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A New Flavone Glycoside From *Lumnitzera littorea* with In Vitro α-Glucosidase Inhibitory Activity

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Abstract

A new flavone glycoside, lumnitzerone (**1**), was isolated from leaves of *Lumnitzera littorea*, together with 9 known flavonoids. Their structures were elucidated by spectroscopic (one-dimensional, two-dimensional nuclear magnetic resonance) and high-resolution mass spectrometry analysis, and comparison with literature data. Extracts and all isolated compounds were evaluated for α-glucosidase inhibitory activity; all the extracts and most of the isolated compounds exhibited better activities than the positive control acarbose.

Keywords

Lumnitzera littorea, flavonoids, α-glucosidase inhibitory activity

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α-Glucosidase is an intestinal enzyme that breaks down α-1,4 linked polysaccharides to α-glucose, which leads to high blood sugar levels. The development of an α-glucosidase inhibitor derived from a natural product could be an important contribution to diabetes prevention.

Lumnitzera littorea (family Combretaceae) grows in Can Gio mangrove forest in Vietnam. There have been 2 reports about the phytochemistry of this species. The antimicrobial activities of *n*-hexane, ethyl acetate, and methanol extracts of the leaves were evaluated against 6 human pathogenic microbes and the first extract was the most active.[1] Recently, we reported the isolation of gallic acid and naringenin.[2] Here, we present the chemical constituents as well as the α-glucosidase inhibitory activity of extracts and compounds isolated from *L. littorea* leaves.

The ethyl acetate extract of *L. littorea* leaves yielded one new and 9 known flavonoids. The new flavonoid glycoside (**1**) was obtained as a light yellow solid and appeared purple on thin-layer chromatography (TLC) under UV light at 365 nm. Its molecular formula was established as $C_{25}H_{24}O_{11}$ through the pseudomolecular ion peak in the high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) at *m/z* 501.1398 $[M+H]^+$ (calculated for $C_{25}H_{24}O_{11}$+H, 501.1397). The ^{1}H and ^{1}H–^{1}H correlation spectroscopy nuclear magnetic resonance (COSY NMR) spectra of **1** revealed signals of AA'BB'-type aromatic protons at δ 7.96 (2H, d, 8.5 Hz,) and 6.94 (2H, d, 8.5 Hz), and 2 *meta* coupled protons at δ 6.43 (1H, d, 2.0 Hz) and 6.78 (1H, d, 2.0 Hz), which were assigned to H-2′/H-6′, H-3′/H-5′, H-6 and H-8, respectively. In addition, an olefinic proton at δ 6.87 (1H, s, H-3), and a chelated hydroxy proton and a phenolic proton at δ 12.96 (1H, s) and 10.40 (1H, s), respectively, were observed. All these data suggested the presence of an apigenin aglycone. Moreover, the ^{1}H NMR spectrum showed the presence of a hexopyranose unit with the anomeric proton at δ 5.13 (d, 7.5 Hz, H-1″) and signals due to sugar protons in the region of δ 3.15 to 5.50. Furthermore, characteristic signals of an (E)-propenyl group were observed at δ 5.83 (1H, dd, 15.5, 1.5 Hz, H-2‴), 6.85 (1H, dq, 15.5, 7.0 Hz, H-3‴), and 1.64 (3H, d, 7.0 Hz, H-4‴). The ^{13}C NMR spectrum of **1** revealed 25 carbon signals, including signals of 2 benzene rings of the apigenin aglycone at δ 164.3 (C-2), 103.1

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Table 1. In Vitro α-Glucosidase Inhibitory Activity of Isolated Compounds From *Lumnitzera littorea*.

Extract	Concentration (μg/mL)	**0.1**	**0.25**	**0.5**	**1.0**	IC_{50} (μg/mL)
Crude ethanol	Inh. per. (%)	99.1 ± 0.1	>100	>100	>100	<0.10
n-Hexane		37.4 ± 0.3	57.3 ± 0.8	71.2 ± 0.3	>100	0.21
Ethyl acetate		5.3 ± 0.6	25.6 ± 0.4	50.5 ± 0.3	91.5 ± 0.9	0.53
Ethanol		78.0 ± 1.0	85.5 ± 0.8	95.0 ± 0.2	>100	<0.10
Acarbose						138.2

IC_{50}, half-maximal inhibitory concentration.

(C-3), 182.0 (C-4), 161.4 (C-5), 99.4 (C-6), 162.6 (C-7), 94.7 (C-8), 156.9 (C-9), 105.4 (C-10), 121.0 (C-1′), 128.6 (C-2′/ C-6′), 116.0 (C-3′/C-5′), and 161.1 (C-4′), of a hexopyranose unit at δ 99.5 (C-1″), 72.9 (C-2″), 76.2 (C-3″), 70.0 (C-4″), 73.8 (C-5″), and 63.4 (C-6″), and of a butenoyl group at δ 165.3 (C-1‴), 122.0 (C-2‴), 145.3 (C-3‴), and 17.4 (C-4‴).

The known compounds were identified from spectroscopic analysis and comparison with literature data as quercetin (**2**),[3] quercitrin (**3**),[4] myricetin (**4**),[5] myricitrin (**5**),[4] naringenin (**6**),[6] chrysoeriol (**7**),[7] pilloin (**8**),[8] afzelin (**9**),[9] and myricetin 3-*O*-(4″-*O*-galloyl)-*α*-L-rhamnopyranoside (**10**)[10] (Figure 1).

The in vitro α-glucosidase inhibitory activity was evaluated of the extracts and purified compounds (Tables 1 and 2). All the extracts and most of the isolated compounds exhibited better activities than the positive control acarbose. The flavonoid-type structure, the position, and the number of hydroxy groups are determining factors for α-glucosidase inhibition. The A-ring 7-OH and the B-ring 4′-OH groups play an important role in the inhibitory effect. This observation was proved by the most potent inhibitors such as naringenin (**6**), quercetin (**2**), and afzelin (**9**), which possess half-maximal inhibitory concentration (IC_{50}) values of 1.87, 3.42, and 6.26 μg/mL, respectively, compared with that of lumnitzerone (**1**) (IC_{50} 11.31 μg/

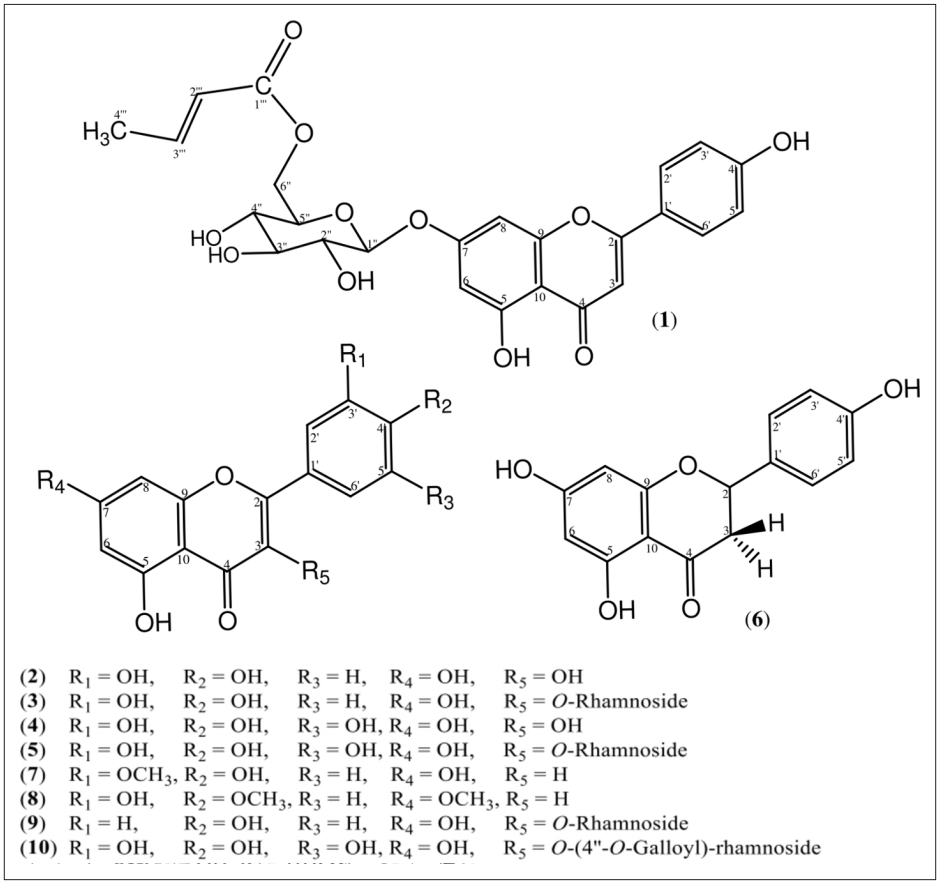

Figure 1. Structure of compounds isolated from *Lumnitzera littorea*.

Table 2. In Vitro α-Glucosidase Inhibitory Activity of Isolated Compounds From *Lumnitzera littorea*.

Compound	**Concentration** (μg/mL)	**1**	**5**	**10**	**15**	**20**	IC_{50} (μg/mL)
1	Inh. per.	17.8 ± 0.7	29.5 ± 0.4	45.5 ± 0.7	61.6 ± 0.3	77.9 ± 0.9	11.3
2		47.0 ± 0.5	52.0 ± 0.6	65.5 ± 0.9	82.9 ± 0.4	100.0 ± 0.3	3.4
3		28.3 ± 0.9	43.1 ± 0.4	53.7 ± 0.3	74.9 ± 1.0	89.4 ± 0.6	7.7
6		39.9 ± 1.0	96.8 ± 0.6	>100	>100	>100	1.9
9		33.4 ± 0.6	44.8 ± 0.5	64.8 ± 0.6	75.8 ± 0.5	91.5 ± 0.5	6.3
	Concentration (μg/mL)	60	80	100	120	140	IC_{50} (μg/mL)
4	Inh. per. (%)	21.1 ± 0.3	38.9 ± 0.7	51.6 ± 0.4	65.8 ± 0.4	80.4 ± 0.3	97.8
5		-	7.8 ± 0.5	19.1 ± 0.7	28.2 ± 0.6	41.6 ± 0.9	153.5
7		59.0 ± 0.9	79.3 ± 0.8	>100	>100	>100	53.4
8		18.1 ± 0.4	40.5 ± 0.5	63.4 ± 0.6	85.8 ± 0.5	98.7 ± 0.7	89.1
10		37.6 ± 0.7	70.2 ± 0.4	>100	>100	>100	67.4
Acarbose*							138.2

Con., concentration; Inh. per., Inhibition percentage; IC_{50}, half-maximal inhibitory concentration.
Values are expressed as mean ± SD. (-) Inhibition percentage value (% I) <1.
* positive control

mL). On the contrary, enhancement of the number of hydroxy groups on the B ring or if the hydroxy group is methylated reduces the inhibitory activity, as seen with chrysoeriol (**7**), pilloin (**8**), and myricitrin (**5**) (IC_{50} 53.4, 89.1, and 153.5 μg/mL, respectively). The presence of a sugar unit did not show a clear effect on the inhibition.

Careful analysis of the chemical shifts, multiplicities, and coupling constant magnitudes in the ^{1}H NMR spectrum, along with the ^{1}H-^{1}H COSY and ^{13}C NMR spectra of **1** (Table 3) indicated that the sugar was a β-D-glucopyranose unit. The nature of the sugar was further confirmed from the anomeric proton data of the sugar obtained after acid hydrolysis.[11]

The multiplicity of the protons H_3-4‴, H-3‴, and H-2‴ and the ^{1}H-^{1}H COSY spectrum confirmed their contiguous arrangement. The heteronuclear multiple bond correlation (HMBC) experiments showed cross-peaks (Figure 2) of the propenyl protons at δ 5.83 (H-2‴) and 6.85 (H-3‴), as well as the methylene protons at δ 4.41 (H-6″a) and 4.08 (H-6″b) to the carbonyl carbon C-1‴ (δ 165.3) suggesting that the butenoyloxy group was attached at C-6″ of the D-glucose. The attachment of this sugar to C-7 in the apigenin nucleus was confirmed by the HMBC cross-peak of the anomeric proton at δ 5.13 (1H, d, 7.5 Hz) to carbon C-7 (δ 162.6). Accordingly, **1** was identified as apigenin 7-*O*-[6″-(*E*)-butenoyl-β-D-glucopyranoside], for which the trivial name lumnitzerone is proposed.

Experimental

General Experimental Procedure

The NMR spectra were recorded on a Bruker Avance III spectrometer at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR spectra. HR-ESI-MS were obtained on a Shimadzu +IDA time-of-flight MS. TLC was performed on silica gel 60 F_{254} (Merck, Darmstadt, Germany). Gravity column chromatography was performed on silica gel 60 (0.040-0.063 mm, Merck) and Sephadex LH-20 (GE Healthcare Bio-Science AB, Uppsala, Sweden). α-Glucosidase (EC 3.2.1.20) from *Saccharomyces cerevisiae* (750 UN) and *p*-nitrophenyl-α-D-glucopyranoside were purchased from Sigma Chemical Co. (St Louis, MO, USA). Acarbose and dimethyl sulfoxide were obtained from Merck. Other chemicals were of the highest grade available.

Plant Material

Leaves of *Lumnitzera littorea* (Jack) Voigt (Combretaceae) were collected at Can Gio mangrove forest, Ho Chi Minh City, Viet Nam in August of 2014. The scientific name of the plant was authenticated by Dr Pham Van Ngot, Faculty of Biology, Ho Chi Minh City University of Pedagogy. A voucher specimen (No US-B012) was deposited in the herbarium of the Department of Organic Chemistry, University of Science.

Extraction and Isolation

The fresh leaves were washed under running tap water to remove all sand partitcles and epiphytes and then were dried and ground into fine powder. The powder (15 000 g) was exhaustively extracted with ethanol at room temperature by maceration. After filtration the ethanol solution was evaporated to dryness under reduced pressure to yield a crude ethanol

Table 3. Nuclear Magnetic Resonance Data of Compound **1**.

	Compound **1** (DMSO-d_6)		
Position	δ_H (multiplicity)	δ_C	HMBC ($^1H \rightarrow {}^{13}C$)
2		164.3	
3	6.87 (1H, s)	103.1	C-2, 4, 10, 1′
4		182.0	
5		161.4	
5-OH	12.96 (1H, s)		C-5, 6, 10
6	6.43 (1H, d, 2.0)	99.4	C-5, 7, 8, 10
7		162.6	
8	6.78 (1H, d, 2.0)	94.7	C-4, 6, 7, 9, 10
9		156.9	
10		105.4	
1′		121.0	
2′, 6′	7.96 (2H, d, 8.5)	128.6	C-2′, 3′, 4′, 5′, 6′
3′, 5′	6.94 (2H, d, 8.5)	116.0	C-1′, 2′, 3′, 4′, 5′
4′		161.1	
4′-OH	10.40 (1H, s)		C-3′, 4′, 5′
1″	5.13 (1H, d, 7.5)	99.5	C-7
2″	3.28 (*)	72.9	C-1″, 3″
2″-OH	5.48 (1H, d, 5.0)		C-2″
3″	3.30 (*)	76.2	C-5″
3″-OH	5.24 (1H, d, 4.5)		C-3″
4″	3.18 (1H, m)	70.0	C-5″
4″-OH	5.35 (1H, d, 5.5)		C-3″, 4″, 5″
5″	3.79 (1H, t, 8.0)	73.8	C-1″, 3″, 4″, 6″
6″a	4.41 (1H, d, 11.0)	63.4	C-1‴
6″b	4.08 (1H, dd, 11.0, 8.0)		C-1‴, 5″
1‴		165.3	
2‴	5.83 (1H, dd, 15.5, 1.5)	122.0	C-1‴, 3‴, 4‴
3‴	6.85 (1H, dq,15.5, 7.0)	145.3	C-1‴, 2‴, 4‴
4‴	1.64 (3H, d, 7.0)	17.4	C-2‴, 3‴

HMBC, heteronuclear multiple bond correlation; DMSO, dimethyl sulfoxide.
(*) overlapped in the solvent signal.

residue (1000 g). This was applied to a silica gel solid phase column and eluted consecutively with *n*-hexane, ethyl acetate, and finally with ethanol. After evaporation under reduced pressure, 3 extracts were obtained, *n*-hexane (100 g), ethyl acetate (250 g), and ethanol (550 g). The ethyl acetate extract (250 g) was fractionated by silica gel column chromatography using a mixture of EtOAc−MeOH (99:1 to 0:100) to yield 8 fractions (EA1-EA8). These were then continuously separated using silica gel and Sephadex LH-20 and eluted with appropriate solvent systems of EtOAc−MeOH to give 10 compounds. As a result, fraction EA2 afforded **2** (50 mg), **3** (15 mg), **4** (10 mg),

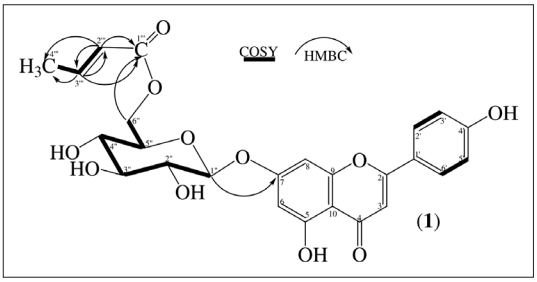

Figure 2. Key COSY and HMBC correlations of compound **1**.

COSY: correlation spectroscopy; HMBC:heteronuclear multiple bond correlation.

and **5** (10 mg), fraction EA3 gave **6** (3 mg) and **7** (5 mg), EA5 gave **1** (5 mg) and **10** (10 mg), and EA7 **8** (5 mg) and **9** (8 mg).

Bioactivity Assay

The α-glucosidase inhibitory activity was determined according to the method of Apostolidis et al[12] The inhibitory activity was calculated using the following equation:

$$\% \text{ Inhibition} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}$$

The IC_{50} values were determined from plots of percent inhibition vs log inhibitor concentration and calculated by non-linear regression analysis from the mean inhibitory values.

Lumnitzerone (1)

Light yellow solid.
^1H, ^{13}C NMR and HMBC (DMSO-d_6): Table 3.
HR-ESI-MS: *m/z* $[M+H]^+$ calculated for $C_{25}H_{24}O_{11}$+H, 501.1397; found: 501.1398.

Acid Hydrolysis of 1

Compound **1** (5 mg) was treated with HCl 0.2 M (dioxane/H_2O, 1/1, *v/v*, 200 µL) at 95°C for 3 hours. After cooling, the reaction mixture was extracted with chloroform (3 × 2 mL) to eliminate the aglycone component. The remaining solution was evaporated to dryness. The obtained residue was dissolved in D_2O for subsequent ^1H NMR analysis. The anomeric ratios were obtained by manual integration with δ_H 5.23 (d, J = 3.5 Hz, 36.4%) and 4.64 (d, J = 8.0 Hz, 63.6%). These values were highly reminiscent of those of glucose.[11]

Declaration of Conflicting Interests

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Supplemental Material

Supplemental material for this article is available online.

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