Journal of Multidisciplinary Engineering Science and Technology (JMEST)
ISSN: 2458-9403
Vol. 7 Issue 3, March - 2020

Isolation of flavonoids from the leaves of *Schisandra perulata*

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***Abstract*—*Schisandra perulata* is a medicinal plant in Vietnam. It produces edible fruits. Its leaves have been used in traditional medicine with tonic property. Phytochemical studies on *Schisandra* genus revealed a lot of nortriterpenes and phenolic compounds in type of lignans and flavonoids. This work describes identification of five flavonoids from the leaves of *S. perulata* including nicotiflorin (1), hyperin (2), isoquercetin (3), catechin (4), and epicatechin (5). Their chemical structures were determined by ESI-MS, NMR spectral data, and comparison with those reported in the literature.**

Keywords*— *Nicotiflorin; Kaempferol glycoside; quercetin glycoside; flavan; Schisandra perulata.

I. INTRODUCTION

Schisandra genus comprises around 30 species and notably distributes in the North America and the East Asia. Several *Schisandra* species were used in the oriental medicines such as *S. chinensis* [1], *S. sphenanthera* [2], *S. henryi*, and *S. rubriflora* [3]. They are known in traditional medicines with the valuable properties such as hepatoprotective, anti-inflammation, antiulcer, antioxidant, tonic and restorative properties [3]. Chemical studies indicate that lignans and triterpenoids are the most common secondary metabolites for the genus *Schisandra*. Approximately 150 lignan derivatives having dibenzocyclooctadiene skeleton were isolated from *Schisandra* species [4]. The triterpenes from *Schisandra* species were reported in three subgroups including lanostane-type triterpenes, cycloartane-type triterpenes, and nortriterpenes [5, 6]. The *Schisandra* nortriterpenes were highlighted by unique structure which are rarely found in natural source. The *S. perulata* species is a medicinal plant and considered as a pandemic plant in Vietnam [7]. Very little chemical study on this plant has been documented up to date. In our course to study chemical constituent of *Schisandra* species, this paper reports the isolation and identification of five flavonoids from the leaves of *S. perulata*.

II. MATERIALS AND METHODS

A. General experiment procedures

NMR spectra were recorded on a Bruker AVANCE 500 MHz (Bruker BioSpin, Bremen, Germany). ESI-MS

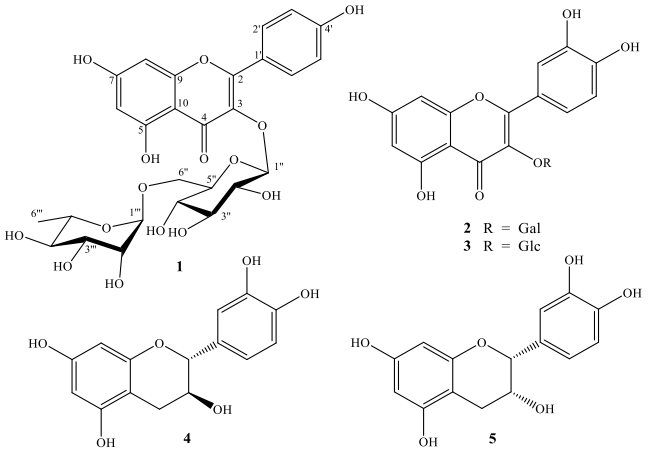

Fig. 1. *Structure of flavonoids **1**-**5** from the leaves of Schisandra perulata*

were obtained from an Agilent 6530 Accurate Mass QTOF LC/MS system (Agilent technology, Santa Clara, CA, USA). Flash Column chromatography was carried out using silica gel (Merck, Whitehouse Station, NJ, USA), sephadex LH20 (GE Healthcare, Uppsala, Sweden), and reverse phase C18 resins (YMC Ltd., Kyoto, Japan) as stationary phase. Thin layer chromatography was performed using pre-coated silica gel 60 F_{254} and RP-C18 F_{254S} plates (Merck, Darmstadt, Germany). Compounds were detected by UV radiation (254 nm) and by spraying with aqueous solution of H_2SO_4 solution (5%, w/w) followed by heating on a hot plate.

B. Plant materials

The plant materials were collected at Lao Cai Province, Vietnam in December of 2018. Its scientific name, *Schisandra perulata* Gagnep., was determined by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, Hanoi, Vietnam. Voucher specimen (NCCT-P81) is kept at the Institute of Marine Biochemistry, Hanoi, Vietnam.

C. Extraction and isolation

The dried leaves of *S. perulata* was pulverized and extracted with methanol. The methanol extract (200 g) was suspended with water (2 L) and partitioned in turn with dichloromethane and ethyl acetate. The ethyl acetate soluble fraction (35 g) was load on a silica gel column chromatography and eluted with gradient solvent system of dichloromethane and methanol (40/1, 20/1, 10/1, 5/1, 3/1, 1/1, v/v) to give six fractions SPE1-SPE6. Fraction SPE2 was chromatographed on

a silica gel column, eluting with hexane/acetone (2/1, v/v) to give five fractions SPE3A-SPE3E. Further purification fraction SPE3B obtained compounds **4** (38 mg) and **5** (21 mg) using reverse phase C-18 column chromatography and methanol/water (1/3, v/v) as eluent. Fraction SPE4 was separated on a silica gel column chromatography, eluting with dichloromethane/methanol/water (5/1/0.1, v/v/v) to give four fractions SPE4A-SPE4D. Fraction SPE4B and SPE4C were purified on a sephadex LH20 column chromatography, eluting with methanol/water (1/2, v/v) to yield compounds **3** (47 mg) and **2** (65 mg), respectively. Compound **1** (18 mg) was isolated from fraction SPE4D by reverse phase C-18 column chromatography with acetone/water (1/3, v/v) as an eluent.

- Nicotiflorin (**1**): Molecular formula: $C_{27}H_{30}O_{15}$; Yellow amorphous powder; ESI-MS *m/z*: 617 $[M+Na]^+$; ^{1}H-NMR and ^{13}C-NMR data are given in the Table 1 and Table 2.

- Hyperin (**2**): Molecular formula: $C_{21}H_{20}O_{12}$; Yellow amorphous powder; ESI-MS *m/z*: 487 $[M+Na]^+$; ^{1}H-NMR and ^{13}C-NMR data are consisted with literature [8].

- Isoquercetin (**3**): Molecular formula $C_{21}H_{20}O_{12}$: Yellow amorphous powder; ESI-MS *m/z*: 487 $[M+Na]^+$; ^{1}H-NMR and ^{13}C-NMR data are given in the Table 1 and Table 2.

- Catechin (**4**): Molecular formula $C_{15}H_{14}O_6$; Yellow amorphous powder; ESI-MS *m/z*: 291 $[M+H]^+$; ^{1}H-NMR and ^{13}C-NMR data are given in the Table 1 and Table 2.

- Epicatechin (**5**): Molecular formula $C_{15}H_{14}O_6$; Yellow amorphous powder; ESI-MS *m/z*: 291 $[M+H]^+$; ^{1}H-NMR and ^{13}C-NMR data are given in the Table 1 and Table 2.

III. Results and Discussion

The leaves of *S. perulata* was extracted with methanol and successively separated with dichloromethane and ethyl acetate. The ethyl acetate soluble fraction usually expected rich of flavonoids which was then attempted to isolation using combination of chromatographic methods to give five compounds **1-5** (Fig. 1).

Compound **1** was isolated as a yellow amorphous powder. The ^{1}H-NMR spectrum of **1** showed six aromatic protons with an AX coupled system [δ_H 6.23 and 6.42 (each 1H, d, *J* = 2.0 Hz)] and an AA′BB′ coupled system [δ_H 6.91 and 8.08 (each 2H, d, *J* = 9.0 Hz)] and two anomeric protons [δ_H 5.14 (1H, d, J = 7.5), 4.54 (1H, br s)]. The ^{13}C-NMR spectrum of **1** observed signals of 27 carbons including a carbonyl carbon (δ_C 179.4), 14 olefinic carbons and two anomeric carbons (δ_C 94.9~166.2), 9 carbinol carbons (δ_C 68.5~78.1), and one methyl group (δ_C 17.9). Above NMR data suggested that compound 1 to be a flavone

TABLE I. ^{13}C-NMR SPECTROSCOPIC DATA OF COMPOUNDS **1-5**

C	δ_C (ppm)			
	1	**2**	**4**	**5**
2	158.5	158.5	82.9	79.8
3	135.5	135.8	68.8	67.4
4	179.4	179.5	28.5	29.1
5	163.0	163.0	157.6	157.5
6	100.0	100.0	96.4	96.4
7	166.2	166.4	157.9	157.7
8	94.9	94.8	95.6	95.9
9	159.4	158.9	156.9	157.2
10	105.6	105.5	100.9	100.1
1′	122.7	122.9	132.3	132.2
2′	132.3	117.8	115.3	115.3
3′	116.1	145.8	146.3	145.7
4′	161.5	150.0	146.3	145.8
5′	116.1	116.1	116.1	115.9
6′	132.3	122.9	120.0	119.4
1″	104.6	105.5		
2″	75.7	73.2		
3″	78.1	75.1		
4″	71.4	70.0		
5″	77.2	77.2		
6″	68.5	62.0		
1‴	102.4			
2‴	72.0			
3‴	72.3			
4‴	73.9			
5‴	69.7			
6‴	17.9			

Measured in CD_3OD, 125 MHz.

glycoside. Particularly, the presence of an AX and and AA′BB' coupled aromatic protons indicated kaempferol backbone flavonoid. A broad singlet anomeric proton (δ_H 4.54) and a doublet proton signal of methyl group (δ_H 1.14) suggested for a α-L-rhamnopyranosyl moiety. Anomeric carbon at δ_C 104.6 and five oxygenated carbon δ_C 78.1, 77.2, 75.7, 71.4, 68.5 indicated for a glucopyranosyl group. Furthermore, *J* coupling constant of anomeric proton Glc H-1″ (*J* = 7.5 Hz) was expected for β-D-glucopyranosyl group. Connection between sugar units and aglycone were further clarified by HMBC spectrum. The HMBC correlation between between Rham H-1‴ (δ_H 4.54) and Glc C-6″ (δ_C 68.5) revealed connection of α-L-rhamnopyranosyl group at C-6″ of glucose moiety to form rutinose disaccharide fragment. The HMBC correlation between Glc H-1 (δ_H 5.14) and C-3 (δ_C 135.5) indicated *O*-glycosidic linkage of disaccharide moiety at C-3 of kaempferol aglycone. Thus, compound **1** was determined as kaempfrerol 3-*O*-rutinoside. This compound was reported abundantly in the flowers of *Nicotiana* species and called nicotiflorin [9]. However, this is the first time that nicotiflorin is isolated from *Schisandra perulata* species. The NMR data of **1** was well agreed with those reported for nicotiflorin in the literature [10].

Compound **2** was isolated as yellow amorphous powder. Like compound **1**, the ^{1}H- and ^{13}C-NMR spectra of **2** characterized for a flavone glycoside. In the ^{1}H-NMR spectrum of **2**, signal of an ABX coupled

TABLE II. ^{1}H-NMR DATA OF COMPOUNDS **1**-**5**

H	δ_H (mult. *J* in Hz)		H	δ_H (mult. *J* in Hz)	
	1	**2**		**4**	**5**
6	6.23 (d, 2.0)	6.22 (d, 1.5)	2	4.57 (d, 7.5)	4.82 (br s)
8	6.42 (d, 2.0)	6.38 (d, 1.5)	3	3.99 (m)	4.29 (m)
2′	8.08 (d, 9.0)	7.85 (d, 1.5)	4	2.53 (dd, 16.0, 8.5)	2.76 (dd, 2.5, 17.0)
3′	6.91 (d, 9.0)	-		2.88 (dd, 16.0, 5.5)	2.88 (dd, 4.5, 17.0)
5′	6.91 (d, 9.0)	6.88 (d, 8.0)	6	5.87 (d, 2.5)	5.95 (br s)
6′	8.08 (d, 9.0)	7.61 (dd, 8.0, 1.5)	8	5.94 (d, 2.5)	5.98 (br s)
1″	5.14 (d, 7.5)	5.17 (d, 8.0)	2′	6.86 (d, 1.5)	7.00 (d, 1.5)
2″	3.45 (dd, 7.5, 9.0)	3.49 (dd, 9.0, 8.0)	5′	6.77 (d, 8.0)	6.79 (d, 8.5)
3″	3.43 (m)	3.56 (br d, 9.0)	6′	6.74 (dd, 8.0, 1.5)	6.82 (dd, 8.5, 1.5)
4″	3.26 (t, 9.0)	3.84 (br s)			
5″	3.36 (m)	3.41 (m)			
6″	3.40 (m)	3.66 (dd, 12.0, 5.0)			
	3.83 (m)	3.84 (dd, 12.0, 2.0)			
1‴	4.54 (br s)				
2‴	3.65 (br d, 3.5)				
3‴	3.54 (dd, 3.5, 9.5)				
4‴	3.30 (t, 9.5)				
5‴	3.47 (m)				
6‴	1.14 (d, 6.0)				

Measured in CD_3OD, 500 MHz.

aromatic protons [δ_H 7.85 (1H, d, *J* = 1.5 Hz), 7.61 (1H, dd, *J* = 8.0, 1.5 Hz), 6.88 (1H, d, *J* = 8.0 Hz)] suggested for the presence of quercetin backbone instead of kaempferol backbone as in the structure of compound **1**. Additionally, one anomeric proton [δ_H 5.17 (1H, d, J = 8.0 Hz)] and six oxygenated carbons (δ_C 105.5, 77.2, 75.1, 73.2, 70.0, 62.0) indicated for a hexose unit in the sugar moiety of **2**. The broad singlet multiplicity of H-4″ suggested that sugar moiety to be galactose. Moreover, *J* coupling constant of anomeric proton (*J* = 8.0 Hz) also indicated for *β*-D-galactopyranosyl group. Finally, HMBC correlation between Gal H-1″ (δ_H 5.17) and C-3 (δ_C 135.8) revealed location of *β*-D-galactopyranosyl group at C-3. Therefore, compound **2** was identified as quercetin 3-O-β-D-galactopyranoside. This compound also has trivial name as hyperin [11]. The NMR data of **2** was consisted with that reported the literature [11, 12].

The NMR data of compound **3** was similar with that of **2** except resonant signals corresponding for *β*-D-glucopyranosyl group instead of *β*-D-galactopyranosyl group in compound **2**. This compound was then determined as quercetin 3-O-β-D-glucopyranoside. A compound had trivial name as isoquercetin and previously isolated from *C. prunifolium* in one of our report [8].

Compound **4** was isolated as yellow amorphous powder. In the ^{13}C-NMR spectrum of **4**, signal of 15 carbons including 12 sp^2 hybridized carbons (δ_C 96.4~157.9) and three sp^3 hybridized carbons (δ_C 82.9, 68.8, 28.5) suggested that compound **4** to be a flavan. A pair of AX coupled aromatic protons [δ_H 5.87 (1H, d, *J* = 2.5 Hz) and 5.94 (1H, d, *J* = 2.5 Hz)] in the ^{1}H-NMR spectrum were typically assigned for H-6 and H-8 of a flavan. Three aromatic protons showing ABX coupled system [δ_H 6.86 (1H, d, *J* = 1.5 Hz), 6.77 (1H, d, *J* = 8.0 Hz), and 6.74 (1H, dd, *J* = 8.0, 1.5 Hz)] indicated B-ring of flavan to be 1,3,4-trisubstituted benzene ring. HMBC correlations between H-2′ (δ_H 6.86)/ H-6′ (δ_H 6.74) and C-4′ (δ_C 146.3)/C-2 (δ_C 82.9), H-5′ (δ_H 6.77) and C-3′ (δ_C 146.3) confirmed the presence of hydroxy groups at C-3′ and C-4′. Doublet signal of H-2 (δ_H 4.57), HMBC correlation of H-3 (δ_H 3.99)/C-1′ (δ_C 132.3), and carbon chemical shift of C-3 (δ_C 68.8) indicated location of hydroxy group at C-3. Remaining methylene signal (δ_C 28.5 /δ_H 2.53 and 2.88) was assigned for C-4. Because the large of *J* coupling constant between H-2 and H-3 (*J* = 7.5 Hz), relative configuration between H-2 and H-3 was deduced to be *axial-trans*. Consequently, structure of compound **4** was determined as catechin, a well-known flavan compound naturally occurring in *Camellia* species. The NMR spectra data of **4** was agreed with that of catechin in the previous reports [13, 14].

The ^{1}H- and ^{13}C-NMR spectral data of compound **5** were recognized in close similarity with those of compound **4** except slight difference in resonant signals at C-2, C-3, and C-4. This evidence indicated that compounds **4** and **5** are a pair of stereoisomers. Difference with compound **4**, broad singlet proton signal of H-2 (δ_H 4.82) suggested *cis* relative orientation between H-2 and H-3. Thus, compound **5** was identified as epicatechin. Its NMR data was compared and found in good agreement with reported data [13, 14].

In summary, five flavonoids including nicotiflorin (**1**), hyperin (**2**), isoquercetin (**3**), catechin (**4**), and epicatecin (**5**) were isolated from the leaves of *Schisandra perulata*. Their chemical structures were determined by ESI-MS, 1D- and 2D-NMR spectral data, and comparison with those reported in the literature. The identification of those flavonoids in *S. perulata* may support for explanation of potential

antioxidant and anti-inflammatory properties of this medicinal plant.

ACKNOWLEDGMENT

This research was supported by Ministry of Education and Training under grant number B2019-GHA-562-01.

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