Antioxidant Activities Correlation Analysis of Procyanidins from China Cultivars Litchi Pericarp

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To cite this article:
Weijing Zhou, Xiaoming Jiang, Ping He, Zhida Sun, Bijun Xie. Antioxidant Activities Correlation Analysis of Procyanidins from China Cultivars Litchi Pericarp. Science Journal of Analytical Chemistry. Vol. 7, No. 1, 2019, pp. 27-31. doi: 10.11648/j.sjac.20190701.14

Received: February 13, 2019; Accepted: March 18, 2019; Published: April 3, 2019

Abstract: Ethanol extracts of litchi pericarp procyanidins (LPPC) were purified from 7 Chinese litchi cultivars. The procyanidin contents in LPPC were ranged from 659.7 mg to 1163.2 mg grape seed procyanidins/g equivalent, while the concentrations of (-)-epicatechin, procyanidin dimmer and trimer were further investigated as well. Antioxidation and free radical scavenging activities of LPPC from different sources were evaluated and compared in vitro. The results showed that LPPC for ‘Huaizhi’ owned the highest free radical scavenging activity on hydrogen peroxide (H₂O₂), superoxide radical (O₂⁻) and ferric reducing; ‘Feizixiao’ exhibited the highest scavenging capacity on 2,2-diphenyl-1-picrylhydrazyl radical (DPPH·), hydroxyl radical (·OH) and lipid peroxidation, however, ‘Guiwei’ possessed the highest value of antioxidant activity based on β-carotene bleaching and ferrous chelating assay. Furthermore, the compositions of LPPC suggested positive correlations with their antioxidant activities significantly.

Keywords: Litchi Cultivars, Pericarp, Procyanidins, Oligomer, Antioxidant Activity

1. Introduction

Litchi (Litchi chinensis Sonn.), which is a tropical fruit of the Sapindaceae family originating from South-east Asia, is a white aril surrounded by a bright red attractive pericarp. Since 2005, litchi cultivated area was more than 6×10⁵ hectare, and its yield was more than 1.3×10⁶ ton per year, of which Guangdong province denominated ‘The Flora of Guangdong Litchi’[1]. They are Guiwei, Feizixiao, Jinfeng, Sanyuehong, Heiye Nuomici and Huaizhi, respectively. The names of litchi are based upon the shape of tortoise-shell-like cracking segments the pericarp, the shape of leaves, inflorescence and fruit, maturity time and the quality of fruit [2].

Litchi extracts have attracted attention in the field of nutrition and pharmaceutical value due to their various active compounds such as anthocyanins, flavanols, phenolic and polysaccharide in aril, flower, seed and pericarp. These compounds have exhibited a dose-dependent on free-radical-scavenging activity [3]. In our previous studies, the main oligomeric procyanidins (such as epicatechin-(4β→8,2β→O→7)-epicatechin, epicatechin-(4β→8,2β→O→7)-epicatechin-(4β→8)-epicatechin) from litchi pericarp were isolated and identified [4]. However, there are still limited publishing studies concerning a completed antioxidant properties of the procyanidins extracts from pericarp of different litchi cultivars in vitro. Therefore, the objective of present study was to analyze the procyanidin compositions of seven well-known litchi pericarp extracts by HPLC method, and then evaluate their antioxidant and free radicals scavenging activities. The relation of the antioxidant activities of LPPC and the procyanidins content in litchi pericarp extract were also discussed, which are essential for predicting their bioactivities and subsequent end-use in food.

2. Material and Methods

2.1. Plant Materials

Seven litchi (Litchi Chinensis Sonn.) cultivars, at
commercial maturation were obtained from Guangdong province in China. These litchi cultivars named ‘Sanyuehong’, ‘Feizixiao’, ‘Guimei’, ‘Nuomici’, ‘Heiye’, ‘Huazhi’ and ‘Jinfeng’ (Figure 1) were collected according to the representative and the fruit maturation period (from May to August). All cultivars litchi were freshly-pickinged fruits. The fruit arrived in the laboratory within 24 h of harvest. Fruits were peeled by handwork and their pericarps were stored at -20°C for further use.

Figure 1. Samples of seven well known cultivars litchi.

2.2. Chemicals and Reagents

Chemicals and reagents were obtained from the following commercial sources: (-)-epicatechin, DPPH, β-carotene, luminal, deoxyribose and pyrogallol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The grape seed procyanidins standard was obtained from Jianfeng Co., Ltd (Tianjin, PRC). AB-8 resin was produced by Nankai Hecheng Science & technology Co., Ltd (Tianjin, PRC). A MDA (Malondialdehyde) kit was purchased from Nanjing Jiancheng Institute of Biology and Engineering (Nanjing, PRC). All other chemicals were of analytical grade.

2.3. Extraction and Purification of Procyanidins

Frozen litchi pericarp (50g) was extracted using 70% ethanol (750 mL) in a water bath at 70 °C for 2 h. The extract was filtered through a vacuum filtering system. The filtrates were concentrated under vacuum by a rotary evaporator at 50°C. The concentrated sample (250 mL) was loaded onto an AB-8 resin column (30×2.5 cm, ID; 25-40 µm particle size), and the fraction eluted by ethanol-water (80:20, v/v), and was collected at a flow rate of 3 mL/min. The eluates were evaporated under vacuum and the residue was lyophilized to obtain LPPC.

2.4. Determination of Procyanidins Content

2.4.1. Butanol-HCl Assay

The method used to determine the contents of procyanidins were according to Li [5]. The grape seed procyanidins was used as standard to calculate the LPPC content by the equation of Y=4.1268X+0.0161, R²=0.9995 from the standard curve. The LPPC content was expressed as the grape seed procyanidins equivalents (GSPC) in mg/g of fresh-frozen weight.

2.4.2. Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) Analysis

The analysis of procyanidins of litchi pericarp extract by RP-HPLC was performed on a Varian liquid chromatogram, and detection was carried out using a photodiode array detector. The chromatographic separation was carried out on a column VP-ODS column (150 mm×4.6 mm ID, 5 µm particle size, SHIMADZU, Japan). The column oven temperature was set at 20°C throughout the whole analytical procedure. The mobile phase was delivered at a flow rate of 1.0 mL/min consisting of 0.4% v/v aqueous acetic acid (A) and acetonitrile (B). A 10µl sample solution was injected and the elution gradient program was as follows: 0-15 min, 5%-15%B; 15-45 min, 15%-50%B. The column was then equilibrated with 5% B for 5 min before the next injection. The absorbance of the eluates was monitored at 280 nm. Before being injected, the extracts were filtered through a 0.45 µm millipore membrane.

The procyanidins trimer epicatechin-(4β→8)-β-Carotein-(4β→8)-epicatechin and A2 dimmer can be identified according to compare with the retention time of the standard afforded by our lab in RP-HPLC chromatographic peak from our laboratory authentic analytic results and publication paper [4, 6]. It can evaluate the concentration of trimer and A2 dimmer according to compare the peak area percentage.

2.4.3. Antioxidant Activity Determination

Antioxidative properties of procyanidins contributed to their capacities on free radical scavenging, metal ion chelating and reducing, lipid peroxidation inhibition. The purpose of this experiment is to evaluate and compare the “in vitro” antioxidant activity of seven cultivars LPPC in different aspect according to use the following assays. DPPH free radical scavenging assay [4]. DPPH radical scavenging activity was assessed according to the method of Shimada. β-Carotene-linoleate bleaching assay. The scavenging ability of LPPC on hydroxide peroxide was measured by a chemiluminescence (CL) method in a luminol-H₂O₂ system by BPLC-4 Ultra-weak luminescence Analyzer [7]. Hydroperoxide scavenging assay. The scavenging ability of LPPC on hydroxide peroxide was measured by a chemiluminescence (CL) method in a luminol-H₂O₂ system by BPLC-4 Ultra-weak luminescence Analyzer [8]. Hydroxy radical scavenging assay. The hydroxyl radical (OH) scavenging ability was determined according to the method [9]. Superoxide anion radical scavenging assay. The scavenging activities of superoxide free radical were determined with some modifications [10]. Ferric ion reducing assay (Fe³⁺ to Fe²⁺). The ferric ion reducing capacity was determined according to the method of Wang [12]. Inhibition of lipid peroxidation assay. The inhibition of lipid
peroxidation of LPPC was evaluated using MDA kit [13]. Statistical analysis. Statistical analysis was done with SPSS 13.0 using a one-way analysis of variance.

3. Results and Discussion

3.1. Isolation and Determination of LPPC

The RP-HPLC analysis of LPPC showed 3 mainly compounds eluting between 15 and 40 min (Figure 2). Most of those compounds had a maximal absorbance wavelength at 280 nm, corresponding to the characteristic absorbance spectrum of flavan-3-ols. Compound 1 was tentative identified as (-)-epicatechin by co-injection with standards, the compound 2 and 3 were identified as procyanidin trimer epicatechin-(4β→8, 2β→O→7)-epicatechin and procyanidin A2 dimer respectively, according to the laboratory authentic analytic results and paper published [4]. The chromatograms of 7 LPPC were similar, in which (+)-epicatechin, A-type dimer and trimer were dominant, but differed from peak intensity.

The contents of procyanidins from 7 LPPC was listed in Table 1. The higher contents were found in ‘Huaizhi’ ‘Feizixiao’ and ‘Guiwei’, and the lowest content was observed in ‘Jinfeng’. Furthermore, (-)-epicatechin content in LPPC was examined. ‘Guiwei’ showed the highest value, followed by ‘Huaizhi’ and ‘Feizixiao’. The (-)-epicatechin content detected in ‘Jinfeng’ was also the lowest. However, differences occurred in A-type dimer content, ‘Feizixiao’ was 14.16%, which was slightly higher than ‘Huaizhi’ and ‘Guiwei’, and the others were less than 10%. We supposed that ‘Huaizhi’ and ‘Feizixiao’ LPPC might be of interest to be used as alternative cultivars for preparing antioxidants.

The LPPC content, yield, pericarp weight rate and harvest time as shown in Table 1. It was found that higher LPPC content also have the higher yield. LPPC content decreased with the harvest time delay in order except ‘Huaizhi’. However, LPPC content has not showed a significant correlation with the color and the shape of litchi pericarp (Figure 1).

### Table 1. Nine litchi cultivars pericarps characteristics and procyanidins contents.

| Cultivar name | Maturity time | Pericarp weight rate (% of fresh fruit) | Yield (% of fresh pericarp) | Procyanidins Contents (mg GSPC/ g extracts)* | (-)-Epicatechin^b (%) | A-type dimer^b (%) | Trimeric procyanidins^b (%) |
|---------------|---------------|----------------------------------------|-----------------------------|-----------------------------------------------|-----------------------|-----------------------|---------------------------|
| Sanyuehong    | Middle May    | 20.94±2.9                             | 1.02±1.7                    | 985.2±9.9                                     | 30.34±0.93            | 13.23±0.23            | 9.25±0.07                 |
| Feizixiao     | Early June    | 20.25±5.1                             | 1.30±1.2                    | 1076.1±14.8                                   | 29.01±0.46            | 14.16±0.14            | 10.58±0.16               |
| Guiwei        | Middle June   | 17.31±17                              | 1.40±1.7                    | 1075.2±10.5                                   | 35.97±0.73            | 5.25±0.12             | 10.01±0.06               |
| Heiye         | Late June     | 14.91±2.3                             | 0.95±1.2                    | 827.5±15.6                                    | 16.28±0.59            | 6.08±0.06             | 5.91±0.08                |
| Nuomici       | Early July    | 17.67±5.9                             | 0.80±1.0                    | 697.9±16.3                                    | 16.34±0.64            | 3.89±0.14             | 6.27±0.12                |
| Jinfeng       | Early July    | 16.17±1.6                             | 0.63±1.6                    | 659.7±23.4                                    | 11.39±0.91            | 2.81±0.36             | 6.03±0.23                |
| Huaizhi       | Middle July   | 17.01±2.9                             | 1.51±2.0                    | 1163.2±141                                    | 31.21±0.48            | 13.96±0.23            | 13.24±0.18               |

^a Procyanidins contents expressed as mg of grape seed procyanidins equivalents/g litchi pericarp extracts.
^b (-)-Epicatechin, Trimeric procyanidins and A-type dimer contents expressed as area percent of epicatechin peak in HPLC chromatogram.

3.2. Comparative Antioxidant Activity of LPPC

Antioxidation and free radical scavenging activities of LPPC from different sources were evaluated and compared in these tests. Figure 3 showed the free radical scavenging activities of 7 cultivars LPPC at various concentrations, and Table 2 showed the effective half inhibition concentration (IC₅₀) of LPPC and ascorbic acid on DPPH radicals. The procyanidins significantly inhibited the activity of free radicals in a dose-dependent manner. The results showed that LPPC for ‘Huaizhi’ owned the highest free radical scavenging activity on hydrogen peroxide (H₂O₂), superoxide radical...
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(\text{O}_2^{\cdot -})$ and ferric reducing; ‘Feizixiao’ exhibited the highest scavenging capacity on 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), hydroxyl radical (OH) and lipid peroxidation, however, ‘Guiwei’ possessed the highest value of antioxidant activity based on β-carotene bleaching and ferrous chelating assay.

Figure 3. Antioxidant activity of seven cultivars LPPC at different system.

Table 2. Antioxidant activity of seven cultivars LPPC and standard antioxidants ascorbic acid and BHT in different aspect.

| Varieties      | Scavenging free radicals | Chelating ferric ion | Reducing ferric ion | Inhibit lipid peroxidant |
|----------------|--------------------------|----------------------|---------------------|-------------------------|
|                | DPPH IC_{50} (µg/mL)     | O_2^{\cdot -} IC_{50} (µg/mL) | H_2O_2 IC_{50} (µg/mL) | OH• IC_{50} (µg/mL) | O_2^{\cdot -} IC_{50} (µg/mL) | Absorbance (A_{700}) | MDA content (nmol/mgprot) |
|----------------|--------------------------|---------------------|---------------------|---------------------|--------------------------|-------------------|-------------------------|
| Sanyuehong     | 9.02±0.93                | 0.227±0.013         | 260.76±16.13        | 42.15±1.56          | 17.12±1.04               | 2.83±0.15         | 2.33±0.15               |
| Feizixiao      | 7.32±1.12                | 0.165±0.006         | 162.69±15.71        | 28.93±0.95          | 15.30±0.61               | 3.42±0.12         | 1.78±0.08               |
| Guiwei         | 9.18±0.69                | 0.191±0.004         | 249.98±8.54         | 36.11±2.04          | 21.28±0.97               | 3.18±0.16         | 1.99±0.07               |
| Heiye          | 10.27±3.18               | 0.283±0.002         | 265.81±11.26        | 44.78±3.20          | 13.55±1.02               | 2.58±0.04         | 1.83±0.11               |
| Nuomici        | 14.15±1.58               | 0.325±0.013         | 296.24±12.54        | 49.95±0.63          | 13.37±0.92               | 2.44±0.08         | 2.36±0.10               |
| Jingfeng       | 14.85±1.83               | 0.364±0.005         | 350.67±13.96        | 50.01±1.19          | 14.01±1.56               | 2.37±0.05         | 2.41±0.09               |
| Huaizhi        | 7.58±0.94                | 0.148±0.009         | 199.51±9.25         | 23.03±1.75          | 18.52±1.30               | 3.96±0.10         | 2.34±0.03               |
| Positive control | 18.75±2.27              | 0.692±0.013         | 408.34±9.01         | 58.91±0.73          | 13.01±0.69               | 1.03±0.09         | 2.46±0.17               |

Values are the means of there replicates ± SD.

Using ascorbic acid as the positive control.

Using BHT as the positive control.
3.3. Correlation Analysis

In these studies, the Pearson correlation coefficient for relationship between the content of procyanidins or oligomeric procyanidins and antioxidant activities from different litchi cultivars named ‘Sanyuehong’, ‘Feizixiao’, ‘Guiwei’, ‘Nuomici’, ‘Heiye’, ‘Huazhi’ and ‘Jinfeng’ are shown in Table 3. The excellent correlations were found between procyanidins content and IC_{50} on scavenging DPPH, H\textsubscript{2}O\textsubscript{2}, OH, O\textsubscript{2}• or A\textsubscript{50} of reducing ferric ion (r\textsuperscript{2} = -0.925, -0.984, -0.879, -0.970 and 0.952, respectively). However, a correlation appeared between the (-)-epicatechin content and the scavenging H\textsubscript{2}O\textsubscript{2} ability and chelating ferric ion rate with excellent correlation coefficient (r\textsuperscript{2} = -0.919, 0.886), IC\textsubscript{50} on scavenging DPPH and A\textsubscript{50} of reducing ferric ion with negative correlation coefficient (r\textsuperscript{2} = -0.840, -0.819). But there was no correlation between either the LPPC content or the O\textsubscript{2} scavenging data and lipid peroxidation activities as for the interaction with biomembrane. Overall, the contributions to antioxidant activities were mainly attributed to procyanidins and its oligomeric procyanidins contents.

Table 3. Correlations coefficients of the procyanidins compounds to antioxidant activities in different system.

| Procyanidins | DPPH (Scavenging rate) | OH\textsubscript{2}O (Scavenging rate) | OH (Scavenging rate) | O\textsubscript{2}• (Scavenging rate) | Rate (Chelating ferric ion) | A\textsubscript{50} (Reducing ferric ion) | MDA content (lipid peroxidation) |
|--------------|------------------------|--------------------------------------|----------------------|-------------------------------------|-----------------------------|---------------------------------|----------------------------------|
| procyanidins | -0.925**                | 0.698                                | -0.984**             | -0.879**                            | -0.970**                    | 0.751                           | 0.932**                          | 0.384                            |
| epicatechin | -0.840**                | 0.706                                | -0.919**             | -0.724                              | -0.819**                    | 0.886**                         | 0.801*                           | 0.102                            |
| A-type dimmer | -0.846*                | 0.323                                | -0.807'              | -0.826'                             | 0.796'                      | 0.303                           | 0.756'                           | -0.164                           |
| trimmer     | -0.820**                | 0.639                                | -0.929**             | -0.790'                             | -0.935**                    | 0.727                           | 0.973**                          | -0.056                           |

** Correlations significant at a 0.01 level (1-tailed).
* Correlations significant at a 0.05 level (1-tailed)

4. Conclusions

The present study strongly suggested that litchi is a new plant source of A-type procyanidins and natural antioxidant, and highlighted the antioxidation dose-effect relationship effect of the litchi pericarp, which have been regarded as waste in the past. More specifically, procyanidin oligomers in litchi pericarp were recorded from 7 Chinese litchi cultivars, ‘Huazhi’ and ‘Feizixiao’ LPPC, compared to other well known cultivars, showed the highest level of antioxidant and free radical scavenging activities based on different methods in vitro. The results also showed that the antioxidant activities partially depend on the procyanidins composition and cultivars, therein ‘Huazhi’, ‘Feizixiao’ were the optimal cultivars for preparing nature antioxidant.

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