

Antioxidant activities of fractions from longan pericarps

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Abstract

The antioxidant activities of ethanolic crude extract (LPCE) and its four different solvent sub-fractions (namely, diethyl ether fraction (LPDF), ethyl acetate fraction (LPEF), n-butyl alcohol fraction (LPBF) and residue fraction (LPR)) from longan pericarps were investigated employing various systems including 2,2-diphenyl-1-picrylhydrazyl (DPPH)/ 2,2'-amino-di(2-ethyl-benzothiazoline sulphonic acid-6)ammonium salt (ABTS)/hydroxyl radical scavenging activity, total phenolic content and reducing power. Each extract showed concentration-dependent antioxidant activity. LPEF showed the highest scavenging activity against DPPH, ABTS and hydroxyl radicals with EC₅₀ values of 0.506, 0.228 and 4.489 mg/mL, respectively. LPEF showed the highest reducing power with EC₅₀ values of 0.253 mg/mL. The next was LPDF with EC₅₀ values of 0.260 mg/mL. LPEF possessed the highest total phenolic content (230.816 mg/g, expressed as gallic acid equivalents), followed by LPDF, LPBF, LPCE and LPR. The results suggested that longan pericarp fractions possessed significant antioxidant activities and could be a promising source of natural antioxidant.

Keywords: longan pericarp; extract; free radicals; antioxidant activity.

1 Introduction

Reactive oxygen species (ROS), such as the superoxide anion (H₂O₂), hydroxyl radicals (OH⁻) and superoxide (O₂⁻), are generated by normal cellular metabolism and exogenous agents (Babior, 2000). They are highly unstable, and attack certain biological molecules, leading to many diseases such as cancer, heart diseases, Parkinson's disease (Je et al., 2005; Deghrigue et al., 2013). However, the action of free radicals and active oxygen can be balanced by a system of antioxidant defenses, including antioxidant compounds and enzymes (Gutteridge & Halliwell, 1992). Synthetic compounds such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiarybutylhydroquinone (TBHQ), are widely employed as preservatives in the food and pharmaceutical industry nowadays (Buenger et al., 2006). But there are numerous reports on adverse effects of synthetic antioxidants such as toxicity and carcinogenicity (Chotimarkon et al., 2008). Many researchers hence focused on exploration of effective and economical natural antioxidants such as plant polyphenols, flavonoids, vitamin C or E, etc.

Longan (*Dimocarpus longan* Lour) is a member of the Sapindaceae family which is widely grown in Southern China, India, and Southeast Asia (Morton, 1987). In recent years, productions and consumption of longan in China have dramatically increased due to the development of planting technology and improvement of agricultural management. Longan pericarp, which account for approximately 20% by weight of the whole fresh fruit, has previously been shown to possess significant amount of phenolic compounds which are promising as functional food ingredients or natural preservatives (Prasad et al., 2010; Soong & Barlow, 2005). However, few comprehensive analysis of their antioxidant property has been conducted while longan pericarps were mainly discarded.

To make the pericarps as good candidate sources for natural antioxidant instead of wastes, the study was designed to assay the antioxidant activities of different longan pericarp fractions.

2 Materials and methods

2.1 Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, 2,2'-amino-di(2-ethyl-benzothiazoline sulphonic acid-6) ammonium salt (ABTS) and ascorbic acid were purchased from Sigma and Aldrich (Fujian, China). All chemicals and reagents were of analytical grade.

2.2 Preparation of extracts and fractions

Powdered longan pericarp was extracted with 50% ethanol at room temperature for 6 h. The supernatant was collected after filtered, and then was evaporated under reduced pressure using a rotavapor to obtain crude extract. Crude extract was extracted with diethyl ether after which the pH value of aqueous fractions was adjusted to 8-9 with 20% NaHCO₃. The aqueous parts were extracted with chloroform to obtain chloroform fraction, insoluble fraction, and aqueous fraction, then the pH value was adjusted to 3-4 by adding 6 N HCl and then extracted with ethyl acetate to obtain ethyl acetate fraction. The aqueous fraction was finally extracted with n-butyl alcohol. Following crude extract and four sub-fractions were obtained: crude extract (LPCE), diethyl ether fraction (LPDF), ethyl acetate fraction (LPEF), n-butyl alcohol fraction (LPBF) and residue fraction (LPR). All of these crude extract and four sub-fractions were dried with a freezer dryer to obtain dry extracts and kept at -4 °C for further research.

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2.3 Determination of total polyphenol contents (TPC)

Total polyphenol contents of crude extract and four sub-fractions were determined by Folin-Ciocalteu method with some minor modifications (Marques et al., 2012). Briefly, 1 mL of sample was mixed with 1 mL of Folin-Ciocalteu's phenol reagent. After 3 min, 1 mL of 35% Na₂CO₃ was added and the volume of reaction system was adjusted to 10 mL with distilled water. The mixture was allowed to stand for 90 min at room temperature in the dark. The absorbance was measured at 725 nm using the spectrophotometer. Total polyphenol contents were expressed as mg gallic acid equivalents, GAE/mg of extract. Calibration curve was built with different concentrations of gallic acid (1-6 ug/mL) as the standard.

2.4 Radical scavenging capacity

To assess the radical scavenging capacity of crude extract and four sub-fractions from longan pericarp, DPPH, ABTS and hydroxyl radical scavenging activity were investigated (Villaño et al., 2007; Vissotto et al., 2013; Re et al., 1999). DPPH and ABTS radical-scavenging activity were calculated as Inhibition Ratio(%) = [(A₀-A₁)/A₀] \times 100, hydroxyl radical-scavenging activity was calculated as Inhibition Ratio(%) = [A₀-(A₁-A₂)/A₀] \times 100, where A₀ is the absorbance without sample, A₁ is the absorbance with samples and A₂ is reagent blank. VC was used as positive controls.

2.5 Reducing power

The reducing powers of longan pericarp fractions and VC were measured according to the method of Yen & Chen (1995) with minor modifications. Briefly, 1 mL of each sample with various concentrations was mixed with 2.5 mL of phosphate buffer (200 mM, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was allowed to stand at 50 °C for 20 min. Then 1 mL of 10% trichloroacetic acid was added, and centrifuged at 4000 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride, and then the absorbance at 700 nm was measured using the spectrophotometer.

2.6 Statistical analysis

All measurements were carried out in triplicate and the results were expressed as mean values \pm SD (standard deviations). Regression analysis was performed by Origin 7.5 for windows.

3 Results and discussion

3.1 Total phenolic contents

It is well known that antioxidant activity was closely correlated with the content of phenolic compounds (Bravo, 1998). In present study, a comparative analysis of total phenolic content of longan pericarps fractions using Folin-Ciocalteu phenol method is presented in Table 1. The total phenolic content of five fractions is ranged from 68.327 to 230.816 mg/g, expressed as gallic acid equivalents (P < 0.05). The highest total phenolic content of the extract was obtained from LPEF (230.816 \pm 2.001 mg/g), while the lowest total phenolic content was from LPR (68.327 \pm 1.364 mg/g). TPC for five fractions from longan pericarp was decreased in the following order LPEF > LPDF > LPBF > LPCE > LPR. Several reports (Duan et al., 2007; Li & Jiang, 2007; Prasad et al., 2005) have conclusively shown a close relationship between total phenolic content and antioxidant activity since phenolic compounds serving as hydrogen-donating agents. Sarikurkcu et al. (2008) reported that dietary plants possessing substantial phenolic compounds may be health-benefit due to their antioxidant properties. Thus, our results suggested that total phenols maybe corresponded to the chemical foundation of the potential antioxidant activities of longan pericarp extracts.

3.2 DPPH free radical-scavenging activity

DPPH can accept an electron or hydrogen radical to become a stable diamagnetic molecule (Soares et al., 1997). DPPH radical scavenging assay is a widely used method to estimate free radical scavenging activities of antioxidants now for its advantage of economical and ease (Pan et al., 2008). A dose-response curve of DPPH radicals scavenging activities of the fractions from longan fruit pericarp was presented in Figure 1. It was observed that the DPPH radical-scavenging activity increased as the concentration of the extract increased. The scavenging activity of LPCE, LPDF, LPEF and LPBF toward to DPPH radicals increased from 0.2 to 2.0 mg/mL and were 91.52%, 95.03%, 93.16% and 72.44% at a concentration of 2.0 mg/mL, respectively. LPR showed the lowest DPPH scavenging ratio of 75.00% at a concentration of 10 mg/mL.

With regard to EC₅₀ as shown in Table 1, amongst all the extracts and fractions examined, the LPEF showed the lowest EC₅₀ (0.506 mg/mL), exhibited the highest DPPH radical-scavenging activity, followed by LPDF, which EC₅₀ value was 0.523 mg/mL. LPR exhibited the lowest DPPH radical-

Table 1. EC₅₀ values and total phenols contents of longan pericarp fractions.

	EC ₅₀ value (mg extract/mL)				Total phenols (mg/g)
	DPPH radical	Hydroxyl radical	ABTS radicals cation	Reducing power	
LPCE	0.794 \pm 0.009	5.694 \pm 0.162	0.495 \pm 0.005	0.498 \pm 0.006	89.511 \pm 1.681
LPDF	0.523 \pm 0.004	4.752 \pm 0.087	0.296 \pm 0.003	0.260 \pm 0.020	132.209 \pm 4.070
LPEF	0.506 \pm 0.010	4.489 \pm 0.027	0.228 \pm 0.003	0.253 \pm 0.004	230.816 \pm 2.001
LPBF	1.061 \pm 0.022	7.980 \pm 0.042	0.447 \pm 0.016	0.407 \pm 0.009	124.128 \pm 3.467
LPR	5.190 \pm 0.043	16.833 \pm 0.067	2.926 \pm 0.044	2.172 \pm 0.030	68.327 \pm 1.364
VC	0.088 \pm 0.001	0.986 \pm 0.009	0.082 \pm 0.000	0.055 \pm 0.001	

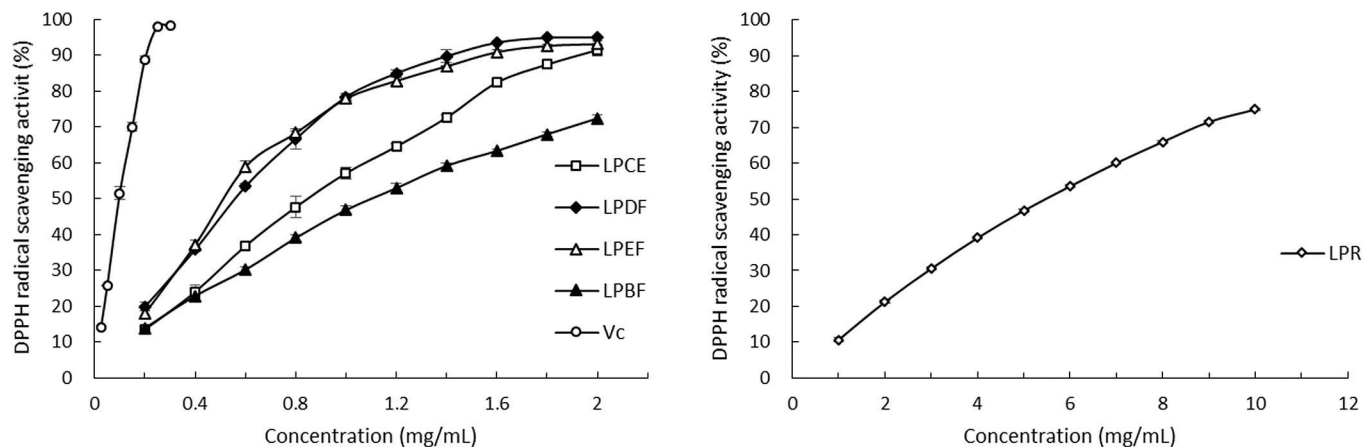


Figure 1. DPPH radical scavenging activities of the extracts from longan fruit pericarp. VC was used as reference antioxidant. Each value is expressed as a mean \pm S.D. (n=3).

scavenging activity with highest EC_{50} value (5.190 mg/mL). Meanwhile, VC showed EC_{50} value of 0.088 mg/mL. Based on the EC_{50} values, the order of DPPH radical-scavenging activity for VC and crude extract and four sub-fractions from longan pericarp was as follows: VC>LPEF>LPDF>LPCE>LPBF>LPR. Our results showed that both LPDF and LPEF possess the excellent activity at reducing the stable radical among all of five fractions. For other fruit extracts, Kui Zhong et al. (2010) has been determined the scavenging effect of longan pulp extracts reached to 85.74% at 20 mg/mL. Free radical scavenging activity of sweet orange peel extracts and sub-fractions expressed as EC_{50} ranged from 0.5 to 8.9 mg dry extract/mg DPPH (Anagnostopoulou et al., 2006). For potato peels, the scavenging effect increased with increasing concentration up to a certain extent (3.75 mg) and then leveled off with further increased (Singh & Rajini, 2004). The result of the present study indicated that longan pericarp extracts possess antioxidant activity comparable to those of fruit extracts.

3.3 Hydroxyl radicals-scavenging activity

Hydroxyl radical is the most reactive radical among all oxygen radicals. It can induce severe damage bio-molecules in the body, which result in cell damage that causes ageing, cancer and several other diseases (Valentão et al., 2003). The removal of hydroxyl radicals is probably one of the most effective ways to defense several diseases. Dose-response curves of hydroxyl radical scavenging activities of crude extract and four sub-fractions from longan pericarp was presented in Figure 2. When at 2 mg/mL, hydroxyl radical scavenging activities were in the following order: LPCE (28.23%) > LPEF (27.19%) > LPDF (25.54%) > LPBF (8.64%). When at 10 mg/mL, the order is LPEF (75.18%) > LPDF (74.25%) > LPCE (67.80%) > LPBF (57.17%). While LPR showed the lowest hydroxyl radical scavenging activity increased from 13.30% to 57.15% when the concentration was 4-20 mg/mL. As positive control, VC's effect on the hydroxyl radical was 69.32% at the concentration of 1.8 mg/mL, showed the highest hydroxyl radical scavenging activity. EC_{50} values of each tested samples were in the following order: LPR (16.833 mg/mL) > LPBF (7.980 mg/mL) > LPCE

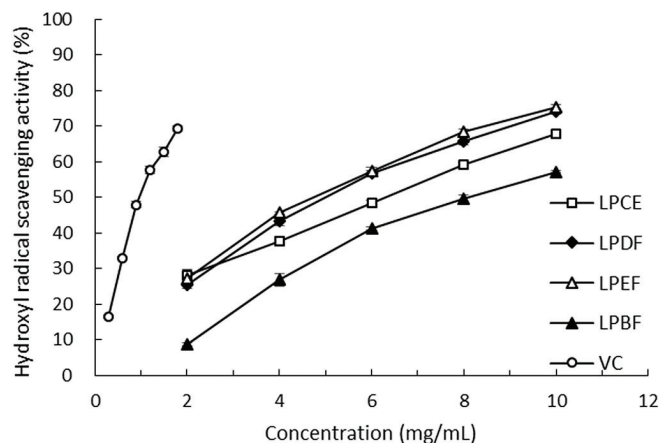


Figure 2. Hydroxyl radical scavenging activity of the extracts from longan fruit pericarp. VC was used as reference antioxidant. Each value is expressed as a mean \pm S.D. (n=3).

(5.694 mg/mL) > LPDF (4.752 mg/mL) > LPEF (4.489 mg/mL) > VC (0.986 mg/mL).

Kui Zhong et al. (2010) investigated the antioxidant activity of extracts from longan pulp with ultrasonic extraction which showed excellent scavenging activity on the hydroxyl radicals and a, a-diphenyl-1-picrylhydrazyl (DPPH) radicals, and obtained the almost complete scavenging effect. Gongming Zheng et al. (2009) isolated and further characterized eight polyphenols from longan seeds and determined the eight polyphenols exhibited scavenging activity towards DPPH radicals with SC_{50} values of 0.80-5.91 μ g/mL and towards superoxide radicals with SC_{50} values of 1.04-7.03 μ g/L. Yuttana Sudjaroen et al. (2012) also pointed out that purified antioxidants from this source may prove useful not only for increasing the shelf life of foods by preventing lipid peroxidation but also for protecting against oxidative damage in living systems by scavenging reactive oxygen radicals. Therefore, longan pericarp can be good candidate sources for natural antioxidant.

3.4 ABTS radical cation-scavenging activity

The effects of longan pericarp fractions and VC on the ABTS radical cation were shown in Figure 3. The ABTS radical cation scavenging activities increased as the concentration of samples increased. At the concentration of 0.1 mg/mL, the ABTS radical cation scavenging was in the order: LPEF (26.37%) > LPBF (18.53%) > LPDF (17.95%) > LPCE (11.96%). At the concentration of 1.2 mg/mL, the order is: LPEF (99.39%) > LPDF (99.35%) > LPBF (88.11%) > LPCE (87.80%), while LPR have an obvious effect on ABTS radical cation when the concentration reached 7 mg/mL. As positive control, VC showed a high activity to ABTS radical at a low concentration, which ranged from 10.87% to 100.00% at the concentration of 0.02 to 0.2 mg/mL. EC_{50} of the extracts in ABTS radical cation activity varied from 0.228 to 2.926 mg/mL (Table 1). The ABTS radical cation activities were ranked in the following order: LPEF > LPDF > LPBF > LPCE > LPR. ABTS assay is widely used to measure the antioxidant activity of variety substances. ABTS and DPPH radical scavenging methods are based on

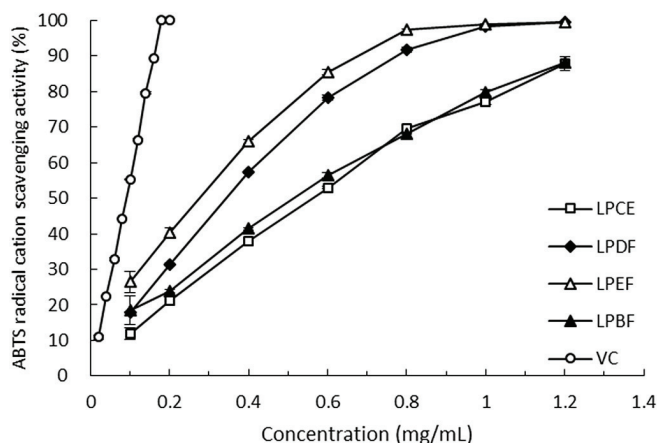


Figure 3. ABTS radical cation scavenging activity of the extracts from longan fruit pericarp. VC was used as reference antioxidant. Each value is expressed as a mean \pm S.D. (n=3).

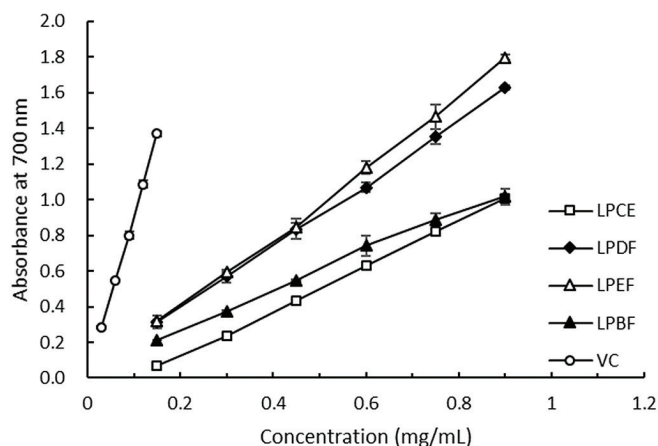


Figure 4. Reducing power of the extracts from longan fruit pericarp. VC was used as reference antioxidant. Each value is expressed as a mean \pm S.D. (n=3).

a similar antioxidant mechanism and both using common spectrophotometric procedures to determine the antioxidant activity of components. Expectedly, the result of ABTS was agreed with the DPPH assays in the present study.

3.5 Reducing power

The reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron because of the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Yildirm et al., 2000). There are some reports pointed that reducing power of plant extracts was associated with their antioxidant activity (Pan et al., 2008). Figure 4 showed the dose-response curves for reducing power of the extracts and fractions from longan fruit pericarp. The reducing power of LPCE, LPDF, LPEF and LPBF increased from 0.072, 0.313, 0.322 and 0.211 at 0.15 mg/mL to 1.004, 1.629, 1.797 and 1.016 at 0.9 mg/mL. LPR showed the lowest reducing power, with an absorbance of 0.932 at the concentration of 4.2 mg/mL. The EC_{50} values of LPEF and LPDF were 0.253 and 0.260 mg/mL, while LPBF and LPCE were 0.407 and 0.498 mg/mL (Table 1). Yingming Pan et al. (2008) revealed the reducing power of Soxhlet extract of longan peel was lower (the absorbance at 700 nm was 1.439 at 1.0 mg/mL) (Sudjaroen et al., 2012).

4 Conclusions

Crude extract and four sub-fractions, diethyl ether fraction (LPDF), ethyl acetate fraction (LPEF), n-butyl alcohol fraction (LPBF) and residue fraction (LPR), were extracted from longan pericarp in the present study, their antioxidant properties were investigated. These results indicated that longan pericarp fractions exhibited antioxidant activities. LPEF possessed the higher phenolic content and shown more significant scavenging activities of free radicals such as DPPH and ABTS radicals. This profound protective effect of longan pericarp suggested that it can be served as a promising source of natural antioxidant. Further work is necessary to promote the development of value-added ingredients from longan pericarp fractions to use in the food and pharmaceutical industry instead of just being waste.

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