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Identification of phenolic compounds and appraisal of antioxidant and antityrosinase activities from litchi (*Litchi sinensis* Sonn.) seeds

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ABSTRACT

Antioxidant and antityrosinase compounds from *Litchi sinensis* Sonn. seeds were extracted with five different types of polar solvents. The five extracts, namely ethanol extract (EE), 50% ethanol extract (50% EE), methanol extract (ME), 50% methanol extract (50% ME), and water extract (WE), were used for the evaluations of total phenolic content, antioxidant capabilities and antityrosinase activity. The 50% EE showed the highest total antioxidant capacity, scavenging the 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical and inhibitory activity against lipid peroxidation, and it was comparable to the activity of the synthetic antioxidant, butylated hydroxyl toluene. Fifty percent EE showed a better antityrosinase activity compared to the other extracts. After application of reverse phase high performance liquid chromatography, coupled to a diode array detector and electrospray ionisation mass spectra, five phenolic compounds, namely, gallic acid, procyanidin B2, (−)-gallocatechin, (−)-epicatechin and (−)-epicatechin-3-gallate were identified from 50% EE. This study suggests that litchi seed can potentially be used as a readily accessible source of natural antioxidants.

1. Introduction

Artificial antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butylhydroquinone (TBHQ), have been widely used in foods for preventing lipid peroxidation, but the usage of these artificial antioxidants is being gradually restricted in the food industry as they are suspected to be toxic and carcinogenic (Namiki, 1990). There has been an increasing demand for antioxidants from plant origins. Epidemiological studies have shown that the consumption of fruits and vegetables with high phenolic content can reduce the risk of cardio- and cerebro-vascular diseases and cancer mortality (Amin & Yazdanparast, 2007). The protective effect of fruits and vegetables against diseases has been attributed to various phenolic compounds possessing antioxidant activity (Jayaprakasha, Girennavar, & Patil, 2008; Prasad, Divakar, Shivamurthy, & Aradhya, 2005).

Tyrosinase (EC1.14.18.1) is involved in melanin production and the melanin production might be responsible for some of the histopathological features exclusive to malignant cancer. Therefore, tyrosinase inhibitors may be clinically helpful in dealing with skin cancer. In recent years, more attention has been paid to use the natural plant extracts such as tyrosinase inhibitors in the cosmetic industry (Momtaza et al., 2008).

The exploration of green technology and of low cost raw materials is important features for the food industry in making improved use of plant resources. As population increases, food production is more intense and a great quantity of waste is generated.

Litchi (*Litchi chinensis* Sonn.) is a subtropical fruit originating from south-east Asia. The fruit is accepted by consumers because of its delicious taste and attractive colour. Litchi by-products consist mainly of litchi pericarp and litchi seeds which are discarded as a waste. The previous studies on biochemical activities of litchi fruit are mainly focussed on its pericarp, because it has been found to be a rich source of a multitude of potential antioxidants (Duan, Wu, & Jiang, 2007; Zhang, Quantick, & Grigor, 2000; Zhao, Yang, Wang, Li, & Jiang, 2006). However, there is no information on litchi seeds, which are commonly used in Chinese Traditional Medicine to relieve neuralgic pain. In the present study, the possibility of litchi seeds being used as a natural antioxidant and tyrosinase inhibitor was investigated, while the major phenolic compounds are identified for the first time. This study could help better utilise litchi seeds, which is not only economical but also environmentally friendly, because they could be recycled in the food industry in the form of value-added products.

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2. Materials and methods

2.1. Plant materials

Fresh fruits of litchi (*Litchi sinensis* Sonn.) at the mature stage were collected from an orchard in Guangzhou, China. Fruits were chosen for uniformity in shape and colour. The fruits were carefully washed in potable water. Litchi seeds were manually separated, then dried for 24 h, using a hot air oven at 60 °C, and powdered, using a blender. The moisture content was determined to be 62%.

2.2. Chemicals and reagents

1,1-diphenyl-2-picryl hydrazyl (DPPH), ascorbic acid, L-tyrosine, tyrosinase solution (1000 units/ml), gallic acid, procyanidin B2, (−)-gallocatechin, (−)-epicatechin, (−)-epicatechin-3-gallate and thiobarbituric acid (TBA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Butylated hydroxyly toluene (BHT) was obtained from Sinopharm Chemical Reagent Co. (Shanghai, China). All other chemicals and solvents used in this study were of analytical grade and obtained from Tianjin Reagent Company (Tianjin, China).

2.3. Extraction

The dried powder (10 g) of litchi seeds was extracted for 12 h in a rotary shaker with 100 ml of five different types of polar solvents (ethanol, 50% ethanol, methanol, 50% methanol and water) separately in a conical flask at 30 °C. The extracts were filtered and then evaporated to dryness using a rotary evaporator (RE-52A, Shanghai Woshi Co., Shanghai, China) and lyophilised using a freeze-dryer (Savant, Vapornet VN 100, Labequip Ltd., Markham, Ontario, Canada) to obtain five different freeze-dried extracts, namely ethanol extract (EE), 50% ethanol extract (50% EE), methanol extract (ME), 50% methanol extract (50% ME), and water extract (WE).

2.4. Determination of total phenolic content

Total phenolic contents of litchi seed extracts were determined by the method of Singleton and Rossi (1965) and then expressed as gallic acid equivalents.

2.5. Antityrosinase activity

Inhibition of tyrosinase activity was analysed according to the method of Kobayashi, Kayahara, Tadasa, Nakamura, and Tanaka (1995), with some modifications. L-Tyrosine solution (4 ml) at 0.5 mg/ml was dissolved in 20 mM phosphate buffer (pH 6.8) and then added to 1 ml of litchi seed extract (dissolved in 50% ethanol). After 10 min of incubation at 37 °C, 1 ml of mushroom tyrosinase (50 units/ml, dissolved in 0.2 M phosphate buffer, pH 6.8) was added to the mixture solution. The absorbance was recorded after 10 min at 475 nm using a spectrophotometer (UV-2802, Unico Co. Ltd., Shanghai, China). Fifty percent ethanol solution was used as a blank, while 1 ml of distilled water was used as the control. Percent tyrosinase inhibitory activity was calculated using the following formula: (Control OD − Sample OD/Control OD) × 100.

2.6. 1,1-Diphenyl-2-picryl hydrazyl radical (DPPH?) radical-scavenging activity

The DPPH radical-scavenging activities of litchi seed extracts were appraised by the method of Blois (1958) with some modification. Different concentrations (25, 50, 75 and 100 μg/ml, dissolved in 50% ethanol) of the samples were taken in different test tubes. The volume was adjusted to 100 μl by adding 50% ethanol and mixed with 0.9 ml of 0.2 mM DPPH (dissolved in methanol). The reaction mixture was incubated for 20 min at 28 °C in the dark. The control contained all reagents except the extract sample while methanol was used as blank. The DPPH radical-scavenging activity was determined by measuring the absorbance at 517 nm using a spectrophotometer. The antioxidant activity was expressed as a percentage of scavenging activity of DPPH radicals and calculated using the following formula: (Control OD − Sample OD/Control OD) × 100. The DPPH radical-scavenging activity of BHT was also assayed for comparison.

2.7. Inhibitory activity against lipid peroxidation

Lipid peroxidation was assessed, following the method used by Tsuda et al. (1994) with some modifications. In brief, liposome sample (egg lecithin, 6 mg/ml in 0.2 M phosphate buffer, pH 7.4) was sonicated using an Ultrasonicator (SB-5200DTD, Xinzhi Biotech Co., Ningbo, China, 40 kHz) for 1 h. Aliquots (100 μl) of samples were dissolved in 50% ethanol to obtain different concentrations of 25, 50, 75 and 100 μg/ml and then added to 0.5 ml of the liposome mixture. Lipid peroxidation was induced by adding 10 μl of 0.2 M $FeCl_3$ and 10 μl of 0.2 M L-ascorbic acid. After incubation for 1 h at 37 °C, the reaction was stopped by adding 2 ml of 0.25 M HCl containing 15% trichloroacetic acid (TCA) and 0.375% TBA. The reaction mixture was subsequently boiled for 15 min, cooled and centrifuged, and the absorbance of the supernatant read at 532 nm. The blank consisted of all the reagents without the lipid, while the control contained all the reagents except the test sample. Percent lipid peroxidation inhibitory activity of the sample was calculated as: (1−absorbance of sample/absorbance of control) × 100. The lipid peroxidation inhibitory activity of BHT was also assayed for comparison.

2.8. Total antioxidant activity

The total antioxidant capacity of litchi seed extracts was investigated according to the method of Prieto, Pineda, and Aguilar (1999). In brief, the samples (0.1 ml, dissolved in 50% ethanol) at 25, 50, 75 and 100 μg/ml concentrations were mixed with 0.3 ml of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate solutions). The tubes were capped and the reaction mixture then incubated for 90 min at 95 °C. The absorbance of the cooled mixture was measured at 695 nm against a blank. The blank contained the reagent solution and solvent. The total antioxidant activity was expressed as the absorbance of the sample. The higher absorbance value indicated higher antioxidant activity. The antioxidant activity of BHT was also assayed for comparison.

2.9. Identification of phenolic compounds

Reverse phase high performance liquid chromatography was used to analyse phenolic compounds present in the 50% EE sample, using the separation module (LC-20 AT, Shimadzu Corporation, Japan) equipped with a C_{18} column (Vydac, 218 TP, 250 × 4.6 mm, 5 μm particle size, Sigma–Aldrich, St. Louis, MO, USA) and a diode array detector (Rheodyne, USA). The samples were eluted with a gradient system consisting of solvent A (2% acetic acid, v/v) and solvent B (acetonitrile: methanol, 10:15, v/v), used as the mobile phase, with a flow rate of 1 ml/min. The temperature of the column was maintained at 25 °C and the injection volume was 10 μl. The gradient system started from 90% A at 0 min, to 80% A at 10 min, 70% A at 15 min, 60% A at 25 min, 50% A at 30–40 min, 75% A at 42 min, and 90% A at 44 min. The peaks of the phenolic compounds