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RESEARCH ARTICLE OPEN ACCESS

Quercetin derivatives of the leaves of *Magnolia Lamdongensis*

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ABSTRACT
Three flavonoid glucosides and a sterol were isolated for the first time from the methanol extract of the leaves of *Magnolia lamdongensis* collected in Lamha district, Lamdong province, Vietnam including (1) astragalin, (2) quercetin 3-neohesperidoside, (3) quercetin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside, and (4) stigmasterol. The structure of these compounds was confirmed by 1 D and 2 D NMR experiments and a comparison with those reported .

Keywords - *Magnolia lamdongensis*, astragalin, quercetin 3-neohesperidoside, quercetin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside, stigmasterol

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I. INTRODUCTION

Magnolia L. is a genus belonging the subfamily Magnoliaceae, which was established in 1753 by Linnaeus, based on *Magnolia virginiana* L. It contains approximately 250 species and is widely distributed in the tropical, subtropical, and warm-temperate regions of Asia and America [1]. Some species of the family Magnoliaceae have been used in indigenous medicine in many countries for thousands of years. *M. officinalis* has long been used as a remedy for flatulent dyspepsia, cough, and asthma in terms of pharmacology or bioactivities [2]. *M. liliflora* flower buds have been used in Chinese traditional medicine to treat rhinitis, headaches, chills, and low blood pressure [3].

Magnolia lamdongensis T.V. Tien, N.V. Duy & N.H. Xia, sp. nov. belonging to the genus *Magnolia* were reported in 2015 but there have not been any studies on chemical composition and biological activity. In this paper, the isolation and structural elucidation of four known compounds **1-4** from the leaves of *M. lamdongensis* were reported. This is the first report about isolated compounds from this plant.

II. EXPERIMENTS

2.1. General experimental procedures

NMR spectra were recorded on a Bruker Avance Neo 600 MHz spectrometer using TMS as an internal standard. ESI-MS were measured on an Agilent 1100 LC/MS system. Column chromatography (CC) was carried out on silica gel (230-400 mesh, Merck), C18 reversed-phase silica gel (100 Å pore size, Fluka), and Sephadex LH-20 gel (25-100 µm, Pharmacia Fine Chemical Co. Ltd.). Thin-layer chromatography (TLC) used pre-coated silica gel 60 F_{254} (1.05554.0001, Merck) and RP-18 F254S plates (1.15685.0001, Merck), and compounds were visualized by spraying with 10% aqueous H_2SO_4 and heating for 3–5 min.

2.2. Plant material

The samples of the plant *Magnolia lamdongensis* were collected at Phu Son slope, Lamdong, Vietnam, in September 2020 and identified by Dr. Nong Van Duy from the Tay Nguyen Institute for Scientific Research, VAST. A voucher specimen (TN3/163) was deposited at the Tay Nguyen Institute for Scientific Research, VAST.

2.3. Extraction and isolation

The air-dried and powdered leaves of *M. lamdongensis* (2.0 kg) were extracted three times with methanol at room temperature. The methanol solutions were filtered, combined, and concentrated

under reduced pressure to obtain methanol residue (267 g). This was suspended in water (2L) and partitioned in turn with *n*-hexane, chloroform, and ethyl acetate to give the corresponding extracts: *n*-hexane (H, 15.0 g), $CHCl_3$ (C, 20.8 g), EtOAc (E, 19.9 g), and water layer (W, 2L).

The extract E (19.9 g) was subjected to chromatography on a silica gel column with stepwise gradient elution of $CHCl_3$/MeOH (from 1:0 to 0:1, v/v) to yield 6 subfractions E1-E8. Subfraction E8 (1.4 g) was further separated by sephadex LH-20 CC with MeOH/H_2O (1:1-1:0, v/v) to give four subfractions E8A-E8D. Subfraction E8D (72 mg) was separated by silica gel CC eluting with $CHCl_3$/MeOH/H_2O (3:1:0.1, v/v/v) and purified by RP-18 column using MeOH/H_2O (3:1.5, v/v) as elution to yield compound **1** (6 mg).

The water layer passed through Diaion HP-20 CC and eluted first with water and then with MeOH/H_2O (0:1-1:0, v/v) to obtain five fractions, W1-W5. Fraction W2 (11.8 g) was further separated by column chromatography on silica gel CC using a mixture of $CHCl_3$/MeOH (3:1, v/v) to afford nine subfractions, W2A-W2I. Subfraction W2H (2.6 g) was fractionated by Sephadex LH-20 CC with MeOH/H_2O (1:1, v/v) to yield three subfractions, W2H1-W2H3. Subfraction W2H3 (82 mg) was subjected to chromatography on the RP-C18 column eluted with MeOH/H_2O (3:2, v/v) to yield compounds **2** (7 mg) and **3** (6 mg).

The extract H (15.0 g) was separated on silica gel CC with stepwise gradient elution of $CHCl_3$/MeOH (1:0-0:1, v/v) to yield seven fractions, H1-H7. Fraction H5 (2.8 g) was fractionated by Sephadex LH-20 CC with stepwise gradient elution MeOH/H_2O (4:1-1:0, v/v) to yield six subfractions, H5A-H5F. Subfraction H5F (98 mg) was purified by the silica gel column eluted with CH_2Cl_2:acetone (20:1, v/v) to yield compound **4** (35 mg).

Astragalin (1): Yellow powder; molecular formula $C_{21}H_{20}O_{11}$; ESI-MS *m/z* 447.5 $[M-H]^-$; ^{1}H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) see table 1.

Quercetin 3-neohesperidoside (2): Yellow powder; molecular formula $C_{27}H_{30}O_{16}$; ESI-MS *m/z* 611.4 $[M+H]^+$; ^{1}H NMR (600 MHz, CD_3OD) and 13C NMR (150 MHz, CD_3OD) see table 1.

Quercetin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside (3): Yellow powder; molecular formula $C_{27}H_{30}O_{16}$; ESI-MS *m/z* 611.5 $[M+H]^+$; ^{1}H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) see table 1.

Stigmasterol (4): White needle; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H: 5.35 (1H, brd, *J* = 3.5 Hz, H-6), 5.16 (1H, dd, *J* = 8.5, 15.0 Hz, H-20), 5.02 (1H, dd, *J* = 8.5, 15.0 Hz, H-21), 3.52 (1H, tdd, *J* = 5.5, 11.5, 16.5 Hz, H-3), 2.29 (1H, dd, *J* = 2.5, 8.0 Hz, H-4_a), 2.23 (1H, dd, *J* = 2.0, 11.0 Hz, H-4_b), 2.04 (1H, m, H-18), 2.00 (1H, m, H-12_a), 1.99 (1H, m, H-12_b), 1.85 (1H, m, H-1_a), 1.83 (1H, m, H-7_a), 1.70 (1H, m, H-16_a), 1.55 (1H, m, H-15_a), 1.54 (1H, m, H-8), 1.53 (1H, m, H-22), 1.49 (1H, m, H-7_b), 1.48 (2H, m, H-2_b, H-11_a), 1.46 (1H, m, H-11_b), 1.44 (1H, m, H-25), 1.42 (1H, m, H-23_a), 1.26 (1H, m, H-16_b), 1.16 (1H, m, H-23_b), 1.14 (1H, m, H-17), 1.09 (1H, m, H-1_b), 1.02 (3H, d, *J* = 6.5 Hz, H-26), 1.01 (1H, s, H-27), 0.99 (1H, m, H-14), 0.92 (1H, m, H-9), 0.84 (3H, d, *J* = 6.5 Hz, H-19), 0.80 (3H, s, H-29), 0.79 (3H, s, H-28), 0.70 (3H, s, H-24). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C: 37.29 (C-1), 31.93 (C-2), 71.83 (C-3), 42.25 (C-4), 140.79 (C-5), 121.72 (C-6), 31.70 (C-7), 31.90 (C-8), 50.21 (C-9), 36.55 (C-10), 21.10 (C-11), 39.72 (C-12), 42.34 (C-13), 56.90 (C-14), 24.38 (C-15), 28.92 (C-16), 56.01 (C-17), 40.48 (C-18), 19.41 (C-19), 138.32 (C-20), 129.32 (C-21), 51.26 (C-22), 25.41 (C-23), 12.07 (C-24), 31.90 (C- 25), 21.23 (C-26), 19.41 (C-27), 19.00 (C-28), 12.25 (C-29).

III. RESULTS AND DISCUSSION

Compound **1** was isolated as a yellow powder with the molecular formula $C_{21}H_{20}O_{11}$ determined by ESI-MS *m/z* 447.5 $[M-H]^-$. The ^{1}H-NMR spectra revealed the signals of four AABB-type protons [δ_H 8.08 (2H, *d*, *J* = 9.0 Hz) and 6.91 (2H, *d*, *J* = 9.0 Hz)] of B ring and meta-coupled protons at δ_H 6.23 (*d*, *J* = 2.1 Hz) and 6.43 (*d*, *J* = 2.1 Hz) of the A ring, which indicated the presence of a kaempferol derivative as an aglycone. Moreover, the signals of one sugar anomeric proton displayed at δ_H 5.26 (*d*, *J* = 7.8 Hz, H-1″) indicating a *β*-configuration. The ^{13}C-NMR and DEPT spectra showed the presence of 21 carbon signals, including nine quaternary carbons, eleven methine carbons, and one methylene. The ^{13}C-NMR chemical shifts of their attached carbons could be assigned unambiguously using the HSQC spectrum. The chemical shifts of sugar moiety are revealed by COSY spectra analysis. Besides, the correlation between anomeric proton H-1 and carbon C-3 (C 135.49) in the HMBC spectrum confirmed that the sugar moiety was attached to the *O*-atom of the aglycone. Compound **1** was identified as astragalin or kaempferol 3-*O*-*β*-D-glucopyranosyl based on spectroscopic evidence and comparison with reported values in the literature [4].

Compound **2** was obtained as a yellow powder. The molecular formula $C_{27}H_{30}O_{16}$ was deduced from ESI-MS *m/z* 611.4 $[M+H]^+$. The signals of three ABX-type protons were visible in the ^{1}H-NMR spectrum of **2**, δ_H 7.63 (*dd*, *J* = 1.8, 8.4 Hz, H-2′), 7.60 (*dd*, J = 2.4, 8.4 Hz, H-6′) and 6.89 (*d*, *J* = 8.4 Hz, H-3′, 5′). As a result, the aglycone of **2** was identified as quercetin. Furthermore, the

signals of two sugar anomeric protons could be discerned, one at δ_H 5.76 (*d*, *J* = 7.8 Hz, H-1″) indicating a *β*-configuration and another one at δ_H 5.24 (*d*, *J* = 1.5 Hz, H-1‴) pointing to an *α*-configuration, as well as a methyl signal at δ_H 0.95 suggested that one of the sugars was rhamnose. The ^{13}C-NMR and DEPT spectra showed the presence of 27 carbon signals, including fifteen carbons of the aglycone and twelve carbons of the sugar moieties. In the HMBC spectrum, the anomeric proton H-1‴ correlated with carbon C-2″ (δ_C 80.13), indicating that the *α*-L-rhamnose was located at the C-2″ position of the glucose moiety. The HMBC data also confirmed the correlation between H-1″ proton with carbon C-3 (δ_C 134.56). Detailed analysis of the NMR spectra, compound **2** was identified as quercetin 3-neohesperidoside when compared to the published data [5].

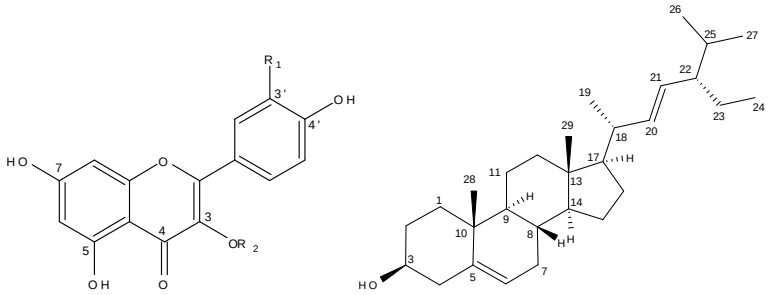
Compound **3** was isolated as a yellow amorphous powder. On the basis of an ion peak $[M+H]^+$ at *m/z* 611.5 in ESI-MS, its molecular formula was determined to be $C_{27}H_{30}O_{16}$. Detailed analysis of the ^{13}C-NMR and DEPT spectra revealed the presence of 27 carbon signals, including ten quaternary carbons, fifteen methines, one methyl, and one methylene. A comparison of the ^{1}H- and ^{13}C-NMR data of **3** with those of **2** revealed that the structures of both compounds were similar, except for the replacement of *β*-glucose moiety in **2** with a *β*-galactose in **3** [δ_C 100.83 (CH, C-1″), 77.59 (CH, C-2″), 75.74 (CH, C-3″), 79.89 (CH, C-4″), 77.12 (CH, C-5″) and 62.11 (CH_2, C-6″)/δ_H 5.76 (1H, *d*, *J* = 7.8 Hz, H-1″), 3.98 (1H, *dd*, *J* = 7.8, 9.6 Hz, H-2″), 3.74 (1H, *m*, H-3″), 3.87 (1H, *m*, H-4″), 3.51 (1H, *t*, *J* = 6.0 Hz, H-5″), 3.66 (1H, *dd*, *J* = 5.4, 12.0 Hz, H-6_a″) and 3.63 (1H, *dd*, *J* = 5.4, 18.0 Hz, H-6_b″)]. By comparison of the NMR data of **3** with those of the published data [6], **3** was identified as quercetin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside.

Compound **4** was obtained as a white needle. The ^{13}C-NMR and DEPT spectrum indicated that **4** had 29 carbons including six methyls, nine methylenes, ten sp^3 methines, one oxygenated methine, and three quaternary sp^3 carbons. The ^{1}H-NMR spectra of **4** showed signals for six methyl groups at (*d*, *J* = 6.5 Hz, H-19), 0.70 (s, H-24), 1.02 (*d*, *J* = 6.5 Hz, H-26), 1.01 (s, H-27), 0.79 (s, H-28), and 0.80 (s, H-29). The presence of a signal at δ_H 3.52 indicated an oxymethine proton and three trisubstituted olefins at δ_H 5.35 (*brd*, *J* = 3.5 Hz, H-6), 5.16 (*dd*, *J* = 8.5, 15.0 Hz, H-20), and 5.02 (*dd*, *J* = 8.5, 15.0 Hz, H-21). The above data suggested that **1** was a sterol. Detailed analysis of the NMR spectra, compound **4** was identified as stigmasterol when compared to the published data [7].

TABLE 1: The NMR data of **1-3**

	1		**2**		**3**	
Position	**δ_C**	**δ_H**	**δ_C**	**δ_H**	**δ_C**	**δ_H**
2	161.59		158.43		158.43	
3	135.49		134.56		134.56	
4	179.55		179.35		179.35	
5	163.15		163.19		163.19	
6	99.93	6.23 d 2.4	99.71	6.20 d 1.8	99.71	6.20 d 1.8
7	166.06		165.74		165.74	
8	94.76	6.43 d 1.8	94.53	6.39 brs	94.53	6.39 brs
9	159.14		158.39		158.39	
10	105.65		105.94		105.94	
1′	122.83		123.25		123.25	
2′	132.28	8.08 d 9.0	117.20	7.63 dd 1.8, 8.4	117.30	7.71 d 2.4
3′	116.09	6.91 d 9.0	146.01		146.01	
4′	158.56		149.57		149.57	
5′	116.09	6.91 d 9.0	115.19	6.89 d 8.4	116.12	6.89 d 8.4
6′	132.28	8.08 d 9.0	123.49	7.60 dd 2.4, 8.4	123.49	7.60 dd 2.4, 8.4
1″	104.13	5.26 d 7.8	100.35	5.76 d 7.8	100.83	5.76 d 7.8
2″	75.74	3.46 d 7,8	80.13	3.68 d 7.8	77,59	3.98 dd 7.8, 9.6
3″	78.06	3.45 m	78.94	3.57 m	75.74	3.74 m
4″	71.39	3.32 m	71.71	3.38 m	70.89	3.87 m
5″	78.43	3.22 ddd 2.4, 5.4, 9.6	78.32	3.25 ddd 2.4, 5.4, 9.6	77.12	3.51 t 6.0

6″	62.65	3.71 dd 2.4, 12.0 3.55 dd 5.4, 12.0	62.57	3.76 dd 2.4, 9.6 3.55 dd 5.4, 9.0	62.11	3.66 dd 5.4, 12.0 3.63 dd 5.4, 18.0
1‴			102.65	5.24 d 1.5	102.58	5.24 d 1.5
2‴			72.40	4.02 dd 1.5, 3.0	72.40	4.02 dd 1.5, 3.0
3‴			72.31	3.80 dd 3.0, 9.6	72.31	3.80 dd 3.0, 9.6
4‴			74.07	3.34 m	74.07	3.34 m
5‴			69.96	4.05 dt 3.0, 9.6	69.84	4.05 dt 3.0, 9.6
6‴			17.46	0.95 d 6.0	17.36	0.95 d 6.0



(**1**) R_1 = H, R_2 = Glc
(**2**) R_1 = OH, R_2 = Rha-(1→2)-Glc
(**3**) R_1 = OH, R_2 = Rha-(1→2)-Gal

(**4**)

FIGURE 1: Structure of compounds **1-4**

IV. CONCLUSION

To study the chemical composition of the endemic species of Vietnam, we have selected *Magnolia lamdongensis* species for chemical research. From its leaves, four compounds were isolated for the first time including astragalin, quercetin 3-neohesperidoside, quercetin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside, and stigmasterol. Their structures elucidation was confirmed by NMR data as well as comparison with published data.

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