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Full Length Research Paper

A comparative study on antioxidant activity of ten different parts of *Nelumbo nucifera* Gaertn.

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The *in vitro* antioxidant activity of ten different parts of lotus (*Nelumbo nucifera* Gaertn.) was evaluated and compared. Among the ten lotus extracts, the receptacle did not only possessed the highest phenolic, flavonoid and proanthocyanidin contents, but its 2-picrylhydrazyl free (DPPH) and 2, 2'-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activities, reducing power as well as total antioxidant activity were also comparable to, if not better than those of the butylated hydroxytoluene (BHT) control ($p < 0.05$). Compared with the BHT, all ten lotus extracts even exhibited significantly ($p < 0.05$) higher metal chelating activity. Nevertheless, the hydroxyl radical scavenging ability of all ten lotus extracts was significantly ($p < 0.05$) lower than that of the ascorbic acid control. In contrast to the metal chelating ability, phenolic compounds in the ten lotus extracts would likely be responsible for their DPPH and ABTS radical scavenging activities.

Key words: Louts (*Nelumbo nucifera*), total phenolic content, total flavonoid content, total proanthocyanidin content, antioxidant activity.

INTRODUCTION

Antioxidants are capable of inhibiting oxidation reactions by scavenging free radical intermediates, protecting our body against infections and degenerative diseases. Apart from medicinal application, antioxidants are widely used in food industry as food additives to prevent rancidity of oil. Commercial use of synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were restricted in recent years, since they were found to be toxic and associated with many side effects (Hossain et al., 2008). As a result, there is a pressing need to search for naturally occurring antioxidants, which are safe for human consumption with promising medicinal and technological effects (Sreelatha and Padma, 2009). Lotus (*Nelumbo nucifera* Gaertn.) is a perennial aquatic plant grown in wetlands or pond and is widely distributed in temperate and tropical Asia (Laongsri et al., 2009). In China, lotus has been cultivated for more than 2,000 years in provinces like Fujian, Zhejiang, Hubei, Hunan, Jiangsu, Anhui and Jiangxi (Guo, 2009). Because of its pleasant flavour and high nutritional value, lotus is one of the most popular edible aquatic plants in China, especially its seed, rhizome and leaf, which have been widely used in Chinese cuisine and numerous food products like dessert, porridge, soup, drink, and tea bags (Huang et al., 2010). Being as the by-products of lotus food production, a huge amount of inedible lotus parts (such as receptacle, seed epicarp, and rhizome knot etc.) are discarded as waste or used as firewood every year. As reported in numerous previous studies, almost every part of lotus did not only possess substantial amount of phenolic compounds, but they also

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exhibited a wide spectrum of pharmacological activities, including antioxidation activity (Huang et al., 2010), improving learning and memory (Gong et al., 2008), hepatoprotective (Sohn et al., 2003; Ono et al., 2006), anti-obesity (Ono et al., 2006), anti-HIV activity (Kashiwada et al., 2005), anti-tumor effect (Duan et al., 2010), diuretic activity (Pulok et al., 1996), antipyretic activity (Mukherjee et al., 1996), antidepressant (Sugimoto et al., 2010), anti-inflammatory (Mukherjee et al., 1997; Liu et al., 2004), rat lens aldose reductase (RLAR) inhibition property (Jung et al., 2003), and cholinesterase inhibitory activity (Jung et al., 2010). However, the phenolic content and antioxidant activity in different parts of lotus have been rarely contrasted in previous literature. Hence, the evaluation of antioxidant properties of different part of Nelumbins Receptaculum remains an interesting and valuable task, particularly for finding a novel and inexpensive source for natural antioxidants, functional foods and nutraceuticals, In the present study, a comprehensive investigation of both phenolic content and antioxidant activity of various lotus parts was conducted.

MATERIALS AND METHODS

Chemicals

Except butylated hydroxyl toluene (BHT) and aluminium trichloride, which were obtained from Sinopharm Chemical Reagent Co. (Shanghai, China), all other chemicals and reagents like Folin-Ciocalteu reagent, 2,2'-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), ascorbic acid, gallic acid and catechin were purchased from Sigma Chemical Co. (St. Louis, MO USA).

Samples of lotus plant

All intact specimens of lotus plants were collected in Fujian province of China followed by morphological and molecular authentication by a plant biologist. The voucher specimens were then cleaned and subdivided into ten different parts namely, receptacle, leaf, stamen, seed epicarp, flower, embryo, seed, petiole, rhizome knot and rhizome. Consequently, all lotus parts were oven-dried (40°C), pulverized into powers and kept in darkness at -20°C.

Preparation of lotus extracts

After lipid removal by using petroleum ether for 6 h, individual defatted lotus powder was sequentially extracted twice with 70% acetone (1:15; w/v) under ultrasonic conditions for 30 min followed by filtration with Whatman No. 1 filter paper. All filtrates were evaporated under vacuum, while the resulting dried extracts were stored at -20°C prior to further analysis.

Determination of total phenolic content

The total phenolic content in the ten lotus extracts was determined according to the procedures previously reported by Ismail et al. (2009) with slight modifications. Briefly, each lotus extracts were diluted with deionized water to a suitable concentration for analysis. Then, 100 ul of these solutions was mixed with 2.5 ml of 10 fold diluted Folin-Ciocalteau reagent, and 2.0 ml of 7.5% sodium carbonate (Na_2CO_3) followed by incubation at 40°C for 30 min. Consequently, total phenolic content in the reaction mixtures was determined colorimetrically at 760 nm and the results were expressed as mg of gallic acid equivalents per gram of lotus extract.

Determination of total flavonoid content

Total flavonoid content in the lotus extract was determined by the method of Chinese Pharmacopeia Committee (2010), using rutin as a standard. In brief, 6 ml of each lotus extract was sequentially mixed with 1 ml of sodium nitrite (5 g/100 g), 1 ml of aluminium nitrate (10 g/100 g) and 10 ml of sodium hydrate (1 mol/L). After incubation at room temperature for 15 min, total flavonoid content in the mixture was measured colorimetrically at 500 nm and all data were expressed as microgram of rutin equivalents per gram of lotus extract.

Determination of total proanthocyanidin content

Total proanthocyanidin content was determined according to the modified method previously described by Yao et al., (2002), using (+)-catechin as a standard. Briefly, 1.5 ml of each lotus extract was mixed with 6 ml of vanillin-methanol solution (4 g/ 100 ml) and 3 ml of hydrochloric acid solution. After incubation at 30°C for 20 min, total proanthocyanidin content in the reaction mixture was measured colorimetrically at 500 nm and the results were expressed as microgram of catechin equivalents per gram of lotus extract.

Evaluation of antioxidant activity

DPPH radical scavenging activity

The DPPH radical scavenging ability of the ten lotus extracts was determined according to the method previously described by Wang et al. (2008) with slight modifications. In brief, individual methanol diluted lotus extract (ranged from 6.25 to 100 μg/ml) was added to 2 ml of methanol containing DPPH radical (0.1 mM) followed by vigorous shaking. After standing in darkness for 30 min, absorbance of the mixture was measured colorimetrically at 517 nm. The DPPH radical scavenging ability of individual lotus extract was calculated by using following equation:

$$\text{Scavenging ability (\%)} = (1 - A_{sample}/A_{blank}) \times 100\%$$

where A_{blank} is the absorbance of the blank reaction (containing all reagents except the sample extract), and A_{sample} is the absorbance of sample reaction. For each lotus extract, its concentration with 50% scavenging ability on the DPPH radicals (IC_{50}) was determined by interpolation of linear regression analysis. The synthetic compound BHT, which is widely used in food industry as antioxidant, was used as positive control.

ABTS radical scavenging activity

For each lotus extract, its scavenging ability on ABTS radicals was determined by ABTS decolorization assay according to the method previously described by Biglari et al. (2008) with slight modifications. In brief, the ABTS radical cation ($ABTS^+$) was firstly prepared by mixing the ABTS stock solution (7 mM) with potassium persulfate (2.45 mM) followed by incubation at room temperature for 12 to 16 h in darkness. Subsequently, the $ABTS^+$ solution was diluted with 80% ethanol to obtain an absorbance of 0.70 ± 0.02 at

Table 1. Extract yields and contents of total phenolics, flavonoids, and proanthocyanidins in lotus extracts.

Parts of lotus	Extract yield (% w/w)	Total phenolic content (mg/g extract)	Total flavonoid content (mg/g extract)	Total proanthocyanidin content (mg/g extract)
Receptacle	20.70±1.24	455.40±5.34	479.30±10.98	371.88±5.00
Leaf	24.54±1.72	257.90±3.54	245.22±1.51	73.16±0.67
Stamen	10.20±0.34	221.48±0.75	289.34±0.84	163.51±1.07
Seed epicarp	1.16±0.08	305.61±1.57	363.00±1.45	161.84±0.32
Embryo	20.84±0.68	96.33±0.78	69.13±0.74	11.23±0.16
Seed	11.87±0.86	33.59±0.30	47.37±0.73	15.93±0.03
Flower	11.64±1.04	218.13±1.66	193.54±1.94	96.28±4.34
Petiole	9.85±0.32	41.73±0.54	36.59±0.31	13.22±0.80
Rhizome knot	9.14±0.52	71.50±1.19	52.86±0.65	40.75±0.58
Rhizome	10.70±0.39	16.14±0.44	11.38±0.15	3.06±0.12

734 nm before use. For determining the ABTS radical scavenging ability, 100 µl of each lotus extract was added to 3.9 ml of diluted $ABTS^+$ solution. After 6 min incubation, absorbance of the reaction mixture was measured colorimetrically at 734 nm. Similarly, both scavenging ability on ABTS radicals (%) and the IC_{50} were calculated as described in the DPPH radical scavenging assay.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging ability of the ten lotus extracts was determined according to the method previously reported by Wang et al. (2008) with BHT being used as the positive control. Similarly, 1 ml of individual lotus extract (ranged from 0.25 to 2 mg/ml) was mixed with a solution containing 1 ml of $FeSO_4$ (1.5 mM), 0.7 ml of H_2O_2 (6 mM) as well as 0.3 ml of sodium salicylate (20 mM) followed by incubation for 60 min. Absorbance of the reaction mixture was measured colorimetrically at 562 nm, while the hydroxyl radical scavenging ability (%) of each lotus extract was calculated by the equation as described in the DPPH radical scavenging assay.

Reducing power assay

The reducing power assay was conducted as previously described by Wang et al. (2008) with ascorbic acid being used as the positive control. In brief, 2.5 ml of individual deionized water diluted lotus extract (ranged from 100 to 500 mg/ml) was sequentially mixed with equal volume of phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide. After incubation at 50°C for 20 min, 2.5 ml of 10% trichloroacetic acid was then added to the mixture followed by centrifuging at 3000 rpm for 10 min. Consequently, 5 ml of the upper layer was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (0.1%), while absorbance of the resulting solution was measured colorimetrically at 700 nm.

Total antioxidant activity

Total antioxidant activity of the lotus extracts was determined by using the method previously reported by Prieto et al. (1999) with BHT being used as the positive control. Briefly, 0.3 ml of each ethanol diluted lotus extracts (ranged 100 to 500 µg/ml) was mixed with a solution (3 ml) containing 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate followed by incubation at 95°C for 90 min. After cooling to 25°C, absorbance of the resulting solution was measured colorimetrically at 695 nm.

Metal chelating activity

The metal chelating assay was performed according to the method previously described by Chan et al. (2007) with BHT being used as the positive control. In short, 1 ml of individual lotus extract (ranged from 1 to 5 mg/ml) was separately mixed with equal volume of $FeSO_4$ (0.1 mM) and ferrozine (0.25 mM) followed by vigorous shaking. After incubation for 10 min, absorbance of the reaction mixture was measured colorimetrically at 562 nm, while the metal chelating ability (%) of each lotus extract was calculated by the equation as described in the DPPH radical scavenging assay.

Statistical analysis

All data were presented as mean values of three determinations ± S.D. The results of all mean values were analyzed by one-way ANOVA and Turkey-HSD at $p < 0.05$ to detect significant differences among groups.

RESULTS AND DISCUSSION

%Yield of lotus extract and its total phenolic, flavonoid and proanthocyanidin contents

The %yields as well as the total phenolic, flavonoid and proanthocyanidin contents in the ten acetone extracts originated from different parts of lotus were presented in Table 1. As shown in Table 1, the %yield of the ten lotus extracts was varied significantly ($p < 0.05$) in a descending order of leaf > embryo > receptacle > seed > flower > rhizome > stamen > petiole > rhizome knot > seed epicarp (ranged from 1.16 to 24.54%). For the seed epicarp, its exceptionally low %yield of acetone extract might be attributed to its high fat content or low solubility of its components.

Interestingly, the total phenolic (ranged from 16.14 to 455.40 mg/g), flavonoid (ranged from 11.38 to 479.30 mg/g) and proanthocyanidin contents (3.06 to 371.88 mg/g) in the ten lotus extracts shared a very similar pattern in which the receptacle and rhizome exhibited the highest and lowest values, respectively ($p < 0.05$).

Table 2. IC_{50} values of DPPH scavenging and ABTS scavenging activities of lotus extracts.

Parts of lotus	DPPH scavenging (μg)	ABTS scavenging (μg)
Receptacle	9.39	135.20
Leaf	21.55	285.17
Stamen	17.38	390.82
Seed epicarp	22.87	142.10
Embryo	89.68	688.50
Seed	210.58	1318.70
Flower	21.96	216.20
Petiole	144.30	1506.64
Rhizome knot	72.47	696.93
Rhizome	458.58	4917.51
BHT	12.91	60.72

Compared with the total phenolic and flavonoid contents, the total proanthocyanidin content in all lotus extracts was found to be low. Nevertheless, a very high correlation between the total phenolic and flavonoid contents was observed (r = 0.9825), indicating that flavonoids would likely be the major type of phenolic compounds present in the lotus extracts.

DPPH and ABTS radical scavenging activities

DPPH and ABTS radical scavenging assays are widely used methods for evaluating the antioxidant activity of a substance in both food and biological systems (Re et al., 1999). As shown in Table 2, all lotus extracts exhibited various scavenging activity on both DPPH and ABTS radicals with IC_{50} values ranging from 0.009 to 0.458 and 135.20 to 4917.51 μg/ml, respectively. Except the receptacle, which possessed significantly (p < 0.05) stronger DPPH radical scavenging ability than that of the BHT, all lotus extracts only showed significantly (p < 0.05) lower DPPH and ABTS radicals scavenging activities, when compared with those of the BHT controls. These findings suggested that the receptacle might act as an alternative source of natural antioxidants for the fast-growing functional food industry nowadays. Besides, a strong correlation between the DPPH radical scavenging activity and total phenolic content as well as the ABTS radical scavenging activity and total phenolic content were observed, indicating that the phenolic compounds in the ten lotus extracts would likely be responsible for their DPPH and ABTS radical scavenging activities.

Hydroxyl radical scavenging activity

Scavenging hydroxyl radicals is an important antioxidant activity for protecting living cells, since the hydroxyl radicals can easily pass through the cell membrane at specific sites, reacting with most biomolecules and causing tissue damage and cell death (Yang et al., 2008). Similar to other plant phenolic compounds (Sakanaka et al., 2005), the hydroxyl radical scavenging ability of all ten lotus extracts was significantly (p < 0.05) lower than that of the ascorbic acid control in a descending order of ascorbic acid > flower > stamen > receptacle > seed epicarp > rhizome knot > petiole > seed > leaf > rhizome > embryo (p < 0.05) at the concentration of 0.4 to 1 mg/ml (Figure 1). In contrast to the DPPH and ABTS radical scavenging activities, no obvious correlation between the lotus extracts' hydroxyl radical scavenging ability and total phenol content was observed (Figure 1).

Reducing power

Reducing power has been used as an indicator for evaluating a substance's antioxidant activity, since their close relationship has been previously reported in numerous literatures (Gursoy et al., 2009). As shown in Figure 2, receptacle, flower, seed epicarp and stamen exhibited obvious reducing power. For each lotus extracts, the peaks were all observed at the concentration of 500 μg/ml. The highest value (2.17) was observed by seedpod at the concentration of 500 μg/ml, which was higher than BHT. At concentration of 500 μg/ml, the reducing power of all ten lotus extracts is in a descending order of receptacle > flower > seed epicarp > stamen > rhizome knot > petiole > rhizome > leaf > seed > embryo (p < 0.05).

Total antioxidant activity

In this study, the total antioxidant capacity of all ten lotus extracts were determined by evaluating their ability to reduce Mo (VI) to Mo (V) followed by formation of a green phosphate/Mo (V) complex at acidic pH. As shown in Figure 3, most lotus extracts exhibited obvious total

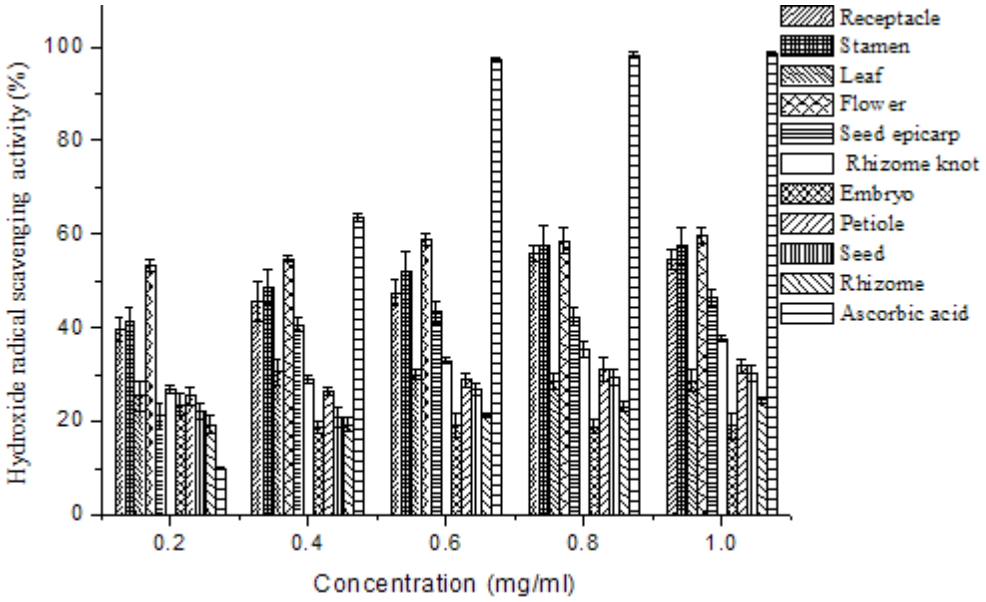

Figure 1. Hydroxide radical scavenging activities of lotus extracts.

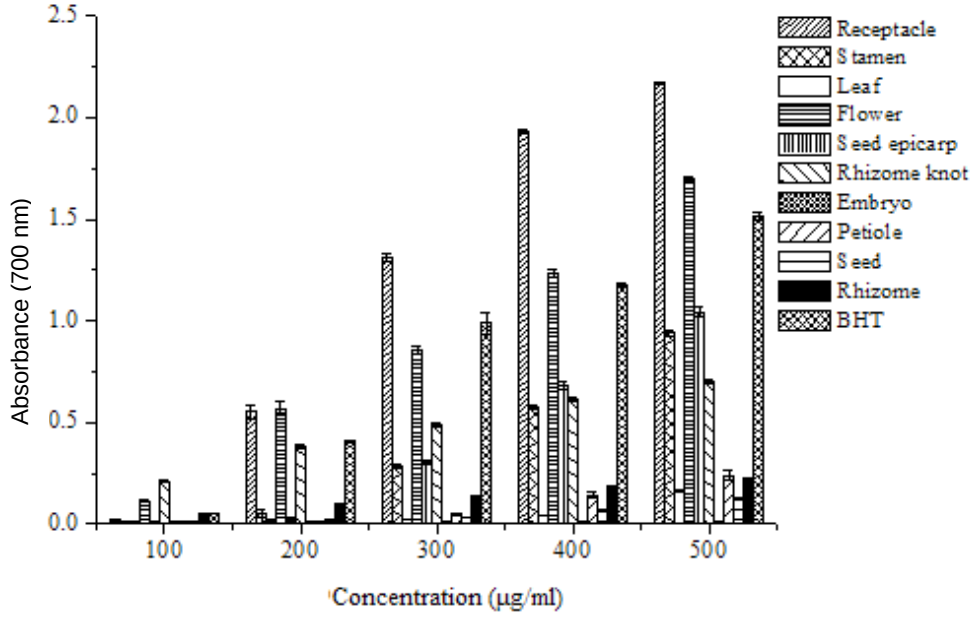

Figure 2. Reducing power of lotus extracts.

antioxidant capacity except rhizome knot, embryo and rhizome. The highest value of receptacle and stamen were observed at the concentration of 500 μg/ml, which was higher than BHT. In addition, the total antioxidant capacity of rhizome knot, embryo and rhizome improved steadily when its corresponding concentrations were gradually increased. At concentration of 500 μg/ml, the total antioxidant activity of all ten lotus extracts is in a descending order of receptacle > stamen > BHT > leaf > flower > seed > seed epicarp > petiole > rhizome knot > embryo > rhizome ($p<0.05$).

Metal chelating activity

Metal chelating is one of the important antioxidant mechanisms which retard metal-catalyzed oxidation. In food systems, ferrous ions are the most effective pro-

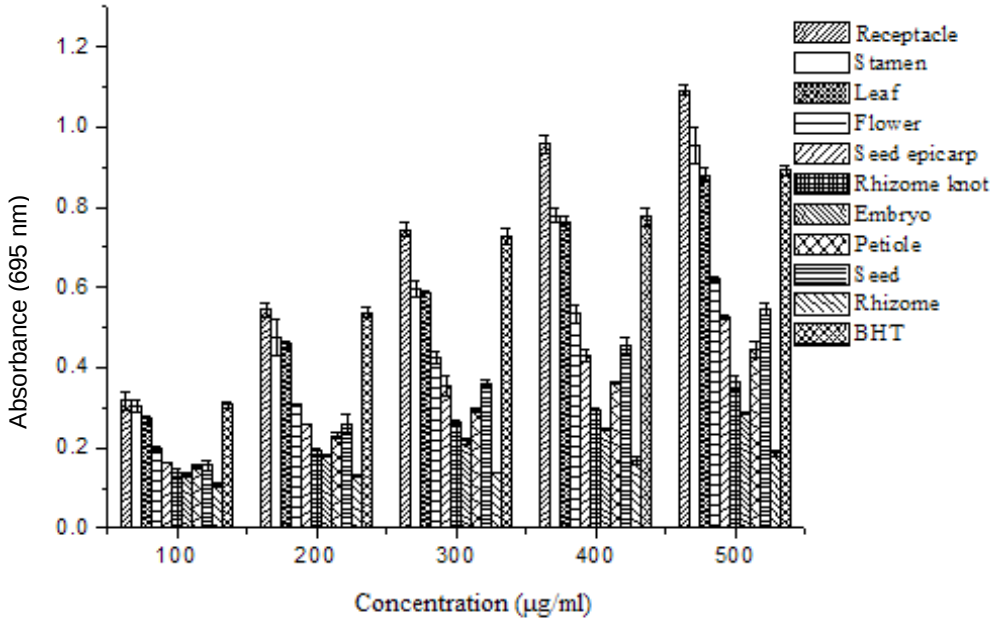

Figure 3. Total antioxidant activities of of lotus extracts.

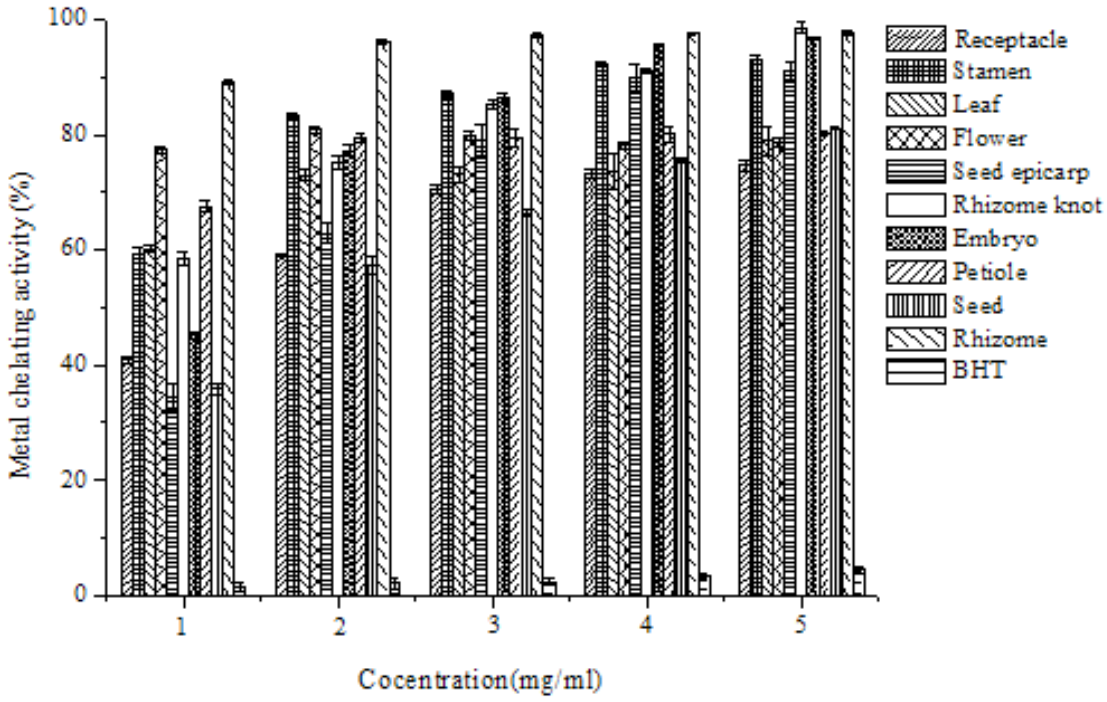

Figure 4. Metal chelating activities of lotus extract.

oxidants (Zha et al., 2009). As shown in Figure 4, all ten lotus extracts showed a significant metal chelating activity. At concentration of 5 mg/ml, the metal chelating activity of all ten lotus extracts is in a descending order of rhizome knot > rhizome > embryo > flower > seed epicarp > seed > petiole > leaf > stamen > receptacle. Compared with the BHT control, all ten lotus extracts even exhibited significantly ($p < 0.05$) higher metal chelating activity. It is interesting to note that for those lotus extracts having remarkable metal chelating ability

(such as rhizome knot, rhizome and embryo extracts), their total phenolic, flavonoids and proanthocyanidins contents were relatively low (Table 1), while their DPPH, ABTS and hydroxyl radical scavenging activities, reducing power as well as total antioxidant activity were also insignificant (Table 2 and Figures 1 to 3). These findings might suggest that instead of those phenolic compounds which are responsible for scavenging the free radicals and reducing power of the lotus extracts, strong metal chelators especially for the ferrous ions would likely be present in the ten lotus extracts.

Conclusions

In the present study, *in vitro* antioxidant activity of ten different parts of the lotus were evaluated and compared. Among the ten lotus extracts, the receptacle did not only possessed the highest phenolic, flavonoid and proanthocyanidin contents, but its DPPH and ABTS radical scavenging activities, reducing power as well as total antioxidant activity were also comparable to, if not better than those of the BHT control, which is a synthetic antioxidant widely used in the food industry. In China, the inedible lotus receptacle is usually discarded as waste or used as firewood. Our findings would not only provide an added value to this regional bio-resource, but could also facilitate its development as a novel and inexpensive source of natural antioxidants especially for the fast growing functional food industry nowadays. Further investigations such as isolation and characterization of the active compounds, as well as mechanistic study of the antioxidant activity exerted by the seedpod at both molecular and cellular levels are underway.

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