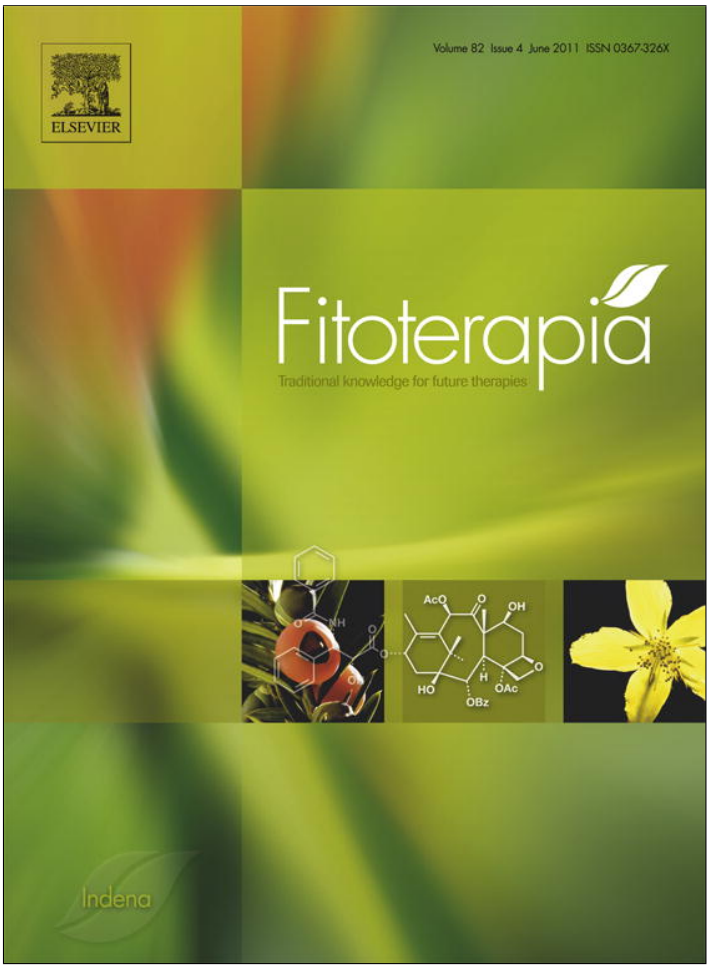

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Antioxidant flavanes from *Livistona chinensis*

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ABSTRACT

Three new flavanes and eight known flavonoids were isolated from the fruits of *Livistona chinensis*. The structure of the new flavanes were established as 2*S*,3*S*-3,5,7,3′,5′-pentahydroxyflavane (**1**), 2*R*,3*R*-3,5,6,7,8,4′-hexahydroxyflavane (**2**) and 2*R*,3*R*-3,5,6,7,8,3′,5′-heptahydroxyflavane (**3**), respectively, on the basis of chemical and spectroscopic data. The antiproliferative activity against four human tumor cell lines (HL-60, Mata, HepG2 and CNE-1) was evaluated. **1** had significantly antiproliferative effects against HL-60 and CNE-1 with the IC_{50} of 0.2 ± 0.01 and 1.0 ± 0.1 μM, respectively, overpowering the reference compound in the assay (cisplatin). Most compounds also exhibited potent antioxidant activity.

1. Introduction

The genus *Livistona* is widely cultured in tropical areas as a landscaping tree, which has three species growing in South China [1]. Every year they produce a large amount of berry-like fruits borne in clusters on long stems, which have been traditionally used for analgesic, hemostatic, antinasopharyngeal carcinoma, antichoriocarcinoma, antiesophageal cancer, and antileukemia [2,3]. The fruits showed significant antiproliferative effects against human myeloid leukemia cell (L1210, P388, HL-60), gastric cancer cell (SGC7901), cervical cancer cell (HeLa), human liver cancer cell (HepG2, Hele7404), melanoma cell (B16), colon cancer cell (HT-29), and bladder cancer cell (T24) [4–9]. They also showed antibacterial and anti-HIV-1 activities [10,11]. Previous chemical investigations on this genus reported some of flavonoids, steroids, amino acids, vitamins, fatty acids and phenolics [12–16].

In our continuing search for natural bioactive agents from high plants, bioactivity-guided isolation and fractionation of the fruits of *L. chinensis* were carried out and three new flavanes, 2*S*,3*S*-3,5,7,3′,5′-pentahydroxyflavane (**1**), 2*R*,3*R*-3,5,6,7,8,4′-hexahydroxyflavane (**2**) and 2*R*,3*R*-3,5,6,7,8,3′,5′-heptahydroxyflavane (**3**), together with four known flavanes and four known flavones were isolated and identified. In addition, their antiproliferative activities against four human tumor cell lines (HL-60, Mata, HepG2 and CNE-1), and antioxidant assays of the scavenging activities of DPPH and superoxide anion free radical (O_2), were evaluated.

2. Experimental

2.1. General

All chemicals were analytical or HPLC grade and obtained from Shanghai Chemical Reagents Co., Ltd, Shanghai, China. Sephadex LH-20 (Pharmacia, Sweden), silica gel (Qingdao Ocean Chemical Co., Qingdao, China), and ODS (40–63 μm, Merck, Darmstadt, Germany) were used for column chromatography. TLC was carried out on preparative Silica gel 60 F_{254} and RP-18 F_{254} plates (Merck, Darmstadt, Germany), and spots were visualized by spraying with 15% H_2SO_4 followed by heating at 105 °C. Preparative HPLC was performed using an ODS column (XTerra®, 19 × 250 mm, 10 μm, Waters, Milford, MA).

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Optical rotations were measured using a JASCO P-1030 automatic digital polarimeter (Tokyo, Japan). NMR spectra were recorded on a Bruker DPX-400 spectrometer using standard Bruker pulse programs. Chemical shifts were showed as the δ-value using tetramethylsilane (TMS) as an internal standard. ESI-MS data were recorded on an Agilent 1200 HPLC/6410B TripleQuad mass spectrometer (Santa Clara, CA), and HR-ESIMS were measured on a Bruker APEX II mass spectrometer.

2.2. Plant material

The fruits of *L. chinensis* were collected in Jiangmen, Guangdong Province, China, in September 2008, which were identified by Prof. Xiangjiu He, College of Pharmacy, Wuhan University. A voucher specimen (No. 20080920) is available at College of Pharmacy, Wuhan University, Wuhan (430071), China.

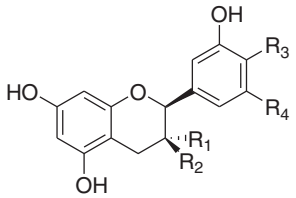
2.3. Extraction and isolation

The air-dried fruits (20.0 kg) were crushed into 20 meshes and were extracted with 70% EtOH (150 L×3). The solvent was removed under vacuum at 55 °C to yield a crude extract (3000.0 g). The extract was resuspended in water and partitioned with petroleum ether (3 L×3), chloroform (3 L×3), ethyl acetate (3 L×3) and n-butanol (3 L×3) gradually to afford 90.2, 91.7, 55.0 and 554.3 g of fractions, respectively. Bioactivity-guided fractionation was applied for further isolations. On the basis of the result of their antiproliferative and antioxidant activities, the ethyl acetate fraction was further fractionated through a silica gel column (200–300 mesh, 10×80 cm) eluted with a gradient of $CHCl_3$-MeOH (100:1, 50:1, 30:1, 20:1, 15:1, 10:1, 5:1, 2:1, 1:1, each 40 L) to afford 23 fractions. Fraction 11 was further applied to a silica gel column (200–300 mesh; 3×58 cm) and eluted with $CHCl_3$/MeOH 100:1 to yield **4** (120 mg). Fraction 15 was applied to an ODS MPLC column and eluted with MeOH/H_2O (10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 80:20, 90:10, each 1 L) to yield 7 subfractions. Subfraction 1 (450 mg) was purified by a preparative Rp-HPLC using 25% methanol (containing 0.1% CF_3COOH) as mobile phase to get **8** (5 mg), **9** (20 mg), and **10** (60 mg), respectively. Subfraction 3 (450 mg) was purified by a Sephadex LH-20 column eluted with MeOH/H_2O (10:90, 20:80, 30:70, 40:60, 60:40, 80:20, each 500 mL) to give **5** (70 mg) and **7** (20 mg). Fraction 17 was applied to an ODS column eluted with MeOH/H_2O (10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30,90:10, each 1 L), followed by a Sephadex LH-20 column eluted with MeOH/H_2O (50:50, 3 L) to obtain **6** (430 mg). Fraction 21 was applied to an ODS column using MeOH/H_2O (10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 90:10, each 1 L) as elution to yield 7 subfractions. Subfraction 1 (450 mg) was purified by a preparative Rp-HPLC eluted with 22% methanol (containing 0.1% CF_3COOH, pH 3.0) to get **1** (20 mg), **2** (60 mg), **3** (5 mg) and **11** (5 mg). The structures of compounds **1**–**11** were showed in Fig. 1.

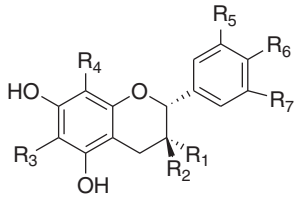
Compound **1**: 2*S*,3*S*-3,5,7,3′,5′-pentahydroxyflavane. Amorphous colorless powder; $[\alpha]^{25}_D$ +38.0 (c 0.20, MeOH). HRESIMS (positive-ion mode) $[M+Na]^+$ *m/z* 313.0677 (calcd. for $C_{15}H_{14}NaO_6$, 313.0688). ^{1}H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz), see Table 1.

Compound **2**: 2*R*,3*R*-3,5,6,7,8,4′-hexahydroxyflavane. Amorphous colorless powder; $[\alpha]^{25}_D$-48.0 (c 0.30, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz), see Table 1.

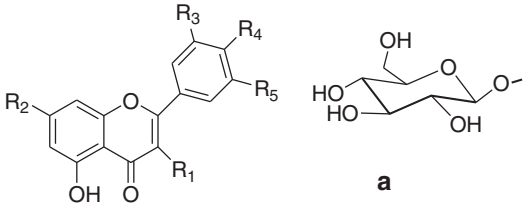
Compound **3**: 2*R*,3*R*-3,5,6,7,8,3′,5′-heptahydroxyflavane. Amorphous colorless powder; $[\alpha]^{25}_D$-20.0 (c 0.26, MeOH). ^{1}H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz), see Table 1.



1, R_1 = H, R_2 = OH, R_3 = H, R_4 = OH
9, R_1 = OH, R_2 = H, R_3 = OH, R_4 = H



2, R_1 = OH, R_2 = H, R_3 = OH, R_4 = OH, R_5 = H, R_6 = OH, R_7 = H
3, R_1 = OH, R_2 = H, R_3 = OH, R_4 = OH, R_5 = OH, R_6 = H, R_7 = OH
8, R_1 = OH, R_2 = H, R_3 = H, R_4 = H, R_5 = H, R_6 = OH, R_7 = H
10, R_1 = OH, R_2 = H, R_3 = H, R_4 = H, R_5 = OH, R_6 = H, R_7 = OH
11, R_1 = H, R_2 = OH, R_3 = H, R_4 = H, R_5 = OH, R_6 = OH, R_7 = H



4, R_1 = H, R_2 = OH, R_3 = OCH_3, R_4 = OH, R_5 = OCH_3
5, R_1 = a, R_2 = OH, R_3 = OH, R_4 = OH, R_5 = H
6, R_1 = a, R_2 = OH, R_3 = OCH_3, R_4 = OH, R_5 = H
7, R_1 = a, R_2 = OCH_3, R_3 = OCH_3, R_4 = OH, R_5 = H

Fig. 1. Flavonoids isolated from *L. chinensis*.

2.4. Antioxidant activity

2.4.1. Chemicals and reagents

DPPH (1,1-diphenyl-2-picrylhydrazyl), NBT (nitrobluetetrazolium dye), PMS (phenazine methosulphate), NADH (reduced form of nicotinamideadenine dinucleotid) were purchased from Sigma (St. Louis, MO).

2.4.2. Antioxidant assay using DPPH· and superoxide anion free radical (O_2^-) methods

DPPH radical assay was determined according to the reported method [17]. DPPH (50 mg/L) was dissolved in methanol. The samples were dissolved in DMSO. The DPPH solution (995 μl) was mixed with 5 μl of the sample solution.

Table 1
^{1}H and ^{13}C NMR Data for compounds **1**–**3** [δ in ppm, mult. (J in Hz)].

Position	1 [a]		2 [b]		3 [b]	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
2	78.0	4.72 (s)	79.9	4.75 (br s)	79.8	4.80 (br s)
3	64.9	4.00 (s)	67.4	4.07 (br s)	67.5	4.23 (br s)
4	28.2	2.68 (dd, 16.0, 4.0)	29.4	2.77 (dd, 16.8, 4.4)	29.3	2.86 (dd, 16.8, 4.4)
		2.48 (m)		2.64 (dd, 16.8, 2.4)		2.74 (dd, 16.8, 2.4)
5	155.7		158.0		157.3	
6	95.0	5.89 (d, 2.0)	157.9		157.5	
7	156.5		157.8		157.6	
8	94.1	5.72 (d, 2.0)	157.6		157.9	
9	156.1		157.5		158.0	
10	98.5		100.1		100.2	
1′	130.6		131.6		132.3	
2′	114.8	6.65 (m)	129.2	7.21 (d, 8.5)	116.0	6.75 (m)
3′	144.4		115.8	6.68 (d, 8.5)	145.9	
4′	118.4	6.89 (d, 0.8)	157.3		119.5	6.98 (s)
5′	144.4		115.8	6.68 (d, 8.5)	145.7	
6′	114.7	6.65 (m)	129.2	7.21 (d, 8.5)	115.4	6.75 (m)

[a] Measured in DMSO-d_6.
[b] Measured in methanol-d_4.

The mixture was shaken and allowed to stand at room temperature in the dark for 20 min. The absorbance was measured spectrophotometrically at 517 nm.

Superoxide anion radical scavenging activity was assayed by the NBT reduction method according to a described procedure [18] with some modification. The reaction mixture used for the O_2^- scavenging activity assay containing Tris–HCl (pH 8.1, 50 mM, 445 μl), NADH (0.15 mM, 250 μl), PMS (0.03 mM, 50 μl), NBT (0.10 mM, 250 μl) and sample solution (5 μl), in the final volume of 1000 μl. All components, except the tested sample dissolved in DMSO, were dissolved in Tris–HCl 50 mM, pH 8.1. The reaction was conducted at 37 °C for 5 min, and initiated by the addition of PMS. The absorbance was measured spectrophotometrically at 560 nm.

The test samples were prepared five dilutions in triplicate assays. Quercetin was used as standard for evaluation of the antioxidant capacity of the extracts and pure compounds.

2.5. Antiproliferative assay

The antiproliferative activity against four human tumor cell lines, human myeloid leukemia HL-60, human myeloid leukemia Mata, human liver cancer HepG2 and human nasopharyngeal carcinoma CNE-1 was performed according to the reported protocol [19]. All the cell lines were cultured in RPMI-1640 medium (Hyclone, Logan, UT), supplemented with 10% fetal bovine serum (Hyclone) and antibiotic (100 units/ml penicillin and 100 μg/ml streptomycin) in 5% CO_2 at 37 °C. The antiproliferative assay was performed according to the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (Sigma, St Louis, MO) method in 96-well microplate. Briefly, 200 μl adherent cells were seeded into each well of 96-well cell culture plate and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition with initial density of 5×10^4 cells/ml. Each tumor cell line was exposed to the test samples at a series of concentrations in quadruples for 48 h. A linear regression was established to calculate IC_{50}. Cisplatin was used as positive control for the evaluation of the inhibitive power of the test samples.

2.6. Quantification of total flavonoids

The $NaNO_2$-$Al(NO_3)_3$-NaOH chromomeric method [20] was applied to quantify the total flavonoids. 2*R*,3*R*-3,5,7,3′,5′-penthahydroxyflavane (compound **10**) was used as standard flavane to make the calibration curve.

3. Results and discussion

3.1. Structure elucidation of compounds 1, 2 and 3

Compound **1** was obtained as an amorphous red powder. The positive electrospray ionization mass spectrum (ESI-MS) gave an ion $[M+Na]^+$ at *m/z* 313 and the molecular formula of $C_{15}H_{14}O_6$ was drawn combined with ^{1}H and ^{13}C NMR. Its molecular formula was confirmed by HRESIMS, which showed an ion $[M+Na]^+$ at *m/z* 313.0677 (calcd. for $C_{15}H_{14}NaO_6$, 313.0688). The ^{1}H NMR spectrum exhibited three meta-coupled doublets at δ_H 6.65 (1 H, m, H-2′, H-6′) and 6.89 (1 H, d, $J=0.8$ Hz, H-4′), which were consistent with a 3′,5′-disubstituted B ring of flavane. Two meta-coupled doublets at δ_H 5.72 (1 H, d, $J=2.0$ Hz, H-8) and 5.89 (1 H, d, $J=2.0$ Hz, H-6) were consistent with a 5,7-dioxygenated A ring of flavane. These resonances together with δ_H 2.48 (1 H, dd, $J=16.5$, 3.2 Hz, H-4_{eq}), 2.68 (1 H, dd, $J=16.5$, 4.0 Hz, H-4_{ax}), 4.00 (1 H, brs, H-3) and 4.72 (1 H, brs, H-2) and their corresponding carbon signals in HMQC spectrum revealed that **1** was 3,5,7,3′,5′-pentahydroxyflavane. The following spectroscopic analysis made the absolute configurations at C-2 and C-3 assignable. The small coupling constant of H-2 and H-3, which was consistent with $J_{2,3}$ of (−)-epicatechin and disagreed with large value of (+)-catechin (6.7 Hz) [21], was indicative of the 2,3-*cis* configuration with the 2-phenyl group in a pseudo-equatorial orientation and the 3-hydroxy group in a pseudo-axial orientation, that's to say, the

configurations were (2*S*,3*S*) or (2*R*,3*R*) [22]. By comparing with the literature [23] of 2*R*,3*R*-3,5,7,3′,5′-pentahydroxyflavane, the obtained optical rotation value of 2*R*,3*R*-3,5,7,3′,5′-pentahydroxyflavane {$[\alpha]^{25}_D = -54.8$ (c 0.25, MeOH)} indicated an absolute configuration of 2*R* and 3*R* for 2*R*,3*R*-3,5,7,3′,5′-pentahydroxyflavane. In the same manner, the optical rotation value of **1** {$[\alpha]^{25}_D = +38.0$ (c 0.20, MeOH)} suggested the absolute configurations of C-2 and C-3 were (2*S*, 3*S*). Thus, **1** was elucidated to be 2*S*,3*S*-3,5,7,3′,5′-pentahydroxyflavane.

Compound **2** was isolated as an amorphous red powder. The positive ESI-MS exhibited an ion $[M+H]^+$ at *m/z* 307 indicating a molecular weight of 306 was compatible with the molecular formula of $C_{15}H_{14}O_7$. The ^{1}H NMR spectrum exhibited an AA′BB′ spin coupling system at δ_H 6.68 (2 H, d, J = 8.5 Hz, H-3′, H-5′) and 7.21 (2 H, d, J = 8.5 Hz, H-2′, H-6′) due to a 4′-substituted pattern of the B ring of flavane. Two oxymethine protons at 4.75 (1 H, brs, H-2) and 4.07 (1 H, brs, H-3), and a pair of deshielded methylene protons at δ_H 2.77 (1 H, dd, J = 16.8, 4.4 Hz, H-4_{ax}) and 2.64 (1 H, dd, J = 16.8, 2.4 Hz, H-4_{eq}), indicated **2** was a 3-hydroxy flavane. The ^{1}H NMR spectrum showed eight protons at δ_H 2.64 (1 H, dd, J = 16.8, 2.4 Hz), 2.77 (1 H, dd, J = 16.8, 4.4 Hz), 4.07 (1 H, brs), 4.75 (1 H, brs), 6.68 (2 H, d, J = 8.5 Hz) and 7.21 (2 H, d, J = 8.5 Hz), which gave correlations with carbon resonances at 29.4, 29.4, 67.4, 79.9, 115.8, and 129.2 in the HMQC spectrum, respectively. These spectroscopic data suggested that **2** was a 3,5,6,7,8,4′-hexahydroxyflavane. The small coupling constant of H-2 and H-3 was indicative of the 2,3-*cis* relative configuration with the 2-phenyl group in a pseudo-equatorial orientation and the 3-hydroxy group in a pseudo-axial orientation, that's to say, the (2*S*,3*S*) or (2*R*,3*R*) configurations [21,22]. The optical rotation value {$[\alpha]^{25}_D = -48.0$ (c 0.30, MeOH)} suggested the absolute configurations of C-2 and C-3 to be (2*R*, 3*R*) [23]. Thus, **2** was elucidated to be 2*R*,3*R*-3,5,6,7,8,4′-hexahydroxyflavane.

Compound **3** was obtained as an amorphous red powder. The positive ESI-MS exhibited an ion $[M+H]^+$ at *m/z* 323, which was compatible with the molecular formula of $C_{15}H_{14}O_8$. The ^{1}H NMR spectrum exhibited three meta-coupled doublets at δ_H 6.75 (1 H, m, H-2′, H-6′) and 6. 98 (1 H, d, J = 0.8 Hz, H-4′) due to a 3′,5′-disubstituted pattern of the B ring of flavane. Two oxymethine protons at 4.80 (1 H, brs, H-2) and 4.23 (1 H, brs, H-3), and a pair of deshielded methylene protons at δ_H 2.74 (1 H, dd, J = 16.8, 2.7 Hz, H-4_{ax}) and 2.84 (1 H, dd, J = 16.8, 2.4 Hz, H-4_{eq}), suggested that **3** was a 3-hydroxyflavane. The ^{1}H NMR spectrum of **3** showed seven protons at δ_H 2.64 (1 H, dd, J = 16.8, 2.4 Hz), 2.77 (1 H, dd, J = 16.8, 4.4 Hz), 4.07 (1 H, brs), 4.75 (1 H, brs), 6.75 (1 H, m, H-2′), 6.75 (1 H, m, H-6′) and 6.98 (1 H, d, J = 0.8 Hz, H-4′), which gave correlations with carbon atom resonances at 29.3, 29.3, 67.5, 79.8, 119.5, 116.0 and 115.4 in the HMQC spectrum, respectively. These data suggested that **3** was a 3,5,6,7,8,3′,5′-heptahydroxyflavane. The 2,3-*cis* configuration [(2*S*,3*S*) or (2*R*,3*R*)] with the 2-phenyl group in a pseudo-equatorial orientation and the 3-hydroxy group in a pseudo-axial orientation was concluded by a small coupling constant of H-2 and H-3 [21,22]. The optical rotation value of **3** {$[\alpha]^{25}_D = -20.0$ (c 0.26, MeOH)} suggested the absolute configuration of C-2 and C-3 to be (2*R*, 3*R*) [23]. Therefore, **3** was assigned as 2*R*,3*R*-3,5,6,7,8,3′,5′-heptahydroxyflavane.

The known compounds were identified by comparison of their spectroscopic data with the literatures as tricin (**4**) [24], quercetin-3-O-β-D-glucopranoside (**5**) [25], isorhamnetin-3-O-β-D-glucopranoside (**6**) [26], rhamnazin-3-O-β-D-glucopyranoside (**7**) [27], (−)-epiafzelechin (**8**) [28], (+)-catechin (**9**) [29], 2*R*,3*R*-3,5,7,3′,5′-penthahydroxyflavane (**10**) [23], and (−)-catechin (**11**) [29], respectively.

3.2. Bioactivity of the extracts and isolated flavonoids

From the quantification results of total flavonoids, ethyl acetate fraction concentrated most of the flavonoids of fruits. The DPPH scavenging activity of the extracts and 11 flavonoids isolated from *L. chinensis* was shown in Table 2. From the results, the ethyl acetate fraction exhibited the highest scavenging activity among all of the extracts. Also, all flavonoids showed potential free radical scavenging activity on DPPH. Among them, **5** showed the highest DPPH radical scavenging activity with the IC_{50} of 0.8 ± 0.03 μM, overpowering the reference compound in the assay (quercetin).

The superoxide anion free radical (O_2^-) assay has been applied to evaluate the free radical scavenging ability of the extracts and 11 flavonoids (Table 2). The ethyl acetate fraction also showed the most powerful scavenging ability. All the pure flavonoids isolated from *L. chinensis* showed potent superoxide anion free radical (O_2^-) scavenging activity. **5** showed the highest radical scavenging activity, which its IC_{50} was 0.1 ± 0.004 μM.

Free radical has been hypothesized to be a major factor in the developing of several degenerative chronic diseases. Oxidative stress can cause oxidative damage to large biomolecules such as lipids, proteins, and DNA, resulting in an increased risk for inflammatory diseases, cardiovascular disease, cancer, diabetes, Alzheimer's disease, cataracts, and age-related functional decline [30,31]. In this research, it found that the ethyl acetate fraction exhibited potent free radical scavenging activity, which might be the main

Table 2
Antioxidant activity of the extracts and compounds **1**–**11**.

Sample	IC_{50} (μM for 1–11 and quercetin, μg/mL for extract)	
	DPPH radical	Superoxide anion free radical (O_2^-)
1	4.9 ± 0.1	5.4 ± 0.4
2	5.4 ± 0.2	6.4 ± 0.3
3	8.1 ± 0.3	3.4 ± 0.1
4	75.8 ± 2.4	43.9 ± 2.3
5	0.8 ± 0.03	0.1 ± 0.004
6	38.8 ± 1.9	3.5 ± 0.1
7	14.9 ± 0.9	4.2 ± 0.2
8	19.6 ± 0.9	20.5 ± 1.2
9	6.3 ± 0.6	3.4 ± 0.2
10	5.0 ± 0.2	4.9 ± 0.3
11	6.7 ± 0.4	3.9 ± 0.2
Ethanol extract	2.3 ± 0.1	2.1 ± 0.2
Petroleum ether fraction	9.8 ± 0.7	8.7 ± 0.8
Chloroform fraction	12.4 ± 0.3	11.6 ± 0.9
Ethyl acetate fraction	1.4 ± 0.1	1.1 ± 0.07
n-Butanol fraction	2.4 ± 0.1	2.3 ± 0.2
Water fraction	15.4 ± 1.2	14.6 ± 1.2
Quercetin	5.4 ± 0.3	4.6 ± 0.2

Table 3
Cytotoxic activity of the extracts and compounds **1**–**11** against HL-60, Mata, HepG-2 and CNE-1 cells.

Sample	IC_{50} (μM for **1**–**11** and cisplatin, μg/mL for extract)			
	HL-60	Mata	HepG-2	CNE-1
1	0.2 ± 0.01	>100	69.7 ± 4.0	1.0 ± 0.1
2	>100	67.9 ± 3.3	45.6 ± 1.8	2.3 ± 0.1
3	87.4 ± 5.6	>100	>100	66.4 ± 3.3
4	1.3 ± 0.1	0.7 ± 0.03	>100	>100
5	>100	>100	46.8 ± 2.0	39.4 ± 2.3
6	0.1 ± 0.01	0.3 ± 0.01	>100	>100
7	0.1 ± 0.005	0.2 ± 0.01	0.01 ± 0.002	>100
8	1.2 ± 0.1	24.3 ± 1.0	0.3 ± 0.01	>100
9	87.5 ± 6.7	>100	>100	>100
10	>100	>100	>100	>100
11	67.4 ± 4.6	>100	>100	56.4 ± 4.2
Ethanol extract	31.2 ± 2.9	43.5 ± 3.1	70.7 ± 6.4	25.3 ± 1.9
Petroleum ether fraction	>100	>100	30.5 ± 1.7	33.6 ± 2.8
Chloroform fraction	>100	>100	56.2 ± 3.2	40.3 ± 2.2
Ethyl acetate fraction	13.2 ± 0.8	16.1 ± 1.2	26.1 ± 1.7	10.5 ± 0.7
n-Butanol fraction	78.5 ± 6.3	80.2 ± 6.5	90.1 ± 7.9	>100
Water fraction	>100	>100	>100	>100
Cisplatin	1.6 ± 0.1	4.7 ± 0.2	3.5 ± 0.2	2.5 ± 0.1

antioxidant contributor to the *L. chinensis*. Also, the configurations of flavanes affected their scavenging activities. For the structure–activity relationship of flavanes, further experiments are needed in the coming research.

The extracts and eleven flavonoids were evaluated for antiproliferative activities against four human tumor cell lines, HL-60 (human myeloid leukemia), Mata (human myeloid leukemia), HepG2 (human liver cancer) and CNE-1 (human nasopharyngeal carcinoma) using a MTT assay and the results were shown in Table 3. The ethyl acetate fraction showed the most potent inhibitive activity among all of the extracts. All of the flavonoids except **10** showed potent antiproliferative activity against the selected cell lines. **1** had significant antiproliferative effects against HL-60 and CNE-1 with the IC_{50} of 0.2 ± 0.01 and 1.0 ± 0.1 μM, overpowering the reference compound in the assay (cisplatin). **6** showed strong inhibitory activity against HL-60 and Mata, which the IC_{50} were 0.1 ± 0.01 and 0.3 ± 0.01 μM. **7** also had significant inhibitory activity against HL-60, Mata, and HepG2, which the IC_{50} were 0.1 ± 0.005, 0.2 ± 0.01 and 0.01 ± 0.002 μM, respectively. These results also suggested that the antiproliferative activity of flavanes had some relation with their configurations.

4. Conclusions

Historically, the fruits of *L. chinensis* have been used in traditional Chinese medicines for many medical conditions. In this research, the flavonoids in the fruits of *L. chinensis* showed potent antiproliferative activities, and some of them showed stronger inhibitory activities against the selected cell lines than cisplatin. These data could provide some scientific evidences for their widely use in clinic. Those flavonoids may be practically responsible for the antioxidant and anticancer activities of the fruits. The mechanisms of those compounds in the cancer prevention are worth of further investigation.

The DPPH and superoxide anion free radical assays were known to give reliable information concerning the antioxidant ability of the tested compounds. The flavonoids isolated from the fruits of *L. chinensis* showed potent free radical and superoxide anion (O_2^{-}) scavenging activities. These results indicated the fruits could be served as cancer preventing agents, which were in accordance with their traditional usage.

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