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Extraction Methods and Antioxidant Properties of Polysaccharide from Longan (Dimocarpus Longan Lour) Seed

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Abstract **- Present study includes extraction and antioxidant properties of polysaccharide from Longan (Dimocarpus longan Lour) seed. The polysaccharide from longan seed (PL) was extracted by hot water, complex enzymes and the microwave-assisted systems. The antioxidant activites of PL were determined by using various test methods, including scavenging of hydroxyl radicals, 2,2-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity, chelating ability on ferrous ions. The data showed that the highest yield of polysaccharide from longan seed was achieved with enzyme extraction (51 %). There was no significant difference in yield of PL extracted by microwave assisted and hot water. The three PL extracts have effective free radical scavenging, hydroxyl radical scavenging, and scavenging on ferrous ions. This antioxidant capacities depends on concentration and increasing with increased amount of sample. This results indicate that polysaccharides from longan seed can be obtained to use as a natural antioxidants.**

Key words: Longan seed, polysaccharide, antioxidant capacities, extraction methods.

1. INTRODUCTION

Longan (Dimocarpus longan Lour.), one of most comon non-climacteric fruit in Southeast Asian countries, is planted widely in both the south and the north of Vietnam due to their high commercial value. Longan fruit, in my country is consumed as fresh, canned and dry products. Longan seed is seen as waste materials from production factories where seasonly remove thousands of tons of seed. This sourse is currently used around 20% by fertilizer producers, other 80% be seen as inductrial waste. Some research in the world revealed that longan seed obtain natural polyphenol [Zheng et al., 2009]; antifatigue properties [Zheng et al, 2010] and be used in traditional medicine [Xiao et al., 2004]. Gongming Zheng et al (2012) found a new lactone from this source. In recent year, polysaccharides from flant and seed have been paying attention due to their unique bioactivities such as anti-tyrosinase [Rout S et al, 2007], antitumor [M. Zhanga, 2007], anti-microbial activity [Attachai K., 2010] and antioxidant activities. There were few reports about polysaccharides from longan seeds. Li and Ding (2007) and Guoxiang et al (2013) investigated extraction methods of polysaccharides from longan by hot water and by ultrasonic-assisted, respectively. Meanwhile, the seed is around 40% of longan fruit weight and the carbonhydrate is around 65% of dry matters [Nianghui Li, 2012]. Therefore, further researches on this seed are necessary to take advantage of this source. The aim of this study is that comparision the antioxidant properties of the polysaccharides extracted in different methods including hot water, complex enzyme and the microwave-assisted systems. The antioxidant activities of PL were evaluated by various tests scavenging of hydroxyl radcals, 2,2-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity and chelating ability on ferrous ions.

2. MATERIAL AND METHODS

2.1. Sample preparation

The fresh seeds of longan were obtained as waste material from the drying longan processing company, Thu Nga, Hung Yen province in the month of July. The fresh seeds were washed and air dried in shade to moisture content of around 3% and kept in clean place for futher use.

Cellulase, pectinase and protease with specific activity (15,000 IU/g) were purchased from Phuong Tram Co., the 2,2-diphenyl-2-picrylhydrazyl (DPPH) and ascorbic acid, d-glucose, Ethylenediaminetetraacetic acid (EDTA) were obtained from Merck (Germany). Other chemicals at analysis standards were bought from Vietnam.

2.2. Polysaccharide extraction

Dry seed was mechanical removed coat and the endosperm was broken into one-quarter to one-third its size by a blender. The broken sample was pretreated in ethanol (purity 99.5%) in proportion 1:3 (seeds: ethanol) at 70^{o}C during 15 min to remove low molecular weight, polyphenol, pigments and inactive the enzymes. The ethanol was decanted and sample let dry at room condition. This pretreated sample was directly extracted polysaccharides by different methods, including hot water extraction (HW), microwave assisted extraction (MA) and complex enzyme extraction (CE).

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First was hot water extraction method, distilled water was added in a proportion of 1:3 (pretreat sample:water), the sample was left to rest for approximately 20 h. Then the water was removed and added a new proportion of 1:5, (endosperm:water). After that, the sample was cooked in water bath at 100ºC for 15 min. Let the sample cooling down and then mixed in a blender for 5 min. The mixed endosperm was centrifuged at 6000 rpm during 15 min at 20ºC. The precipitation of the polysaccharides was achieved by adding the supernatant to ethanol (purity 99.5%) at a ratio of 1:1.5. After being washed two times with chloroform: n-butanol (3:1, v/v) in 20 min to remove protein and two times with anhydrous ethanol, the precipient was lyophilized and kept in -24ºC until further use.

Second was the microwave-assisted extraction method, distilled water was added in a proportion of 1:5 (pretreat sample:water) and heated for 10 min in a microwave oven (540 W). The extract was centrifuged, washed and dried as the same steps that were taken in the boiling water extraction.

Third was enzyme extraction method, the sample was treated with 1% complex enzyme (cellulose: pectinase: protease = 1:1:1) and the ratio of endosperm to water was 1:10. The reactions were carried out at a temperature of 50°C, for 120min. The following procedure was in same with the above method of hot water extraction.

The polysaccharides yield (%) is calculated as follows:

$$\text{Yield (\%)} = W_1 / W_0 \times 100 \quad (1)$$

W_1 = The weight of extracted polysaccharide

W_0 = The weight of broken sample

2.3. Analysis procedures

Determinationon the purity of polysaccharide

The polysaccharide content was determined according to the phenol–sulfuric acid method ((Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Standard curve was built base on glucose. Standard glucose (10 mg) was dissolved with distilled water (100 ml), then the solution was diluted as concentration of 0.05; 0.1; 0.15; 0.2; 0.25; 0.3, 0.4 mg/mL. Each diluted solution (1ml) was added 5% phenol (1 mL) and sulfuric acid (5 mL) then mixed and vortexed. The absorbance of the solution was measured at 490 nm with an ultraviolet-visible spectrophotometer, using water as a blank.

Determination on the purity of polysaccharide: 1 ml of sample (dissolved 0.5 mg powder in to 100ml distilled water) was used. The purity was calculated according to its OD value and standard curve.

DPPH radical scavenging assay

The DPPH free radical scavenging activity of each sample was determined according to according to procedure of Brand-Williams (1995) with some modifications. Briefly, 2 mL of sample dissolved in deionized water in different concentrations (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6 and 1.8 mg/ml) and 2 mL of freshly prepared 0.1mM DPPH ethanolic solutions were thoroughly mixed and kept for 30 min in the dark. The absorbance of the reaction mixture at 517 nm was read with a spectrophotometer. Ascorbic acid was used as a standard. The IC50 value (concentration providing 50% inhibition) was graphically calculated using a calibration curve in the linear range by plotting the extract concentration against the corresponding scavenging effect. The DPPH radical scavenging effect was calculated using the following equation:

$$\text{scavenging effect(\%)} = 1-(A- A_i)\, A_0 \times 100\%$$

Where A is the absorbance of the sample mixed with DPPH solution, A_i is the absorbance of the polysaccharide without DPPH solution and A_0 is the absorbance of DPPH solution.

Hydroxyl radical assay

The antioxidant activity of scavenging hydroxyl radical was determined according to the method of Li et al [2006]. The samples were firstly dissolved in deionized water in different concentrations (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6 and 1.8 mg/ml). Then, the sample (1 ml) mixed with phenanthroline (2.5 mM, 1 ml), phosphate buffer (0.5M, PH 7.4, 1 ml), ferrous sulfate (5 mM, 1 ml), and H_2O_2 1%, 0.5 ml) at 37°C for 1 h. The absorbance of the mixture was measured at 510 nm with a spectrometer (CECIL/CE 1011, 101S, 120554). In the control group, samples were replaced by solvent. The ability to scavenge hydroxyl radicals was calculated as follows:

$$\text{Scavenging effect (\%)} = [(F_2-F_1)/(F_0-F_1)] \times 100$$

WhereF_1 is the absorbance of the control, F_2 is the absorbance of the polysaccharide and F_0 is the absorbance of the solution without polysaccharide and H_2O_2.

Chelating ability on ferrous ions

Chelating ability was determined according to the method of Dinis, Madeira, and Almeida (1994). The samples were firstly dissolved in deionized water in different concentrations (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6 and 1.8 mg/ml). Then, 1 ml of sample was mixed with 3 ml of methanol and 0.1 ml of 2 mM ferrous chloride. The reaction was initiated by the addition 0.2 ml of 5 mM ferrozine. After 10 min at room temperature, the absorbance of the mixture was determined at 562 nm against a blank. A lower absorbance indicates a higher chelating power. Ethylenediaminetetraacetic acid were used for comparison.

2.4. Statistical Analysis

All analysis was performed in triplicate and data were expressed as mean ± standard deviation. Statistical analysis was performed using Statistica 8.0. The data were analyzed by using variance analysis (ANOVA) and statistical differences with p-values under 0.05 were considered significant. Tukey's test was performed to analyze differences among means by software and the graphs were decribed by using Microsoft Excel 2010.

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3. RESULT AND DISCUSSION

3.1. Extraction yields

Of three mentioned extraction methods, the yield of polysaccharide extracted by complex enzymes was highest (51%), following by microwave-assisted, and hot water extraction with 45.5% and 43.6%, respectively (table 1). It seems that enzyme treatment was effective in degrading the cell wall and water insoluble materials into water soluble components, resulting in high extraction effiency [Dasaesamoh, 2014; Junchen Chen et al 2013]. However, after purity, small components in three extracts were removed and there was no significant differences (P>0.05) in purity polysaccharide contents from these extraction methods.

Table 1: Comparision for extraction efficiency among various methods

Extraction methods	Yield (%)	Purity (%)	Extraction time (hrs)
Hot water	43.6[a]	80.7[a]	22
Microwave-assist	45.5[ba]	81.2[a]	5
Enzyme complex	51[c]	82.6[a]	6.5

Hot water extraction is a traditional and conventional method. It takes longer extraction time than others but the facilities request simple, running cost low, impact to environment friendly and products safely use to humankind [Junchen Chen et al 2013]. The polysaccharide is polarity macromolecule tend to easily resolve in water, especially in hot water. Be immersing the seed in 20 hrs in HW extraction could help remove nicely the molecular weights, hence the yield of polysaccharide was lowest but the purity was impressive comparing to CE and MA methods.

Comparing with result of hot water extraction, the extraction efficiency with microwave-assist method was higher. The principle of heating using microwave is based upon its direct impacts on polar materials (Letellier and Budzinski, 1999) and heating function of microwave that can rupture the cell wall and help for polysaccharides molecule released easily. The advantage of this method is time saving, however the facilities must be invested. In recent years, this technique has been applied widely and rapidly, especially in research of naturals compounds extractions [Letellier et al, 1994; Paré et al, 1999; Junchen Chen et al 2013; Dam Sao Mai, 2015].

With complex enzyme method, the yield of crude polysaccharide was highest, about 51% and the time consumed for extraction reduced to 1/4 comparing to hot water extraction. Enzyme-assist extraction revealed obviously in its advantages that over the conventional hot water extraction. The contemporary action of three kind of enzymes help rapidly hydrolysis. Most of works on applying enzyme extraction methods of bioactives from plants showed that this method was effective on yield and product quality [Junchen Chen et al (2013); Dam Sao Mai, et al (2015); Xiulian Yin., (2011); Yang, Y-C. et al., (2010); Barzana, E. et al. (2002); Passos, C.P. et al., (2009); Wu, Y. et al., (2005); Ruiz-Teran, F. et al. (2001); Dehghan-Shoar, Z. et al. (2011).

3.2. Scavenging ability

Scavenging ability on 1,1-diphenyl-2-picrylhydrazyl radicals

The DPPH radical scavenging activities increased with increased concentrations of PL solutions up to 1.8 mg/ml (Fig.1). This result was similar to the early report on antioxidant properties of crude polysaccharide and purified polysaccharide of longan seed [Guoxiang J, 2013]. At 1.4 mg/ml, scavenging effect of HW, MA, and CE extracted polysaccharides and ascorbic acid on DPPH radicals were 75, 78, 80 and 95% respectively. However, the ability to scavenge DPPH radicals did not increased concentrations above 1.4 mg/mL. This trend was agreement with research of Jun-Yi Jin (2010) due to the present of solvent in sample solution leading to precipitate polysaccharides when its concentration was high enough. The value of solution concentration required to decrease the initial DPPH concentration by 50% (IC50) of CE polysaccharides (IC50 0.52 mg/mL) exhibited a higher level of radical-scavenging activity than MA (IC50 0.69 mg mL) and HW (IC50 0.82 mg mL) but they were all weaker than ascorbic acid (< 0.2 mg/ml).

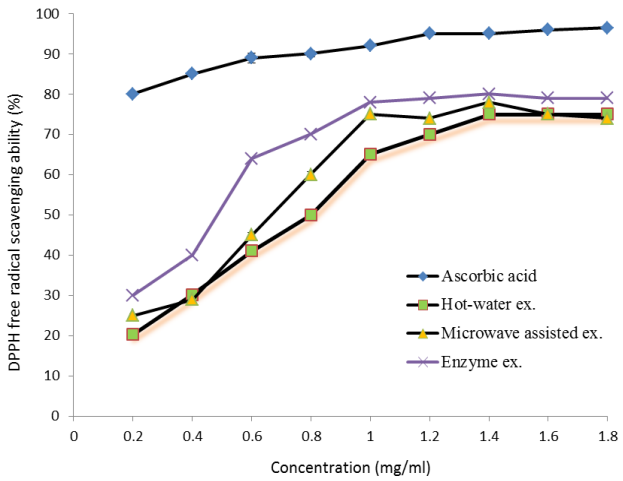

Fig 1. Scavenging ability of hot water extracted, microwave assisted extracted and complex enzyme extracted polysaccharides on 2,2-diphenyl-2-picrylhydrazyl radicals. Each value is expressed as mean ± S.D (n=3)

Scavenging ability on hydroxyl radicals

It is known that the hydroxyl radical is a powerful oxidant that can react with nearly all biological molecules such as proteins, lipids and carbohydrates [Santanam N, 1998]. The scavenging effect increased with increasing concentration.

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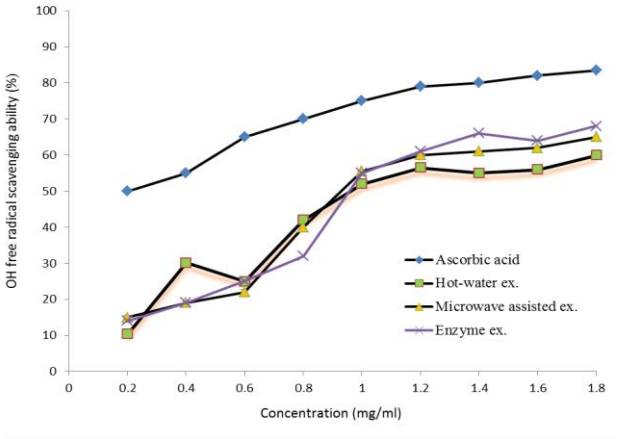

Fig. 2. Scavenging ability of hot water extracted, microwave assisted extracted and complex enzyme extracted polysaccharides on hydroxyl free radicals. Each value is expressed as mean ± S.D (n=3)

Compared with CE and MA polysaccharide, the scavenging activity of PL from HW extract was the strongest at concentrations 0.4 mg/mL. There was no significant differences (P>0.05) in hydroxyl radical capacity between three extraction samples with concentration from 0.6 mg/ml to 1.8 mg/ml and they were lower than those of ascorbic acid. Further investigation on longan seed is needed to confirmed which extraction methods effectively on antioxidant properties of these polysaccharides.

Chelating ability on ferrous ions

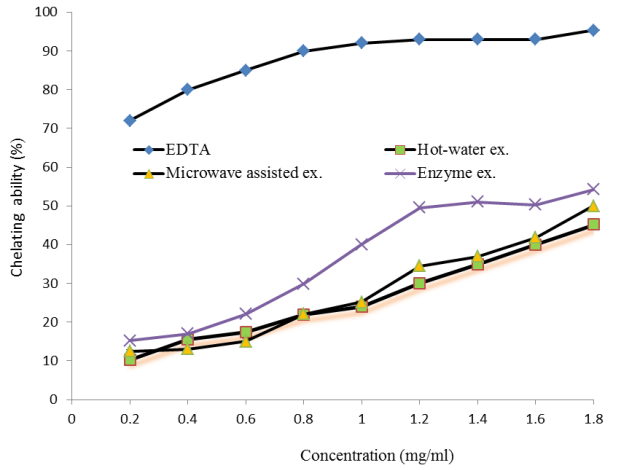

Fig. 3. Chelating ability of hot water extracted, microwave assisted extracted and complex enzyme extracted polysaccharides on ferrous ions. Each value is expressed as mean ± S.D (n=3)

Regarding to chelating ability on ferrous ions, CE extracted polysaccharides were more effective than HW, MC extracts at solution concentration ranged from 0.2 to 1.8 mg/ml (Fig.3). At 0.2 mg/ml, chelating abilities of polysaccharides from HW, MC, EA were 10.3, 12.5 and 15.2 %, respectively. At 1.8 mg/ml, chelating abilities of polysaccharides of these three samples reached highest value, 45.2, 50 and 54.2%, respectively. EDTA showed an excellent chelating ability of 72% at 0.2 mg/ml. Metal chelating capacity are the most effective pro-oxidants in the food system (Yamaguchi, 2000) since they reduced the concentration of the catalysing transition metal in lipid peroxidation (Duh et al., 1999).

4. CONCLUSION

With comparison between three extraction methods (traditional hot water, microwave-assisted and complex-enzyme) used for polysaccharide of longan seed, the complex enzyme extraction methods tends to be a efficiency one. The further studies need optimization of polysaccharides extraction by both the enzyme method and traditional hot water method to give flexible techniques to utilize this byproducts from fruit processing industry.

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