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Polyphenols from longan seeds and their radical-scavenging activity

Gongming Zheng [a,b], Liangxiong Xu [a], Ping Wu [a], Haihui Xie [a], Yueming Jiang [a], Feng Chen [c], Xiaoyi Wei [a,*]

[a] Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Xingke Road 723, Tianhe District, Guangzhou 510650, China
[b] Faculty of Chemistry and Life Science, Maoming College, Guanduerlu 139, Maoming 525000, China
[c] Department of Food Science and Human Nutrition, Clemson University, Clemson, SC 29634, USA

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ABSTRACT

Five previously uncharacterised polyphenols, ethyl gallate (**2**), 1-β-*O*-galloyl-D-glucopyranose (**3**), methyl brevifolin carboxylate (**4**), brevifolin (**5**) and 4-*O*-α-L-rhamnopyranosyl-ellagic acid (**8**), and three previously identified polyphenols, gallic acid (**1**), corilagin (**6**) and ellagic acid (**7**), were isolated from longan seeds. Their structures were identified by spectroscopic and chemical methods including HRESIMS and NMR. Eight polyphenols exhibited scavenging activity towards DPPH radicals with SC_{50} values of 0.80–5.91 μg/ml and towards superoxide radicals with SC_{50} values of 1.04–7.03 μg/ml. The radical-scavenging activity of the newly characterised polyphenols was comparable to that of gallic acid, corilagin and ellagic acid.

1. Introduction

Longan is an evergreen tree, *Dimocarpus longan* Lour. (syn. *Euphoria longana* Lam.), of the Sapindaceae family, which is widely grown in Southern China, India, and Southeast Asia (Jiang, Zhang, Joyce, & Ketsa, 2002). Longan fruit is one of the most favoured tropical fruits in China (Cai et al., 2002). In recent years, production of longan fruits in China has dramatically increased due to continuous development of plantations and improvement of agricultural management. Currently, longan arils are consumed as fresh and processed fruits while the seeds, which account for about 17% of the fresh weight of whole fruits (Xiao et al., 2004), are discarded as waste or burned as fuel. However, longan seeds have been found to be a rich source of antioxidant phenolic compounds which are promising as functional food ingredients or natural preservatives. Soong and Barlow (2005) reported longan seeds contained high levels of gallic acid, corilagin and ellagic acid, which have been proven to possess strong free radical-scavenging activity (Rangkadilok et al., 2007). However, Rangkadilok et al. (2007) reported that the aforementioned three characterised polyphenols might not be the only contributors for the high antioxidant activity of longan seeds. Instead, other phenolic constituents might also play important roles. On the other hand, besides gallic acid and ellagic acid, many other phenolic glycosides such as monogalloyl-glucose, monogalloyl-diglucose, digalloyl-diglucose, penta- to heptagalloyl-glucose, ellagic acid-pentose conjugate, galloyl-hexahydroxydiphenoyl (HHDP)-glucopyranose, pentagalloyl-HHDP-glucopyranose, etc., were found in longan seeds by HPLC–ESIMS analysis (Soong and Barlow, 2005). However, neither the complete structures of these polyphenols nor their individual antioxidant activities have been determined. Therefore, further investigation of the characterisation of the phenolic constituents in longan seeds and their antioxidant activities is necessary in order to elucidate the bioactive constituents in longan seeds. The objectives of the present study were to purify and characterise the potentially bioactive polyphenols in the longan seeds, and evaluate their antioxidant activity.

2. Materials and methods

2.1. Plant material

Longan seeds were collected from a commercial longan orchard at Maoming, Guangdong, China, in September 2005. The seeds were sundried, and ground to powder.

2.2. Phytochemical study

2.2.1. General procedures

Optical rotations were obtained on a Perkin-Elmer 343 spectropolarimeter (Perkin-Elmer, Boston, MA). Melting points were determined with a micromelting point apparatus (Yanagimoto Seisakusho Ltd., Kyoto, Japan). UV spectra were recorded in MeOH on a Perkin-Elmer Lambda 25 UV–Vis spectrophotometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker DRX-400 instrument (Bruker BioSpin, Ettlingen) in

* Corresponding author. Fax: +86 20 37252537.
E-mail address: wxy@scbg.ac.cn (X. Wei).

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DMSO-d_6 with the residual solvent peak (δ_H 2.49 and δ_C 39.51) as reference. HRESIMS data were obtained on a Bruker Bio TOF IIIQ mass spectrometer (Bruker Daltonics, MA). ESIMS were collected on a MDS SCIEX API 2000 LC/MS/MS instrument (AB MDS Sciex, Toronto). For column chromatography, silica gel 60 (100–200 mesh, Qingdao Marine Chemical, Qingdao), polyamide (80–100 mesh, Taizhou Luqiao Biochemical Corp., Taizhou, China), and Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala) were used. TLC was performed on precoated silica gel plates (GF_{254}, Qingdao Marine Chemical, Qingdao, China) with detection under fluorescent light (λ = 254 nm), exposure in I_2 vapour, and spray of H_2SO_4 solution (10%) in EtOH, followed by heating.

2.2.2. Extraction and isolation

The powder of longan seeds (10.5 kg), after defatting with petroleum ether, was extracted with 95% EtOH three times at room temperature. The EtOH solution was combined and concentrated under vacuum. The residue was suspended in H_2O and partitioned successively with petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH to obtain petroleum ether- (181.2 g), $CHCl_3$- (39.0 g), EtOAc- (120.5 g), and *n*-BuOH-soluble (102.3 g) extracts.

The $CHCl_3$-soluble extract was subjected to a silica gel column and eluted with $CHCl_3$–MeOH mixtures with increasing polarities (49:1–4:1). The fraction obtained on elution with $CHCl_3$–MeOH (19:1) was further separated by polyamide CC eluted with 5–80% aqueous MeOH. The subfraction obtained by elution with 10% aqueous MeOH was subjected to a Sephadex LH-20 column and eluted with MeOH to yield compound **2** (262 mg).

The EtOAc-soluble extract was subjected to a polyamide column. Using gradient elution the following fractions were obtained: fractions I and II with water, fractions III and IV with 10% MeOH, fractions V and VI with 30% MeOH, and fractions VII and VIII with MeOH. Fraction I was further separated by Sephadex LH-20 CC using MeOH to afford compound **3** (9 mg). Each of fractions II–V was dissolved in hot MeOH. The solution was immediately filtered. The collected filtrate was allowed to stand at room temperature for 1–7 days to slowly evaporate the solvent and give crystals, which yielded compounds **1** (6.72 g) from fraction II, **4** (22.4 mg) from fraction III, **5** (124 mg) from fraction IV, and **6** (356 mg) from fraction V. When MeOH was added to fractions VI and VII, they yielded precipitates which were collected by filtration and washed with MeOH to give **8** (37 mg) and **7** (242 mg), respectively.

2.3. Antioxidant assays

2.3.1. Chemicals and reagents

2,2′-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan); riboflavin, methionine, and nitro blue tetrazolium were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Other chemicals were purchased from China National Medicine Group Shanghai Corporation (Shanghai, China). All chemicals and solvents used were of analytical grade.

2.3.2. DPPH radical-scavenging activity

Scavenging activity of the isolated polyphenols towards DPPH radicals was assessed by using the method described by Duan, Jiang, Su, Zhang, & Shi (2007) with minor modifications. Briefly, 0.1 ml of various concentrations (1, 2, 4, 8 and 16 μg/ml) of the isolated polyphenols and l-ascorbic acid (positive control) in methanol was added to 0.1 ml of DPPH (0.2 mM) in MeOH. This yielded the final test concentrations (0.5–8 μg/ml). After a 30 min incubation period at 25 °C in darkness, the decrease in the absorbance at 517 nm was measured. The control contained methanol instead of the compound solution and blanks contained methanol in place of DPPH solution. The inhibition of DPPH radical by the samples was calculated according to the following equation: DPPH-scavenging activity (%) = [1 − (absorbance of sample − absorbance of blank)/absorbance of control] × 100.

2.3.3. Superoxide anion radical-scavenging activity

The superoxide anion radical-scavenging activity was measured by the method of Sun, Shi, Jiang, Xue, and Wei (2007) with some modifications. Briefly, 0.2 ml of various concentrations (8, 16, 32, 64 and 128 μg/ml) of the polyphenols in methanol were added to 3 ml of reaction solution (1.3 μM riboflavin, 13 mM methionine, 63 μM nitro blue tetrazolium, and 100 μM EDTA in 0.05 M sodium phosphate buffer). This brought the compound dilutions to the final test concentrations (0.5–8 μg/ml). The control contained methanol in place of the compound solution. After incubation for 15 min under the 4000 lux illumination, the absorbance of samples was measured against blanks at 560 nm. The scavenging activity was calculated as follows: scavenging activity (%) = (1 − absorbance of sample/absorbance of control) × 100.

2.3.4. Data analysis

All determinations of the radical-scavenging activity were performed in triplicate. The results were expressed as means ± standard error (SE). SC_{50} (the concentration scavenges 50% of the radical) was determined by applying Curveexpert 1.3 software.

3. Results and discussion

3.1. Isolation and structure determination

The ethanolic extract of longan seeds was fractionated with petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH. Separation by a combination of silica gel, polyamide and Sephadex LH-20 column chromatography and recrystallization afforded the compound **2** from the $CHCl_3$–soluble fraction and the compounds **1** and **3**–**8** from the EtOAc-soluble fraction.

The structures of the eight compounds (see Fig. 1), gallic acid (**1**) (Lee, Chiou, Lee, & Kou, 2005), ethyl gallate (**2**) (Hussain, Modan, Shabbir, & Zaidi, 1979; Zhang, Chen, Pei, & Hua, 2001), 1-*O*-galloyl-β-D-glucopyranoside (**3**) (Kashiwada, Nonaka, & Nishioka, 1984), methyl brevifolin carboxylate (**4**) (Yao & Zuo, 1993), brevifolin (**5**) (Mahmoud, Sahar, & Irmgard, 1994; Sha, Liu, Wang, & Xu, 2000), corilagin (**6**) (Mahmoud et al., 1994; Sha et al. 2000), ellagic acid (**7**) (Mahmoud et al., 1994) and 4-*O*-α-L-rhamnopyranosylellagic acid (**8**) (Yang et al., 1998), were determined by interpretation of their spectroscopic data (see supplementary data) as well as by comparison with reported data. The structures were further supported by co-TLC with authentic compound samples or hydrolysis followed by co-TLC with authentic compound samples (see supplementary data).

Although a few polyphenolic constituents of longan seeds have been characterised (Soong and Barlow, 2005; Rangkadilok, Worsuttayangkurn, Bennett, & Satayavivad, 2005), many others have not been characterised due to the technique limitations in the previous studies. Among the isolated compounds in the present study, gallic acid (**1**), corilagin (**6**) and ellagic acid (**7**) were previously identified as predominant polyphenols in longan seeds by HPLC and HPLC–ESIMS (Rangkadilok et al., 2005; Soong and Barlow, 2005). However, the presence of ethyl gallate (**2**), methyl brevifolin carboxylate (**4**), brevifolin (**5**) and 4-*O*-α-L-rhamnopyranosylellagic acid (**8**) in longan seeds is reported for the first time. In addition, this study has fully characterised the complete structure of 1-*O*-galloyl-β-D-glucopyranoside (**3**) that was temporarily determined as monogalloyl-glucose by HPLC–ESIMS in a previous study (Soong and Barlow, 2005).

3.2. Antioxidant activity

Longan seeds have shown strong antioxidant activities due to their polyphenols. Although three antioxidant polyphenols, (i.e., gallic acid, corilagin and ellagic acid), have been found to be abundant in longan seeds, previous investigation indicated other uncharacterised polyphenolic constituents might also be the important contributors of the high antioxidant activity of longan seeds. The present study obtained five previously uncharacterised polyphenols (compounds **2**–**5**, and **8**), and determined their respective antioxidant activities by two different antioxidant assays, i.e., the DPPH radical-scavenging assay and the superoxide anion radical-scavenging assay. In both assays, all the eight purified polyphenols (**1**–**8**) showed radical-scavenging activities in a dose-dependent manner (see supplementary data) but in different intensities. In the DPPH radical-scavenging assay, SC_{50} values of the compounds **1**–**8** ranged from 0.80 to 5.91 μg/ml (Table 1) in a decreasing antioxidant order of **1** > **2** > **4** > **6** > **7** > **5** > **3** > **8**, of which the first four compounds (**1**, **2**, **4** and **6**) showed better scavenging activity (SC_{50} = 0.80–2.01 μg/ml) than vitamin C (SC_{50} = 2.13 μg/ml). In the superoxide anion radical-scavenging assay, the SC_{50} values of the eight compounds were between 1.04 and 7.03 μg/ml (Table 1) in a similar order (**1** > **2** > **6** > **7** > **4** > **3** > **5** > **8**) to that in the DPPH radical-scavenging assay. Compound **1** was again the best scavenger, followed by compound **2**. Compounds **6**, **7**, and **4** came next with SC_{50} values within the range of 2.37–2.62 μg/ml, followed by compounds **3** and **5**, while compound **8** was again the weakest scavenger among the eight compounds. The results showed that the antioxidant activities of the newly characterised polyphenols were comparable with those of three known antioxidants, i.e., gallic acid, corilagin and ellagic acid.

It is noteworthy that there is a significant change in the radical-scavenging activity of compounds **4** and **5**, two structurally close polyphenols that both bear a galloyl residue with three free hydroxyl groups. The antioxidant activities of compound **4** are higher than those of compound **5**. A possible explanation for this phenomenon is as follows: both compounds **4** and **5** possess a ketone carbonyl (C-10) group at C-2, which has an electron-withdrawing effect on the conjugative system (Nie & Xia, 2000; Hansch et al., 1973) and therefore decreases the radical-scavenging capacity of the compounds. However, since compound **4** has a methoxycarbonyl group attached at its C-8, it might have two additional resonances, i.e., methoxy-enol and enol structures as shown in Fig. 2. Both resonances have an extra hydroxyl group at C-10 or C-11, which can be electron-donating (Nie & Xia, 2000; Hansch et al., 1973). The electron donation by the enol hydroxyl group might counteract the electron-withdrawing effect of the carbonyl group at C-2 or C-8 (Fig. 2), and increase the electron density in the aromatic ring of galloyl residue, as well as the scavenging activity as that found in compound **4**. This assumption has also obtained an unambiguous support from the fact that the ^{13}C NMR signals of C-3, C-4, C-5 and C-10 in compound **4** were shifted upfield by 1.8, 0.7, 1.2 and 2.5 ppm, respectively, compared with those of them in compound **5** (see supplementary data), and from the optical inactivity of compound **4** (see supplementary data).

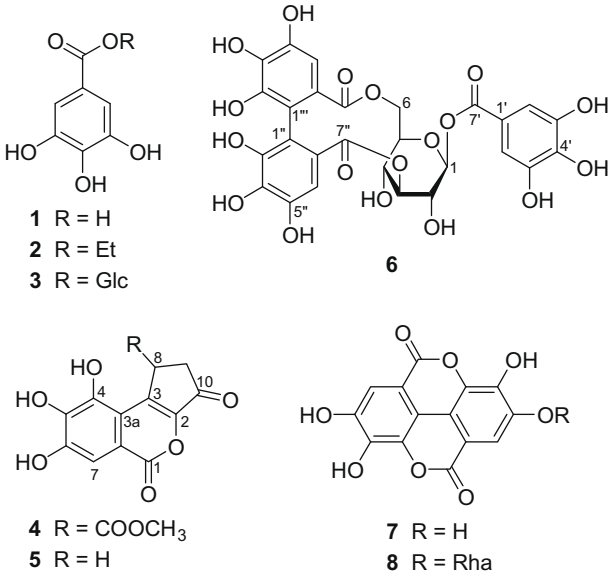

Fig. 1. Structures of polyphenols from longan seeds (**1**, gallic acid; **2**, ethyl gallate; **3**, 1-β-*O*-galloyl-D-glucopyranose; **4**, methyl brevifolin carboxylate; **5**, brevifolin; **6**, corilagin; **7**, ellagic acid; and **8**, 4-*O*-α-L-rhamnopyranosyl-ellagic acid).

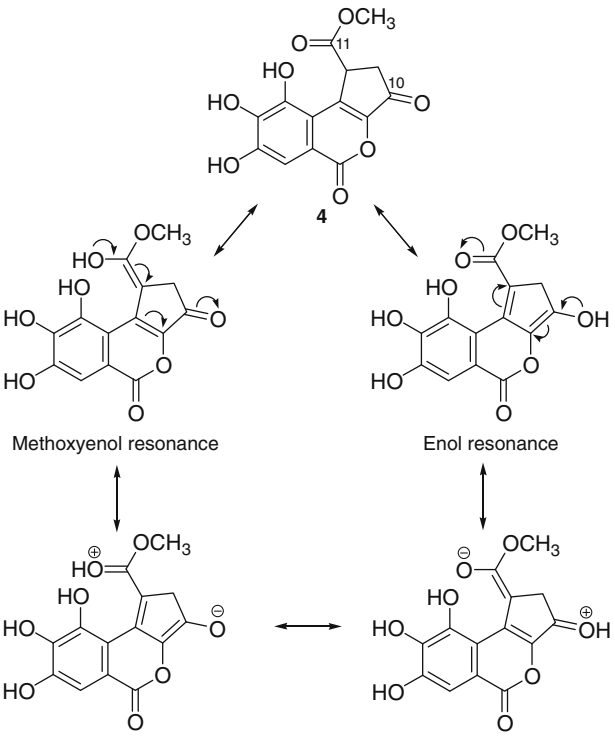

Fig. 2. Methoxy-enol and enol resonance structures of brevifolin carboxylate (**4**).

Table 1
SC_{50} values of compounds **1**–**8** towards DPPH and superoxide radicals.

Compound	μg/ml (μM)	
	DPPH radical	Superoxide radical
1	0.80 ± 0.02 (4.71 ± 0.10)	1.04 ± 0.11 (6.12 ± 0.67)
2	1.09 ± 0.03 (5.53 ± 0.13)	1.42 ± 0.16 (7.17 ± 0.80)
3	3.84 ± 0.10 (11.56 ± 0.31)	3.24 ± 0.43 (9.76 ± 1.31)
4	1.79 ± 0.01 (5.84 ± 0.03)	2.62 ± 0.26 (8.56 ± 0.84)
5	2.84 ± 0.02 (11.45 ± 0.06)	3.40 ± 0.29 (13.71 ± 1.17)
6	2.01 ± 0.03 (3.17 ± 0.05)	2.37 ± 0.27 (3.74 ± 0.42)
7	2.20 ± 0.10 (7.29 ± 0.32)	2.56 ± 0.35 (8.48 ± 1.16)
8	5.91 ± 0.20 (13.19 ± 0.44)	7.03 ± 0.79 (15.69 ± 1.76)
Vitamin C	2.13 ± 0.02 (12.10 ± 0.09)	Not determined

4. Conclusions

In the present study, five previously uncharacterised polyphenols, ethyl gallate (**2**), 1-β-*O*-galloyl-D-glucopyranose (**3**), methyl brevifolin carboxylate (**4**), brevifolin (**5**) and 4-*O*-α-L-rhamnopyranosyl-ellagic acid (**8**), along with three previously identified

polyphenols, gallic acid (**1**), corilagin (**6**) and ellagic acid (**7**), were obtained in purity from longan seeds. Their structures were identified by spectroscopic and chemical means including HRESIMS and NMR. The antioxidant activities of five newly characterised pholyphenols were comparable to those of gallic acid (**1**), corilagin (**6**) and ellagic acid (**7**) in both the *in vitro* DPPH and superoxide radical-scavenging assays.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2009.02.059.

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