The antioxidant activities of litchi pericarp among different cultivars

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Abstract. The contents of total phenol and total flavonoid of 7 litchi pericarp cultivars were determined. Their antioxidant activities were also evaluated by 1,1-diphenyl-2-pireyhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP). Correlations between total flavonoid and DPPH scavenging capacity as well as FRAP were also analyzed. The results showed that litchi pericarp was rich in natural antioxidant compounds and the antioxidant abilities were different among different cultivars, and this was useful in the utilization of litchi processing waste.

1. Introduction
Litchi ((litchi chinensis Sonn.) is a fruit tree belonging to the Sapindaceae family, native from southern China, cultivated extensively in China and southeast Asian countries. The fruit of litchi is rich in minerals, dietary fiber, and phytochemicals [1]. The litchi deep processing including juicing and wine making, large amounts of by-products such as pericarp and seed were produced during the processing of litchi. Previous studies indicated that litchi pericarp were abundant in phenolic compounds and showed powerful antioxidant and hepatocellular carcinoma[2,3]. Studies on litchi pericarp were in abundance about bioactive compounds and activities. However, only a certain litchi cultivars were analysed in previous research.

In this paper, the contents of total phenol and total flavonoid of 7 litchi pericarp cultivars were determined. Their antioxidant activities were also evaluated by 1,1-diphenyl-2-pireyhydrazyl (DPPH) radical scavenging and ferroc reducing antioxidant power (FRAP). Correlations between total flavonoid and DPPH scavenging capacity as well as FRAP were also analyzed. The results showed that litchi pericarp were rich in natural antioxidant compounds the antioxidant abilities were different among different cultivars, and this was useful in the utilization of litchi processing waste.

2. Materials and methods
2.1 Materials and reagents
Ripen fruits of 7 litchi cultivars in China, namely ‘Caomi’, ‘Guifenhong’, ‘Lingshanxiang’, ‘Lingfengnuo’, 'Jingganghong', ‘Yingshanhong’, and ‘Ziniangxi’ (designed as 1-7 in this research) were collected just from the trees planted in South Subtropical Crop Research Institute. 10 fruits of each cultivar were picked and cleaned with water, then the pericarp were collected and then ground using a stainless-steel grinder. They were stored in vacuum-packaged polyethylene pouches at -20°C
until required for analysis.

Folin-Ciocalteu’s (FC) phenol reagent and gallic acid (GA) were purchased from Fluka. The 1,1-diphenyl-2-pireyhydrazyl (DPPH) and rutin and all other standards were received from Sigma-Aldrich.

2.2 Sample extraction

1 g litchi pericarp of different cultivars was weighed and refluxed with 30 ml of 70% methanol at 60 °C for 2 h under magnetic stirring. The filtrate was separated by centrifugation, and the extraction was repeated for 3 times. All the filtrate was collected and concentrated under reduced pressure at 40 °C with a final volume of 30 ml and the solution used in the following evaluation and detection.

2.3 Determination of total phenol and total flavonoid in the extracts

The total phenol content (TPC) was determined using the FC assay described before with some modifications [4]. Typically, 0.025 ml of the extract of different cultivar was introduced into test tubes and followed by the addition of 2.0 ml of FC reagent (diluted 10 times with water in advance) and 5.975 ml of water. The solutions were allowed to stand 5 min at room temperature before the addition 2 ml of sodium carbonate solution (7.5% w/v). After reacting in dark for 30 min at room temperature, the absorbance of the solutions were measured at 760 nm on a UV–vis spectrophotometer (Shimadzu UV-2700, Japan). The calibration curve was prepared using a standard solution of gallic acid. The results were expressed as milligram gallic acid equivalents (GAE)/g (fresh weight, FW).

Total flavonoid content was determined based on the method described by Kim et al [5]. One milliliter of extract solution of different cultivar was mixed with 0.3 ml of 5% NaNO₂ and 4 ml of distilled water. Then 0.3 ml of Al(NO)₃ was added to the mixture followed by adding 2 ml of 1 M NaOH. The solution was immediately diluted to 10 ml using distilled water. The absorbance of the solution was measured at 506 nm and the total flavonoid content was calculated by using a calibration curve of rutin standard and expressed as mg rutin equivalent (QR Equiv)/g FW.

2.4 Antioxidant activities of litchi pericarp

2.4.1 DPPH radical scavenging activity assay. The free radical scavenging activity of the extract was performed by measuring the decrease in absorbance of DPPH solution at 517 nm in the presence of the extracts by the method proposed by Liyana-Pathirana et al [6] with minor changes. The solution of 0.5 mM was prepared by dissolving DPPH in methanol. For the evaluation of free radical scavenging activity, 3 ml of DPPH was added into 0.5 ml of the extracts with different concentrations. The mixture was then allowed to stand at room temperature for 30 min in dark before the absorbance at 517 nm was read. The control was prepared as above without extract. The antioxidant activity could be expressed as the following equation:

\[
\text{Scavenging activity} = \left( \frac{A_0 - A_s}{A_0} \right) \times 100\%
\]

where \( A_0 \) and \( A_s \) were the absorbance at 517 nm of the control and sample solution, respectively.

2.4.2 Ferric reducing antioxidant power (FRAP). This assay was determined according to the method reported [7]. For the determination of FRAP, the extracts (10 μL) were mixed with 1 ml distilled water and 1.8 ml of the FRAP solution. Then the mixture was reacted at 37 oC for 10 min. The absorbance of the reaction solution was recorded at 593 nm. Trolox standard solution was used to perform the calibration curves and the results were expressed as μM trolox/ml (original extract solution).
3. Results and discussion

3.1 Contents of total phenol and total flavonoid

The contents of total phenol and total flavonoid in each cultivar was summarized in Table 1. It can be concluded from the table that the total phenol and total flavonoid ranged from 5.1-7.4 and 5.8-7.8 mg/g, respectively. The cultivars of ‘Caomili’ and ‘Ziniangxi’ possessed the highest and lowest content of total phenol and total flavonoid. This indicated that the bioactive compounds in litchi pericarp varied greatly different cultivars. Similar results have been reported by Wang et al [8].

|         | 1  | 2  | 3  | 4  | 5  | 6  | 7  |
|---------|----|----|----|----|----|----|----|
| Total phenol (mg/g) | 7.4 | 7.1 | 6.5 | 5.9 | 5.7 | 5.6 | 5.1 |
| Total Flavonoid (mg/g) | 7.8 | 7.5 | 7.55 | 6.4 | 7.2 | 7.6 | 5.8 |

3.2 Antioxidant activities of litchi pericarp

Due to its operating simplicity, DPPH is one of the most popular methods employed for the evaluation of antioxidant ability, especially in plant extract. DPPH is a kind of stable organic radical. In the radical form, the molecule of DPPH has an absorbance at 517 nm, which will disappear after the acceptance of an electron or hydrogen radical from an antioxidant in the solution to become a stable diamagnetic molecule [9].

The DPPH scavenging abilities of the pericarp of different litchi cultivars were given in Figure 1. Similar to the results of total phenol and total flavonoid, obvious difference were also detected among those values. This indicated the radical scavenging abilities were also different among different cultivars.

The values of FRAP were given in Figure 2. It can be seen that FRAP of litchi pericarp extract of different cultivars ranged from 847 to 1089 μM/ml, and the trend was almost the same as that of total flavonoid. Besides, the trend of FRAP was the same as that of the DPPH scavenging abilities.

3.3 Correlations

Correlations were made in order to determine the contribution of total phenol and total flavonoid to the antioxidant activities of litchi pericarp. The correlation coefficients between total phenol and DPPH scavenging abilities, FRAP were very low (not shown here). However, Both FRAP and DPPH scavenging ability correlated positively with total flavonoid (Figure 3). This suggested that flavonoid s
represent a major part of antioxidant capacity in litchi pericarp. The result was different with that of apple and other litchi cultivars [8, 10].

4. Conclusions
The contents of total phenol and total flavonoid of litchi pericarps of 7 different cultivars, namely ‘Caomi’, ‘Guifenhong’, ‘Lingshanxiang’, ‘Lingfengnuo’, ‘Jingganghong’, ‘Yingshanhong’, and ‘Ziniangxi’ were determined and compared in this research. Their antioxidant abilities were also evaluated by DPPH radical scavenging and FRAP. Results showed that the total phenol and total flavonoid ranged from 5.1–7.4 and 5.8–7.8 mg/g, respectively. The cultivars of ‘Caomili’ and ‘Ziniangxi’ possessed the highest and lowest content of total phenol and total flavonoid. The values of FRAP varied from 847 to 1089 μM Trolox/ml. Correlation study showed that both FRAP and DPPH scavenging ability were positively correlated with total flavonoid, and this indicated that flavonoids were the mainly bioactive compounds in the pericarp of those litchi cultivars. The research showed that litchi pericarps were rich in natural antioxidant compounds and the antioxidant abilities were different among different cultivars, and flavonoids represent a major part of antioxidant capacity. The results were also helpful in the utilization of litchi waste.

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