity or did not show any effect on both enzymes.

4. CONCLUSIONS

The isolation procedure performed on crude extract of *Ampelopsis cantoniensis* had led to obtain five flavonoids: myricitrin, myricetin, quercetin, dihydromyricetin, and phloretin. All five flavonoids had strong DPPH radical scavenging activity and four of them (myricetin, myricitrin, quercetin and phloretin) are α-glucosidase and α-amylase excellent inhibitors at varying effectiveness.

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Conflict statements: There is no conflict of interest.

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