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Polyphenols from Longan Leaf and Their Radical-Scavenging Activity

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Abstract. The extract from leaves of longan was subjected to separation using column chromatography. Eight polyphenolic compounds from the extract were purified and identified as ellagic acid (1), 3, 4-O-di-methyl ellagic acid (2), (+)-catechin (3), ethyl gallate (4), gallic acid (5), kaempferol (6), quercetin (7) and kaempferol-3-O-α-L-rhamnoside (8), respectively. Barring quercetin, the other compounds were isolated from the leaves of longan for the first time. Furthermore, the antioxidant activities of purified compounds were compared using free radical scavenging activities and Fe^{2+}-ferrozine test system for metal chelating ability. All the purified compounds showed strong radical-scavenging activities and the chelating ability on ferrous. Evaluation for stereochemistry-activity relationship suggested that the antioxidant activities of these phenolic compounds depended largely on the frame of stereochemistry, the number of hydroxyls and the degree of polymerization.

Keywords: *Dimocarpus longan Lour*, leaf, stereochemistry, polyphenol, antioxidant activity.

1. Introduction

Longan (*Dimocarpus longan* Lour.) is a kind of subtropical fruit of high nutritive and economic value, widely distributed in South China. Longan leaf has been traditionally used as a folklore medicine. Up to now, most of papers reported the bioactive component from flesh, seed and pericarp of longan fruit [1] and the bioactive compounds were identified as a few terpenoids, sterols and flavones [2]. In recent years, a great deal of attention has been paid to antioxidants from plants to replace synthetic additives. Unfortunately, the polyphenolic compounds from longan leaf are unclear. This objective of this study was to separate the polyphenolic compounds from the longan leaf, identify their structures and then evaluate their antioxidant activities. Furthermore, the structure-activity relationship of the purified compounds was determined.

2. Materials and Methods

2.1. Plant materials

The leaves of longan (*Dimocarpus longan Lour*.) were obtained from Guangzhou, Guangdong province, China. After selected, these leaves were dried for 2 h in an oven at 105 ℃ and then shattered to screen through a 20-mesh sieve.

2.2. Extraction and isolation procedure

The powder of leaves (7 kg) was extracted 3 times with 60 l of 70% ethanol at 80 ℃ for 2 h. The total filterate was concentrated by a rotary vacuum evaporator and then lyophilized. Eventually, the dried extract (1006 g) obtained was dissolved in water, then passed through NKA macroporous adsorption resin column, and finally eluted successively with water, 30, 60 and 100% (v/v) ethanol, which yielded four subfractions

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(A-D). Fraction B was subjected to a silica gel column (60 cm in length and 8 cm in diameter) and then gradiently eluted with EtOAc-methanol to yield Compounds 1 (45 mg), 2 (68 mg), 5 (20. 9 mg) and 8 (19 mg). Fraction C was loaded on to a silica gel column and then eluted with acet-EtOAc to yield Compounds 3 (18.5 mg) and 7 (53 mg). Fraction D was separated through a silica gel column and gradiently eluted with acet-chloroform to yield Compounds 4 (104 mg) and 6 (15.1 mg).

2.3. Determination of total phenolics content

Total phenolics content of the extract was assayed using Folin-Ciocalteu method [3].

2.4. UV and infrared spectrometry

The purified compounds (0.1 mg/ml in methanol) were recorded the UV-spectrum at 200-800 nm in a U-3010 spectrophotometer (Hitachi). IR spectra were recorded on a Nicolet Magna 750 FT-IR spectrometer using pellets in the 400-4000 cm^{-1} range.

2.5. Molecular weight estimation

ESI-MS analysis was taken on a MAT295 mass spectrometer (Thermo, Finland). The purified compound (1 mg) was dissolved in 10 ml of methanol and the solution (100 μl) was injected into the MS system. Mass spectra was recorded with a heat capillary voltage of 4.5 kV, a heat capillary temperature of 280 ℃, sheath gas flow rate of 70 units and auxiliary gas flow rate of 10 units. The scan range of *m/z* was 200-1200.

2.6. NMR spectroscopy

^{1}H NMR and ^{13}C NMR spectra were obtained on Bruker AV-600 and Bruker AMX-500 instruments (Bruker Biospin Co., Germany) with TMS as an internal standard. HMQC, DEPT and HMBC spectra were obtained on a Bruker AV-600 instrument and recorded using standard pulse sequences.

2.7. Radical scavenging activities and metal chelating ability assay

DPPH radical scavenging activity was measured following by the method of Williams *et al* [4]. Superoxide anion radical scavenging activity was measured according to the method of Yang *et al* [5]. The hydroxyl free radical scavenging assay was assayed by using the 1, 10-phenanthroline-Fe^{2+} oxidative method [6]. The chelating ability of ferrous ions was estimated following by the method of Tung et al. [7].

2.8. Radical scavenging activities and metal chelating ability assay

Data were presented as mean ± standard deviation (SD) of three determinations. Statistical analyses were performed using a one-way analysis of variance.

3. Results and Discussion

3.1. Total phenolic content

The extract from longan leaves was firstly analysed for total phenolic content (TPC). The TPC value was 25.8 ± 0.03 mg GAE /g dry sample.

3.2. Identification of the purifed compounds

Compound 1: yellow amorphous powder; UV (MeOH) λ_{max} nm: 214, 273; IR (KBr) υ_{max} cm^{-1}: 3557, 3424, 1700, 1619, 1583, 1507, 1448, 1397, 1342, 1195, 1112, 1057, 923, 758; APCI-MS: *m/z* 301.5 $[M-1]^-$, 303.4 $[M+1]^+$; ^{1}H NMR (DMSO-d_6, 600MHz) δ: 7.46 (2H, s, H-2, 6); ^{13}C NMR (DMSO-d_6, 150MHz) δ: 107.5 (C-5, 5′), 110.1 (C-1, 1′), 112.2 (C-6, 6′), 136.2 (C-3, 3′), 139.5 (C-2, 2′), 147.9 (C-4, 4′), 159.4 (C-7, 7′). Its ^{13}C NMR and DEPT spectroscopic data revealed seven carbon signals which consisted of one carbonyl (δ_C 159.01) and six aromatic carbon (1 CH and 5 C). The only proton (δ_H 7.46) exhibited HMBC correlations with four aromatic carbons (δ_C 107.5, 112.2, 139.5, 147.9) and one carbonyl (δ_C 159.02). The data were in agreement with the reported literature values [8]. Based on the above evidence, compound 1 was identified as ellagic acid (Fig. 1).

Compound 2: white powder; ESI-MS: *m/z* 329.3 $[M-1]^-$; ^{1}H NMR (acetone-d_6, 600MHz) δ: 7.63 (1H, s, H-5), 7.65 (1H, s, H-5′), 4.19 (1H, s, 3-OCH_3), 4.09 (3H, s, 4-OCH_3); ^{13}C NMR (acetone-d_6, 150MHz) δ: 109.3 (C-5, 5′), 112.7(C-1, 1′), 115.0 (C-6, 6′), 141.6 (C-3, 3′), 144.7 (C-2, 2′), 153.2 (C-4, 4′), 159.4 (C-7,

7′), 57.3 (3- OCH_3), 61.9 (4-OCH_3). The above spectrum data were in accordance with reports of 3, 4-O-dimethyl ellagic acid [8], as shown in Fig. 1.

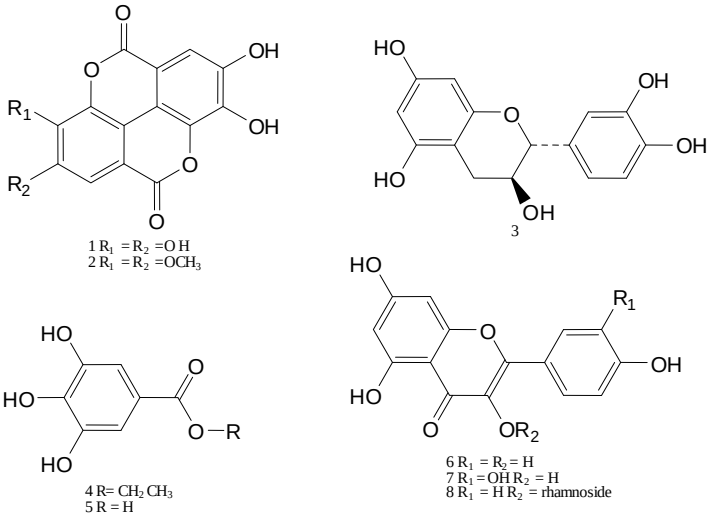

Fig. 1: Chemical structures of compounds 1-8

Compound 3: yellowy powder; ESI-MS: *m/z* 289.1 $[M-H]^-$; ^1H NMR (acetone-d_6, 600MHz) δ: 7.05 (1H, d, *J* = 1.8Hz, H-2′), 6.84 (1H, dd, *J* = 8.4, 2.4Hz, H-6′), 6.79 (1H, d, *J* = 8.4Hz, H-5′), 6.02 (1H, d, *J* = 2.4Hz, H-8), 5.86 (1H, d, *J* = 2.4Hz, H-6), 4.88 (1H, d, *J* = 2.4Hz, H-2), 4.20 (1H, m, H-3), 2.85 (1H, dd, *J* = 11.8, 4.8Hz, H-4α), 2.50 (1H, dd, *J* = 13.2, 3.0Hz, H-4β); ^{13}C NMR (acetone-d_6, 150MHz) δ: 157.9 (C-9), 157.6 (C-5), 156.9 (C-7), 146.3 (C-4′), 146.2 (C-3′), 132.3 (C-1′), 120.0 (C-6′), 116.1 (C-5′), 115.3 (C-2′), 100.9 (C-10), 96.4 (C-8), 95.6 (C-6), 82.9 (C-2), 68.9 (C-3), 28.5 (C-4). The NMR data were identical to those reports of Jin, et al. [9]. ^{13}C NMR and DEPT spectroscopic data revealed fifteen carbon signals which consisted of twelve aromatic (5 CH and 7 C), one sp^3 methylene and two sp^3 methine carbon signals. The C-ring protons (δ_H 2.85, 2.50) exhibited HMBC correlations with three aromatic carbons including C-5, C-9 and C-10, with two sp^3 methine carbon containing C-2 and C-3. The C-ring proton (δ_H 4.20) exhibited HMBC correlations with C-2, C-4, C-5 and C-1′. The C-ring proton (δ_H 4.88) exhibited HMBC correlations with C-3, C-4, C-9, C-1′, C-3′ and C-6′. The A-ring proton (δ_H 5.86) exhibited HMBC correlations with C-5, C-7, C-8, and C-10. The A-ring proton (δ_H 6.02) exhibited HMBC correlations with C-6, C-7, C-9 and C-10. The B-ring proton (δ_H 6.79, 7.05) exhibited HMBC correlations with C-1′, C-3′, C-4′and C-6′. The B-ring proton (δ_H 6.84) exhibited HMBC correlations with C-1′, C-2′, C-4′ and C-5′. Based on the above evidence, compound 3 was confirmed as (+)-catechin (Fig. 1).

Compound 4: colorless needle crystal; IR (KBr) υ_{max} cm^{-1}: 3500, 3299, 1706, 1610, 1535, 1315, 1270, 763; ^1H NMR (methanol-d_4, 600MHz) δ: 7.04 (2H, s, H-2, 6), 4.25 (2H, q, *J* = 7.8Hz, -OCH2-), 1.32 (3H, t, *J* = 7.8Hz, -CH3); ^{13}C NMR (methanol-d_4, 150MHz) δ: 168.6 (-COO-), 146.4 (C-3, 5), 139.6 (C-4), 121.9 (C-1), 110.1 (C-2, 6), 61.6 (-OCH_2-), 14.6 (-CH_3). The IR and NMR data were identical to those reported literature values of ethyl gallate [10], as shown in Fig. 1.

Compound 5: colorless needle crystal; IR (KBr) υ_{max} cm^{-1}: 3496, 3066, 2660, 1705, 1619, 1542, 1452, 1385, 1338, 1250, 1208, 956, 905, 867; ^1H NMR (methanol-d_4, 600MHz） δ: 7.05 (2H, s, H-2, 6); ^{13}C NMR (methanol-d_4, 150MHz) δ: 121.9 (C-1), 110.3 (C-2), 146.3 (C-3), 139.6 (C-4), 146.3 (C-5), 110.3 (C-6), 170.4 (-COOH). This compound showed navy blue on the TLC plates by spraying with 3% $FeCl_3$ in an ethanol solution. The IR and NMR data were identical to those reported literature values of gallic acid [10], as presented in Fig. 1.

Compound 6: yellow powder; UV (MeOH) λ_{max} nm: 265, 365; ESI-MS: *m/z* 287 $[M+H]^+$; IR (KBr) υ_{max} cm^{-1}: 3318, 1660, 1615, 1566, 1510; ^1H NMR (methanol-d_4, 600MHz) δ: 12.41 (1H, s, 5-OH), 7.8 (2H, dd, *J* = 4.8, 2.4Hz, H-2′, 6′), 7.00 (2H, dd, *J* = 4.8, 1.8Hz, H-3′, 5′), 6.43 (1H, d, *J* = 2.4Hz, H-8), 6.25(1H, d, *J* = 2.4Hz, H-6); ^{13}C NMR (methanol-d_4, 150MHz) δ: 179.2 (C-4), 165.5 (C-7), 160.9 (C-5), 158.3 (C-4′), 158.0 (C-9), 135.7 (C-2), 131.6 (C-3), 129.4 (C-2′, 6′), 122.6 (C-1′), 115.2 (C-3′, 5′), 102.7 (C-10), 99.6 (C-6), 94.4

(C-8). The UV, IR, ESI-MS and NMR data were identical to those reported literature values [11]. The structure of compound 6 was determined as kaempferol (Fig. 1).

Compound 7: yellow powder; UV (MeOH) λ_{max} nm: 258, 365; ESI-MS: *m/z* 287 $[M+H]^+$; ^{1}H NMR (methanol-d_4, 500MHz) δ: 6.17 (1H, d, *J* = 2.5Hz, H-6), 6.38 (1H, d, *J* = 2.5Hz, H-8), 7.73 (1H, d, *J* = 2.5Hz, H-2′), 6.88 (1H, d, *J* = 8.4Hz, H-5′), 7.63 (1H, dd, *J* = 8.4, 2.5Hz, H-6′); ^{13}C NMR (methanol-d_4, 125MHz) δ: 148.0 (C-2), 137.2 (C-3), 177.3 (C-4), 158.2 (C-5), 99.2 (C-6), 165.6 (C-7), 94.4 (C-8), 162.5 (C-9), 104.5 (C-10), 124.1 (C-1′), 116.0 (C-2′), 146.2 (C-3′), 148.8 (C-4′), 116.2 (C-5′), 121.7 (C-6′). The data were in agreement with the reported literature values [12], [13]. So compound 7 was identified quercetin (Fig. 1).

Compound 8: yellowy powder; ^{1}H NMR (methanol-d_4, 600MHz) δ: 7.84 (2H, dd, *J* = 4.8, 2.4Hz, H-2′, 6′), 7.01 (2H, dd, *J* = 4.8, 1.8Hz, H-3′, 5′), 6.46 (H, d, *J* = 2.4Hz, H-8), 6.25 (H, d , *J* = 2.4Hz, H-8), 5.47 (H, d, *J* = 1.8Hz, H-1″), 4.2 (H, dd, *J* = 4.8, 1.8Hz, H-5″), 3.68 (H, d, *J* =1.8Hz, H-2″), 3.31 (H, m, H-3″,4″), 0.89 (3H, d, *J* = 6.0Hz, H-6″); ^{13}C NMR (methanol-d_4, 150MHz) δ: 179.2 (C-4), 165.5 (C-7), 160.9 (C-5), 158.3 (C-4′), 158.0 (C-9), 135.7 (C-3), 131.7 (C-2′, 6′), 122.5 (C-1′), 116.3 (C-3′, 5′), 105.6 (C-2), 102.7 (C-10, C-1″), 99.6 (C-6), 94.6 (C-8), 72.9 (C-3″), 72.1 (C-5″), 71.4 (C-2″), 71.3 (C-4″), 17.7 (-CH_3). The NMR data were identical to those reported [12]. ^{13}C NMR and DEPT spectroscopic data revealed twenty-one carbon signals which consisted of one carbonyl (δ_C 179.2), twelve aromatic (6 CH and 6 C), five methane, one methyl and two sp^3 quaternary carbon signals. The protons (δ_H 0.89, 3.31, 3.68, 4.20, 5.47, 6.25, 6.46, 7.01, 7.84) exhibited HMQC correlation with -CH_3, C-3″/C-4″, C-2″, C-5″, C-1″, C-8, C-3′/C-5′, C-2′/C-6′, respectively. The only proton (δ_H 5.47) of rhamnose moiety revealed HMBC correlations with C-3 (δ_C 135.7). This result indicated the linkage of this rhamnose at C-3. Based on the above evidence, the structure of compound 8 was determined as kaempferol-3-O-α-L-rhamnoside (Fig. 1)

3.3. Radical scavenging activities and metal chelating ability

Lower IC_{50} value corresponded to a greater scavenging activity, and vice versa. As shown in table 1, DPPH scavenging activity of samples descended in the order of quercetin > ellagic acid > kaempferol > Gallic acid > 3, 4-O-di-methyl ellagic acid > ethyl gallate > (+)-catechin > kaempferol-3-α-L-O-rhamnoside. The superoxide radical scavenging activity of samples descended in the order of ellagic acid > 3, 4-O-di-methyl ellagic acid > quercetin > kaempferol > (+)- catechin > gallic acid > ethyl gallate > kaempferol-3-O-rhamnoside. The hydroxyl radical scavenging activity of samples descended in the order: (+)-catechin > quercetin > ellagic acid > kaempferol > gallic acid > 3, 4-O-di-methyl ellagic acid > ethyl gallate > kaempferol-3-O-α-L-rhamnoside.The chelating ability on ferrous ions was in descending order: ellagic acid > 3, 4-O-di-methyl ellagic acid > gallic acid > ethyl gallate > quercetin > kaempferol > kaempferol-3-O-α-L-rhamnoside > (+)-catechin.

Table 1: DPPH radical, superoxide anion radical and hydroxyl radical scavenging activity and ferrous ions chelating activity of polyphenolic compounds from the leaves of longan.

Sample	IC_{50} ± SD (μg/ml)			
	DPPH radical scavenging activity	$O_2^{\cdot-}$ radical scavenging activity	Hydroxyl radical scavenging activity	Ferrous ions (Fe^{2+}) chelating activity
Ellagic acid	19.79 ± 0.33	0.5054 ± 0.0014	69.58 ± 0.76	0.94 ± 0.12
3,4-O-di-methyl ellagic acid	24.77 ± 0.56	1.0316 ± 0.0031	93.20 ± 0.65	1.46 ± 0.13
(+)-catechin	43.66 ± 0.44	1.4458 ± 0.0131	40.08 ± 1.21	288.67 ± 0.79
Ethyl gallate	28.65 ± 0.22	1.8770 ± 0.0146	95.18 ± 0.65	22.82 ± 0.77
Gallic acid	22.60 ± 0.35	1.8040 ± 0.0214	90.13 ± 0.33	21.94 ± 0.62
Kaempferol	21.31 ± 0.69	1.1933 ± 0.0157	79.53 ± 0.41	60.44 ± 0.97
Quercetin	8.72 ± 0.39	1.0754 ± 0.0097	56.88 ± 0.97	24.62 ± 0.61
Kaempferol-3-O-α -L-rhamnoside	70.37 ± 1.11	2.5306 ± 0.0254	197.46 ± 1.44	112.95 ± 1.45
EDTA	——	——	——	0.17 ± 0.01

The evaluation for structure-activity relationships of the purified compounds suggested the antioxidant activities of these phenolic compounds depended largely on the frame of stereochemistry, the degree of

polymerization and the number of hydroxyls. For example, kaempferol possessed higher DPPH scavenging activity than kaempferol-3-O-rhamnoside. This conclusion confirmed that O-rhamnoside at C-3 had a passive effect on antioxidant activity and could reduce the antioxidant activity. Dimeric gallic (ellagic acid) acid possessed the highest superoxide radical scavenging activity. This result revealed the $O_2^{\cdot-}$ scavenging capacities of isolated compounds were positively related with their degree of polymerization. Ellagic acid possessed higher hydroxyl radical scavenging activity than 3, 4-O-di-methyl ellagic acid, and quercetin possessed good chelating activity in comparison to kaempferol. Those results might confirm radical scavenging activity and chelating activity had quantitative relations with free hydroxyl groups in the molecule.

4. Results and Discussion

Eight polyphenolic compounds from longan leaf were obtained and then identified as ellagic acid (1), 3, 4-O-di-methyl ellagic acid (2), (+)-catechin (3), ethyl gallate (4), gallic acid (5), kaempferol (6), quercetin (7) and kaempferol-3-O-α-L-rhamnoside (8). Barring compound 7, the other compounds were separated from the leaves of longan for the first time. All the purified compounds showed strong chelating ability on ferrous ions and radical scavenging activities on hydroxyl radicals, superoxide anion radicals and DPPH radicals. Moreover, the antioxidant activities of these phenolic compounds depended largely on the frame of stereochemistry, the number of hydroxyls and the degree of polymerization. Thus, longan leaf exhibits good potential as a source for natural antioxidants.

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