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Research Article

A new flavonoid from leaves of *Avicennia officinalis* L.

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ABSTRACT

Avicennia officinalis L., Avicenniaceae (or Acanthaceae), is a wide spread plant in mangrove forest in Vietnam, Cambodia, Thailand, Indonesia and so on. Some parts of this plant such as barks, leaves, and fruits of *A. officinalis* have been traditionally used as an aphrodisiac, diuretic, hepatitis, and leprosy treatment. *Avicennia officinalis's* chemical compositions remain mostly unknown and have not even been studied in Vietnam. Therefore, we now report on the isolation and the structural elucidation of four flavonoids from this plant growing in Can Gio mangrove forest in Ho Chi Minh City. The crude extract was obtained by the maceration of air–dried powder of leaves with methanol and then evaporation at reduced pressured. This crude extract was separated to three difference extracts including *n*-hexane, ethyl acetate and remaining aqueous residue by liquid-liquid partition. The ethyl acetate extract was applied to normal and reversed phrase RP-18 silica gel column chromatography and preparative thin layer chromatography to give four compounds. The chemical structures of these compounds were elucidated by 1D-, 2D-NMR spectroscopic and HR-MS analysis as well as compared with data in the literature. They are chrysoeriol 6"-(3"',5"'-dimethoxycoumaroyl)-7-*O*-β-D-glucopyranoside **(1)**, luteolin 7-*O*-β-D-glucopyranoside **(2)**, 3'-methylluteolin 4'-*O*-β-D-glucopyranoside **(3)**, and flavogadorinin **(4)**. Among them, **(1)** is a new compound. Further studies on this species are in progress.

1. INTRODUCTION

Avicennia officinalis L. (Avicenniaceae or Acanthaceae) wildly grows in many mangrove forests in Vietnam. The barks, leaves, and fruits of *A. officinalis* have been traditionally used as an aphrodisiac, diuretic, hepatitis, and leprosy treatment[1]. A methanol leaf extract of *A. officinalis* showed significantly acetylcholinesterase and butyrylcholinesterase inhibitions with the IC_{50} values of 1.24 and 0.91 mg/mL, respectively, compared to donepezil with IC_{50} values of 3.96 and 8.87 mg/mL, respectively[2]. *A. officinalis* showed anti–HIV property by inhibiting the virus by two different mechanisms including interference with the gp120/CD4 interaction and inhibition of viral reverse transcriptase[3].

A number of compounds have been isolated from leaves and roots of *A. officinalis*[4-7]. In the early of 2017, we formerly

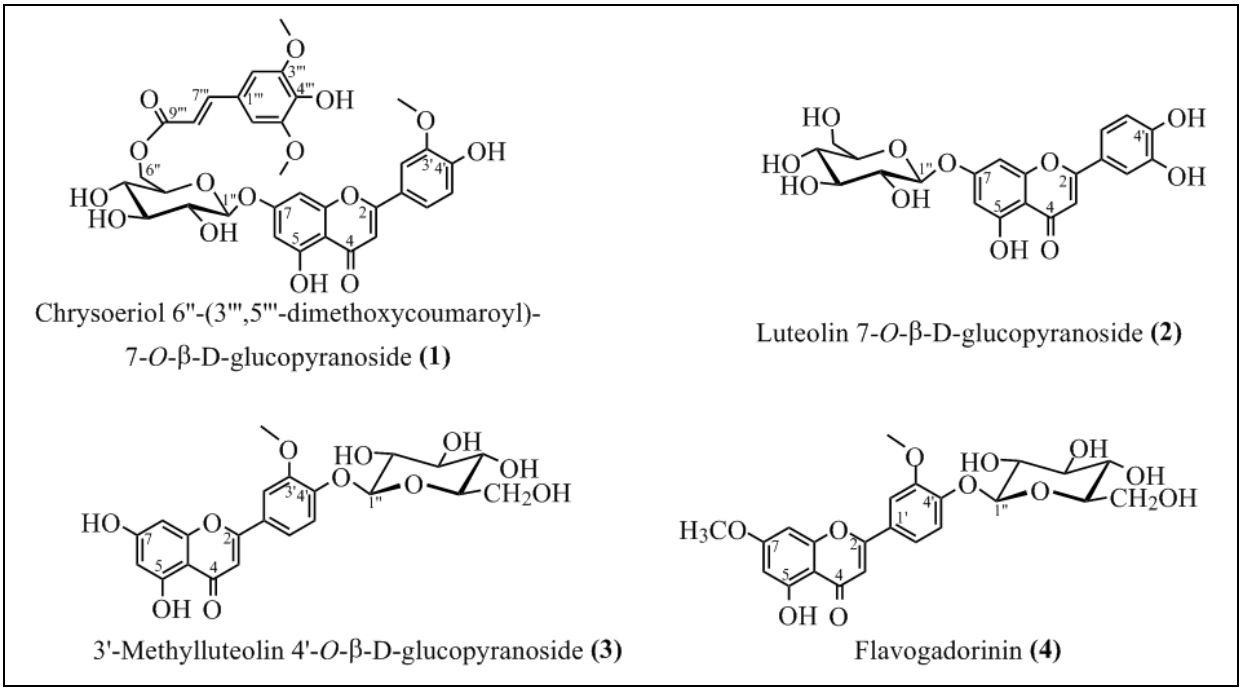

Figure 1. The chemical structure of isolated compounds.

examined the leaves of *A. officinalis* collected at Can Gio mangrove forest in Ho Chi Minh city, Vietnam, and reported the isolation of six compounds including kaempferol, kaempferol 3-*O*-*β*-D-glucopyranoside, isorhamnetin 6"-*O*-*α*-L-rhamnopyranosyl-3-*O*-*β*-D-glucopyranoside, ursolic acid, betulinic acid, and benzyl alcohol *β*-D-glucopyranoside[8]. In this paper, we display the structural elucidation of four compounds (Figure 1) isolated from this species.

2. EXPERIMENTALS

2.1. General experimental procedures

The NMR spectra were recorded on a Bruker Avance 500 (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR) at the Center of Analysis, University of Science, Vietnam National University – Ho Chi Minh City.

2.2. Plant material

Leaves of *Avicennia officinalis* were collected at Can Gio mangrove forest in Ho Chi Minh city, Vietnam in March 2012. The scientific name of the plant was authenticated by the botanist PhD. Vo Van Chi. A voucher specimen (N^{o} US–B008) was deposited in the herbarium of the Department of Organic Chemistry, University of Science, Vietnam National University – Ho Chi Minh City.

2.3. Extraction and isolation

The air–dried powder of leaves (11,205 g) was macerated with methanol (50 L x 3) at room temperature for 48 hours and after filtration the methanol solution was concentrated at reduced pressure to yield a residue of 1,317 g. This crude extract was suspended in water with 10% of methanol, and was partitioned first with *n*-hexane and then with ethyl acetate. After evaporation at reduced pressure, three types of extracts were obtained: *n*-hexane (405 g), ethyl acetate (350 g) and remaining aqueous residue (512 g).

The ethyl acetate residue was subjected to silica gel column chromatography (CC) (column: 120 x 6 cm), eluted with a solvent system of *n*-hexane–ethyl acetate (1:4, 0:1), and then ethyl acetate–methanol (stepwise, 9:1, 4:1, 1:1, 0:1) to give fourteen fractions (A1–A14). Fraction A12 (15.5 g) was subjected to a silica gel CC and eluted with ethyl acetate–methanol (stepwise, 9:1, 4:1, 1:1, 0:1) to give five subfractions (A12.1–A12.5). The subfraction A12.2 (6.2 g) was further separated by reversed–phase RP–18 silica gel CC and eluted with water–methanol (stepwise, 20:1, 9:1, 4:1, 1:1) to obtain **1** (10.0 mg). The same procedure was applied on the subfraction A12.3 (4.0 g) to afford **2** (80.0 mg), **3** (7.0 mg), and **4** (12.0 mg).

Table 1. ^{1}H-NMR data of isolated compounds in DMSO-d_6

Pos.	(1)	(2)	(3)	(4)
3	6.88 (1H, *s*)	6.75 (1H, *s*)	6.98 (1H, *s*)	7.05 (1H, *s*)
6	6.51 (1H, *d*, 2.0)	6.44 (1H, *d*, 2.1)	6.21 (1H, *d*, 2.0)	6.38 (1H, *d*, 2.0)
8	6.81 (1H, *d*, 2.0)	6.79 (1H, *d*, 2.1)	6.54 (1H, *d*, 2.0)	6.85 (1H, *d*, 2.5)
2'	7.51 (1H, *d*, 2.0)	7.42 (1H, *d*, 2.1)	7.60 (1H, *d*, 2.0)	7.64 (1H, *d*, 2.0)
5'	6.91 (1H, *d*, 8.0)	6.92 (1H, *d*, 8.1)	7.25 (1H, *d*, 8.5)	7.25 (1H, *d*, 9.0)
6'	7.53 (1H, *dd*, 8.0, 2.0)	7.46 (1H, *dd*, 8.1, 2.4)	7.63 (1H, *dd*, 8.5, 2.0)	7.68 (1H, *dd*, 8.5, 2.5)
1"	5.17 (1H, *d*, 7.5)	5.09 (1H, *d*, 7.2)	5.06 (1H, *d*, 7.5)	5.07 (1H, *d*, 7.5)
2"	3.25–4.50 (*m*)	3.15–3.73 (*m*)	3.00–3.60 (*m*)	3.29 (1H, *m*)
3"	3.25–4.50 (*m*)	3.15–3.73 (*m*)	3.00–3.60 (*m*)	3.29 (1H, *m*)
4"	3.25–4.50 (*m*)	3.15–3.73 (*m*)	3.00–3.60 (*m*)	3.18 (1H, *m*)
5"	3.25–4.50 (*m*)	3.15–3.73 (*m*)	3.00–3.60 (*m*)	3.38 (1H, *m*)
6"	4.14 (*m*) 4.53 (*m*)	3.15–3.73 (*m*)	3.00–3.60 (*m*)	3.68 (1H, *m*) 3.46 (1H, *m*)
2'''/6'''	6.84 (2H, *s*)			
7'''	7.50 (1H, *d*, 16.0)			
8'''	6.47 (1H, *d*, 16.0)			
5–OH	13.02 (1H, *s*)	12.99 (1H, *s*)	12.90 (1H, *s*)	12.90 (1H, *s*)
7–OMe				3.88 (3H, *s*)*
3'–OMe	3.89 (3H, *s*)		3.90 (3H, *s*)	3.90 (3H, *s*)*
3'''/5'''–OMe	3.72 (6H, *s*)			

Note: * interchangeable signals

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a yellowish powder. The HR-ESI-MS showed a pseudo-molecular ion peak at *m/z* 691.1620 $[M+Na]^+$, corresponding to the molecular formula of $C_{33}H_{32}O_{15}$, calcd. for $C_{33}H_{32}O_{15}$+Na, 691.1639. This compound was identified as a flavonoid by analyzing its NMR spectra. The ^{1}H-NMR spectrum of **1** showed a down field signal at δ_H 13.02 (1H, *s*) indicating the presence of a chelated hydroxyl group at C–5 position. Two *meta*–coupled doublet proton signals at δ_H 6.51 and 6.81, each integrated for one proton, were assigned to H-6 and H-8, respectively, of ring A of 5,7-dihydroxyflavonoid. The presence of an ABX system at δ_H 7.53 (dd, 8.0, 2.0 Hz, H-6'), 7.51 (d, 2.0 Hz, H-2') and 6.91 (d, 8.0 Hz, H-5') was the characteristic of a 1,3,4-trisubstituted phenyl group. The singlet at δ_H 6.88, integrated for one proton, was assigned to H-3. These spectral data revealed the presence of a luteolin skeleton (Table 1).

Besides, at low magnetic zone, its proton spectrum also showed signals of a coumaroyl unit including a singlet at δ_H 6.84 (2H, s, H–2''', H–6'''), two doublet proton signals at δ_H 7.50 (H–7''') and 6.47 (H–8''') with a large coupling constant of *J*=16.0 Hz of an *E*–double bond. It corresponded to the ^{13}C–NMR spectrum with signals resonating from 95.0 to 182.0 ppm of 15 carbons of the luteolin skeleton and 9 carbons of one coumaroyl group (Table 1).

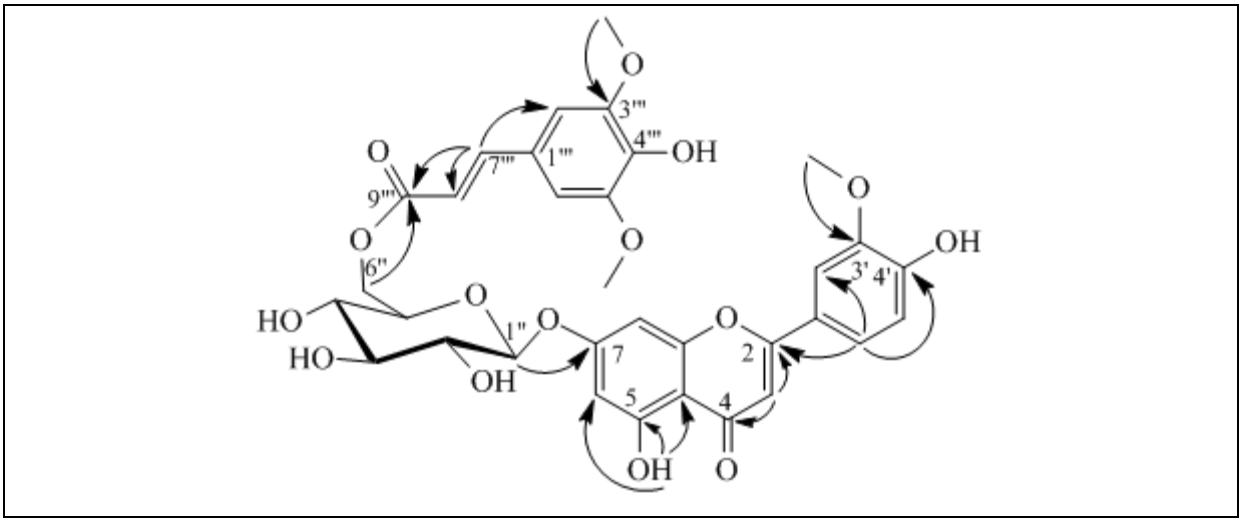

Figure 2. HMBC correlations of compound 1.

At higher magnetic field, the ^{1}H–NMR spectrum of **1** showed signals of three methoxy groups at δ_H 3.89 (3H, s, 3'–OMe), 3.72 (6H, s, 3'''–OMe, 5'''–OMe). A signal at δ_H 5.17 (1H, d, 7.5 Hz, H-1'') and signals with δ_H 3.25–4.50 were assigned to a *β*-D-glucose. It corresponded to the ^{13}C-NMR spectrum with signals at δ_C 99.6 (C-1''), 73.0 (C-2''), 73.8 (C-3''), 70.1 (C-4''), 76.3 (C-5''), and 63.2 (C-6'') of the sugar unit, two signals of three methoxy groups at δ_C 55.8 và 55.9, in which the signal at δ_C 55.9 appeared as double intensity (Table 1).

The position of three methoxy groups were determined at C-3', C-3''' and C-5''' via the HMBC correlations of methoxy protons with carbons at δ_C 148.0 (C-3'), 147.9 (C-3''', C-5''') (Figure 2). The β-D-glucopyranosyl unit was attached to the luteolin skeleton at C-7 which was confirmed by the HMBC cross-peak of the anomeric proton with a carbon at δ_C 162.7 (C-7). The HMBC correlations of two methylene protons H-6''a and H-6''b with a carboxyl carbon at δ_C 166.4 (C-9''') suggested the attachment of the coumaroyl group at C-6'' of the sugar unit (Table 2).

All data in the preceding text suggested that **1** was chrysoeriol 6''-(3''',5'''-dimethoxycoumaroyl)-7-*O*-β-D-glucopyranoside.

The ESI-MS of **2** showed a pseudomolecular ion peak at *m/z* 446.97 $[M-H]^-$, corresponding to the molecular formula of $C_{21}H_{20}O_{11}$, calcd. for $[C_{21}H_{20}O_{11}-H]^-$, 447.09. The NMR data analysis of **2** showed that its structure also possessed the luteolin skeleton and a sugar unit as that of **1**. However, **2** differed from **1** in the absence of the coumaroyl unit and three methoxy groups. This was evidenced by the presence of only 21 carbon signals including 15 carbons of luteolin and 6 carbons of a sugar moiety. This corresponded to the upfield shift of carbon C-6'' at δ_C 60.6 instead of at δ_C 63.2 (C-2) as in **1**. The coupling constant (J = 7.2 Hz) of the anomeric proton located at δ_H 5.09 and the ^{13}C-NMR chemical shift values of oxygenated carbons at δ_C 99.9, 77.2, 76.4, 73.1, 69.5 and 60.6 revealed the presence of a *β*-D-glucopyranosyl unit. The comparison of NMR data of **2** with those reported in the literature assigned the structure of **2** to be luteolin 7-*O*-*β*-D-glucopyranoside[9] (Table 1 and 2).

Table 2. ^{13}C-NMR data of isolated compounds in DMSO-*d*$_6$

Pos.	(1)	(2)	(3)	(4)
2	164.1	164.5	163.1	163.4
3	103.2	103.2	104.1	104.2
4	182.0	181.9	181.8	181.9
5	161.2	161.1	161.4	161.1
6	99.4	99.5	99.0	98.0
7	162.7	162.9	164.6	165.2
8	95.0	94.7	94.2	92.8
9	156.8	156.9	157.4	157.2
10	105.4	105.3	103.7	104.7
1'	121.2	121.4	124.1	123.9
2'	110.2	113.6	110.2	110.4
3'	148.0	145.8	149.2	149.2
4'	150.9	150.0	149.7	149.9
5'	115.7	116.0	115.0	115.1
6'	120.4	119.2	119.7	119.8
1''	99.6	99.9	99.5	99.6
2''	73.0	73.1	73.1	73.1
3''	73.8	76.4	76.8	76.8
4''	70.1	69.5	69.6	69.6
5''	76.3	77.2	77.1	77.1
6''	63.2	60.6	60.6	60.6
1'''	124.2			
2'''/6'''	105.9			
3'''/5'''	147.9			
4'''	138.3			
7'''	145.5			
8'''	114.5			
9'''	166.4			
7–OMe				56.1*
3'–OMe	55.8		56.0	56.0*
3'''/5'''–OMe	55.9			

Note: * interchangeable signals

The ESI-MS of **3** showed a pseudomolecular ion peak at *m/z* 460.94 $[M-H]^-$, corresponding to the molecular formula of $C_{22}H_{22}O_{11}$, calcd. for $[C_{22}H_{22}O_{11}-H]^-$, 461.10.

Similar to NMR data of **2**, the ^1H and ^{13}C-NMR spectra of **3** also possessed the signals of a luteolin skeleton and a β-D-glucospyranosyl moiety. However, the ^1H-NMR spectrum of **3** displayed one more proton signal at δ_H 3.90 (3H, s) of a methoxy group. It corresponded to the ^{13}C-NMR spectrum revealing of 22 carbon signals, including a methoxy carbon signal at δ_C 56.0. These analyses suggested that **3** contained the luteolin skeleton, the β-D-glucopyranoside and the methoxy group in its chemical structure.

According to the comparison of ^{13}C-NMR data of luteolin with those of its derivative possessing two substituents (*β*-D-glucopyranosyl moiety and a methoxy group) in the same deuterated solvent (DMSO-d_6)[10-14], if they possess a *β*-D-glucopyranosyl or a methoxy moiety at C-7, the chemical shift of C-10 resonates at lower magnetic zone at about 104.5 ppm comparing to that of luteolin at δ_C 103 (C-10). If there is a methoxy group at C-3' [10,13] carbons C-2' and C-5' resonate at δ_C 110 and 115, respectively and if there is a methoxy group at C-4' [11,12], these carbons resonate at δ_C 112 and 113, respectively. In the case of compound **3**, its ^{13}C-NMR spectrum showed signals at δ_C 110.2 (C-2') and 115.0 (C-5'), therefore, **3** possessed a methoxy group at C-3'. Besides, the quaternary carbon C-10 resonated at δ_C 103.7, which was similar to that of luteolin, therefore **3** was suggested to possess a hydroxyl group at C-7. It meant that the *β*-D-glucopyranosyl moiety was attached to the aglycone at C-4' (Table 1 and 2).

Based on all the aforementioned analysis and the comparison of the NMR data of **3** with those reported in the literature[13], **3** was determined to be 3'-*O*-methylluteolin 4'-*O*-*β*-D-glucopyranoside.

The ESI-MS of **4** showed a pseudomolecular ion peak at *m/z* 474.93 $[M-H]^-$, corresponding to the molecular formula of $C_{23}H_{24}O_{11}$, calcd. for $[C_{23}H_{24}O_{11}-H]^-$, 475.12. The NMR data analysis of **4** showed that it had one more methoxy group comparing to the chemical structure of **3**. This was evidenced by the presence of a further three–proton singlet signal at δ_H 3.88 of a methoxy group. It corresponded to the ^{13}C-NMR spectrum of **4** possessing one more carbon signal at δ_C 56.1 than that of **3**. The comparison of its NMR data with those reported in the literature[15] showed good compatibility, therefore **4** was determined to be flavogadorinin (Table 1 and 2).

4. CONCLUSION

From the ethyl acetate extract of the leaves of *Avicennia officinalis*, four flavonoids including chrysoeriol 6"-(3"',5"'-dimethoxycoumaroyl)-7-*O*-*β*-D-glucopyranoside **(1)**, luteolin 7-*O*-β-D-glucopyranoside **(2)**, 3'-methylluteolin 4'-*O*-*β*-D-glucopyranoside **(3)**, and flavogadorinin **(4)** were isolated. Their structures were identified by comparing their NMR and MS data as well as physical properties with those in literatures. To the best of own knowledge, **(1)** is a new compound.

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