

Full Length Research Paper

Antioxidant activity of lotus (*Nelumbo nucifera* Gaertn.) receptacles of eleven cultivars grown in China

Li-Jun Zheng^{1,2#}, Yan-Bin Wu^{1#}, Jian-Guo Wu¹, Chun-Jiang Tan¹, Jun Yi³, Ti-Qiang Chen⁴ and Jin-Zhong Wu^{1*}

¹Academy of Integrative Medicine, Fujian University of Traditional Chinese Medicine, Fuzhou, Fujian 350108, P. R. China.

²Department of Pharmacy, Fujian University of Traditional Chinese Medicine, Fuzhou, Fujian 350108, P. R. China.

³Department of Chemistry and Life Science, Fujian Institute of Education, Fuzhou, Fujian 350001, China.

⁴Institute of Edible and Medicinal Fungi, Fujian Academy of Agricultural Sciences, Fuzhou 350013, China.

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Receptacles of 11 lotus (*Nelumbo nucifera* Gaertn) cultivars [ShiLiHe 1 (L-1), Taikong 1 (L-2), GanLisn 62 (L-3), DaHongLian (L-4), Taikong 3 (L-5), JianXuan 17 (L-6), CunSanLian (L-7), JianLian (L-8), Taikong 36 (L-9), Zajaolian 8236 (L-10) and Jianjibaihualian (L-11)] were analyzed for the contents of total phenolic, flavonoid and proanthocyanidin, and tested for antioxidant activity by some different assays. Extracts from L-8, L-9, L-10 and L-11 showed higher total phenolic, flavonoid, proanthocyanidin contents and antioxidant activities, which were measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH), diammonium salt (ABTS), superoxide anion radical scavenging activities and total antioxidant activity assays, than the other cultivars. Moreover, the total phenolic, flavonoid and proanthocyanidin contents correlates well with the antioxidant activities as measured by the DPPH, ABTS, superoxide anion scavenging activity, reducing power, and total antioxidant assays, but does not correlate with metal chelating activity and β -carotene bleaching activity.

Key words: Lotus (*Nelumbo nucifera*), phenolic, flavonoid, proanthocyanidin, antioxidant activity.

INTRODUCTION

Lotus (*Nelumbo nucifera* Gaertn.), one of aquatic vegetable, has been cultivated for more than 2,000 years, and now is widely cultivated in almost all provinces in China 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 vegetables 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 the by-product of lotus food production, lotus receptacle is usually discarded as waste or used as firewood, except when sometimes used as a traditional medicine. As reported in numerous previous studies, lotus receptacle contains moderate but significant quantities of

phenolics. These key secondary metabolites exhibited a wide spectrum of biopharmacological effects, including antioxidation (Ling et al., 2005), improving learning and memory abilities (Gong et al., 1992), protective effects against experimental myocardial injury and ischemia (Zhang et al., 2004), radio protective activity (Duan et al., 2004), and anti-tumor effect (Duan et al., 2010). However, all research about the lotus receptacle only focused on the LSPC, a procyanidin-rich fraction isolated from the lotus receptacle. In fact, scientific information on antioxidant properties of the ethanol extract of lotus receptacle is still rather scarce. Even though it has already been demonstrated that the LSPC have antioxidant potential, no scientific information is available that the other compositions of lotus receptacle does not necessarily correlate with its antioxidant activity. Moreover, little information was available on a comparison of antioxidant activity of lotus receptacle from various lotus cultivars. It is known that the amount of each antioxidant in fruits and vegetables is strongly influenced by genotype differences and external factors, such as agro-technical

*Corresponding author. E-mail: jinzhongfj@126.com.

#These authors contributed equally to this work.

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

Sample	Palace of collection (province)	Extract yield (% w/w)	Total phenolic content (mg GAE/g extract)	Total flavonoid content (mg RE/g extract)	Total proanthocyanidin content (mg CAE/g extract)
L-1	Zhejiang	5.85	255.65 ± 1.18 ^a	353.31 ± 2.22 ^a	112.41 ± 0.780 ^a
L-2	Zhejiang	10.10	297.22 ± 3.78 ^b	369.78 ± 1.68 ^b	137.41 ± 1.08 ^b
L-3	Zhejiang	9.31	297.22 ± 2.96 ^b	377.54 ± 1.45 ^c	170.05 ± 0.00 ^d
L-4	Zhejiang	12.23	320.75 ± 3.59 ^c	405.64 ± 1.68 ^d	158.59 ± 0.00 ^c
L-5	Zhejiang	12.00	359.18 ± 2.04 ^d	441.02 ± 2.22 ^e	200.96 ± 1.67 ^f
L-6	Zhejiang	16.13	399.96 ± 2.45 ^e	468.64 ± 4.67 ^f	270.34 ± 0.30 ^g
L-7	Hunan	16.41	430.55 ± 3.60 ^f	479.78 ± 3.03 ^g	175.09 ± 1.97 ^e
L-8	Fujian	32.50	435.26 ± 0.680 ^g	501.11 ± 1.45 ^h	296.79 ± 0.80 ⁱ
L-9	Fujian	24.26	472.51 ± 1.36 ^h	537.45 ± 2.52 ⁱ	280.47 ± 1.56 ^h
L-10	Fujian	32.54	473.69 ± 1.36 ^{h,i}	540.84 ± 0.84 ^j	358.42 ± 2.17 ^k
L-11	Fujian	33.40	477.61 ± 0.68 ⁱ	509.34 ± 3.66 ⁱ	346.09 ± 2.08 ^j

Each value is expressed as the mean ± SD of three replicate determinations. Values with different superscript letters within the same column are statistically different ($p < 0.05$).

processes and environmental conditions. Hence, the evaluation of antioxidant properties of lotus receptacle remains an interesting and valuable task, particularly for finding new sources for natural antioxidants, functional foods and nutraceuticals.

Accordingly, in this work, the antioxidant properties of lotus receptacle from 11 lotus cultivars grown in China were evaluated through several chemical and biochemical assays: 2,2'-diphenyl-1-picrylhydrazyl (DPPH), diammonium salt (ABTS), superoxide anion radical scavenging activities, reducing power, total antioxidant activity, metal chelating activity and β -carotene bleaching activity.

MATERIALS AND METHODS

Chemicals and reagents

Except butylated hydroxyl toluene (BHT) and aluminium trichloride, which were obtained from Sinopharm Chemical Reagent Co. (Shanghai, China), all other chemicals and reagents used in antioxidant activity assay 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). And the rest chemicals and solvents are of analytical grade.

Samples of lotus seedpod

Lotus receptacles of the cultivars, ShiLiHe 1 (L-1), Taikong 1 (L-2), GanLian 62 (L-3), DaHongLian (L-4), Taikong 3 (L-5), JianXuan 17 (L-6), CunSanLian (L-7), JianLian (L-8), Taikong 36 (L-9), Zaijiaolian 8236 (L-10) and Jianjibaihualian (L-11) were collected in different locations of China in August 2010 (Table 1). All samples were kept in darkness at -20°C prior to usage.

Preparation of lotus extracts

The open-air dried lotus receptacles were powered and then

extracted for 60 min under ultrasonic conditions with 50% ethanol at the ratio of 1:30 (w/v). These extracts were filtered through Whatman No. 1 filter paper and the entire extraction process was repeated again on the residue obtained from the filtration process. The filtrates were individually evaporated under vacuum for obtaining dried products which were kept at -20°C before further studies.

Photometric determination of total phenols, flavonoids and proanthocyanidin

The total phenolics content in lotus receptacles were determined using Folin-Ciocalteu according to the procedures previously reported by Ismail et al. (2009) with some modifications. Gallic acid (GA) was used as standard and the results were calculated as gallic acid equivalents (mg/g) of lotus receptacle extract through the calibration curve of gallic acid. The linearity range of the calibration curve was 1 to 9 $\mu\text{g/ml}$ ($r = 0.9998$). The total flavonoid content was determined using a colorimetric method reported by Yang et al. (2009) with some modifications. The results were calculated and expressed as rutin equivalents (mg) per gram of lotus receptacle extract using the calibration curve of rutin. The linearity range of the calibration curve was 7 to 44 $\mu\text{g/ml}$ ($r = 0.9999$). The total proanthocyanidin content was determined according to the modified method previously described by Duan et al. (2009), using (+)-catechin as a standard and the results were expressed as (+)-catechin equivalents in microgram per gram of lotus receptacle extract. The linearity range of the calibration curve was 20 to 280 $\mu\text{g/ml}$ ($r = 0.9998$).

Evaluation of antioxidant activity

DPPH radical-scavenging activity

The DPPH radical scavenging activities of the eleven lotus receptacle extracts were determined according to the method previously described by Wang et al. (2008) with slight modifications. In brief, 2 ml of each extract at various concentrations (2.5 to 40 $\mu\text{g/ml}$) were respectively mixed with 2 ml of DPPH methanolic solution (0.2 mM). Then, the mixtures were shaken vigorously and left to stand in the dark for 30 min. Consequently, the absorbance of

these mixtures was measured using a spectrophotometer at 517 nm. The DPPH radical scavenging activity of each lotus extract was calculated by the following formula:

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

where A_{blank} is the absorbance of the blank reaction (using 2ml of methanol instead of the tested sample) and A_{sample} is the absorbance of the tested sample.

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 for comparison.

ABTS radical-scavenging activity

The effect on scavenging ABTS radicals in the extracts of lotus receptacle were determined according to ABTS decolorization assay according to the method previously described by Biglari et al. (2008) with slight modifications. Firstly, the ABTS radical was generated by mixing 7 mM ABTS solution and 2.45 mM potassium persulphate solution and left to stand in the dark at room temperature for 12 to 16 h. Then, this ABTS radical solution was diluted with distilled water to get an absorbance of 0.7 (± 0.02) at 734 nm before usage. For determining the ABTS radical scavenging capacity, 3.9 ml of the ABTS radical solution was added to 100 μ l of the tested samples (50 to 400 μ g/ml) and mixed vigorously. The mixture was allowed to react for 6 min and the absorbance at 734 nm was immediately measured. BHT was used as positive control. The ability of individual lotus receptacle extract to scavenge ABTS radicals (%), and the IC_{50} were calculated as described in the DPPH radical scavenging assay.

Superoxide anion scavenging activity

Superoxide anion scavenging activities of the lotus receptacle extracts were based on the method previously described by Deliang et al. (2009) with slight modifications. Briefly, 1 mL of NBT solution (156 μ M in 100 mM phosphate buffer, pH 7.4), 1 ml of NADH solution (468 μ M in 100 mM phosphate buffer, pH 7.4) and 1 ml of lotus extract solution (25 to 200 μ g/ml) were mixed. The reaction was started by adding 1 ml of PMS solution (60 μ M in 100 mM phosphate buffer, pH 7.4) to the mixture. Then the reaction mixture was incubated at room temperature for 5 min, and the absorbance at 560 nm was measured against a blank (using 1 ml of water and 1 ml of 100 mM phosphate buffer, taking place of the tested sample and NBT solution, respectively). The inhibition percentage of superoxide anion was calculated using the following formula:

$$\text{Inhibition rate (\%)} = (1 - (A_1 - A_2)/A_0) \times 100\%$$

where A_0 is the absorbance of the control (1 ml of water instead of the tested sample), A_1 is the absorbance of the sample and A_2 was the absorbance of the mixture with 100 mM phosphate buffer instead of NBT solution. Rutin was used as positive control.

Reducing power assay

The determination of reducing power was performed according to a literature procedure previously described by Wang et al. (2008) with slight modifications. In brief, 2.5 ml of various concentrations (12.5 to 100 μ g/ml) of each extract were respectively mixed with 2.5 ml of 200 mM PBS (pH 6.6) and 2.5 ml of 1% $K_3Fe(CN)_6$. After the mixture was incubated at 50°C for 20 min, 2.5 ml of 10% trichloroacetic acid

(w/v) was added and the mixture was centrifuged at 3000 rpm for 10 min. Consequently, the upper layer of solution (5 ml) was mixed with 5 ml of deionised water and 1 ml of 0.1% $FeCl_3$, and then the mixture was left to stand for 10 min. Finally, the absorbance was measured at 700 nm against a blank. The reducing power of BHT was determined for comparison.

Total antioxidant activity

Total antioxidant activities of the lotus receptacle extracts were analyzed using the method previously reported by Prieto et al. (1999). In brief, 0.3 ml of various concentrations (31.25 to 500 μ g/ml) of the extracts in 70% ethanol were combined with 3 ml of reagent solution (containing 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Then, the tubes were incubated at 95°C for 90 min. After the samples were cooled to room temperature, the absorbance was measured at 695 nm against a blank. The reaction mixture without sample was used as the blank. BHT was also used for comparison.

Metal chelating activity

Metal chelating capacities of the lotus receptacle extracts were determined by the method previously described by Nisha et al. (2009) with some modifications. Briefly, 2.4 ml of various concentrations (100 to 600 μ g/ml) of each extract dissolved in 60% ethanol were mixed with a solution of 1 mM ferrous chloride (200 μ l). The reaction was initiated by the addition of 1 mM ferrozine (400 μ l). Then, the mixture was shaken vigorously and allowed to stand at room temperature for 10 min. The absorbance was measured at 562 nm. Metal chelating effect (%) was calculated by the following equation:

$$\text{Metal chelating effect (\%)} = (1 - (A_s - A_c)/A_b) \times 100\%$$

where A_s is the absorbance of the tested sample, A_c is the absorbance of the mixture with 200 μ l of distilled water instead of the solution of 1 mM ferrous chloride, and A_b is the absorbance of the blank reaction (containing all reagents except the tested sample), respectively. In addition to BHT, EDTA was also used as a comparison.

β -Carotene bleaching inhibition activity

The β -carotene bleaching assay previously reported by Liu et al. (2007) and Kulisic et al. (2004) were adapted with some modifications. β -carotene/linoleic acid emulsion was prepared as follows: 1 ml of β -carotene [0.5 mg/ml β -carotene chloroform solution (HPLC grade)] was added to 25 μ l of linoleic acid and 200 mg of Tween-40. Next, chloroform was completely evaporated under vacuum and 100 ml of oxygenated distilled water was added with vigorous shaking. Then 4 ml of this emulsion was added to 200 μ l of different concentrations (10 to 800 μ g/ml) of each lotus extract. Consequently, the reaction system was incubated for 2h at 50°C. Both BHT and ascorbic acid were used as positive controls, and a blank with 200 μ l of 70% ethanol taking place the tested sample was used as a negative control. The absorbance of the mixtures was measured at 470 nm. The inhibition rate (%) of β -carotene bleaching was calculated with the following equation:

$$\text{Inhibition rate (\%)} = 100 \times (A_{A(120)} - A_{C(120)}) / (A_{C(0)} - A_{C(120)})$$

where $A_{A(120)}$ was the absorbance of the tested sample after 120 min, $A_{C(120)}$ and $A_{C(0)}$ were the absorbances of the negative control before and 120 min after incubation, respectively.

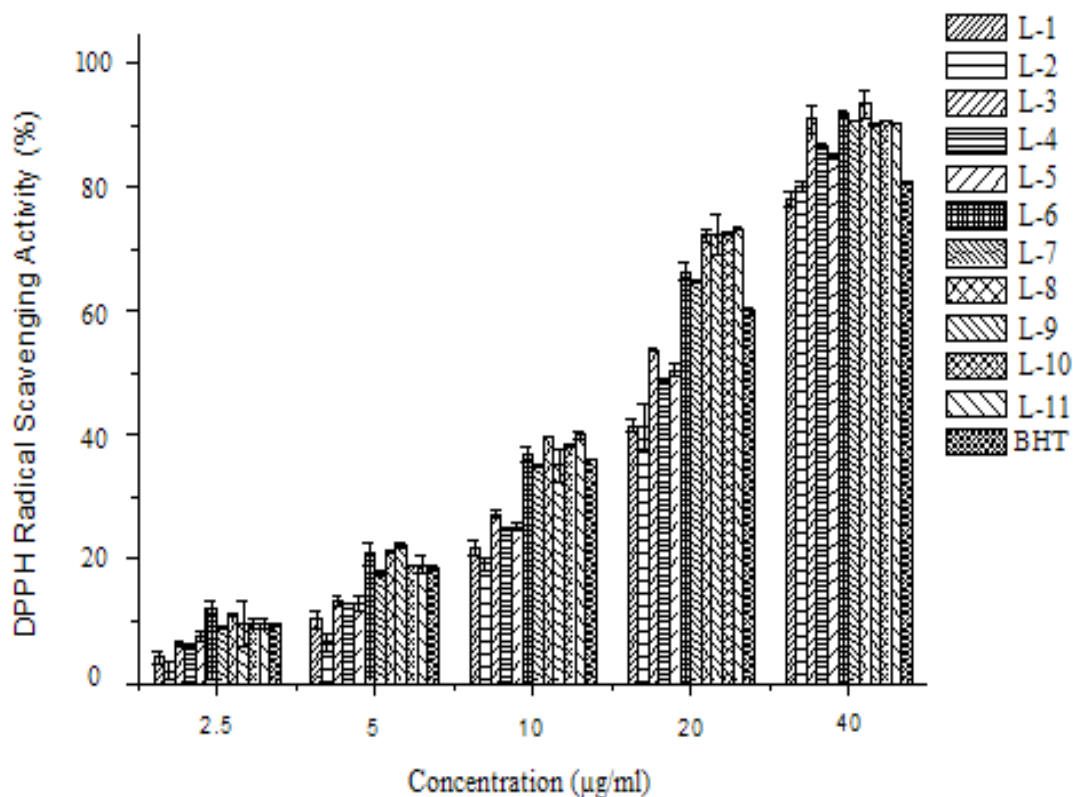


Figure 1. DPPH radical scavenging activity of lotus receptacle extracts.

Statistical analysis

All the experiments were conducted in triplicates, and the data were presented as mean \pm S.D. Microsoft Excel, SPSS 16.0 and Origin 8.0 were used to process the results, which were analyzed by one-way ANOVA and Tukey-HSD at $p < 0.05$ to detect significant differences among groups.

RESULTS AND DISCUSSION

Extraction yield and contents of total phenolics, flavonoids and proanthocyanidins

Table 1 exhibits the extraction yield, contents of total phenolics, flavonoids and proanthocyanidins of the eleven lotus receptacle extracts. The yield of the lotus receptacle extracts varied from 5.9 to 33.4%. Among all tested extracts, the highest and the lowest yields were respectively obtained from the L-11 and L-1 ($p < 0.05$).

The amounts of total phenolics, flavonoids, and proanthocyanidins in lotus receptacle extracts from 11 different lotus cultivars varied widely, ranging from 255.65 to 477.61 mg/g of extract, from 353.31 to 540.84 mg/g of extract, and from 112.41 to 358.42 mg/g of extract, respectively. It can be noted that the total phenolics contents of L-8, L-9, L-10 and L-11 from Fujian cultivars were similar and higher than the other cultivars. Similar

distribution was found in the total flavanoid and total proanthocyanidin contents in receptacles among the lotus cultivars studied. This suggests that variation in the levels of phenolics between extracts could be partly due to differences in growing conditions and cultivars.

Moreover, the contents of total phenolics and flavonoids of lotus receptacle are much higher than the proanthocyanidins content. This means that the antioxidant activity of lotus receptacle could not be solely explained on the basis of the LSPC, which also needs other phenolic compounds or their synergetic effects.

Radical scavenging activities

The radical scavenging activities of lotus receptacle extracts were evaluated using the methods of DPPH, ABTS and superoxide anion scavenging activity assays, which have been widely used to test radical scavenging activity. Results of this evaluation at various concentrations of extracts and the reference compounds BHT and rutin are shown in Figures 1, 2 and 3, and showed a concentration-dependent activity. Meanwhile, the concentrations required to inhibit each radical by 50% (IC_{50}) are shown in Table 2. It can be noted that most of lotus receptacle extracts showed appreciable free radical scavenging activities in the DPPH assay. Moreover, L-6,

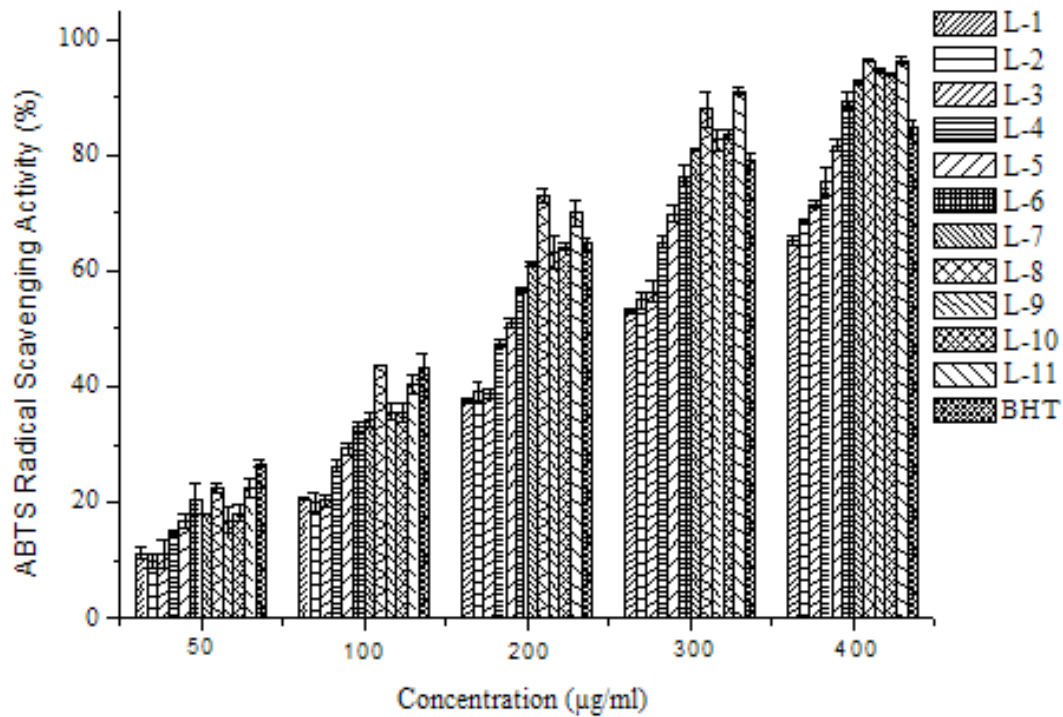


Figure 2. ABTS radical scavenging activity of lotus receptacle extracts.

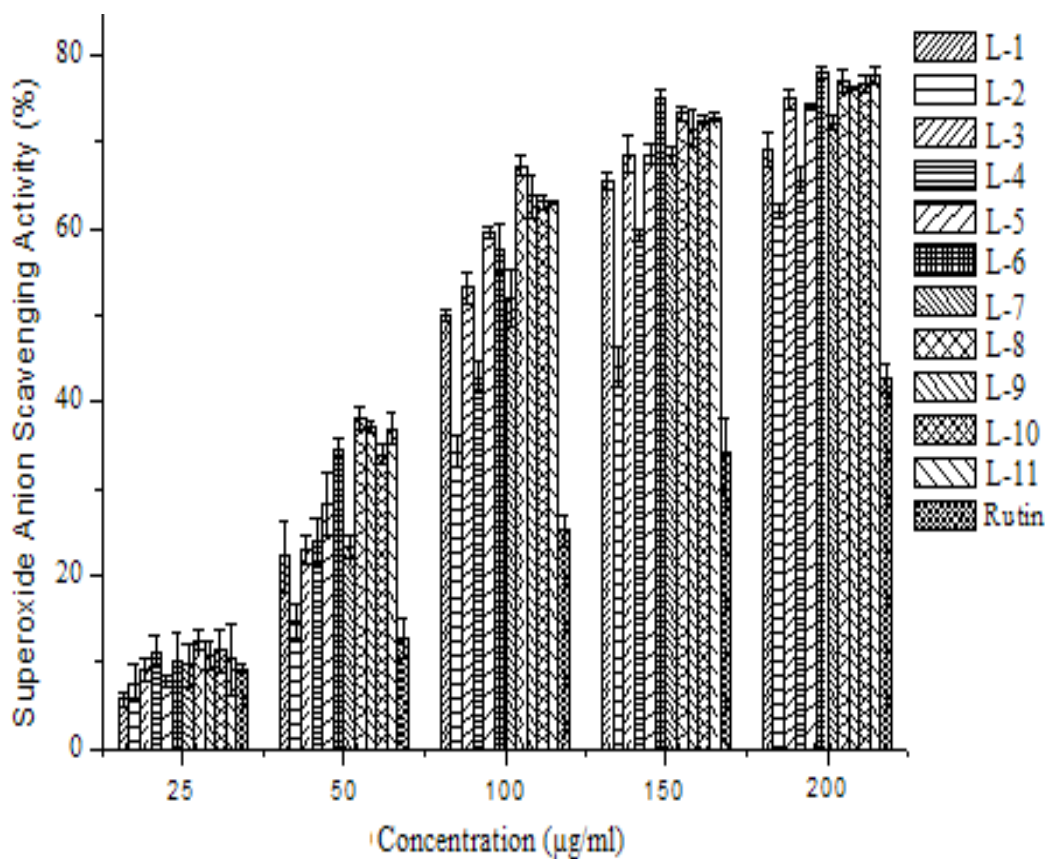


Figure 3. Superoxide anion scavenging activity of lotus receptacle extracts.

Table 2. IC₅₀ values(μg/ml) obtained in the antioxidant assays of DPPH scavenging, ABTS scavenging, superoxide scavenging, metal chelating activities and β-carotene bleaching inhibition activities of the receptacle extracts of the lotus cultivars.

Sample	DPPH radical scavenging activity	ABTS radical scavenging activity	Metal chelating activity	Superoxide radical scavenging activity	β-carotene bleaching inhibition activity
L-1	20.90 ± 0.84 ^e	268.98 ± 3.92 ^j	243.58 ± 2.76 ^e	110.65 ± 2.51 ^d	86.19 ± 3.91 ^e
L-2	21.06 ± 0.59 ^e	252.63 ± 3.36 ⁱ	166.16 ± 2.05 ^c	159.71 ± 3.76 ^f	97.94 ± 2.56 ^f
L-3	14.31 ± 0.67 ^c	241.30 ± 3.76 ^h	251.25 ± 1.47 ^f	98.43 ± 2.49 ^{b,c}	70.65 ± 1.25 ^c
L-4	16.47 ± 0.14 ^d	193.54 ± 0.93 ^g	155.59 ± 1.05 ^b	119.51 ± 3.83 ^e	86.30 ± 2.34 ^e
L-5	16.51 ± 0.27 ^d	166.57 ± 3.31 ^f	324.26 ± 3.79 ^g	94.37 ± 3.43 ^b	55.00 ± 2.07 ^b
L-6	11.42 ± 0.40 ^a	135.93 ± 3.20 ^e	244.18 ± 2.72 ^e	83.64 ± 3.14 ^a	58.10 ± 2.50 ^b
L-7	12.16 ± 0.06 ^b	129.19 ± 0.65 ^d	178.77 ± 2.85 ^d	100.73 ± 1.08 ^c	54.56 ± 3.54 ^b
L-8	10.25 ± 0.49 ^a	103.58 ± 2.91 ^a	327.59 ± 4.96 ^g	76.87 ± 1.05 ^a	76.32 ± 2.64 ^d
L-9	11.30 ± 0.59 ^a	124.79 ± 3.28 ^{c,d}	158.47 ± 3.43 ^b	82.32 ± 3.14 ^a	106.28 ± 3.79 ^g
L-10	11.23 ± 0.11 ^a	123.18 ± 2.64 ^c	364.57 ± 4.78 ^h	82.58 ± 2.85 ^a	82.92 ± 4.43 ^e
L-11	11.13 ± 0.06 ^a	104.95 ± 2.12 ^a	240.20 ± 2.59 ^e	79.88 ± 2.69 ^a	69.81 ± 3.36 ^c
BHT	14.48 ± 0.10 ^c	114.77 ± 2.76 ^b	—	—	7.03 ± 0.11 ^a
EDTA	—	—	15.39 ± 0.25 ^a	—	—
Rutin	—	—	—	306.57 ± 3.48 ^g	—

Each value is expressed as the mean ± SD of three replicate determinations. Values with different superscript letters within the same column are statistically different ($p < 0.05$).

L-8, L-9, L-10 and L-11 had the strongest activities, which is higher than that of BHT and other lotus cultivars, whereas the activities of L-1, L-2, L-3, L-4, L-5 and L-7 samples were lower than BHT. Positive correlation was found between the DPPH free radical scavenging activity (IC₅₀) and total phenolic content ($r = 0.9529$).

Regarding ABTS method, the result showed that the cultivars L-8 (IC₅₀ = 103.58 μg/ml) and L-11 (IC₅₀ = 104.95 μg/ml) had the strongest activities, higher than that of BHT (IC₅₀ = 114.77 μg/ml). The activities of extracts from L-5, L-6, L-7, L-9, and L-10 were also comparable with that of BHT, whereas the L-1, L-2, L-3, and L-4 samples exhibited lower activity. Positive correlation was found between the ABTS free radical scavenging activity (IC₅₀) and total phenolic content ($r = 0.8729$). Similar tendency was found in the superoxide anion scavenging activity assay. The highest activities were obtained from the cultivars L-8 (IC₅₀ = 76.87 μg/ml) and L-11 (IC₅₀ = 79.88 μg/ml), higher than that of other cultivars. Meanwhile, all of lotus receptacle extracts showed higher activities than that of rutin (IC₅₀ = 306.57 μg/ml) control. Antioxidants are usually employed to prevent chronic and degenerative diseases by scavenging free radical intermediates or depressing the generation of free radicals (Rodriguez et al., 2007; Prior and Gu, 2005). It has been demonstrated that the long-term treatment with lotus receptacle can prevent the age-related increases in oxidation products, and age-related deficits in cognitive functions may be due to its free radical-scavenging and antioxidant activity, resulting from the presence of phenolic compounds in the extracts (Gong et al., 2008). In addition, the antioxidant properties of lotus receptacle were believed to contribute

to restore acetylcholine contents and acetylcholinesterase activities in hippocampus and cerebral cortex of aged impaired animals (Xu et al., 2009).

Reducing power

In the reducing power assay, Fe³⁺/ferricyanide complex is reduced to the ferrous form by antioxidants in the tested samples and this generated Fe²⁺ with navy blue color can be monitored by measuring the reaction mixture at 700 nm (Gupta and Prakash, 2009). The reducing power of different lotus cultivars was depicted in Figure 4. In this assay, the concentration-dependent profile of reducing power was obvious for all the tested samples. For each lotus cultivar extracts, the peaks were all observed at the concentration of 100 μg/ml. At this concentration, L-10 and L-11 showed highest activities, higher than that of BHT and other cultivars, but the L-1, L-2 and L-3 samples exhibited lower activity.

Total antioxidant activity

In this study, the total antioxidant activity was evaluated by the phosphomolybdenum method through which the Mo (VI) was reduced to Mo (V) by the antioxidant compounds and the formation of a green Mo (V) complex at a low pH with a maximal absorbance at 695 nm was measured (Pan et al., 2008). As shown in Figure 5, lotus cultivar extracts also showed a concentration-dependent activity, ranked in a similar order to ABTS radical scavenging activity.

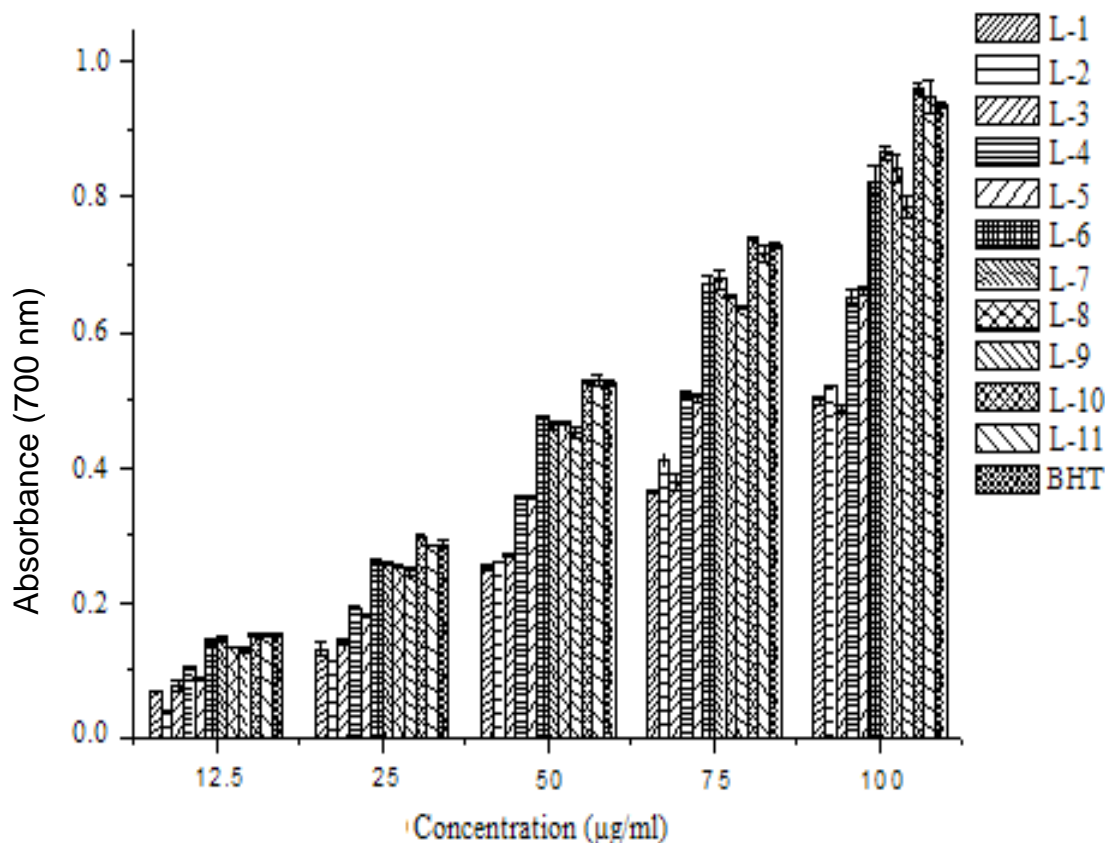


Figure 4. Reducing power of lotus receptacle extracts.

Metal chelating activity

Metal chelating is one of the important antioxidant mechanisms which retard metal-catalyzed oxidation. Ferrous ions are the most effective pro-oxidants (Zha et al., 2009), since iron can stimulate lipid peroxidation by the Fenton reaction, and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxy and alkoxy radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation (Halliwell, 1991).

As shown in Table 2 and Figure 6, all of the extracts showed high, concentration-dependent metal chelating activity, higher than that of BHT but lower than that of EDTA. The metal chelating activity of L-2, L-4, L-7 and L-9 was stronger than other cultivars, but there were no obvious differences between L-4 and L-9 samples ($P > 0.05$).

β -carotene bleaching activity

The β -carotene-linoleic acid method was used to determine the antioxidant activity of samples in the lipid system. In this assay, the free radicals generated by

linoleic acid oxidation in an emulsion could attack the highly unsaturated β -carotene, leading to the degradation of its orange coloured chromophore, and the result could be monitored spectrophotometrically. While the presence of antioxidants can hinder the extent of β -carotene-bleaching by neutralizing the linoleate free radical and other free radicals formed in the system. As shown in Table 2 and Figure 7, all of the extracts showed high, concentration-dependent β -carotene-bleaching activity, higher than that of ascorbic acid but lower than that of BHT. Notably, L-5 ($IC_{50} = 50.00 \mu\text{g/ml}$), L-6 ($IC_{50} = 58.10 \mu\text{g/ml}$) and L-7 ($IC_{50} = 54.56 \mu\text{g/ml}$) had the strongest activities; higher than other cultivars, but there were no obvious differences between L-5, L-6 and L-7 samples ($P > 0.05$). The result was similar to the previous studies that there was no correlation between phenolic contents and β -carotene bleaching inhibition activity (Amarowicz et al., 1993; Othman et al., 2007; Sun and Ho, 2005). This is probably due to the polarity of the bioactive compounds, just as the well known antioxidant ascorbic acid with hydrophilic property showed little antioxidant activity. This interesting phenomenon formulated as the "polar paradox" has been reported earlier (Koleva et al., 2002). The polar compounds with antioxidant properties are difficult to dissolve in the lipid phase of the emulsion and

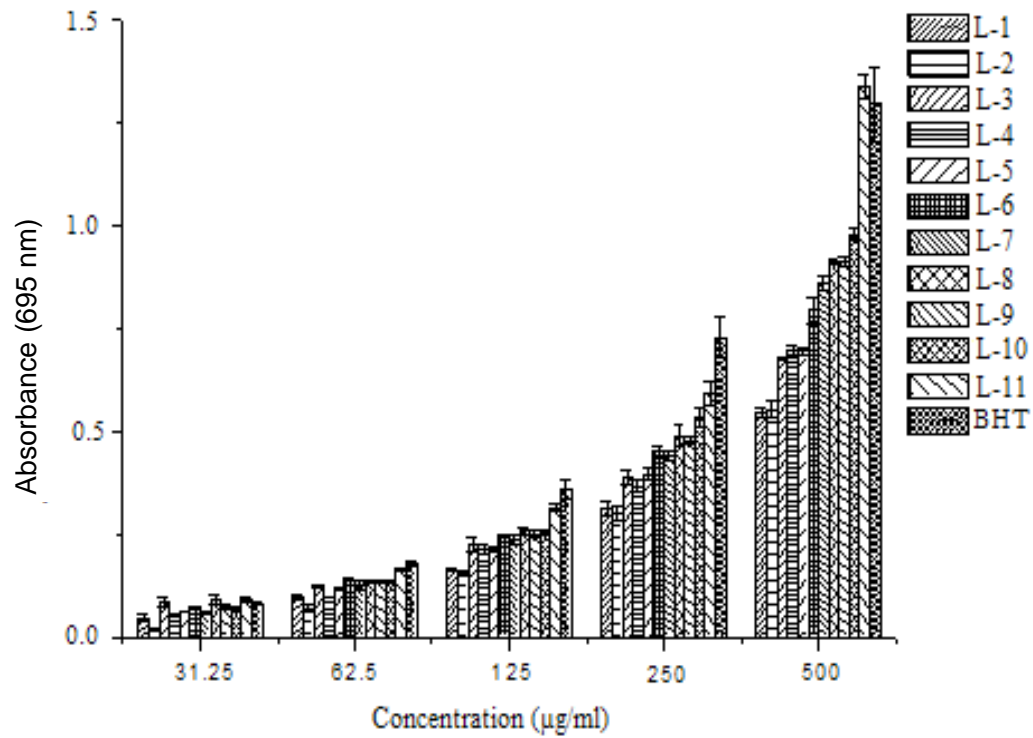


Figure 5. Total antioxidant activity of lotus receptacle extracts.

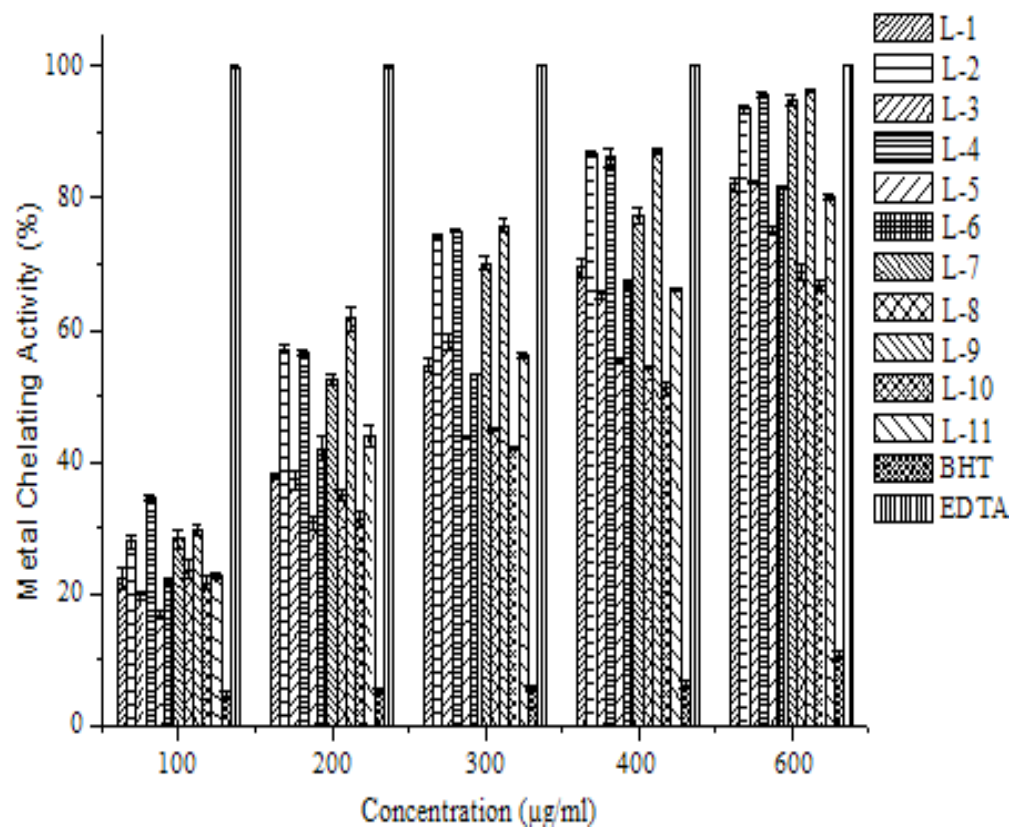


Figure 6. Metal chelating activity of lotus receptacle extracts.

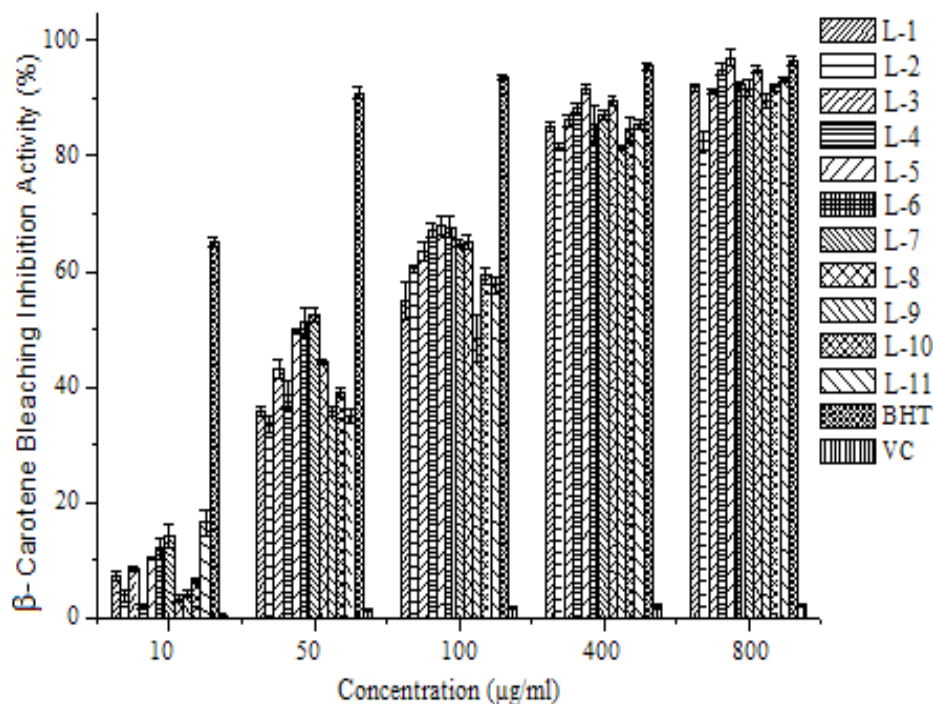


Figure 7. β -carotene bleaching inhibition activity of lotus receptacle extracts.

thus make little contribution to preventing the highly unsaturated β -carotene from being attacked in the lipid phase.

Conclusion

According to the result of this study, there was a considerable variation in the total phenolic, flavonoid, proanthocyanidin contents and antioxidant activity of eleven lotus cultivars receptacle, which indicated that genotype and external factors such as climate and agricultural conditions might be the main influences on the active constituents and bioactivity. The obvious antioxidant activity in lotus receptacle confirms its important role in the bioactivity of whole lotus, and this suggests that lotus receptacle removal may induce a more significant nutrient loss.

It is concluded that our observations may enhance the potential application of the lotus receptacle 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 are underway.

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