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Flavonoid Glycosides from the Seeds of *Litchi chinensis*

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ABSTRACT: Seven flavonoid glycosides, including one new (**1**) and five previously uncharacterized (**3**−**7**), were obtained from the seeds of lychee (*Litchi chinensis* Sonn. cv. Heiye) by means of repetitive column chromatography and high-performance liquid chromatography (HPLC) preparation. They were identified as litchioside D (**1**), (−)-pinocembrin 7-*O*-neohesperidoside (**2**), (−)-pinocembrin 7-*O*-rutinoside (**3**), taxifolin 4′-*O*-*β*-D-glucopyranoside (**4**), kaempferol 7-*O*-neohesperidoside (**5**), tamarixetin 3-*O*-rutinoside (**6**), and phlorizin (**7**) on the basis of spectroscopic analysis and comparison of their data to the values reported in the literatures. Among them, compounds **1**, **4**, and **5** showed *in vitro* antitumor activity against A549, LAC, Hep-G2, and HeLa cell lines in the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay.

KEYWORDS: *Litchi chinensis*, lychee seed, flavonoid glycoside, litchioside D, cytotoxic activity

INTRODUCTION

Lychee (*Litchi chinensis* Sonn.), the only member of the genus *Litchi* in the family Sapindaceae, is a subtropical tree native to China and now cultivated in many parts of the world. Its fruits are popular in domestic and oversea markets because of their edible and delicious arils and attractive appearance. The annual output of lychee fruits in China exceeds 1.3×10^9 kg. As a byproduct of the fruits, lychee seeds are mainly discarded as waste, although they are used as a traditional Chinese medicine for the treatment of epigasteric pain and testicular swelling and pain.[1,2] Pharmacological studies revealed that lychee seeds possessed antihyperglycemic, antihyperlipidemic, antiplatelet aggregation, antitumor, antiviral, and antioxidant effects.[3−5] However, reports concerning their biologically active components remain very scarce.[6,7] To clarify the structures and bioactivities of uncharacterized components, we carried out further chemical investigation on the seeds. In previous papers, we reported four eudesmane sesquiterpene glucosides and their cytotoxic activity[8] and seven A-type proanthocyanidins and two related compounds and their antioxidant and antiviral activities.[9] As a continuation of this work, we describe the isolation and identification of seven flavonoid glycosides and their *in vitro* antitumor activity in the present paper.

MATERIALS AND METHODS

General Methods. Optical rotations were measured on a Perkin-Elmer 343 polarimeter (Perkin-Elmer, Inc., Waltham, MA). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker DRX-400 NMR spectrometer (Bruker Biospin Gmbh, Rheistetten, Germany) with the solvent residual peaks of dimethyl sulfoxide (DMSO)-d_6 at δ_H 2.50 and δ_C 39.52 as references. High-resolution electrospray ionization mass spectrometry (HRESIMS) was obtained on a Bruker Bio TOF IIIQ mass spectrometer (Bruker Daltonics, Inc., Billerica, MA), and electrospray ionization mass spectrometry (ESIMS) data were acquired on a MDS SCIEX API 2000 liquid chromatography/tandem mass spectrometry (LC/MS/MS) instrument (Applied Biosystems, Inc., Forster, CA). Column chromatography was performed over silica gel (100−200 mesh, Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), Develosil ODS (S-75 μm, Nomura Chemical Co., Ltd., Seto, Japan), macroporous resin Diaion HP-20 (Mitsubishi Chemical Corporation, Tokyo, Japan), and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden). High-performance liquid chromatography (HPLC) was carried out on a Shimadzu LC-6AD liquid chromatograph equipped with a Shimadzu RID-10A refractive index detector (Shimadzu Corp., Kyoto, Japan). YMC-Pack ODS-A columns (S-5 μm, 250 × 4.6 mm and 250 × 20 mm inner diameter, YMC Co., Ltd., Kyoto, Japan) were used for analytical and preparative purposes, respectively. Thin-layer chromatography (TLC) was conducted on precoated silica gel $HSGF_{254}$ plates (Yantai Jiangyou Silica Gel Development Co., Ltd., Yantai, China) and reversed-phase (RP)-18 F_{254S} plates (Merck Japan Ltd., Tokyo, Japan), and spot detection was performed under fluorescent light (λ = 254 and 365 nm) and then spraying 10% H_2SO_4 in EtOH, followed by heating. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and naringinase were purchased from Sigma Chemical Co. (St. Louis, MO). Roswell Park Memorial Institute (RPMI)-1640 medium and admycin were purchased from Gibco BRL (Gaithersburg, MD) and Shenzhen Main Luck Pharmaceutical, Inc. (Shenzhen, China), respectively. Fetal calf serum was obtained from Guangzhou Jinan Biomedicine Research and Development Center (Guangzhou, China).

Plant Material. Lychee (*L. chinensis* cv. Heiye) fruits at commercial maturity were collected from an orchard in Luogang District, Guangzhou, China, in July 2007. A voucher specimen was deposited at the Herbarium of South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, China. The seeds were manually separated and ground to pieces with a Santronic multifood processor.

Extraction and Isolation. Fresh lychee seed pieces (6,150 g) were extracted 3 times with 95% EtOH (10 L × 3) at room temperature (25−32 °C) for 4 days each time. The concentration of the solution under vacuum gave a reddish solid (934.86 g), of which 906.34 g was

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dissolved in 0.8 L of water and then successively partitioned with petroleum ether (0.8 L × 5), ethyl acetate (EtOAc, 0.8 L × 5), and *n*-butanol (*n*-BuOH, 0.8 L × 5) to afford petroleum-ether-soluble (38.80 g), EtOAc-soluble (91.65 g), and *n*-BuOH-soluble (151.60 g) fractions after condensation to dryness *in vacuo*. The petroleum-ether-soluble fraction (38.80 g) was subjected to silica gel (822 g) column (72 mm inner diameter × 490 mm) chromatography eluted with $CHCl_3$/MeOH [10:0, 9.5:0.5, 9:1, 8.5:1.5, 8:2, and 7:3 (vol/vol) at 9.0, 11.0, 12.0, 17.5, 10.0, and 7.5 L, respectively] to gain fractions P1−P11 after pooling according to their TLC profiles. Fraction P8 (2.10 g), obtained from the elution of $CHCl_3$/MeOH of 8.5:1.5, was passed through a Develosil ODS (4.2 g) precolumn, and the 80% MeOH/H_2O (vol/vol) eluate (0.29 g) was separated by Develosil ODS (5.8 g) column (12 mm inner diameter × 105 mm) chromatography eluted with MeOH/H_2O [1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, and 8:2 (vol/vol) at 60 mL each] to gain fractions P8-1−P8-8. Fraction P8-4 (28 mg) was submitted to Sephadex LH-20 column chromatography eluted with MeOH to yield compound **7** (10.7 mg, 0.000 18%). The *n*-BuOH-soluble fraction (141.20 g) was dissolved in water and passed through a HP-20 (2800 g) column sequentially eluted with H_2O and MeOH. The MeOH eluate (80.80 g) was subjected to silica gel (1600 g) column (92 mm inner diameter × 590 mm) chromatography eluted with $CHCl_3$/MeOH [9:1, 8.5:1.5, 8:2, 7:3, and 6:4 (vol/vol) at 14 L each] to attain fractions B1−B6. Fraction B3 (2.23 g), obtained from the elution of $CHCl_3$/MeOH of 8.5:1.5 and 8:2, was separated by Develosil ODS (45.0 g) column (18 mm inner diameter × 360 mm) chromatography eluted with MeOH/H_2O [1:9, 2:8, 3:7, 4:6. 5:5, 6:4, and 7:3 (vol/vol) at 450 mL each] to give fractions B3-1−B3-35. Fractions B3-21 and B3-22 (178.9 mg) were purified by HPLC using 49% MeOH/H_2O (v/v) as the mobile phase at the flow rate of 5 mL/min to offer compound **2** (t_R at 59.8 min, 8.2 mg, 0.000 15%), compound **3** (t_R at 54.1 min, 8.6 mg, 0.000 15%), and compound **6** (t_R at 41.4 min, 21.6 mg, 0.000 39%). Fraction B5 (1.43 g), obtained from the elution of $CHCl_3$/MeOH of 8:2 and 7:3, was separated by Develosil ODS (28.8 g) column (18 mm inner diameter × 230 mm) chromatography eluted with MeOH/H_2O [1:9, 2:8, 3:7, 4:6. 5:5, and 6:4 (vol/vol) at 300 mL each] to afford fractions B5-1−B5-30. Fractions B5-8 and B5-9 (76.9 mg) were separated by Sephadex LH-20 column chromatography eluted with MeOH to yield compound **4** (39.0 mg, 0.000 70%). Fractions B5-18−B5-21 (348.2 mg) were separated by Sephadex LH-20 column chromatography eluted with MeOH to supply compound **5** (7.1 mg, 0.000 13%) and a residue, which was purified by HPLC using 48% MeOH/H_2O (vol/vol) as the mobile phase at the flow rate of 5 mL/min to provide compound **1** (t_R at 51.9 min, 15.3 mg, 0.000 28%).

Litchioside D (1). Yellowish powder. $[\alpha]_D^{20}$ − 95.3 (*c* 0.60, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 5.66 (1H, dd, *J* = 2.8, 12.1 Hz, H-2), 3.17 (1H, dd, *J* = 12.1, 16.9 Hz, H-3a), 2.84 (1H, dd, *J* = 2.8, 16.9 Hz, H-3b), 12.00 (1H, br s, OH-5), 6.12 (1H, d, *J* = 1.8 Hz, H-6), 6.14 (1H, d, *J* = 1.8 Hz, H-8), 7.54 (2H, dd, *J* = 1.8, 7.6 Hz, H-2′, H-6′), 7.44 (2H, dd, *J* = 7.6, 7.6 Hz, H-3′, H-5′), 7.38 (1H, m, H-4′), 5.17 (1H, d, *J* = 7.6 Hz, H-1″), 3.44 (1H, m, H-2″), 3.52 (1H, dd, *J* = 8.9, 9.1, H-3″), 3.20 (1H, m, H-4″), 3.62 (1H, m, H-5″), 3.44 (1H, m, H-6″a), 3.79 (1H, dd, *J* = 3.8, 10.2 Hz, H-6″b), 5.07 (1H, d, *J* = 2.8 Hz, H-1‴), 3.58 (1H, m, H-2‴), 3.47 (1H, d, *J* = 3.0 Hz, H-3‴), 3.44 (1H, m, H-4‴), 3.89 (1H, q, *J* = 6.4 Hz, H-5‴), 1.03 (3H, d, *J* = 6.5 Hz, H-6‴), 4.52 (1H, br s, H-1⁗), 3.65 (1H, m, H-2⁗), 3.44 (1H, m, H-3⁗), 3.15 (1H, dd, *J* = 3.2, 8.4 Hz, H-4⁗), 3.40 (1H, m, H-5⁗), 1.08 (3H, d, *J* = 5.7 Hz, H-6⁗). ^{13}C NMR (DMSO-d_6, 100 MHz): see Table 1. ESIMS (+) *m*/*z*: 711 $[M+H]^+$, 733 $[M+Na]^+$; (−) *m*/*z*: 255 [M − glucosyl − rhamnosyl − rhamnosyl − H]$^-$, 709 $[M-H]^-$, 745 $[M+Cl]^-$. HRESIMS (−) *m*/*z*: 745.2134 $[M+Cl]^-$ (calculated for $C_{33}H_{42}ClO_{17}^-$, 745.2116).

(−)-Pinocembrin 7-*O*-Neohesperidoside (2). Yellowish powder. $[\alpha]_D^{20}$ − 34.0 (*c* 0.05, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 5.66 (1H, dd, *J* = 2.8, 12.5 Hz, H-2), 3.20 (1H, dd, *J* = 12.5, 17.2 Hz, H-3a), 2.84 (1H, dd, *J* = 2.8, 17.2 Hz, H-3b), 12.02 (1H, br s, OH-5), 6.10 (1H, d, *J* = 2.0 Hz, H-6), 6.18 (1H, d, *J* = 2.0 Hz, H-8), 7.53 (2H, dd, *J* = 1.6, 7.5 Hz, H-2′, H-6′), 7.44 (2H, dd, *J* = 7.5, 7.5 Hz, H-3′, H-5′), 7.39 (1H, m, H-4′), 5.15 (1H, d, *J* = 7.7 Hz, H-1″), 5.07 (1H, d, *J* = 3.6 Hz, H-1‴), 1.03 (3H, d, *J* = 6.5 Hz, H-6‴). ^{13}C NMR (DMSO-d_6, 100 MHz): see Table 1. ESIMS (+) *m*/*z*: 257 [M − glucosyl − rhamnosyl + H]$^+$, 587 $[M+Na]^+$; (−) *m*/*z*: 255 [M − glucosyl − rhamnosyl − H]$^-$, 563 $[M-H]^-$, 599 $[M+Cl]^-$.

(−)-Pinocembrin 7-*O*-Rutinoside (3). Yellowish powder. $[\alpha]_D^{20}$ − 31.4 (*c* 0.53, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 5.66 (1H, dd, *J* = 3.0, 12.2 Hz, H-2), 3.22 (1H, dd, *J* = 12.2, 17.2 Hz, H-3a), 2.85 (1H, dd, *J* = 3.0, 17.2 Hz, H-3b), 11.96 (1H, br s, OH-5), 6.14 (2H, br s, H-6, H-8), 7.54 (2H, dd, *J* = 1.6, 7.7 Hz, H-2′, H-6′), 7.44 (2H, dd, *J* = 7.7, 7.7 Hz, H-3′, H-5′), 7.39 (1H, m, H-4′), 4.98 (1H, d, *J* = 7.4 Hz, H-1″), 4.52 (1H, br s, H-1⁗), 1.08 (3H, d, *J* = 6.2 Hz, H-6⁗). ^{13}C NMR (DMSO-d_6, 100 MHz): see Table 1. ESIMS (+) *m*/*z*: 587 $[M+Na]^+$; (−) *m*/*z*: 255 [M − glucosyl − rhamnosyl − H]$^-$, 563 $[M-H]^-$, 599 $[M+Cl]^-$.

Taxifolin 4′-*O*-β-D-Glucopyranoside (4). Yellowish powder. $[\alpha]_D^{20}$ − 20.4 (*c* 0.58, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 5.82 (1H, dd, *J* = 11.2 Hz, H-2), 5.04 (1H, d, *J* = 11.2 Hz, H-3), 11.89 (1H, br s, OH-5), 5.86 (1H, d, *J* = 1.8 Hz, H-6), 8.71 (1H, br s, OH-7),

Table 1. ^{13}C NMR Data for Compounds 1−3 and (−)-Pinocembrin 7-*O*-β-D-Glucopyranoside (1a) in DMSO-d_6

C	1	1a[11]	2	3
2	78.52	78.67	78.64	79.07
3	42.05	42.20	42.18	42.29
4	196.82	196.89	196.83	196.46
5	162.55	162.58	162.92	162.40
6	96.48	96.66	96.53	96.62
7	164.90	165.37	165.06	165.02
8	95.21	95.54	95.21	95.38
9	163.07	162.98	162.63	162.35
10	103.47	103.31	103.39	103.39
1′	138.47	138.50	138.48	138.39
2′	126.82	126.80	126.74	126.61
3′	128.64	128.64	128.63	128.44
4′	128.64	128.72	128.70	128.47
5′	128.64	128.64	128.63	128.44
6′	126.82	126.80	126.74	126.61
1″	97.60	99.58	97.77	99.41
2″	79.76	73.04	79.92	72.94
3″	76.54	76.33	77.06	76.26
4″	69.33	69.48	69.20	69.62
5″	75.50	77.11	76.60	75.56
6″	65.94	60.57	60.43	65.99
1‴ (2″-Rha)	100.73		100.73	
2‴ (2″-Rha)	68.60		68.62	
3‴ (2″-Rha)	69.70		69.71	
4‴ (2″-Rha)	71.65		71.65	
5‴ (2″-Rha)	66.39		66.38	
6‴ (2″-Rha)	16.54		16.52	
1⁗ (6″-Rha)	100.65			100.52
2⁗ (6″-Rha)	70.29			70.19
3⁗ (6″-Rha)	70.72			70.67
4⁗ (6″-Rha)	72.09			72.05
5⁗ (6″-Rha)	68.37			68.20
6⁗ (6″-Rha)	17.85			17.68

5.90 (1H, d, J = 1.8 Hz, H-8), 6.96 (1H, d, J = 1.8 Hz, H-2′), 7.12 (1H, d, J = 8.4 Hz, H-5′), 6.87 (1H, dd, J = 1.8, 8.4 Hz, H-6′), 4.70 (1H, d, J = 7.1 Hz, H-1″). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 82.68 (C-2), 71.51 (C-3), 197.67 (C-4), 163.35 (C-5), 96.11 (C-6), 166.92 (C-7), 95.08 (C-8), 162.48 (C-9), 100.48 (C-10), 131.83 (C-1′), 115.64 (C-2′), 146.55 (C-3′), 145.61 (C-4′), 116.36 (C-5′), 119.33 (C-6′), 102.24 (C-1″), 73.34 (C-2″), 77.24 (C-3″), 69.86 (C-4″), 75.93 (C-5″), 60.80 (C-6″). ESIMS (+) m/z: 288 $[M - \text{glucosyl} + H]^+$, 489 $[M + Na]^+$; (−) m/z: 465 $[M - H]^-$, 501 $[M + Cl]^-$.

Kaempferol 7-*O*-Neohesperidoside (5). Yellowish powder. $[\alpha]_D^{20}$ − 57.2 (c 0.14, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 12.50 (1H, br s, OH-5), 6.37 (1H, br s, H-6), 6.79 (1H, br s, H-8), 8.06 (2H, d, J = 8.6 Hz, H-2′, H-6′), 6.94 (2H, d, J = 8.6 Hz, H-3′, H-5′), 5.24 (1H, d, J = 7.6 Hz, H-1″), 5.09 (1H, d, J = 3.3 Hz, H-1‴), 1.02 (3H, d, J = 6.3 Hz, H-6‴). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 147.65 (C-2), 136.11 (C-3), 176.14 (C-4), 160.42 (C-5), 98.63 (C-6), 162.47 (C-7), 94.16 (C-8), 155.82 (C-9), 104.82 (C-10), 121.55 (C-1′), 129.70 (C-2′), 115.56 (C-3′), 159.46 (C-4′), 115.56 (C-5′), 129.70 (C-6′), 98.13 (C-1″), 80.21 (C-2″), 77.17 (C-3″), 69.28 (C-4″), 76.67 (C-5″), 60.51 (C-6″), 100.89 (C-1‴), 68.69 (C-2‴), 69.74 (C-3‴), 71.67 (C-4‴), 66.48 (C-5‴), 16.52 (C-6‴). ESIMS (+) m/z: 595 $[M + H]^+$, 617 $[M + Na]^+$; (−) m/z: 285 $[M - \text{glucosyl} - \text{rhamnosyl} - H]^-$, 593 $[M - H]^-$, 629 $[M + Cl]^-$.

Tamarixetin 3-*O*-Rutinoside (6). Yellowish powder. $[\alpha]_D^{20}$ − 65.3 (c 0.48, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 12.59 (1H, br s, OH-5), 6.22 (1H, d, J = 1.5 Hz, H-6), 10.97 (1H, br s, OH-7), 6.43 (1H, d, J = 1.5 Hz, H-8), 7.50 (1H, d, J = 1.8 Hz, H-2′), 7.03 (1H, d, J = 8.5 Hz, H-5′), 7.71 (1H, dd, J = 1.8, 8.5 Hz, H-6′), 3.85 (3H, s, OCH_3), 5.37 (1H, d, J = 7.0 Hz, H-1″), 4.40 (1H, br s, H-1‴′), 0.98 (3H, d, J = 6.0 Hz, H-6‴′). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 156.42 (C-2), 133.64 (C-3), 177.46 (C-4), 161.23 (C-5), 98.81 (C-6), 164.30 (C-7), 93.71 (C-8), 156.51 (C-9), 104.04 (C-10), 121.53 (C-1′), 111.42 (C-2′), 150.10 (C-3′), 145.91 (C-4′), 115.69 (C-5′), 122.52 (C-6′), 55.66 (OCH_3), 101.31 (C-1″), 74.10 (C-2″), 76.44 (C-3″), 69.81 (C-4″), 75.80 (C-5″), 66.86 (C-6″), 100.81 (C-1‴′), 70.40 (C-2‴′), 70.65 (C-3‴′), 71.86 (C-4‴′), 68.29 (C-5‴′), 17.77 (C-6‴′). ESIMS (+) m/z: 625 $[M + H]^+$, 647 $[M + Na]^+$; (−) m/z: 315 $[M - \text{glucosyl} - \text{rhamnosyl} - H]^-$, 623 $[M - H]^-$, 659 $[M + Cl]^-$.

Enzymtic Hydrolysis of Compound 1. A solution of compound **1** (2.8 mg) and naringinase from *Penicillium decumbens* (Product N1385, 390 IU/g, 2 mg) in 1 mL of acetate buffer (pH 4.4) was stirred at 38 °C for 4 h. The reaction solution was extracted with ethyl ether (1 mL × 3) after neutralization with 0.1 M NaOH. The concentration of the combined ethyl ether solution yielded a residue, which was separated by silica gel column chromatography eluted with $CHCl_3$/MeOH (9:1, vol/vol) to afford compound **1a** (1.0 mg),[10] which was identified as (−)-pinocembrin by a comparison of its ESIMS and ^{1}H NMR data and $[\alpha]_D^{20}$ − 46.8 (c 0.08, $CHCl_3$) to those reported in the literature.[11]

Cytotoxic Assay. Human lung cancer A549, human pulmonary carcinoma LAC, human hepatoma Hep-G2, and human cervical carcinoma HeLa cell lines were generously provided by Guangzhou Jinan Biomedicine Research and Development Center, Guangzhou, China. The cells were maintained in RPMI-1640 medium plus 10% heat-inactivated fetal bovine serum in a humidified atmosphere with 5% CO_2 at 37 °C. The cytotoxic activity of compounds **1**−**7** was determined using the MTT colorimetric assay as previously described by Mosmann.[12] Briefly, cancer cells were seeded into wells of a 96-well flat-bottom microtiter plate at 5 × 10^4 cell/mL in 195 μL of culture medium. A serial 2-fold dilution of test compounds was made in DMSO. Each serial solution of 5 μL was added to a well, including 195 μL of cell culture medium. The final concentrations of each test compound were 6.25, 12.5, 25, 50, and 100 μM. The plate was incubated at 37 °C in a humidified atmosphere with 5% CO_2. After 72 h, 10 μL of 5 mg/mL MTT solution was added to each well and incubated for 4 h. The supernatant was carefully removed by pipet, and then 200 μL of DMSO solution was added to each well and shaken for 15 min to dissolve formazan crystals. The absorbance was measured on a Bio-Rad model 550 microplate reader (Bio-Rad Laboratories, Hercules, CA) at the wavelength of 570 nm. MTT solution with DMSO (without both cells and medium) was used as a blank control, and admycin was used as a reference control. The cytotoxic activity of test compounds to proliferation of A549, LAC, Hep-G2, and Hela cells was calculated by SPSS 16.0 analytic software according to the following formula: cytotoxic activity (%) = (A_{570} of control cells − A_{570} of treated cells)/A_{570} of control cells × 100%. The 50% inhibitory concentration (IC_{50}) was defined as the concentration required to reduce the viability of untreated cell cultures by 50%.

RESULTS AND DISCUSSION

The EtOH extract of lychee seeds was dissolved in water and then sequentially partitioned with petroleum ether, EtOAc, and *n*-BuOH. The resulting petroleum-ether- and *n*-BuOH-soluble fractions were separated by repetitive column chromatography over silica gel, Develosil ODS, and Sephadex LH-20 and preparative HPLC to yield seven flavonoid glycosides (**1**−**7**).

Compound **1** was obtained as a yellowish powder with negative optical rotation. Its molecular formula was deduced to be $C_{33}H_{42}O_{17}$ from quasi-molecular ion peaks at m/z 711 $[M + H]^+$ and 709 $[M - H]^-$ in the ESIMS spectrum in combination with its NMR data as well as a negative-ion peak at 745.2134 $[M + Cl]^-$ in the HRESIMS spectrum. The ^{1}H NMR spectrum showed two *meta*-coupling doublets at δ 6.12 and 6.14 (1H each, J = 1.8 Hz, H-6, H-8)], five aromatic protons at δ 7.38 (1H, m, H-4′), 7.44 (2H, dd, J = 7.6, 7.6 Hz, H-3′, H-5′), and 7.54 (2H, dd, J = 1.8, 7.6 Hz, H-2′, H-6′), and three aliphatic doublets at δ 5.66 (1H, J = 2.8, 12.1 Hz, H-2), 3.17 (1H, J = 12.1, 16.9 Hz, H-3a), and 2.84 (1H, J = 2.8, 16.9 Hz, H-3b), suggesting the presence of a pinocembrin moiety.[11] Moreover, the spectrum exhibited three anomeric protons at δ 5.17 (1H, d, J = 7.6 Hz, H-1″), 5.07 (1H, d, J = 2.8 Hz, H-1‴), and 4.52 (1H, br s, H-1‴′) and two methyls at δ 1.03 (3H, d, J = 6.5 Hz, H-6‴) and 1.08 (3H, d, J = 5.7 Hz, H-6‴′), suggesting a β-glucosyl and two α-rhamnosyl moieties. As shown in Table 1, the ^{13}C NMR spectrum presented signals of a carbonyl at δ 196.82 (C-2), 12 aromatic carbons at δ 95.21 (C-8), 96.48 (C-6), 103.47 (C-10), and 126.82−164.90, 3 anomeric carbons at δ 100.73 (C-1″), 100.65 (C-1‴), and 97.60 (C-1‴′), 14 oxygenated carbons ranging from δ 65.94 to 79.76, an aliphatic methylene at δ 42.05 (C-3), and 2 methyls at δ 17.85 and 16.54. These data indicated that compound **1** was a pinocembrin triglycoside. This was supported by enzymatic hydrolysis of compound **1** with naringinase, which yielded (−)-pinocembrin. In comparison to the data of (−)-pinocembrin 7-*O*-β-D-glucopyranoside,[11] carbon resonances for the pinocembrin skeleton in compound **1** were consistent with the literature data, whereas values of C-1″−C-6″ for the glucosyl moiety were shifted by −1.98, +6.72, +0.21, −0.15, −1.61, and +5.37 ppm, respectively (Table 1). These findings suggested that the glucosyl moiety was linked to C-7 of the pinocembrin skeleton and two rhamosyl moieties were connected to C-2″ and C-6″ of the gulcosyl moiety.[13] In the heteronuclear multiple-bond correlation (HMBC) spectrum, long-range correlations from H-1″ (δ_H 5.17) to C-7 (δ_C 164.90), from H-1‴ (δ_H 5.07) to C-2″ (δ_C 79.76), and from H-1‴′ (δ_H 4.52) to C-6″ (δ_C 65.94) were observed, confirming the aforementioned connections. Furthermore, the proton and carbon values of three sugar moieties in

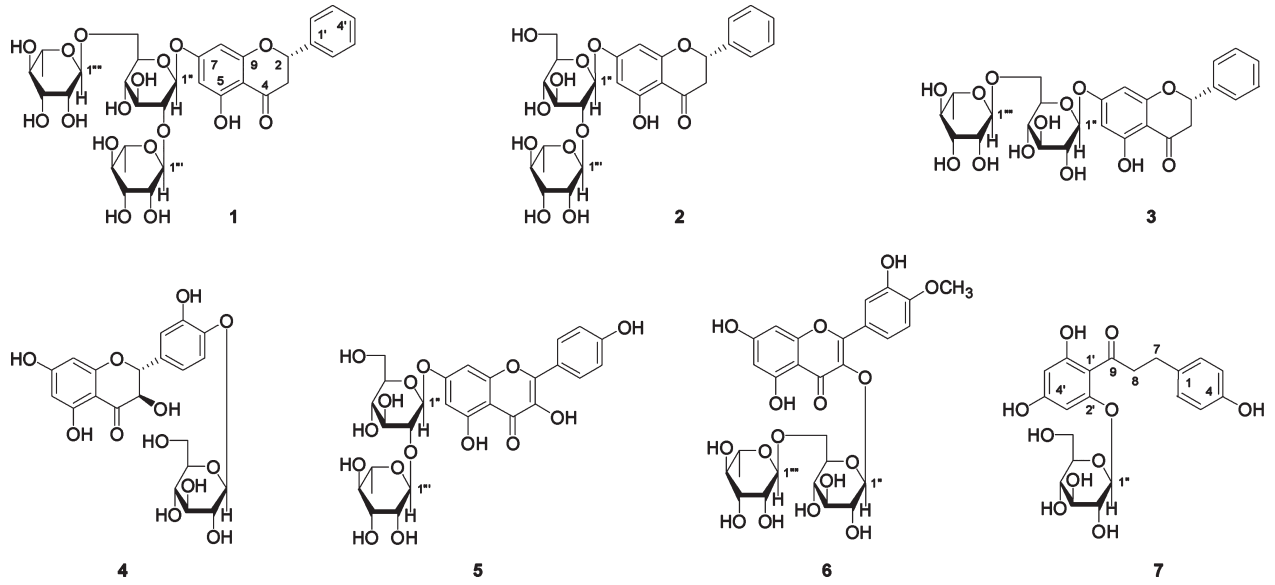

Figure 1. Structures of compounds **1**−**7** from the seeds of *L. chinensis*.

Table 2. Cytotoxic Activity of Compounds 1−7 (IC_{50}, μM)

compounds	A549	LAC	Hep-G2	HeLa
1	57.38	0.79	0.030	23.98
2	>100	>100	>100	37.64
3	>100	>100	>100	>100
4	17.50	11.58	4.22	1.82
5	0.53	7.93	0.020	0.051
6	>100	>100	>100	>100
7	>100	>100	>100	>100
admycin	15.15	20.48	79.50	22.64

compound **1** were consistent with those of apigenin 7-*O*-(2″,6″-di-α-L-rhamnopyranosyl)-β-D-glucopyranoside.[13] Accordingly, compound **1** was determined to be (−)-pinocembrin 7-*O*-(2″,6″-di-α-L-rhamnopyranosyl)-β-D-glucopyranoside (Figure 1), which was a new flavonone glycoside and trivially named litchioside D.

The isolated known compounds were identified as (−)-pinocembrin 7-*O*-neohesperidoside (**2**),[14,15] (−)-pinocembrin 7-*O*-rutinoside (**3**),[16] taxifolin 4′-*O*-β-D-glucopyranoside (**4**),[17] kaempferol 7-*O*-neohesperidoside (**5**),[18] tamarixetin 3-*O*-rutinoside (**6**),[19] and phlorizin (**7**)[20,21] by interpretation of their spectroscopic data (^{1}H and ^{13}C NMR and ESIMS) and by comparison to reported data. Among them, compounds **3**−**7** were isolated from this species for the first time.

Cytotoxic Activity. The *in vitro* cytotoxic activity of seven isolated flavonoid glycosides was evaluated against human lung cancer A549, human pulmonary carcinoma LAC, human hepatoma Hep-G2, and human cervical carcinoma HeLa cell lines using the MTT colorimetric assay.[12] Their IC_{50} values on the viability of aforementioned cancer cell lines after 72 h of incubation were presented in Table 2. Kaempferol 7-*O*-neohesperidoside (**5**) exhibited significant cytotoxic activity against all of the test cell lines, with IC_{50} values of 0.53, 7.93, 0.020, and 0.051 μM, respectively, which were more potent than the reference compound admycin. Litchioside D (**1**) also showed potent activity against LAC and Hep-G2 cells (IC_{50} = 0.79 and 0.030 μM). Taxifolin 4′-*O*-β-D-glucopyranoside (**4**) demonstrated moderate activity against all four cell lines, with IC_{50} values ranging from 1.82 to 17.58 μM. However, the other flavonoid glycosides (**2**, **3**, **6**, and **7**) were inactive (IC_{50} > 100 μM), except that compound **2** showed a weak activity against the HeLa cell line. It is noted that the moderate activity of compound **2** against P-388 lymphocytic leukemia, with an IC_{50} value of 2.58 μg/mL (= 4.57 μM), was previously reported,[22] while the activity of compounds **1**, **4**, and **5** against A549, LAC, Hep-G2, and HeLa cancer cells is reported for the first time. The discovery of the cytotoxic compounds **1**, **4**, and **5** suggested that they might also be involved in the antitumor activity of lychee seeds.

It is worth noting that the isolated known compounds were found to have other biological activities according to literature reports. (−)-Pinocembrin 7-*O*-neohesperidoside (synonym: onychin, **2**) showed biological activities of inhibiting the proliferation of vascular smooth muscle cells and preventing H_2O_2-induced apoptosis in ECV304 endothelial cells.[23,24] Tamarixetin 3-*O*-rutinoside (**6**) demonstrated inhibitory activity against osteoclast differentiation by 40 and 63% at the concentrations of 0.1 and 1.0 μg/mL.[25] The dihydrochalcone phlorizin (**7**) was a natural product and dietary constituent found in a number of fruit trees, and its principal pharmacological action was to produce renal glucosuria and block intestinal glucose absorption through inhibition of the sodium−glucose symporters located in the proximal renal tubule and mucosa of the small intestine.[26]

In conclusion, seven flavonoid glycosides were isolated in purity from the seeds of lychee (*L. chinensis* var. Heiye). Their structures were identified by the spectroscopic method, including NMR and HRESIMS. Litchioside D (**1**) was a new flavonone triglycoside. The known ones, except (−)-pinocembrin 7-*O*-neohesperidoside (**2**), were obtained from this species for the first time. Among these flavonoid glycosides, kaempferol 7-*O*-neohesperidoside (**5**) possessed more potent *in vitro* cytotoxic activity against A549, LAC, Hep-G2, and HeLa cancer cells than admycin and litchioside D (**1**) and taxifolin 4′-*O*-β-D-glucopyranoside (**4**) also showed the activity toward the test cell lines, suggesting that they could be contributors to the antitumor activity of lychee seeds.

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