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Phenolics from *Litchi chinensis* Sonn. and Their Potential Antioxidant Effects

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Authors' contributions

This work was carried out in collaboration between all authors. Authors XH, YW and XD designed the study, performed the statistical analysis, wrote the protocol, and first draft of the manuscript. Authors XD and YW managed the analyses of the study. Author XD managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To research phenolics from the seed of *Litchi chinensis* Sonn. and estimate their antioxidant activities.
Study Design: Phenolics were isolated and determined from the seed of *Litchi chinensis* Sonn. Their effects on DPPH radical scavenging were investigated.
Place and Duration of Study: School of Pharmacy, Guangdong Pharmaceutical University, between September 2015 and March 2018.
Methodology: Phenolics were obtained by Silica gel, Sephadex LH-20, ODS as well as HPLC chromatography and characterized by 1D and 2D NMR. The antioxidant abilities of the isolated compounds were measured by the DPPH assay.
Results: Fifteen Phenolics were isolated and confirmed from the seed of *Litchi chinensis* Sonn (**1**–**15**), compounds protocatechuic acid (**1**), ethyl-3,4-dihydroxybenzoic acid (**4**), methyl p-hydroxymandelate (**7**), ethyl 3,4,5-trihydroxybenzoate (**9**) exhibited antioxidant capacities on DPPH radical scavenging.
Conclusion: This study suggests that phenolics from the seed of *Litchi chinensis* Sonn. showed potential antioxidant properties, which mean that lychee seed could be utilized as a natural antioxidant for health care.

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Keywords: Litchi chinensis sonn; phenolics; antioxidant; dpph.

ABBREVIATIONS

NMR : Nuclear Magnetic Resonance spectrum
ODS : Octadecyl Silane
HPLC : High-performance liquid chromatography
DPPH : 1-diphenyl-2-picrylhydrazyl
HMBC : Heteronuclear Multiple Bond Correlation spectroscopy

1. INTRODUCTION

Lychee (*Litchi chinensis* Sonn.), native to southeast of China, is a world-wide spread tropical fruits which belong to the member of the Sapindaceae family. It is chiefly distributed in southeast Asia, particularly in China, Thailand and Philippines. This lovely fruit has white and semilucent flesh aril which was covered by a red and attractive pericarp. It is favoured by the global market due to its delicious arils and abundant nutritional value. Not only a sweet fruit, lychee has been utilized for traditional medicine since ancient times. Over the past ten years, the yearly output of lychee in China is increasing steadily. Lychee flesh is consumed as fresh and deep-processing products, whereas its seeds are mostly discarded as agricultural waste except a small amount of those are applied as traditional Chinese medicine with anti-dysenteric functions and to treat epigastric pain [1-2]. In Chinese and Indian traditional medicines, lychee seeds were served as an analgesic agent that can alleviate the symptoms of coughing, gas-tralgia, and neuralgia [3-4]. In Palau, lychee seeds macerated in alcohol are applied as coughs remedy [5]. Previous pharmacological studies suggested that lychee seed possessed anti-hyperglycemic, hepatoprotective, immune-modulatory, antitumor, antibacterial, antiviral and antioxidant effects [6], as well as some research found that litchi seed could to be available as a source of natural antioxidant [7]. Furthermore, recent research provides that lychee seeds also significantly improving the learning and memory capacities in the model mice [8]. Additionally, previous investigations of lychee seeds have focused on its biological activities, which resulted in the isolation of various bioactive compounds, such as flavonoids, oligosaccharides, sesquiterpene, lignans and triterpenes [9]. Among them, flavonoids were considered as the major constituents responsible for bioactive in lychee seed [10]. Previous chemical studies on lychee seeds reported the isolation and determination of the following flavonoids compounds: (+)-catechin, (-)-epicatechin, rutin, pinocembrin 7-O-neohesperidoside and a type of proanthocyanidins analogues [11-12], which exhibited better radical scavenging capacities and reducing power of DPPH and superoxide radicals than positive control (Vitamin C).[13] However, these flavonoids derivatives might not be the only contributors for the high antioxidant activity of lychee seeds. Further phytochemical investigation on lychee seed is required for better medicine utilization.

The main objective of this research was to isolate and concentrate the components out of flavonoids derivatives from the lychee seed, to assess their antioxidant activities.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Methanol for HPLC was provided by Oceanpak Chemical Co. (Gothenburg, Sweden). Silica gel (200–300 and 300-400 mesh, Anhui Liangchen Silicon Material Co. Ltd. Lu'an, China). Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) and ODS (40–60 μm, Merck KGaA, Darastadt, Germany) were utilized for column chromatography. All other reagents and analytical chemicals were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

2.2 Instrumentation

NMR spectra were measured with a Bruker AV-400 NMR spectrometer (Bruker, Switzerland). Semi-preparative HPLC was performed on a Rainin HPLC system equipping with Shodex RI-201H refractive index detector (Shodex Inc. Tokyo, Japan). A C_{18} column (Cosmosil $5C_{18}$-AR-II, 5 μm, 10ID × 250 mm, Nacalai Tesque, Kyoto, Japan) was applied for HPLC.

2.3 Plant Material

Seeds of lychee (20 kg) were purchased from Qingping herbal market, Guangzhou on Aug. 2015 and were identified to be *Litchi chinensis* Sonn. by Prof. X. J. He of Guangdong Pharmaceutical University. A voucher specimen was deposited at the Lead Compounds Laboratory, Guangdong Pharmaceutical University.

2.4 Extraction and Purification

The dried pieces of lychee seeds (20 kg) were refluxed with 70% ethanol (65 L × 3) and 4 h for each extraction. The ethanol extract was concentrated under vacuum to provid 12 L ethanol-free extract, and then sequentially partitioned with cyclohexane, chloroform, ethyl acetate and n-butanol, respectively. The EtOAc fraction (120.11 g) was subjected to a silica gel C.C. elution with an increasing polarity $CHCl_3$-MeOH (100:0 to 1:1, v/v) to get fraction E1-E17.

Fraction E4 (2.89 g) was isolated via silica gel C.C. elution with a gradient of cyclohexane-ethyl acetate, followed by Sephadex LH-20 column ($CHCl_3$/MeOH, 2:1, v/v), and then purified with the preparative HPLC (46% MeOH/H_2O, v/v) to get compounds **6** (100.3 mg), **12** (66.2 mg). Compound **11** (49.4 mg) was obtained by recrystallized.

Fraction E6 (1.25 g) was separated with a silica gel C.C. elution with cyclohexane-ethyl acetate (100:0 to 2:1, v/v) to get subtraction E6-1–E6-5. Subtraction E6-2 was purified by HPLC (42% MeOH/H_2O, v/v) to yield compound **7** (23.3 mg). Subtraction E6-3 (261.7 mg) was followed by a Sephadex LH-20 column ($CHCl_3$/MeOH 2:1, v/v) and then purified with the HPLC (32% MeOH/H_2O, v/v) to afford compound **8** (5.6 mg) and **13** (81.1 mg). Subtraction E6-4 (550.1 mg) was purified with the HPLC (21% MeOH/H_2O, v/v) to yield compound **5** (10.1 mg) and **9** (12.2 mg).

Fraction E8 (3.92 g) was subjected to a silica gel C.C. eluted with $CHCl_3$-MeOH (100:0 to 2:1, v/v), followed by a Sephadex LH-20 column ($CHCl_3$/MeOH 1:1, v/v), and then purified with the HPLC (35% MeOH/H_2O, v/v) to afford compound **2** (15.4 mg), **3** (19.2 mg), **4** (15.1 mg) and **10** (11.1 mg).

Fraction E9 (14 g) was subjected to a silica gel C.C. elution with $CHCl_3$-MeOH (100:0 to 1:1, v/v) to provided subtraction E9-1–E9-10. Compound **1** (98.5 mg) was recrystallized from subtraction E9-5. E9-8 (1.66 g) was subjected to an ODS elution with MeOH/H_2O (10% to 70%, v/v), and finally purified with the HPLC (20% MeOH/H_2O, v/v) to yield compounds **14** (135.9 mg) and **15** (31.9 mg).

2.5 DPPH Radical Scavenging Activity

Antioxidant ability was evaluated by measuring the DPPH radical scavenging utilizing the DPPH assay as previously described [10]. Briefly, DPPH solution (in methanol, 0.2 mmol/L, 100 μL) was mixed with 100 μL of each of the tested sample (in methanol, 6.25 to 200 μM). The solution system was shaken and incubated in the dark at room temperature for 0.5 h. Finally, the OD value was measured at 510 nm, respectively. Ascorbic acid was implemented as positive control.

3. RESULTS AND DISCUSSION

3.1 Structure Identification of the Purified Phenolics

The dried lychee seeds were extracted with 70% ethanol, fractionated with different solvents, and then successively chromatographed on silica gel, ODS, Sephadex LH-20 and HPLC to obtain fifteen compounds (Fig. 1).

Compound **14** was compiled as a white powder from the EtOH extract of lychee seed. The molecular formula was established to be $C_{17}H_{18}O_8$ on the basis of the ^{1}H NMR and ^{13}C NMR. In ^{1}H NMR spectrum, a set of AA′BB′-type signals at δ_H 7.54 (2H, d, J = 8.3 Hz) and δ_H 6.90 (2H, d, J = 8.3 Hz) as well as two olefinic proton signals at δ_H 7.60 (1H, d, J = 16.0 Hz) and δ_H 6.31 (1H, d, J = 16.0 Hz) indicated the existence of a *trans*-p-hydroxycinnamic acid moiety. As well as a methoxy proton signal at δ_H 3.68 (3H, s). There were seventeen carbon signals in the ^{13}C NMR spectrum. Except for two carboxyl signals, two olefinic carbons, six aromatic carbon signals, one methoxy signals and six cycloparaffin carbons signals. The chemical shifts of δ_C 75.9 (C-1′), 71.5 (C-3′), 73. 2 (C-4′) and 70.9 (C-5′) disclosed that C-1′, C-3′, C-4′and C-5′ were oxygenated. According to a described protocol [14], compound **14** was eventually elucidated to be methyl 5-O-*p*-coumaroylquinic acid methyl ester.

Compound **15** was obtained as a white powder from the EtOH extract of lychee seed. The molecular formula was identified to be $C_{17}H_{18}O_8$ dependence on the ^{1}H NMR and ^{13}C NMR. Although the ^{1}H and ^{13}C NMR spectra of **15** were exactly similar to those of **14**, differences in the chemical shifts of three of cycloparaffin signals were observed: in the ^{13}C NMR spectrum of **15**, carbon signals at δ_C 64.5, 75.7 and 65.5 were

assigned to C-3, C-4 and C-5, respectively. In the HMBC spectrum (Fig. 2), the correlations of δ_H 4.74 (H-4′) with δ_C 64.5 (C-3′)/38.0 (C-6′)/166.3 (C-9), and δ_H 1.99 (H-2′) coupled with δ_C 64.5 (C-3′), as well as δ_H 2.08 (H-6′) correlated with δ_C 65.5 (C-5′), above these indicated that ester linkage between hydroxyl group and *trans*-4-hydroxycinnamic acid moiety at C-4′. Its ^{1}H and ^{13}C NMR spectral data are consistent with literature [15], consequently compound **15** was concluded to be 4-O-*p*-coumaroylquinic acid methyl ester.

Compound 1-13 was determined to be protocatechuic acid (**1**) [16], 4-hydroxybenzoic acid (**2**) [17], protocatechuic acid methyl ester (**3**) [18], ethyl-3,4-dihydroxybenzoic acid (**4**) [19], 3-hydroxy-4-methoxybenzoic acid (**5**) [20], methyl *p*-hydroxy benzeneacetate (**6**) [21], methyl *p*-hydroxymandelate (**7**) [22], *p*-hydroxymandelic acid (**8**) [23], ethyl 3,4,5-trihydroxybenzoate (**9**) [24], (*E*)-p-hydroxycinnamic acid (**10**) [25], (*E*)-ethyl-p-hydroxycinnamate (**11**) [26], *p*-hydroxy-phenylpropionic methyl ester (**12**) [27], O-methyl-2-hydroxy-3-(4-hydroxy)-phenylpropionate (**13**) [28].

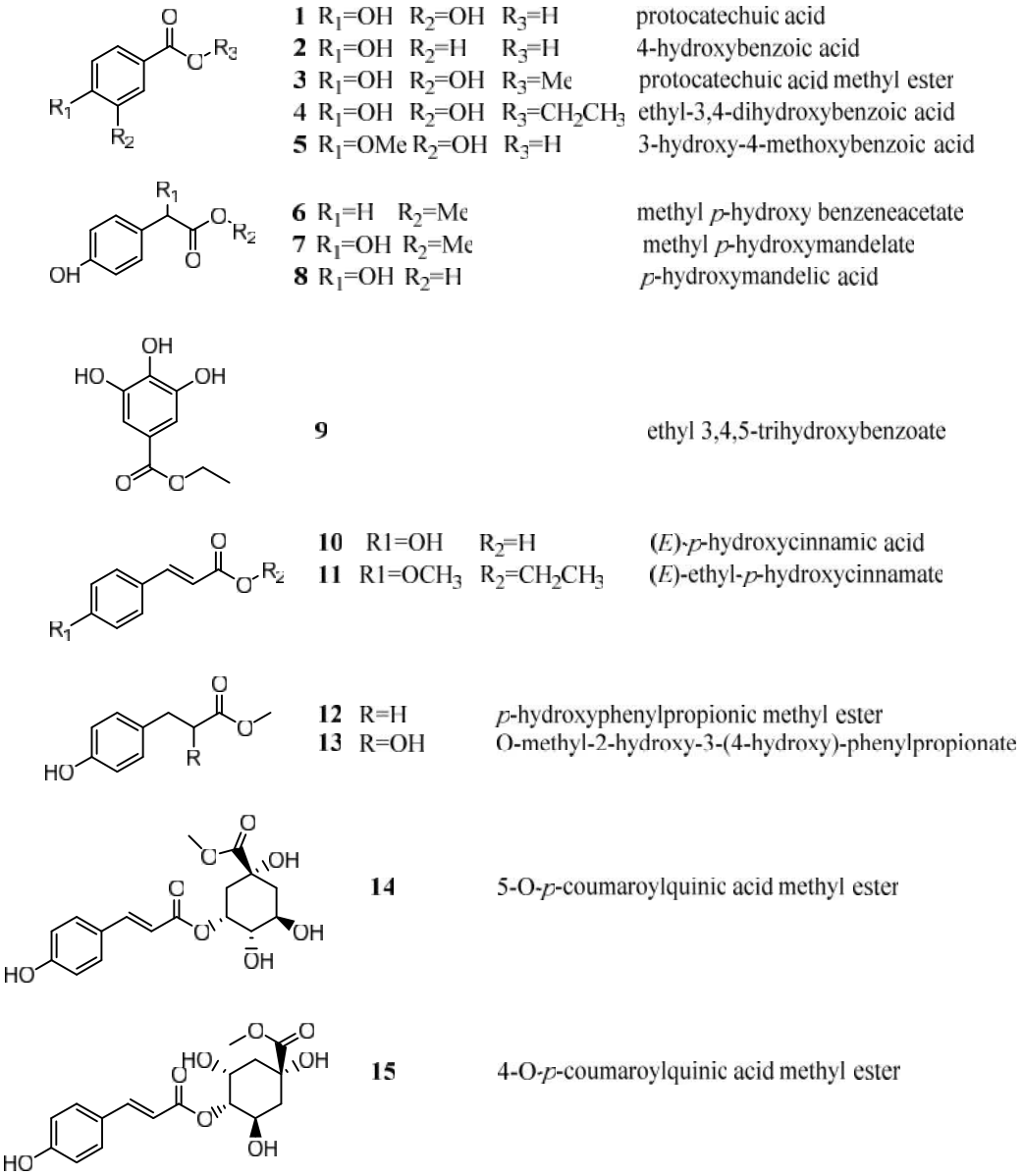

Fig. 1. Structures of phenolics 1-15 isolated from lychee seed

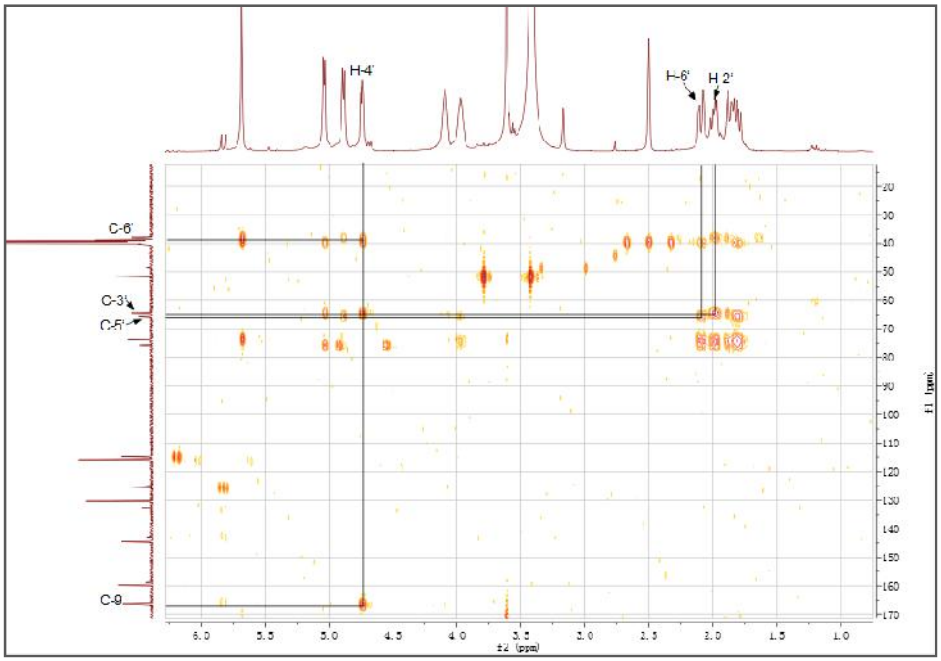

Fig. 2. Enlarged HMBC of compound 15

3.2 Antioxidant Activity

In the present study, the DPPH assay was applied to evaluate the antioxidant capacities of compounds **1**–**15**. The results were summarized in Fig. 3, compounds **9** exhibited noteworthy DPPH radical scavenging activity with the IC_{50} values under 10 μM. Compounds **4**, **7** also exhibited stronger antioxidant capacity and **1** displayed moderate DPPH free radical scavenging activity, compared to ascorbic acid (29.97±1.12 μM). The IC_{50} values of other compounds more than 200μM and were considered feeble activities. Previous pharmacological research demonstrated that lychee seed contains ample flavonoids derivatives which contribute to its excellent antioxidant abilities [13]. The antioxidant effects of compounds **1**, **4**, **7**, **9** displayed that flavonoids derivatives might not be the only member responsible for the high antioxidant activity of lychee seeds, and further study on lychee seed is needed for discovering more multifunctional bioactivity ingredients from it.

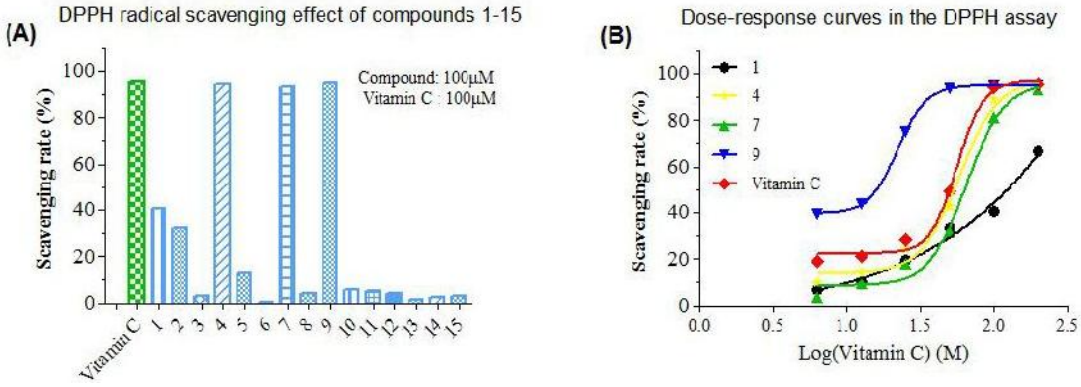

Fig. 3. *Antioxidant actives of compounds 1-15*

*Test concentrations ranged from 6.25 to 200 μM. The IC_{50} values of compound **1** (113.60±4.09 μM), **4** (41.36±2.15 μM), **7** (52.51±1.16 μM) and **9** (9.92±0.36 μM). Vitamin C was utilized as positive control. Values are the mean ± SD, n = 3. **p < 0.01 vs the positive control. *p < 0.05 vs the positive control*

Table 1. ^{13}C NMR (101 MHz) data of compounds 1-15 in DMSO-d_6

	δ_C (ppm)														
No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1	123.1	122.6	122.9	120.7	121.8	124.6	127.6	128.2	119.9	127.1	127.2	130.7	130.3	127.0	125.2
2	117.5	132.8	117.2	116.2	112.8	130.4	130.2	130.3	108.7	130.9	129.8	129.2	127.7	130.7	130.3
3	145.6	116.1	145.6	145.0	147.2	115.3	114.8	114.8	145.8	115.9	114.4	115.2	114.9	115.7	115.9
4	150.8	162.7	150.7	150.4	151.1	156.3	155.8	155.7	138.5	160.5	161.4	155.7	155.9	160.6	159.8
5	115.7	116.1	115.7	115.3	115.0	115.3	114.8	114.8	145.8	115.7	114.4	115.2	114.9	115.7	115.9
6	123.7	132.8	123.3	121.7	123.5	130.4	130.2	130.3	108.7	130.9	129.8	129.2	130.3	130.7	130.3
7	167.8	167.8	167.1	165.7	167.3	39.5	71.6	71.4	166.1	145.6	144.4	35.5	39.4	145.5	144.5
8			51.8	60.9	55.6	172.2	174.0	175.3	60.3	116.7	115.7	29.6	71.7	115.8	114.8
9				14.3		51.7	51.3		14.4	168.3	167.6	172.9	174.1	167.1	166.3
1'											60.5	51.3	51.4	75.9	73.6
2'											14.4			38.7	39.9
3'											55.4			71.5	64.5
4'														73.2	75.7
5'														70.9	65.5
6'														38.0	38.0
7'														174.5	173.8
8'														52.6	51.7

4. CONCLUSION

In this study, fifteen compounds were isolated from the *Litchi chinensis* Sonn. and their structures were identified by the spectroscopic methods. Among them, compounds **4** ,**5**, **7**, **8**, **9**, **11**, **12**, **13**, **15** were first isolated from lychee seed. All of isolated compounds were measured by DPPH assay to assess their antioxidant capacities. Compounds **1**, **4**, **7** and **9** showed potential DPPH radical scavenging properties. These results suggested that other types of compounds could also responsible for the prominent antioxidant activity of lychee seed, not just flavonoids derivatives and phenolic acid. And the further investigation about chemical constituents and biological activities of lychee seed is required for the potential utilization of the seed of *Litchi chinensis* Sonn. as a natural antioxidant in functional foods and healthy care products.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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