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Flavonol glycoside constituents from water extract of *Cratoxylum prunifolium*

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***Abstract*—The leaves of *Cratoxylum prunifolium* have been used as herb tea in Thailand, China and Vietnam. Chemical investigation of *Cratoxylum* genus resulted a lots of xanthone compounds. However, flavonoid constituents have not been extensively focused. In this work, four flavonol glycoside were isolated from water extract of the leaves of *C. prunifolium*. Their chemical structures were determined to be kaempferol 3-O-α-L-arabinopyranoside (1), kaempferol 3-O-α-L-rhamnopyranoside (2), quercetin 3-O-β-D-glucopyranoside (3), and quercetin 3-O-α-L-arabinopuranoside (4) by HR-ESI-MS, NMR spectra, and as well as comparison with literature.**

Keywords*— *Kaempferol arabinoside; kaempferol rhamnoside, quercetin glucoside, quercetin arabinoside, Cratoxylum prunifolium.

I. INTRODUCTION

Cratoxylum genus (Hypericaceae family) is widely distributed in several Southeast Asian countries such as Thailand, Vietnam. They are flowering plants and leaves decoction of several *Cratoxylum* species have been used in traditional medicine for treatment of diuretic, stomachic, tonic effects, and for food poisoning [1, 2]. Leaves of *C. formosum* and *C. prunifolium* was known as herb tea in Thailand, China and Korea. In Vietnam, herb tea made from *C. prunifolium* leaves was expected to prevent risk of cardiovascular diseases. Phytochemical studies on *Cratoxylum* genus revealed the presence of xanthone, prenylated xanthone, and phenolics which exhibited potential antioxidant, anti-bacterial and anti-fungal activities [3-5]. To clarify chemical constituents of *C. prunifolium*, this work describes isolation and chemical structure elucidation of four flavonol glycosides from water extract of this plant.

II. MATERIALS AND METHODS

A. General experiment procedures

NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (Bruker BioSpin, Bremen, Germany) using TMS as an internal standard. High resolution electrospray ionization mass spectra were acquired on an Agilent 6530 Accurate Mass QTOF LC/MS system (Agilent technology, Santa Clara, CA, USA). Column chromatography was performed using silica gel (Merck, Whitehouse Station, NJ, USA) and reverse phase C18 resins (YMC Ltd., Kyoto, Japan). Thin layer chromatography was carried out using pre-coated silica gel 60 F_{254} (0.25 mm, Merck) and RP-C18 F_{254S} plates (0.25 mm, Merck). Spots were visualized under UV radiation (254 and 365 nm) and sprayed with aqueous solution of H_2SO_4 (10%), heating with a heat gun.

B. Plant materials

The leaves of *Cratoxylum prunifolium* were collected at Hoa Binh Province, Vietnam in August 2015. Its scientific name was identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, Hanoi, Vietnam. Voucher specimen is deposited at the Faculty of basic science, University of Transport and Communications.

C. Extraction and Isolation

The dried powdered *C. prunifolium* leaves (5.0 kg) was ultrasonically extracted with methanol in 4 hours (3 times, each 10 L, room temperature). After removal of methanol *in vacuo*, a dark solid methanol extract (450 g) was obtained. This extract was then suspended in 3 L of distilled water and successively partitioned with n-hexane, dichloromethane and ethyl acetate to give corresponding n-hexane extract, dichloromethane extract, ethyl acetate, and water layer. The water layer was passed through HP-20 diaion resins column chromatography (CC), washed with distilled water (2 L) and then eluted with increasing methanol in water (25%, 50%, 75%, and 100% methanol) to give four fractions W1-W4. Fraction W2 (80 g) was roughly separated on a silica gel column chromatography, eluting with gradient solvent system of dichloromethane/methanol (0-100% volume of methanol) to give teen fractions (W2A-W2K). Fraction W2B was chromatographed on a silica gel column, eluting with isocratic solvent system of dichloromethane/methanol/water (7/1/0.05, v/v/v) to give two fractions W2B1 and W2B2. Fraction W2B1 was subjected on a sephadex LH-20 CC, eluting with methanol/water (1/1, v/v) and then purified on a reverse phase C18 resins CC to give compounds **1** (57 mg) and **2** (138 mg). In a similar manner, fraction W2D was loaded on a silica gel CC and eluted with dichloromethane/methanol/water (5/1/0.1, v/v/v) to give sub-fractions W2D1-W2D3. Sub-fraction W2D2 was

first separated on a sephadex LH-20 CC eluting with methanol/water (1/1, v/v) and then further purified on a reverse phase C18 resins CC, eluting with methanol/water (1/2, v/v) to yield compounds **3** (210 mg) and **4** (33 mg).

- Kaempferol 3-O-α-L-arabinopyranoside (**1**): Molecular formula: $C_{20}H_{18}O_{10}$; Yellow amorphous powder; HR-ESI-MS *m/z*: 441.0787 $[M+Na]^+$ (calcd for $C_{20}H_{18}O_{10}Na$, 441.0798); ^{1}H-NMR and ^{13}C-NMR data are given in the Table 1 and Table 2.

- Kaempferol 3-O-α-L-rhamnopyranoside (**2**): Molecular formula: $C_{21}H_{20}O_{10}$; Yellow amorphous powder; HR-ESI-MS *m/z*: 445.0948 $[M+Na]^+$ (calcd for $C_{21}H_{20}O_{10}Na$, 445.0954); ^{1}H-NMR and ^{13}C-NMR data are given in the Table 1 and Table 2.

- Quercetin 3-O-β-D-glucopyranoside (**3**): Molecular formula $C_{21}H_{20}O_{11}$: Yellow amorphous powder; HR-ESI-MS *m/z*: 471.0894 $[M+Na]^+$ (calcd for $C_{21}H_{20}O_{10}Na$, 471.0903); ^{1}H-NMR and ^{13}C-NMR data are given in the Table 1 and Table 2.

- Quercetin 3-O-α-L-arabinofuranoside (**4**): Molecular formula $C_{20}H_{18}O_{11}$; Yellow amorphous powder; HR-ESI-MS *m/z*: 435.0920 $[M+H]^+$ (calcd for $C_{20}H_{19}O_{11}$, 435.0927), 457.0742 $[M+Na]^+$ (calcd for $C_{20}H_{18}O_{11}Na$, 457.0747); ^{1}H-NMR and ^{13}C-NMR data are given in the Table 1 and Table 2.

III. Results and Discussion

C. prunifolium leaves was extracted with methanol and successively separated with n-hexane, dichloromethane, ethyl acetate to give corresponding n-hexane, dichloromethane, ethyl acetate, and water residues. The water layer was subjected to various chromatographic technique to obtain four flavonol glycosides **1-4** (Fig. 1).

Compound **1** was isolated as a yellow amorphous powder. The molecular formula of **1**, $C_{20}H_{18}O_{10}$, was deduced by quasi molecular ion peak 441.0787 $[M+Na]^+$ (calcd for $C_{20}H_{18}O_{10}Na$, 441.0798) in the HR-ESI-MS and ^{13}C-NMR analysis. In the ^{13}C-NMR spectrum of **1** revealed signals of 20 carbons. Among them, signals of 15 sp^2-hybirdised carbons at δ_C 95.4-179.2 indicated that compound **1** to be a flavonoid. Remaining signal five carbinol carbons (δ_C 104.6, 74.1, 72.8, 69.0, 66.8) demonstrated a pentose sugar unit and characterized for an arabinopyranose moiety. The presence of a sugar unit was also indicated by a signal of anomeric proton at δ_H 5.11 (1H, d, *J* = 6.5 Hz). In addition, in the ^{1}H-NMR spectrum of **1**, an AX coupling spin system of a pair aromatic proton at δ_H 6.35 (1H, d, *J* = 2.5 Hz), 6.17 (1H, d, *J* = 2.5 Hz) were assigned for H-8 and H-6 of flavonoid meanwhile an AA′BB′ coupling pattern at δ_H 8.06 (2H, d, *J* = 8.5 Hz), 6.90 (2H, d, *J* = 8.5 Hz) indicated para-disubstituted benzene fragment of B-ring of flavonoid. Finally, HMBC correlation between anomeric proton δ_H 5.11 and C-3 (δ_C 135.5) confirmed a O-glycosidic linkage at C-3. Consequently, compound **1** was determined to be kaempferol 3-O- α-L-arabinopyranoside [6].

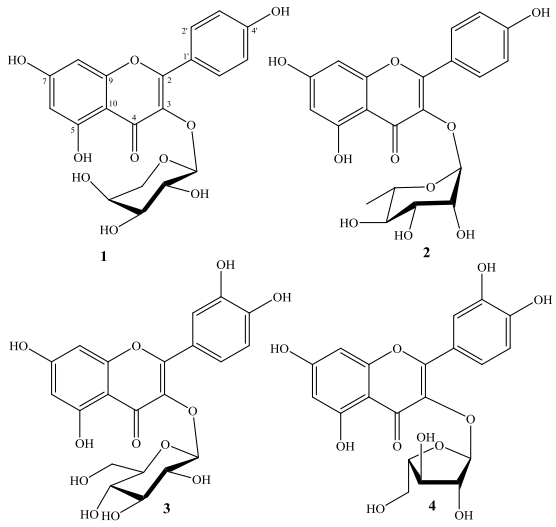

Fig. 1. *Chemical structure of compounds **1**-**4** isolated from water extract of C. prunifolium*

TABLE I. ^{13}C-NMR SPECTROSCOPIC DATA OF COMPOUNDS **1-4**

C	δ (ppm)			
	1[a]	**2**[b]	**3**[a]	**4**[b]
2	158.5	156.5	158.5	157.4
3	135.5	134.1	135.6	133.8
4	179.2	177.6	179.4	178.1
5	162.8	161.2	163.1	161.6
6	100.8	98.9	99.9	99.1
7	168.7	164.9	167.1	164.7
8	95.4	93.8	94.6	94.0
9	158.6	157.1	159.0	156.7
10	104.9	103.9	105.8	104.3
1′	122.7	120.5	123.3	121.3
2′	132.1	130.5	115.9	115.9
3′	116.3	115.4	145.9	145.5
4′	161.5	160.0	149.8	148.9
5′	116.3	115.4	117.6	116.0
6′	132.1	130.5	123.2	122.1
1″	104.6	101.8	104.4	108.2
2″	72.8	70.3	75.7	82.5
3″	74.1	70.6	78.1	77.3
4″	69.0	71.1	71.2	86.2
5″	66.8	70.1	78.3	61.0
6″		17.4	62.6	

Measured in [a]CD_3OD, [b]DMSO-d_6

TABLE II. 1H-NMR DATA OF COMPOUNDS **1**-**4**

H	δ_H (mult. *J* in Hz)			
	1[a]	**2**[b]	**3**[a]	**4**[b]
6	6.17 (d, 2.5)	6.18 (br s)	6.19 (d, 2.0)	6.17 (d, 2.0)
8	6.35 (d, 2.5)	6.38 (br s)	6.37 (d, 2.0)	6.38 (d, 2.0)
2′	8.06 (d, 8.5)	7.75 (d, 8.5)	7.72 (d, 2.0)	7.45 (d, 2.0)
3′	6.90 (d, 8.5)	6.91 (d, 8.5)	-	-
5′	6.90 (d, 8.5)	6.91 (d, 8.5)	6.86 (d, 8.0)	6.82 (d, 8.0)
6′	8.06 (d, 8.5)	7.75 (d, 8.5)	7.58 (dd, 8.0, 2.0)	7.52 (dd, 2.0, 8.0)
1″	5.11 (d, 6.5)	5.30 (br s)	5.24 (d, 7.2)	5.55 (br s)
2″	3.91 (dd, 6.0, 8.0)	3.98 (br s)	3.43 (dd, 7.2, 8.8)	4.12 (br s)
3″	3.65 (dd, 3.0, 8.0)	3.487 (dd, 3.0, 9.0)	3.49 (dd, 8.8, 8.8)	3.68 (br s)
4″	3.81 (m)	3.14 (dd, 9.0, 9.0)	3.35 (dd, 8.8, 8.8)	3.53 (m)
5″	3.82 (dd, 4.0, 13.5) 3.41 (dd, 3.0, 13.5)	3.10 (m)	3.25 (m)	3.28*
6″	-	0.80 (d, 6.0)	3.72 (dd, 2.4, 12.0) 3.59 (dd, 5.6, 12.0)	-

Measured in [a]CD_3OD, [b]DMSO-d_6

Compound **2** was obtained as a yellow amorphous powder. The HR-ESI-MS of **2** revealed quasi-molecular ion peak at *m/z*: 445.0948 $[M+Na]^+$, indicating its molecular formula to be $C_{21}H_{20}O_{10}$ (calcd for $C_{21}H_{20}O_{10}Na$, 445.0954). The ^{1}H- and ^{13}C-NMR of **2** recognized similar spectral pattern with those of **1**, suggesting compound **2** also to be a kaempferol glycoside. The difference between **2** and **1** were resonant signals of sugar moiety. The NMR spectra of compound **2** showed additional signal of a secondary methyl group at δ_H 0.80 (3H, d, *J* = 6.0 Hz) and δ_C 17.4. Moreover, specific carbon chemical shift of sugar moiety δ_C 101.8, 71.1, 70.6, 70.3, 70.1, 17.4 suggested it to be rhamnose, a hexose-type. The HMBC correlation between anomeric proton δ_H 5.30 (br s) and C-3 (δ_C 134.1) indicated location of O-α-L-rhamnopyranosyl moiety at C-3. Therefore, compound 2 was established to be kaempferol 3-O- α-L-rhamnopyranoside [7].

Compound **3** was also obtained as a yellow amorphous powder. Molecular formula of 3 was deduced to be $C_{21}H_{20}O_{11}$ from quasi-molecular ion peak at *m/z* 471.0894 $[M+Na]^+$ (calcd for $C_{21}H_{20}O_{10}Na$, 471.0903) in the HR-ESI-MS spectrum, indicating 11 indices of hydrogen deficiency. Likes **1** and **2** signals of 15 sp^2-hybridised carbons δ_C 94.7-179.5 indicated **3** was a flavonoid. However, in the ^{1}H-NMR spectrum of **3** appeared a pair signals of AX coupling protons at δ_H 6.19 (1H, d, *J* = 2.0 Hz)/ 6.37 (1H, d, *J* = 2.0 Hz) and a set signals of ABX coupling protons at δ_H 6.86 (1H, d, J = 8.0 Hz)/ 7.72 (1H, d, J = 2.0 Hz)/ 7.58 (1H, dd, J = 8.0, 2.0 Hz) suggested B-ring of flavonoid **3** to be a 1,3,4-trisubtituted benzene ring. In addition, carbon signals of sugar moiety δ_C 104.4, 75.7, 78.1, 71.2, 78.3, 62.6 indicated it to be a glucopyranoside moiety. The HMBC correlation between anomeric proton δ_H (5.24) and C-3 (δ_C 135.6) confirmed sugar moiety at C-3. Thus, compound **3** was determined to be quercetin 3- O-β-D-glucopyranoside [8].

Compound **4** had a molecular formula of $C_{20}H_{18}O_{11}$, showing a set of quasi molecular ion peaks at *m/z* 435.0920 $[M+H]^+$ (calcd for $C_{20}H_{19}O_{11}$, 435.0927) and 457.0742 $[M+Na]^+$ (calcd for $C_{20}H_{18}O_{11}Na$, 457.0747) in the HR-ESI-MS spectrum. Analysis of ^{1}H-NMR of **4** indicated that **4** was a quercetin glycoside, showing similar an AX and ABX aromatic proton coupling signals with those of **3**. On the other hand, molecular formula of **4**, $C_{20}H_{18}O_{11}$**,** suggested its sugar moiety containing five carbons. Furthermore, chemical shift of anomeric carbon observed at δ_C 108.2 together with three oxygenated methins δ_C 82.5, 77.3, 86.2 and an oxygenated methylene δ_C 61.0 indicating sugar moiety

of **4** to be arabinofuranose. The HMBC correlation between anomeric proton δ_H 5.55 and C-3 (δ_C 133.8) confirmed location of sugar moiety at C-3. Thus, compound **4** was established to be quercetin 3-α-L-arabinofuranoside [9].

In summary, four flavonol glycosides including kaempferol 3-O- α-L-arabinopyranoside, kaempferol 3-O- α-L-rhamnopyranoside, quercetin 3- O-β-D-glucopyranoside, quercetin 3-α-L-arabinofuranoside were successfully isolated from water extract of *C. prunifolium* leaves. Their chemical structures were approved by HR-ESI-MS, NMR analysis and good consistence with literature. Flavonoids are naturally compound class in the plant. They are reported having broad range of biological activities such as potential antioxidant, anti-inflammatory activities. The presence of flavonols in *C. prunifolium* may support for explanation of potential antioxidant of this plant.

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