EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 9
Issue: 11
13-16
Year: 2022

FLAVONOID GLYCOSIDES FROM THE LEAVES OF *MAGNOLIA TIEPII* (MAGNOLIACEAE)

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Article Received on 08/09/2022 **Article Revised on 28/09/2022** **Article Accepted on 18/10/2022**

ABSTRACT
Magnolia tiepii V. T. Tran & N. V. Duy sp. nov. (Magnoliaceae) is recognized from southern Vietnam, where it occurs in Khanh Vinh slope, Khanh Vinh District, Khanh Hoa Province. Four flavonoid glycosides were isolated from the leaves of *Magnolia tiepii*, including kaempferol 3-neohesperidoside (1), kaempferol 3-*O*-*α*-L -rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside (2), quercetin 3-neohesperidoside (3), and quercetin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside (4). Their chemical structures were elucidated by spectrocopic NMR, MS, and IR analysis as well as comparing their data with the ones in the literature. Although these compounds were known in other plants but this is the first time they are reported in this species.

KEYWORDS: *Magnolia tiepii*, kaempferol, quercetin, flavonoid glycoside.

INTRODUCTION
The majority of Magnoliaceae species have been used as folk medicines. *Magnolia obovata* Thunb. has been used to treat fever, headache, diarrhea, anxiety, and asthma relief.[1] The stem bark of *M. officinalis* L. is used for relieving asthma and treatment of abdominal distention and pain, dyspepsia, and asthmatic cough.[2] *M. delavayi* has traditionally been used to treat indigestion, chronic gastritis, cough, and bronchitis.[3] *M. grandiflora* bark, wood, and other parts have been used in American Indian medicine and are listed as bitter tonics, antimalarials, and diaphoretics in the United States Pharmacopoeia and pharmacognosy texts.[4] *M. biondii*, *M. sprengeri*, and *M. denudate* dried flower buds have been used to treat nasal congestion, empyema, sinusitis, and allergic rhinitis, while *M. officinalis* bark has been used to treat edema, lung disorders, cough, asthma, and intestinal disorders.[5]

M. tiepii, evergreen trees up to 20 m tall and 50 cm in diameter, has been identified in Khanh Vinh slope, Khanh Hoa Province, Southern Vietnam in 2013.[6] Antioxidant activity of the total extract of the leaves of *M. tiepii* were studied based on DPPH free radical scavenging activity, yielding SC_{50} values of 396.30 μg/mL. In this paper, we report the isolation and structural elucidation of four flavonoid glycosides **1-4** from the leaves of *Magnolia tiepii*. This is the first report about isolated compounds from this plant.

EXPERIMENTAL
General experimental procedures
Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70–230 mesh and 230–400 mesh, Merck, Darmstadt, Germany) and reversed-phase silica gel (ODS-A, 12 nm S-150 mm, YMC Co., Ltd., Japan) resins. TLC used pre-coated silica gel 60 F254 (1.05554.0001, Merck) and RP-18 F254S plates (1.15685.0001, Merck), and compounds were visualized by spraying with aqueous 10% H_2SO_4 and heating for 3–5 min. The ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectra were recorded on an AVANCE III HD 600 (Bruker, Germany) FT-NMR spectrometer with tetramethylsilane (TMS) was used as an internal standard. ESI mass spectra were collected on Agilent 1100 LC/MS systems. The IR spectra were recorded on a JASCO FT/IR 4100 FT-IR in KBr.

Plant material
The samples of the plant *Magnolia tiepii* were collected in May 2021 at the Khanh Vinh slope in Khanh Hoa province, Vietnam, and identified by Dr. Nong Van Duy of Tay Nguyen Institute for Scientific Research, VAST.

A voucher specimen (TN3/227) was deposited at Tay Nguyen Institute for Scientific Research, VAST.

Extraction and isolation

Fresh leaves of *M. tiepii* (10.0 kg) were dried at room temperature in shade and ground to fine powder. The air-dried and powdered leaves (4.1 kg) were extracted three times with methanol at room temperature (20 L/time). The methanol solutions were filtered, combined, and concentrated under reduced pressure to obtain methanol residue (760 g). This was suspended in water (3 L) and partitioned in turn with *n*-hexane, chloroform, and ethyl acetate to give the corresponding extracts: *n*-hexane (H, 7.3 g), $CHCl_3$ (C, 7.8 g), EtOAc (E, 14.5 g), and water layer (W, 3 L).

The water layer passed through Diaion HP-20 CC and eluted first with water and then with MeOH/ H_2O (0:100, 25:75, 50:50, 75:25, and 100:0, v/v) to obtain five fractions, W1-W5. Fraction W3 (11.7 g) was repeatedly subjected to silicagel CC, eluted with $CHCl_3$/MeOH (4:1, v/v) to obtained three subfractions, W3A-W3C. Subfraction W3B (2.7 g) was separated by RP-18 column using MeOH/H_2O (2:3, v/v) to give six subfractions, W3B1-W3B6. Subfraction W3B2 (980 mg) was subjected to chromatography on the RP-18 column, eluted with MeOH/H_2O (3:2, v/v) to yield six subfractions, W3B2A-W3B2F. Subfraction W3B2B (86 mg) was chromatographed and purified by the silica gel CC with $CHCl_3$/MeOH/H_2O (3:1:0.1) to afforded compounds **1** (8 mg) and **2** (10 mg). Fraction W2 (32.4 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 **3** (7 mg) and **4** (6 mg).

Kaempferol 3-neohesperidoside (1): Yellow powder; molecular formula $C_{27}H_{30}O_{15}$; ESI-MS *m/z* 595.14 $[M+H]^+$, 449.10 $[(M+H)–Rha]^+$, 287.06 $[(M+H)–Rha–Glc]^+$; IR (KBr): 3349 cm^{-1} (OH), 1661 cm^{-1} (C=O), 1609 cm^{-1} (C=C, aren); ^1H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD): see table 1.

Kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside (2): Yellow powder; molecular formula $C_{27}H_{30}O_{15}$; ESI-MS *m/z* 595.16 $[M+H]^+$, 449.10 $[(M+H)–Rha]^+$, 287.03 $[(M+H)–Rha–Glc]^+$, $C_{27}H_{30}O_{15}$; IR (KBr): 3349 cm^{-1} (OH), 1661 cm^{-1} (C=O), 1609 cm^{-1} (C=C, aren); ^1H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD): see table 1.

Quercetin 3-neohesperidoside (3): Yellow powder; molecular formula $C_{27}H_{30}O_{16}$; ESI-MS: *m/z* 611.15 $[M+H]^+$, 464.98 $[(M+H)–Rha]^+$; IR (KBr): 3399 cm^{-1} (OH), 1661 cm^{-1} (C=O), 1653 cm^{-1} (C=C, aren); ^1H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD): see table 1.

Quercetin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside (4): Yellow powder; molecular formula $C_{27}H_{30}O_{16}$; ESI-MS: *m/z* 611.15 $[M+H]^+$, 464.98 $[(M+H)–Rha]^+$; IR (KBr): 3399 cm^{-1} (OH), 1661 cm^{-1} (C=O), 1653 cm^{-1} (C=C, aren); ^1H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD): see table 1.

RESULTS AND DISCUSSION

Compound **1** was obtained as a yellow powder. The molecular formula $C_{27}H_{30}O_{15}$ was deduced from ESI-MS *m/z* 595.14 $[M+H]^+$. The NMR data of **1** revealed the presence of flavone aglycone and two sugar moieties. The ^1H NMR spectrum of **1** displayed signals of four AABB-type protons [δ_H 8.07 (2H, *d*, *J* = 9.0 Hz, H-2′, H-6′) and 6.91 (2H, *d*, *J* = 9.0 Hz, H-3′, H-5′)], two aromatic protons at δ_H 6.40 (*d*, *J* = 2.1 Hz, H-8) and 6.21 (*d*, *J* = 2.1 Hz, H-6). Also, two anomeric protons presented by doublets at δ_H 5.76 (*d*, *J* = 7.6 Hz, H-1″) and 5.25 (*d*, *J* = 1.6 Hz, H-1‴) were assigned to *β*-D-glucose (Glc) and *α*-L-rhamnose (Rha) units, in turns. The ^{13}C NMR and DEPT spectra of **1** displayed signals of 27 carbons, including 15 carbons of the aglycone and 12 carbons of the sugar moieties. The HMBC data also confirmed the correlation between H-1″ proton with carbon C-3 (δ_C 134.45) and anomeric proton H-1‴ (Rha) with carbon C-2″ (δ_C 80.07, Glc). The comparison with spectral data in the literature confirmed **1** as kaempferol 3-neohesperidoside.[7]

Compound **2** was isolated as a yellow amorphous powder. Detailed analysis of the ^{13}C NMR and DEPT spectra revealed the presence of 27 carbon signals, including nine quaternary carbons, sixteen methines, one methyl, and one methylene. A comparison of the ^1H and ^{13}C NMR data of **2** with those of **1** revealed that the structures of both compounds were similar, except for the replacement of *β*-glucose moiety in **1** with a *β*-galactose in **2** [δ_C 100.64 (CH, C-1″), 77.68 (CH, C-2″), 76.89 (CH, C-3″), 70.72 (CH, C-4″), 75.72 (CH, C-5″) and 62.12 (CH_2, C-6″)/δ_H 5.70 (1H, *d*, *J* = 7.7 Hz, H-1″), 3.95 (1H, *dd*, *J* = 7.7, 9.6 Hz, H-2″), 3.52 (1H, *td*, *J* = 1.5, 6.1 Hz, H-3″), 3.84 (1H, *brd*, *J* = 3.5 Hz, H-4″), 3.73 (1H, *dd*, *J* = 3.5, 9.6 Hz, H-5″), 3.64 (1H, *dd*, *J* = 6.0, 11.5 Hz, H-6a″), and 3.59 (1H, *dd*, *J* = 6.0, 11.5 Hz, H-6_b″)]. By comparison of the NMR data of **2** with those of the published data, **2** was identified as kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside.[8]

Compound **3** was obtained as a yellow powder. The molecular formula $C_{27}H_{30}O_{16}$ was deduced from ESI-MS *m/z* 611.15 $[M+H]^+$. The ^1H NMR spectra revealed the signals of three ABX-type protons [δ_H 7.63 (*d*, *J* = 2.4 Hz, H-2′), 7.60 (*dd*, *J* = 2.4, 8.4 Hz, H-6′), and 6.89 (*d*, *J* = 8.4 Hz, H-5′)] of B ring and meta-coupled protons at δ_H 6.20 (*d*, *J* = 2.0 Hz, H-6) and 6.39 (*d*, *J* = 2.0 Hz, H-8) of the A ring, which indicated the presence of a quercetin derivative as an aglycone. Furthermore, the signals of

two sugar anomeric protons could be discerned at δ_H 5.76 (*d*, *J* = 7.8 Hz, H-1″) and 5.24 (*d*, *J* = 1.5 Hz, H-1‴). The ^{13}C NMR and DEPT spectra showed the presence of 27 carbon signals, in which, a methyl group at δ_C 17.46 and a methylene group at δ_C 62.57 suggested that two of the sugars were rhamnose and glucose, orderly. In the HMBC spectrum, the anomeric proton H-1‴ correlated with carbon C-2″ (δ_C 80.13), indicating that the Rha was located at the C-2″ position of the Glc moiety. The HMBC data also confirmed the correlation between H-1″ (Glc) proton with carbon C-3 (δ_C 134.56). Detailed analysis of the NMR spectra, compound **3** was identified as quercetin 3-neohesperidoside when compared to the published data.[9]

Compound **4** was isolated as a yellow powder. Detailed analysis of the ^{13}C NMR and DEPT spectra revealed the presence of 27 carbon signals. After comparing ^{1}H and ^{13}C spectra, compounds **3** and **4** had structural similarities, except for the substitution of the *β*-glucose in **3** with *β*-galactose in **4** [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-6a″), and 3.63 (1H, *dd*, *J* = 5.4, 12.0 Hz, H-6_b″)]. By comparison of the NMR data of **4** with those of the published data, **4** was identified as quercetin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside.[8]

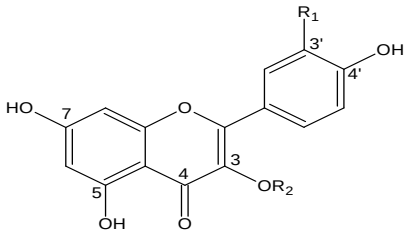

Figure 1: Structure of compounds 1–4.

Compound	R1	R2
1	H	*α*-L-Rha-(1→2)-*β*-D-Glc
2	H	*α*-L-Rha-(1→2)-*β*-D-Gal
3	OH	*α*-L-Rha-(1→2)-*β*-D-Glc
4	OH	*α*-L-Rha-(1→2)-*β*-D-Gal

Table 1: The ^{1}H and ^{13}C NMR data of compounds 1-4.

	1		2		3		4	
Position	**δ_C**	**δ_H**	**δ_C**	**δ_H**	**δ_C**	**δ_H**	**δ_C**	**δ_H**
2	161.30		161.21		158.43		158.43	
3	134.45		134.47		134.56		134.56	
4	179.40		179.44		179.35		179.35	
5	163.20		163.08		163.19		163.19	
6	99.71	6.21 d 2.1	99.68	6.16 d 2.1	99.71	6.20 d 2.0	99.71	6.20 d 2.0
7	165.67		165.59		165.74		165.74	
8	94.58	6.40 d 2.1	94.56	6.37 d 2.1	94.53	6.39 d 2.0	94.53	6.39 d 2.0
9	158.54		158.54		158.39		158.39	
10	105.99		105.90		105.94		105.94	
1′	123.15		123.06		123.49		123.49	
2′	132.10	8.07 d 9.0	132.16	8.07 d 9.0	117.20	7.63 d 2.4	117.30	7.71 d 2.4
3′	116.11	6.91 d 9.0	116.15	6.90 d 9.0	146.01		146.01	
4′	158.44		158.35		149.57		149.57	
5′	116.11	6.91 d 9.0	116.15	6.90 d 9.0	115.99	6.89 d 8.4	116.12	6.89 d 8.4
6′	132.10	8.07 d 9.0	132.16	8.07 d 9.0	123.22	7.60 dd 2.4, 8.4	123.04	7.60 dd 2.4, 8.4
1″	100.30	5.76 d 7.6	100.64	5.70 d 7.7	100.35	5.76 d 7.8	100.83	5.76 d 7.8
2″	80.07	3.64 dd 7.6, 9.5	77.68	3.95 dd 7.7, 9.6	80.13	3.68 d 7.8	77.59	3.98 dd 7.8, 9.6
3″	78.36	3.25 ddd 2.3, 5.7, 9.5	76.89	3.52 td 1.5, 6.1	78.94	3.57 m	75.74	3.74 m
4″	71.85	3.31 d 8.8	70.72	3.84 brd 3.5	71.71	3.38 d 2.0	70.89	3.87 m
5″	78.95	3.58 t 8.8	75.72	3.73 dd 3.5, 9.6	78.32	3.25 ddd 2.4, 5.4, 9.6	77.12	3.51 t 6.0
6″	62.65	3.75 dd 2.3, 12.0 3.53 dd 5.7, 12.0	62.12	3.64 dd 6.0, 11.5 3.59 dd 6.0, 11.5	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, 12.0
1‴	102.62	5.25 d 1.6	102.57	5.23 d 1.8	102.65	5.24 d 1.5	102.58	5.24 d 1.5
2‴	72.41	4.02 dd 1.6, 3.4	72.39	4.01 dd 1.8, 3.3	72.40	4.02 dd 1.5, 3.0	72.40	4.02 dd 1.5, 3.0
3‴	72.32	3.80 dd 3.4, 9.6	72.33	3.79 dd 3.3, 9.6	72.31	3.80 dd 3.0, 9.6	72.31	3.80 dd 3.0, 9.6
4‴	74.06	3.36 t 9.6	74.04	3.35 dt 5.5, 9.6	74.07	3.35 dd 2.0, 9.6	74.07	3.35 dd 2.0, 9.6
5‴	69.92	4.06 dd 6.2, 9.6	69.82	4.04 dd 6.2, 9.6	69.96	4.05 dt 3.0, 9.6	69.84	4.05 dt 3.0, 9.6
6‴	17.53	0.98 d 6.2	17.46	0.95 d 6.2	17.46	0.99 d 6.0	17.36	0.95 d 6.0

CONCLUSION

From *Magnolia tiepii* leaves collected at Khanh Vinh slope, Khanh Hoa province, four flavonoid compounds were isolated, including kaempferol 3-neohesperidoside, kaempferol 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside, quercetin 3-neohesperidoside, and quercetin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside were isolated. Their structures elucidation was confirmed by NMR, MS, and IR as well as comparison with published data. The obtained results showed that the leaves of this species contain many valuable derivatives of quercetin and kaempferol.

ACKNOWLEDGEMENTS

This research is funded by Vietnam national project of the Tay Nguyen Program in the period 2016-2020 (code: TN18/C09).

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