Journal of Multidisciplinary Engineering Science and Technology (JMEST)
ISSN: 2458-9403
Vol. 4 Issue 10, October - 2017

Flavonoids and flavan-3-ol from aerial part of *Agrimonia pilosa* LEDEB.

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***Abstract*—Using various chromatography methods, three flavonoids, quercetin-3-O-rutinoside (1), quercetin-3-O-*β*-D-galactopyranoside (2), quercetin (3), and a flavan-3-ol, catechin (4) were isolated from methanol extract of *Agrimonia pilosa.* Their structures were elucidated by 1D- and 2D-NMR spectroscopic analyses and comparison with those reported in the literature. Compound 2 was reported from *A. pilosa* for the first time.**

Keywords—Agrimonia pilosa; flavonoid; quercetin-3-O-β-D-galactopyranoside; quercetin derivatives.

I. INTRODUCTION

Agrimonia pilosa Ledeb. (Rosaceae) is widely distributed in southwest of Vietnam, and has been used for treatment hemoptysis, hemorrhage, fever, diarrhea, and tuberculosis [1]. Recent study demonstrated that compounds from *Agrimonia pilosa* possessed variety bioactivities. Agrimoniin, a tannin, is a potent antitumor and the effect may be due to this tannin enhancing the immune response and a cytokine inducer [2, 3]. Four active phloroglucinol derivatives were isolated from the antimicrobial active extract of A. *pilosa* and the activity of this plant was dependent on the presence of phloroglucinol derivatives [4]. From the aqueous extract of the roots isolated an hepatoprotective isocoumarin, agrmonolide. This compound showed effects on both tacrine-incuded cytotoxicity in human liver-derived Hep G2 cells and tert-butyl hydroperoxide-induced cytotoxicity in rat with EC_{50} values of 88.2 ± 2.8 and 37.7 ± 1.6 μM, respectively. Moreover, compounds from *A. pilosa* showed neuroprotective effects, anti-inflammatory effects [5-9]. Chemical study to clarify active components were isolated quercetin-3-O-rutinoside (1), quercetin-3-O-*β*-D-galactopyranoside (2), quercetin (3), and a flavan-3-ol, catechin (4) from the methanol extract of *A. pilosa*.

II. MATERIALS AND METHODS

A. General experiment procedures

NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR) with TMS as the internal standard. Column chromatography (CC) 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.). Thin layer chromatography (TLC) was performed using pre-coated silica gel 60 F_{254} (0.25 mm, Merck) and RP-18 $F_{254}S$ 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 aerial parts of *A. pilosa* were collected at Trung Khanh, Cao Bang province, Vietnam in August 2013. Its scientific name was identified by Dr. Pham Thanh Huyen, Institute of Ecology and Biological Resources, VAST. A voucher specimen (6695A) is deposited at the Herbarium of Military Institute of Traditional Medicine.

C. Extraction and Isolation

The dried and powdered of aerial parts of A. pilosa (5.0 kg) were sonically extracted with methanol at 50 °C for three times (10.0 L each). After removal of the solvents, methanol extract (700.0 g) was suspended with distilled water (1.5 L) and successively partitioned with dichloromethane and ethyl acetate (three times, 2.0 L each) to give corresponding soluble extracts, dichloromethane (BAPD, 320.0 g), ethyl acetate (BAPE, 80.0 g), and water layer (BAPW). The BAPE extract (80.0 g) was separated on a silica gel column chromatography, eluting with gradient of dichloromethane/ methanol (100/0, 50/1, 30/1, 20/1, 10/1, 1/1, 0/100, v/v) to give seven fractions, BAP1A - BAP1G). Fraction BAP1D (10.0 g) was continuously separated on a RP-18 column chromatography, eluting with methanol/water (1/3, v/v) to give seven smaller fractions, BAP2A - BAP2G. Fraction BAP2D (2.0 g) was separated on a silica gel column chromatography, eluting with dichloromethane/methanol/water (4/1/0.1, v/v/v) to give three fractions, BAP3A - BAP3C. Fraction BAP3A (0.5 g) was purified on a RP-18 column chromatography, eluting with methanol/water (2/3, v/v) to yield compound **2** (40 mg). Fraction BAP2E was chromatographed on a RP-18 column, eluted with acetone/water (1/2, v/v) to yield 3 sub-fractions, BAP4A-BAP4C. The sub-fraction BAP4B was continuously separated on a RP-18 column, eluted with methanol/water (1/4, v/v) to obtain 2 smaller fractions, BAP4B1-BAP4B2. Compound **4** (7 mg) was obtained from BAP4B2 fraction (0.15 g) using a silica gel column, eluted with dichloromethane/methanol (8/1, v/v). Water layer was passed through a Diaion HP-20 column, washed with distilled water and

TABLE I. NMR DATA FOR COMPOUNDS **1** AND **2**

Comp	1			2			Comp	1			2		
	$^{\#}\delta_C$	$^{a,b}\delta_C$	$^{a,c}\delta_H$ (mult., J in Hz)	$^{\$}\delta_C$	$^{d,b}\delta_C$	$^{d,c}\delta_H$ (mult., J in Hz)		$^{\#}\delta_C$	$^{a,b}\delta_C$	$^{a,c}\delta_H$ (mult., J in Hz)	$^{\$}\delta_C$	$^{d,b}\delta_C$	$^{d,c}\delta_H$ (mult., J in Hz)
2	158.52	158.5	-	156.5	156.1	-	1″	104.69	104.7	5.12 (d, 7.5)	102.0	101.8	5.36 (d, 7.5)
3	135.62	135.6	-	133.7	133.4	-	2″	75.74	75.7	3.50 (dd, 7.5, 9.0)	71.3	71.2	3.58 (t, 9.0)
4	179.44	179.4	-	177.6	177.4	-	3″	78.20	78.2	3.43 (dd, 9.0, 9.0)	73.2	73.2	3.36 *
5	162.98	163.0	-	161.1	161.2	-	4″	71.42	71.4	3.28 *	67.9	67.9	3.65 (d, 2.0)
6	99.94	99.9	6.23 (brs)	98.8	98.7	6.18 (s)	5″	77.25	77.2	3.33 (m)	75.9	75.8	3.35 *
7	166.01	166.0	-	164.2	nd	-	6″	68.56	68.6	3.82 (br d, 11.0) 3.40 (dd, 5.0, 11.0)	60.2	60.1	3.33 *
8	94.87	94.9	6.42 (brs)	93.7	93.5	6.38 (s)	1‴	102.42	102.4	4.54 (brs)			
9	159.35	159.4	-	156.4	156.3	-	2‴	72.12	72.3	3.65 (brs)			
10	105.63	105.6	-	104.0	103.7	-	3″	72.26	72.1	3.56 (dd, 3.0, 9.0)			
1′	123.15	123.1	-	121.3	121.0	-	4‴	73.94	73.9	3.30 (dd, 9.0, 9.0)			
2′	117.69	117.7	7.69 (brs)	116.1	115.9	7.53 (brs)	5‴	69.71	69.7	3.47 (m)			
3′	145.84	145.8	-	144.8	144.8	-	6‴	17.87	17.9	1.14 (d, 6.0)			
4′	149.81	149.8	-	148.4	148.5	-							
5′	116.06	116.1	6.89 (d, 8.5)	115.3	115.3	6.81 (d, 8.5)							
6′	123.55	123.6	7.65 (brd, 8.5)	122.0	121.9	7.66 (dd, 1.5, 8.5)							

*) Overlapped signals.

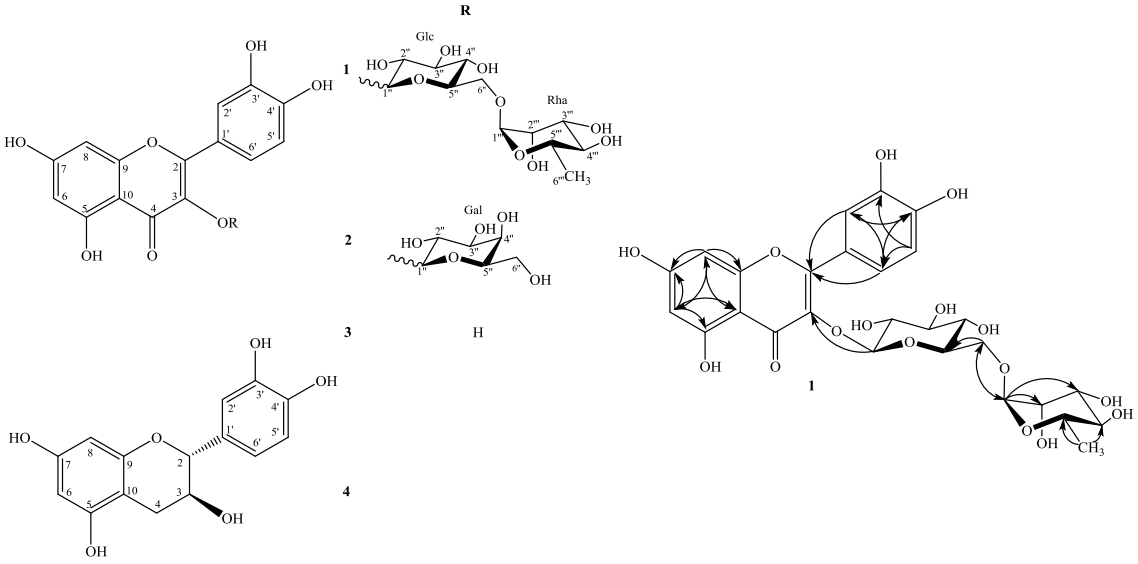

Fig1. Chemical structures of compounds **1**-**4** and key HMBC correlations of **1**

desorbed with methanol/water (25%, 50% and 100% volume of methanol, each 1.0 L stepwise) to give three fractions, BAP6A - BAP6C. Fraction BAP6B (5.0 g) was separated on a silica gel column chromatography eluting with dichloromethane/methanol (20/1, v/v) to give three fractions, BAP8A – BAP8C. Compound **3** (7 mg) was obtained from BAP8A fraction (0.2 g) using a Shephadex LH20 column, eluted with methanol/water (1/1, v/v). Fraction BAP8C (1.2 g) was chromatographed on a RP-18 column, eluting with methanol/water (1/3, v/v) to give compound **1** (10 mg).

- **Quercetin-3-O-rutinoside (1):** yellow amorphous powder, ESI-MS: *m/z* 633 $[M + Na]^+$, $C_{77}H_{30}O_{16}$ (M = 610). ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (CD_3OD, 125 MHz): see Table 1..

- **Quercetin-3-O-*β*-D-galactopyranoside (2):** yellow amorphous powder, ESI-MS: *m/z* 603 $[M + Na]^+$, $C_{18}H_{36}O_{13}$ (M = 580). ^{1}H-NMR (DMSO-d_6, 500 MHz) and ^{13}C-NMR (DMSO-d_6, 125 MHz): see Table 1.

- **Quercetin (3):** yellow amorphous powder, $C_{15}H_{10}O_7$ (M = 302). ^{1}H-NMR (DMSO-d_6, 500 MHz): 6.18 (1H, d, *J* = 2.0 Hz, H-6), 6.38 (1H, d, *J* = 2.0 Hz, H-8), 7.42 (1H, d, *J* = 2.0 Hz, H-2′), 6.88 (1H, d, *J* = 8.5 Hz, H-5′) and 7.64 (1H, d, *J* = 8.5, 2.0 Hz, H-6′). ^{13}C-NMR (DMSO-d_6, 125 MHz): 147.9 (C-2), 137.2 (C-3), 177.3 (C-4), 162.4 (C-5), 99.2 (C-6), 165.5 (C-7), 94.4 (C-8), 158.2 (C-9), 104.5 (C-10), 124.1 (C-1′), 116.0 (C-2′), 146.2 (C-3′), 148.7 (C-4′), 116.2 (C-5′) and 121.7 (C-6′).

- **Catechin (4)**: yellow amorphous powder, ESI-MS: *m/z* 291 $[M + H]^+$, $C_{15}H_{14}O_6$ (M = 290). ^{1}H-NMR (CD_3OD, 500 MHz): 4.58 (d, *J* = 7.5 Hz, H-2), 3.99 (m, H-3), 2.53 (dd, *J* = 5.0, 16.0 Hz, H-4a), 2.87 (dd, *J* = 8.0, 16.0, H-4b), 5.94 (d, *J* = 2.5 Hz, H-6), 5.87 (d, *J* = 2.5 Hz, H-8), 6.85 (d, *J* = 2.5 Hz, H-2′), 6.78 (d, *J* = 8.0 Hz, H-5′) and 6.73 (dd, *J* = 2.0, 8.0 Hz, H-6′). ^{13}C-NMR (CD_3OD, 125 MHz): 82.87 (C-2), 68.83 (C-3), 28.52 (C-4), 157.85 (C-5), 96.32 (C-6),157.58 (C-7), 95.52 (C-8), 155.92 (C-9),100.83 (C-10), 132.24 (C-1′), 115. (C-2′), 146.32 (C-3′), 146.32 (C-4′), 116.09 (C-5′) and 120.04 (C-6′).

III. Results and Discussion

Compound **1** was obtained as yellow amorphous powder. The ^{1}H-NMR showed three signals of a 1,3,4-trisubstituted benzene ring at 7.69 (1H, brs), 7.65 (1H, brd, *J* = 8.5 Hz) and 6.89 (1H, d, *J* = 8.5 Hz); two singlet olefinic protons at 6.42 (1H, brs) and 6.23 (1H, brs); and two anomeric protons at 5.12 (1H, d, *J* = 7.5 Hz) and 4.54 (1H, brs); and a secondary methyl at 1.14 (3H, d, *J* = 6.0 Hz). The ^{13}C-NMR showed 27 signals including 15 signals of quercetin aglycone, 12 signals of two glucose moieties. The protons were assigned to corresponding carbons with the aid of HSQC spectrum (Table 1). The signal of a oxymethylene carbon at 68.2 and a methyl carbon at 17.9 together with two anomeric carbons at 104.7 and 102.4 suggest the presence of a glucose moiety with substitution at C-6 and a rhamnose. The NMR data of **1** were close similar to those of quercetin-3-rutinoside (Table 1) [10]. The HMBC correlations from anomeric proton 5.12 (H-1″) to carbon 135.6 (C-3); from other anomeric proton 4.54 (H-1‴) to carbon 68.6 (C-6″) suggest the presence of α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranose oligosaccharide at position C-3. Considering these data, the structure of **1** was determined to be quercetin-3-rutinoside (or rutin), a flavonoid glycoside found in a wide variety of plants.

Compound **2** was also obtained as yellow amorphous powder. The NMR spectra of **2** were similar to those of **1** excepted the replacement of oligosaccharide in **1** by a monosaccharide in **2** (Table 1). The sugar was determined to be *β*-D-galactopyranoside with the presence of anomeric signal at 101.8/ 5.36 (1H, d, *J* = 7.5 Hz), three oxymethine signals, and oxymethylene signal at 60.1 / 3.33 (2H, overlapped). Based on the spectra evidence and in comparison with previous reported data, compound **2** was determined as quercetin-3-O-*β*-D-galactopyranoside, a compound isolated from *Bidens sulphurea* [11]. In addition, ESI-MS analysis of 2 was also suitable with a molecular formula of $C_{18}H_{36}O_{13}$, by an ion peak at *m/z* 603.

The remaining compounds **3** and **4** were determined as quercetin and catechin, respectively, based on their NMR spectra and in comparison with the previously reported data [12].

In the present study, three flavonoids, quercetin-3-O-rutinoside (**1**), quercetin-3-O-*β*-D-galactopyranoside (**2**), quercetin (**3**), and a flavan-3-ol, catechin (**4**) were isolated from the methanol extract of *A. pilosa*. Absorption and metabolism of quercetin and its derivatives has attracted much attention in relation to their pro-healthy value. Quercetin 3-O-glucosides and rutin contributed to the relaxation of smooth muscles in mammals. Rutin has anti-inflammatory effects which are displayed mostly in respect of chronic diseases and also display chemopreventive properties, acting as an agent blocking carcinogenesis induced by heterocyclic amines [13].

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