Food Sci. Biotechnol. Vol. 18, No. 4, pp. 1051 ~ 1054 (2009)

RESEARCH NOTE

Food Science and Biotechnology

Antioxidant Activity of the Various Extracts from Different Parts of Lotus (*Nelumbo nucifera* Gaertner)

Hae-Yeon Choi, Kyunghee Jung, and Han-Seung Shin*

Department of Food Science and Technology and Institute of Lotus Functional Food Ingredients, Dongguk University, Seoul 100-715, Korea

Abstract This study was conducted to investigate the antioxidant activity of the extracts of lotus (*Nelumbo nucifera* Gaertner). The total phenolic contents in leaf, stem, and root were 165, 74, and 30 tannic acid equivalent mg/g of dried extract or fraction respectively. The butanol and ethylacetate fractions of lotus parts showed higher 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity than other fractions. 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) cation radical scavenging activity also showed the similar result as the DPPH radical scavenging activity. The antioxidative capacity of the ethylacetate fraction was the highest among fractions and its fraction showed higher contents of total polyphenol.

Keywords: lotus, *Nelumbo nucifera* Gaertner, antioxidant activity, radical scavenging activity, total phenolic content

Introduction

Edible plants, such as fruits, vegetables, and whole grains contain many components that are beneficial to human health. Research supports that some of these foods, as part of an overall healthful diet, have the potential to delay the onset of many age-related diseases. These observations have led to continuing research aimed at identifying specific bioactive components in foods, such as antioxidants, which may be responsible for improving and maintaining health (1).

Lotus (*Nelumbo nucifera* Gaertner, Nelumbonaceae family), known by a number of common names including Indian lotus, sacred lotus, bean of India, or simply lotus, is an aquatic perennial grown and consumed all parts such as flowers, seeds, leaves, stems, and roots (rhizomes) throughout asia. In Korea, the leaves and petals are used as tisane or teas. The rhizome is used as a vegetable in soups, deep-fried, stir-fried, and braised dishes (2). Previous studies about lotus have mainly on general considerations such as composition (2) and antioxidative property (3-8).

However, there are little studies on the systematic approaches to evaluate the novel component of lotus for developing a potential nutraceutical source elucidating the antioxidant activity. The objectives of this study were to evaluate the antioxidant activity of extracts from leaves, stems and roots of lotus, and fractions of organic solvents. Antioxidant activity of all parts in different solvents were evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) cation radical scavenging activity.

*Corresponding author: Tel: +82-2-2260-8590; Fax: +82-2-2260-8740
E-mail: spartan@dongguk.edu
Received April 13, 2009; Revised June 3, 2009;
Accepted June 11, 2009

Materials and Methods

Materials Each part of lotus, including the leaves, stems, and roots, was harvested in 2007 and were purchased from a local retailer, Seoul, Korea. Contaminants were removed by washing and drying. After drying (30°C for 48 hr), each part of leaves, stems, and roots was grounded and sieved approximately 30 mesh for use. All solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Extraction and fractionation of lotus Dried leaves, stems, and roots powder (each 25 g) of lotus were refluxed with 80% aqueous enthanol for 3 hr (3×250 mL) at 80°C in the water bath. The extract was filtered, concentrated with a vacuum rotary evaporator (Ilshin Lab., Yangju, Korea) at 40°C under reduced pressure, and lyophilized. The ethanol extract was suspended in distilled water and fractionated with 5 different solvents including *n*-hexane, chloroform, ethyl acetate, and *n*-butanol in a stepwise manner to yield *n*-hexane fraction (HF), chloroform fraction (CF), ethyl acetate fraction (EF), *n*-butanol fraction (BF), and water fraction (WF). Each solvent was extracted 3 times at room temperature, evaporated, and then freeze-dried. All extract and fractions were stored at −20°C until use.

Determination of total phenolic content (TPC) The TPC of the ethanol extracts and several fractions were measured according to the method of Folin-Dennis (9) with slight modification. One mL of sample in dimethyl sulfoxide (DMSO) was mixed with 0.5 mL of Folin-Dennis reagent. After 3 min, 1 mL of saturated Na_2CO_3 and 0.5 mL distilled water were added to the mixture. Finally, the absorbance was measured at 725 nm on UV/VIS spectrophotometer (Optizen 2120 UV; Mecasys, Daejeon, Korea). All the experiments were conducted using tannic acid as a calibration standard and the results were recorded as mg of tannic acid equivalent (TE)/g of dried extract or fraction (TE, mg/g of each extract or fraction). The TE values are expressed as mean±standard deviation (SD) of triplicate experiments.

DPPH radical scavenging activity 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was assessed according to the method of Choi (10). Initially, 0.2 mL of the ethanol extracts or several fractions in distilled water, yielding a series of extracts or fractions with different concentrations (25, 50, 100, 200, 500, and 1,000 μg/mL) in each reaction, were mixed with 0.8 mL of 0.4 mM DPPH solution. After 10 min, the absorbance was measured at 525 nm on UV/VIS spectrophotometer. The percent scavenging activity of free radicals by the sample was calculated using the following the equation: DPPH radicals scavenging activity (%)=[1− (absorbance of sample/absorbance of blank)]×100

ABTS cation radical scavenging activity The scavenging activity of the extracts or fractions on 2,2'-azinobis (3-ethylbenzothi-azoline-6-sulfonic acid) (ABTS) radical cation was measured according to the method of Re *et al.* (11) ABTS radical cation was generated by adding 7.4 mM ABTS diammonium salt to 2.6 mM potassium persulphate stock solution and the mixture was left to stand for overnight in the dark at room temperature. The ABTS radical cation solution was diluted with distilled water to obtain an absorbance of 1.0 at 734 nm. Diluted ABTS radical cation solution (4 mL) was added to 20 μL of the different concentration of extract. After 60 min, the absorbance was measured at 734 nm on UV/VIS spectrophotometer (Optizen 2120 UV; Mecasys). All determinations were performed in triplicate. The ABTS radical scavenging activity was calculated using the following the equation: ABTS cation radicals scavenging activity (%)=[1−(absorbance of sample/absorbance of blank)]×100.

Statistical analysis The results were analyzed by Sigma Stat 2.0 (Jandel Corp., San Rafael, CA, USA). One-way analysis of variance (ANOVA) was performed. Appropriate comparisons were made using the Student-Newman-Keuls test ($p<0.05$) of one-way ANOVA analysis.

Results and Discussion

Yields on different parts of lotus The yields of the extracts and fractions were recorded as % of crude 80% ethanol extract of leaf/25 g of plant material powder as shown in Table 1. The 80% ethanol extract yields from different part of lotus were as follows: leaf (26.68%; 6.671 g 80% ethanol extract/25 g dry leaf powder), stem (4.30%; 1.075 g 80% ethanol extract of stem/25 g dry stem powder), and root (23.48%; 5.870 g 80% ethanol exract of roots/25 g dry root powder). The extraction yield of 70% ethanol extract of lotus leaf was 8.16%, while of the methanol extract was 17% (4,5). Yang *et al.* (6) indicated that the methanol extract yield of lotus rhizome was 4.6%, but its 80% ethanol extract was 9.14%. The variation in the extraction yields may be due to the difference of extraction procedures including extraction method, solvent, extraction time, and temperature. The BF of lotus roots was the highest yield among the fractions of lotus ethanol extracts.

TPC TPC of leaf, stem, and root were 164.97, 73.95, and 29.76 TE mg/g of extract or fraction, respectively shown in Table 1. Yang *et al.* (6) showed 437.5 and 1,266 mg catechin equivalents/100 g phenolic content in methanol and acetone extracts of lotus rhizome, respectively. While, Hu and Skibsted (3) reported 31.9 g/100 g phenolic content in 50% aqueous ethanol extract of lotus rhizome. BF of the leaf part was the highest TPC (341.39 mg/g). The contents of solvent fractions of the leaf part followed the order: BF>EF>WF>CF>HF. In case of the stem part, EF>BF >CF>HF>water. In case of the root part, BF>EF>CF >WF>HF. The TPC of ethylacetate and butanol fraction of the leaf, stem, and root part was higher than the others. BF and EF were good solvents for the fractionation of the leaf part of lotus (4,5,7). Several thousand molecules having a polyphenol structure (i.e., several hydroxyl groups on aromatic rings) have been identified in higher plants, and several hundred are found in edibles plants (9). The

Table 1. Extraction and solvent fraction yields, total phenolic content (TPC), and ABTS cation radical scavenging activity (ABTS) from different parts of lotus

Extract or fraction	Leaf			Stem			Root		
	Yields (%)	TPC (mg/g)	ABTS (%)	Yields (%)	TPC (mg/g)	ABTS (%)	Yields (%)	TPC (mg/g)	ABTS (%)
80% Ethanol extract	26.68 (100)[1)]	164.97±6.86^{c}[2)]	25.14±1.48^{b}	4.30 (100)	73.95±4.34^{c}	10.58±1.06^{a}	23.48 (100)	29.76±1.55^{b}	2.52±1.02^{a}
n-Hexane fraction	3.48 (13.04)	55.83±4.22^{a}	14.03±1.85^{a}	0.80 (18.60)	60.63±4.54^{b}	12.74±2.26^{ab}	1.10 (4.68)	13.62±0.96^{a}	2.42±0.45^{a}
Chloroform fraction	3.83 (14.36)	61.31±6.92^{a}	12.43±1.50^{a}	0.25 (5.81)	123.77±5.60^{d}	24.10±0.99^{c}	0.55 (2.34)	40.09±3.05^{c}	7.11±0.55^{b}
Ethyl acetate fraction	1.55 (5.81)	188.95±4.27^{d}	39.71±3.37^{c}	0.15 (3.49)	139.31±5.11^{e}	28.82±3.31^{d}	0.95 (4.05)	82.09±6.02^{d}	23.57±1.80^{c}
Butanol fraction	3.68 (13.79)	341.39±5.95^{e}	96.67±0.51^{d}	0.76 (17.67)	136.18±6.69^{e}	29.77±3.49^{ed}	18.05 (76.87)	164.35±9.53^{e}	32.28±2.53^{d}
Water fraction	11.83 (44.34)	122.72±7.80^{b}	14.32±3.11^{a}	2.08 (48.37)	47.80±2.93^{a}	8.55±0.91^{a}	0.60 (2.56)	14.49±0.30^{a}	2.24±0.23^{a}

[1)]Parenthesis indicated the ratio of the fractions.
[2)]All values are expressed as mean±SD of triplicate determinations; Means in the same column not sharing a common letter are significantly different ($p<0.05$) by Student-Newman-Keuls.

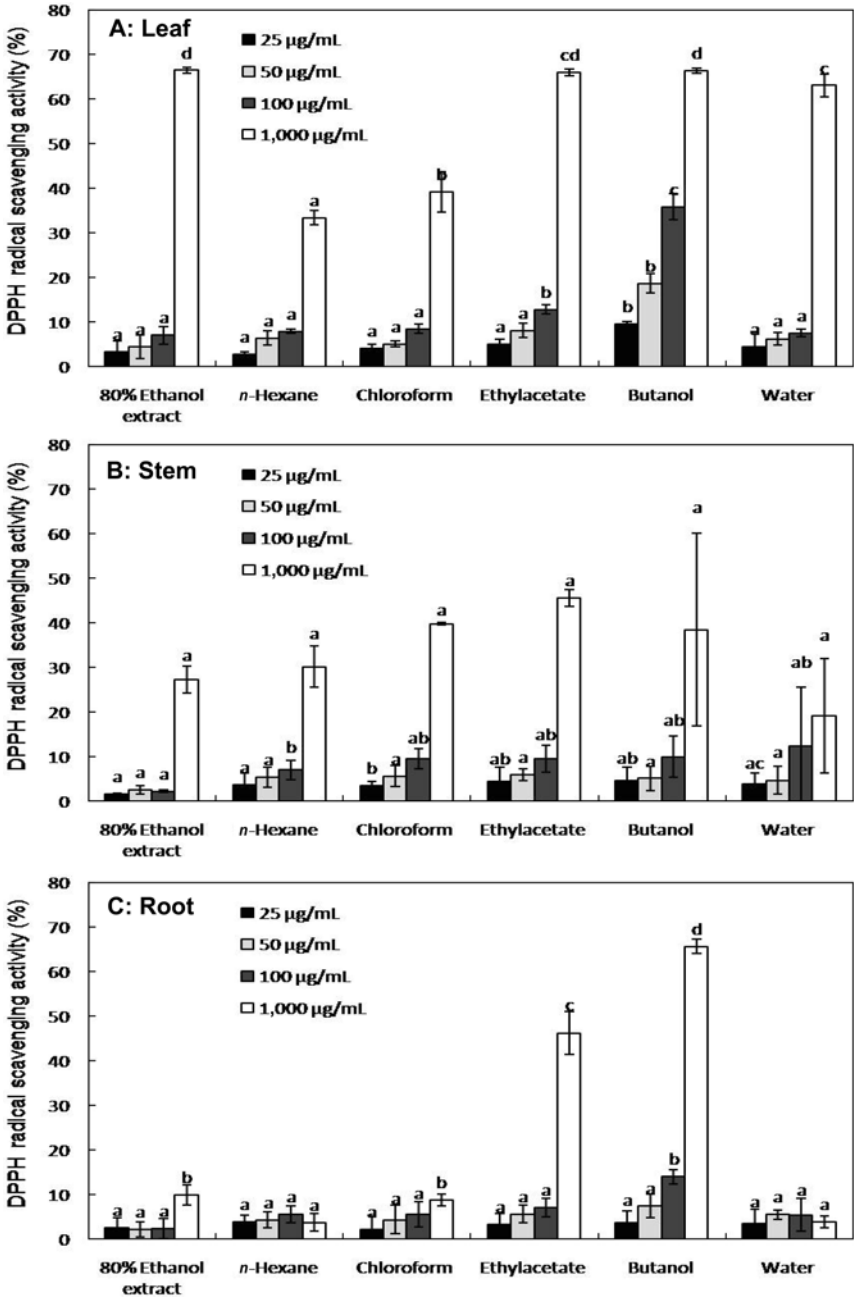

Fig. 1. DPPH radical scavenging activity of the different extract and fractions of lotus leaf (A), stem (B), and root (c).

phenolic compounds were luteolin, which belongs to flavones, and quercetin 3-*O*-β-D-glucopyranoside, which is a kind of flavonols. Jung *et al.* (7) indicated quercetin 3-*O*-β-D-glucopyranoside and quercetin-*O*-β-D-glucuronopyranoside were two of major components in EF of lotus leaf.

DPPH radical scavenging activity All extract and fractions of different parts of lotus showed DPPH radical scavenging activity in Fig. 1. The 80% ethanol extracts exhibited the scavenging activity at concentration of 1,000 μg/mL, showing 66% (leaf), 27% (stem), and 10% (root). Figure 1 showed the DPPH free radical scavenging activity tested with various fractions of leaf part. EF and BF demonstrated strong scavenging activities at the concentrations of 500 and 1,000 μg/mL. Park *et al.* (5) found the phenolic compounds from the leaves of lotus showing DPPH radical scavenging effect. Results of several studies on lotus using different methods have examined that lotus have strong antioxidant activity (4-8). DPPH scavenging activity was increased with concentration for both the extracts and fractions. This agrees with reports of Yang *et al.* (6). They demonstrated a positive correlation between scavenging activity and TPC.

ABTS cation radical scavenging activity The values of ABTS cation radical scavenging activity tested with extracts and various fraction of leaf, stem, and root at

Table 2. Comparing on ABTS cation radical scavenging activity of the extract and fractions of lotus, vitamin C, and tannic acid

(%)

Sample	Concentration (g/mL)					
	25	50	100	200	500	1,000
Ethyl acetate fraction	1.03±0.27$^{a1)}$	5.69±3.29^{a}	2.77±2.59^{a}	8.16±2.54^{a}	25.04±0.67^{a}	45.28±2.57^{a}
Butanol fraction	3.41±2.38^{a}	6.69±2.96^{a}	14.38±2.77^{b}	29.81±2.86^{c}	69.08±1.87^{c}	96.74±0.38^{bcd}
Vitamin C	1.69±0.31^{a}	3.15±0.53^{a}	6.57±2.02^{a}	13.82±1.42^{b}	39.01±1.28^{b}	86.39±9.35^{b}
Tannic acid	7.60±2.09^{b}	12.62±1.36^{b}	23.49±3.87^{c}	49.55±0.83^{d}	96.63±1.18^{d}	93.56±6.58^{bc}

[1)]All values are expressed as mean±SD of triplicate determinations; Means in the same column not sharing a common letter are significantly different ($p<0.05$) by Student-Newman-Keuls.

concentration of 1,000 μg/mL are shown in Table 1. The values of ABTS assay showed a wide variation from 2.24±0.23 (WF of root) to 96.67±0.51 (BF of leaf). The scavenging effect of 80% ethanol extracts was in the order: leaf (24.14%)>stem (10.58%)>root (2.52%). BF and EF of all parts (leaf, stem, and root) had the highest ABTS scavenging activity. Table 2 shows the values of ABTS cation radical scavenging activity tested with 2 fractions (BF and EF of leaf) and positive controls (vitamin C and tannic acid) at various concentrations of 25, 50, 100, 200, 500, and 1,000 μg/mL. BF has higher ABTS scavenging activity compared with activity of vitamin C and tannic acid. The two most widely used chromogen compounds to measure the antioxidant activity of biological material are the ABTS and the DPPH radicals. Both present excellent stability in certain assay conditions but also show several important differences in their response to antioxidants and in their manipulation. DPPH is a free radical that is acquired directly without preparation (read to dissolve), while ABT must be generated by enzymatic (peroxidase, myoglobin) or chemical (manganese dioxide, potassium persulfate, 2,2'-azobis(2-amino propane) (ABAP) reactions. Another important difference is that ABTS can be solubilized in aqueous and in organic media, in which the antioxidant activity can be measured by hydrophilic a lipophilic nature of the compounds in sample. In contrast, DPPH can only be dissolved in organic media (especially in alcoholic media), not in aqueous media, which is an important limitation when interpreting the role of hydrophilic antioxidants. The solubility and stability of ABTS as well as the reactions response to antioxidants have been reported (10,11).

References

1. IFIC Foundation Publications. Functional Foods Fact Sheet: Antioxidants. Available from: http://www.ific.org/publications/factsheets/antioxidantfs.cfm. Accessed Apr. 2, 2009.
2. Sridhar KR, Baht R. Lotus-A potential nutraceutical source. Int. J. Agr. Technol. 3: 143-155 (2007)
3. Hu M, Skibsted LH. Antioxidative capacity of rhizome extract and rhizome knot extract of edible lotus (*Nelumbo nucifera*). Food Chem. 76: 327-333 (2002)
4. Lee KS, Kim MG, Lee KY. Antioxidative activity of ethanol extract from lotus (*Nelumbo nucifera*) leaf. J. Korean Soc. Food Sci. Nutr. 35: 182-186 (2006)
5. Park CH, Hur JM, Song KS, Park JC. Phenolic compounds from the leaves of *Nelumbo nucifera* showing DPPH radical scavenging effect. Korean J. Pharmacogn. 38: 263-269 (2007)
6. Yang DY, Wang QW, Ke L, Jiang J, Ying T. Antioxidant activities of various extract of lotus (*Nelumbo nucifera* Gaertn) rihizome. Asia Pac. J. Clin. Nutr. 16 (S1): 158-163 (2007)
7. Jung HA, Jung YJ, Yoon NY, Jeong DM, Bae HY, Kim DW, Na DH, Choi JS. Inhibitory effects of *Nelumbo nucifera* leaves on rat lens aldose reductase, advanced glycation endproducts formation, and oxidative stress. Food Chem. Toxicol. 46: 3818-3826 (2008)
8. Khattak KF, Simpson TJ, Ihasnullah T. Effect of gamma irradiation on the microbial load, nutrient composition, and free radical scavenging activity of *Nelumbo nucifera* rhizome. Radiat. Phys. Chem. 78: 206-212 (2009)
9. Claudine M, Augustin S, Christine M, Christian R, Liliana J. Polyphenols: Food sources and bioavailability. Am. J. Clin. Nutr. 79: 727-747 (2004)
10. Choi NY. Antioxidant activity and nitrite scavenging ability of olive leaf (*Olea europaea* L.) fractions from different cultivating locations. MS thesis, Dongguk University, Seoul, Korea (2007)
11. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antoxidant activity applying and improved ABTS radical cation decolorization assay. Free Radical Bio. Med. 26: 1231-1237 (1999)