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Full Communication

Bioactive Compounds and Antioxidant and Antiproliferative Activities of Korean White Lotus Cultivars

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ABSTRACT In traditional Korean medicine, lotus (*Nelumbo nucifera* Gaertn) roots have been used as an antidiabetic and an antiproliferative remedy. However, scientific publications on lotus properties are very limited. Therefore, it was decided to investigate the Korean white lotus cultivars in order to find out their bioactivity. It was found that all lotus cultivars (Inchisa, Muan, Garam, and Chungyang) possess high amounts of bioactive compounds: total phenols, between 7.95 ± 0.8 and 4.21 ± 0.3 mg of gallic acid equivalents (GAE)/g dry weight (DW); ascorbic acid, between 15.8 ± 1.1 and 22.3 ± 1.7 mg of ascorbic acid/g DW; and amino acids, between $15.05 \pm 0.82\%$ and $16.62 \pm 0.90\%$ DW. The highest contents of polyphenols (7.95 ± 0.8 mg of GAE/g DW) and the highest levels of antioxidant [by 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) and 1,1-diphenyl-2-picrylhydrazyl assays, 54.27 ± 6.1 and 21.98 ± 2.5 μM Trolox equivalents/g DW, respectively] and antiproliferative activities on both human cancer cell lines (Calu-6 for human pulmonary carcinoma and SMU-601 for human gastric carcinoma, $59.75 \pm 3.99\%$ and $71.21 \pm 2.79\%$ cell viability, respectively) were found in the Chungyang cultivar. Fluorometry and Fourier transform infrared spectroscopy can be applied as rapid methods for determination of bioactive compounds, such as polyphenols. The correlation between the bioactive compounds and the antioxidant activity was high. In conclusion, all Korean white lotus cultivars are valuable medicinal foods, and in order to receive the best results a combination of lotus cultivars has to be consumed.

KEY WORDS: • ***antioxidant and antiproliferative activities • bioactive compounds • Korean white lotus cultivars***

INTRODUCTION

LOTUS (*NELUMBO NUCIFERA* GAERTN) is well known in Far East countries as a sacred aquatic plant for Hinduism and Buddhism. Lotus consists of two species, *N. nucifera* (Indian lotus) and *Nelumbo pentapetara* (American lotus).[1] In traditional Korean medicine lotus has been used as an alleviator of bleeding, blood stagnancy, and thirstiness and antipyretic, antidiabetic, and antiproliferative remedy.[1–4] Different parts of lotus are useful in treatment of diarrhea, tissue inflammation, and hemostasis, and rhizome extract has anti-inflammatory properties due to the presence of asteroidal triterpenoid.[5,6] According to the above-mentioned authors, the impression is that lotus is a panacea for most diseases.

Because of the high nutritional value of fresh lotus, its consumption in recent years has increased rapidly, showing that lotus is a valuable medicinal food.[7–10]

It was also shown in experiments on laboratory animals that lotus could be used as an effective medicine, decreasing platelet aggregation in rabbits.[11]

Unfortunately, both the growing season and shelf life of fresh-cut lotus are very short, and many factors decrease the quality of lotus after harvest, one of the most serious of them being yellowing.[12–15] The tissue browning is caused mainly by activity of polyphenol oxidase (PPO), and there was a positive relationship between the browning index and PPO activity.[15] The enzyme browning is one of the most important reactions impacting quality of fresh-cut lotus root, and the PPO is a key enzyme inducing browning.[14,15] There have been only a few scientific investigations on the

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properties of the Korean white lotus cultivars both as medicinal food and as medicine.

Therefore, it was decided to determine the nutritional value and the medicinal properties of the Korean white lotus cultivars Inchisa, Muan, Garam, and Chungyang.

MATERIALS AND METHODS

Chemicals

Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid), 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), potassium persulfate, Folin-Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, and catechin were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All reagents were of analytical grade. Deionized and distilled water was used throughout. The cell lines were purchased from the Korean Cell Line Bank (Seoul, Republic of Korea) for the 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Preparation of the lotus samples

Cultivars of white lotus (*N. nucifera* Gaertn) were obtained from a local farm (in the country near Muan, Republic of Korea) at commercial maturity on January 15, 2008, transported to the laboratory, and held overnight at 0°C. Uniformly sized roots of Inchisa, Muan, Garam, and Chungyang Korean white lotus cultivars were selected.

Determination of the main nutritional compounds and metals

The main nutritional compounds (proteins, crude fat, carbohydrates, and total fiber) were determined as previously described.[16,17] Flesh firmness (two measurements per slice) was monitored using a firmness tester (model KM, Fruit Test Tech, Tokyo, Japan) with a 5-mm needle tip. Flesh color was measured with a CR-400 Chroma meter and CR-400 utility software version CR-S4w (Minolta, Tokyo) and expressed as an "L" value.

Lyophilized lotus samples of all studied cultivars (0.5 g) were separately mixed with 1 mL of sulfuric acid and 21 mL of 50% $HClO_4$. The mixture was heated at 310–410°C in a furnace for 3–4 hours. After cooling, 100 mL of distilled water was added. The inorganic content (P, K, Ca, Mg, and Na) was measured by the flame atomic absorption spectrometric method (AVANTA spectrophotometer, GBC Scientific Equipment Pty Ltd., Dandenong, VIC, Australia).[3,9]

Determination of amino acids

Lotus powder (10 mg) was mixed with 0.5 *N* perchloric acid, homogenized, centrifuged, neutralized with potassium hydrogen carbonate, centrifuged, divided in two phases, diluted with buffer (0.2 *N* lithium citrate, pH 1.3), and used for liquid chromatography (Waters [Bedford, MA, USA] liquid chromatograph controlled by Multichrom software and equipped with a SYKAM S7131 reagent organizer, connected with the SYKAM s 2100 solvent delivery system [SYKAM, Esering, Germany]). The SYKAM column was LCA K07/Li (particle size, 5 μm; 4.6×150 mm). High-performance liquid chromatography conditions were as follow: mobile phase A, 0.12 *N* lithium citrate buffer (pH 2.9); mobile phase B, 0.30 *N* lithium citrate buffer (pH 4.2); mobile phase C, 0.30 *N* lithium citrate borate buffer (pH 8.0) and regeneration solution (0.5 *N*). The flow rate was set at 0.45 mL/minute, and the column was maintained at 37°C.

Determination of total phenol content

Total phenols were extracted from lyophilized root powder with methanol (concentration, 25 mg/mL) at room temperature for 3 hours twice and then were determined by the Folin-Ciocalteu method with measurement at 750 nm with a spectrophotometer (model 8452A, Hewlett-Packard, Rockville, MD, USA).[17] The results were expressed as mg of gallic acid equivalents (GAE)/g dry weight (DW).

The presence of polyphenols (flavonoids and phenolic acids in the range of the wavenumbers from 1,000 to 1,700 cm^{-1}) in the investigated lotus cultivars was studied by Fourier transform infrared (FTIR) spectroscopy. A Bruker Optic GMBH Vector FTIR spectrometer (Bruker Optic GMBH, Attingen, Germany) was used to record infrared spectra. A potassium bromide microdisk was prepared from the finely ground lyophilized powder produced from 2 mg of lotus sample with 100 mg of KBr.[16]

Fluorescence measurements of polyphenol extracts were done using a Jasco (Tokyo) spectrofluorometer (model FP-6500). Fluorescence emission spectra for all lotus samples at a concentration of 0.25 mg/mL were taken at emission wavelength of 330 nm and recorded from wavelength of 265 nm to 310 nm, at the following emission wavelengths: at 685 nm from 300 to 750 nm; and at excitation of 350 nm from 370 to 650 nm. Standards of 0.01 m*M* quercetin in methanol were used.[18,19]

Determination of the antioxidant activity

The following two tests were used in order to compare the results obtained:

ABTS method. The ABTS diammonium salt ($ABTS^{\cdot+}$) was generated by the interaction of ABTS (7 mmol/L) and $K_2S_2O_8$ (2.45 mmol/L). This solution was diluted with methanol until the absorbance reached 0.7 at 734 nm.

DPPH method. DPPH solution (3.9 mL, 25 mg/L) in methanol was mixed with the sample extracts (0.1 mL). The reaction progress was monitored at 515 nm until the absorbance was stable.

Determination of ascorbic acid content

Lotus samples of all studied cultivars (1 g) were separately mixed with 100 mL of the extract solution (0.1 *M* citric acid plus 0.05% EDTA in 5% methanol). The mixture was centrifuged and filtered. Before injection into the high-performance liquid chromatography system (model 515,

Table 1. Basic Nutritional and Inorganic Compounds in the Four Korean White Lotus Cultivars Studied

	Level in cultivar			
Sample	*Inchisa*	*Muan*	*Chungyang*	*Garam*
Protein	13.0 ± 0.5^{a}	12.9 ± 0.3^{a}	13.1 ± 0.5^{a}	12.8 ± 0.3^{a}
Crude fat	2.2 ± 0.1^{a}	2.3 ± 0.1^{a}	2.2 ± 0.1^{a}	2.3 ± 0.1^{a}
Carbohydrates	65.9 ± 5.6^{a}	65.8 ± 5.5^{a}	65.7 ± 5.4^{a}	66.1 ± 5.9^{a}
Total fiber	2.8 ± 0.1^{a}	2.7 ± 0.1^{a}	2.9 ± 0.1^{a}	2.8 ± 0.1^{a}
K	29.74 ± 1.5^{a}	30.11 ± 1.5^{a}	30.05 ± 1.5^{a}	29.97 ± 1.5^{a}
Ca	28.53 ± 1.4^{b}	27.69 ± 1.4^{b}	21.20 ± 1.1^{a}	30.60 ± 1.5^{b}
P	5.14 ± 0.3^{b}	4.19 ± 0.2^{b}	3.34 ± 0.1^{a}	4.77 ± 0.2^{b}
Na	34.79 ± 1.7^{a}	37.26 ± 1.9^{a}	34.08 ± 1.7^{a}	38.63 ± 1.9^{a}
Mg	29.33 ± 1.5^{b}	30.16 ± 1.5^{b}	30.45 ± 1.5^{b}	24.92 ± 1.3^{a}

Data are mean ± SD values (percentage DW for nutrients and mg/g DW for inorganic compounds) of three measurements.
Means in rows with different superscript letters are significantly different ($P < .05$).

Waters), 1 mL of 1,2-phenylenediamine (3.33 mg/mL) in methanol/water (5:95, vol/vol) was kept at room temperature for 37 minutes in the dark. The standard and extracted samples were analyzed using a high-performance liquid chromatography pump connected with a refractive index detector. The Waters XBridge™ C_{18} column (particle size, 5 μm; 4.6×150 mm) was pre-equilibrated with a mobile phase consisting of methanol/water (5:95, vol/vol) at a flow rate of 1.0 mL/minute with the column temperature at 35°C and the detector temperature at 30°C. The retention time for determining standard ascorbic acid was 5.3 minutes.

Determination of PPO activity

Flesh powders of all studied cultivars were under a nitrogen atmosphere in 10 mL of chilled 0.1 *M* sodium phosphate buffer (pH 4.0) and then centrifuged at 0°C for 20 minutes. The PPO activity was measured by the change of absorbance at 420 nm of the assay mixtures, which contained 20 μL of supernatant (enzyme extract), 0.5 mL of distilled water, and 0.5 mL of assay solution (7.1 g of Na_2HPO_4, 5.25 g of citrate, and 2.76 g of catechol/250 mL), which was added before measurement. PPO activity was calculated as the change in absorbance units (AU) at 420 nm/mg of protein/minute.[12–15]

Determination of browning potential (BP)

Flesh powder (10 g) was homogenized under a nitrogen atmosphere in 10 mL of chilled 0.1 *M* sodium phosphate buffer (pH 4.0) and then centrifuged at 0°C for 20 minutes. The absorbance at 420 nm of the warmed-up supernatant (BP extract) was determined at zero-time and after incubation for 5 hours in a covered water bath at 30°C using a spectrophotometer (model UV 160, Shimadzu, Kyoto, Japan).

Determination of the antiproliferative activity

The antiproliferative activities were measured using the MTT assay. Cells were harvested, counted (3×10^4 cells/mL), transferred into a 96-well plate, and incubated for 24 hours prior to addition of lotus methanol extracts. Serial dilutions of the extracts were prepared by dissolving compounds in dimethyl sulfoxide followed by dilution with RPMI 1640 medium to give final concentrations of 125, 250, 500, 1,000, and 2,000 μg/mL. Stock solutions of samples were prepared for cell lines at 90 μL and for samples at 10 μL and incubated for 72 hours. MTT solution at 5 mg/mL was dissolved in 1 mL of phosphate buffer solution, and 10 μL of it was added to each of the 96 wells. The wells were wrapped with aluminum foil and incubated at 37°C for 4 hours. The solution in each well containing medium, unbound MTT, and dead cells was removed by suction, and 150 μL of dimethyl sulfoxide was added to each well. The plates were then shaken, and optical density (OD) was recorded using a microplate reader at 540 nm. Distilled water was used as the positive control and dimethyl sulfoxide as the solvent control.[20,21] The effect of the lotus extract on the proliferation of cancer and normal cells was expressed as relative cell viability: percentage viability = (OD of lotus extract-treated sample/OD of untreated sample)×100.[22]

Statistical analysis

Data are mean ± SD values of three measurements. Where appropriate, differences between groups were tested by two-way analysis of variance. Values of $P < .05$ were considered significant.

RESULTS

Basic nutritional compounds

There were no significant differences in the content of crude protein, crude fat, carbohydrates, and dietary fiber (Table 1) in the four Korean white lotus cultivars ($P > .05$).

Flesh firmness (L) was determined as follows: Inchisa, 72.48 ± 6.15; Muan, 66.21 ± 5.78; Garam, 67.46 ± 5.98; Chungyang, 65.46 ± 6.09. A more negative number indicates an increase in browning. If the L value is higher, then lotus is more beneficial in quality compared to a cultivar with a lower value.

The contents of Ca and P in Chungyang and of Mg in Garam were significantly lower than in the other cultivars ($P < .05$).

Table 2. Contents of Amino Acids in the Four White Lotus Cultivars Studied

Amino acid	Level (mg/g DW) in cultivar			
	Inchisa	Muan	Garam	Chungyang
Phosphoserine	7.14 ± 0.35^{a}	8.37 ± 0.42^{b}	7.24 ± 0.36^{a}	7.49 ± 0.37^{a}
Aspartic acid	7.74 ± 0.38^{a}	8.14 ± 0.41^{a}	7.97 ± 0.4^{a}	7.44 ± 0.37^{a}
Hydroxyproline	3.01 ± 0.15^{a}	3.49 ± 0.17^{a}	3.11 ± 0.16^{a}	3.02 ± 0.15^{a}
Threonine	8.41 ± 0.42^{a}	9.21 ± 0.46^{a}	8.22 ± 0.41^{a}	8.74 ± 0.44^{a}
Serine	20.13 ± 1.01^{a}	21.41 ± 1.07^{a}	23.35 ± 1.17^{a}	22.55 ± 1.13^{a}
Asparagine	14.25 ± 0.71^{b}	12.47 ± 0.62^{a}	15.41 ± 0.77^{b}	16.65 ± 0.83^{c}
Proline	7.15 ± 0.36^{a}	8.07 ± 0.4^{a}	7.61 ± 0.38^{a}	7.63 ± 0.38^{a}
Glycine	5.74 ± 0.29^{b}	4.16 ± 0.21^{a}	5.95 ± 0.3^{b}	3.93 ± 0.2^{a}
Citrulline	4.08 ± 0.20^{a}	4.11 ± 0.21^{a}	4.02 ± 0.2^{a}	4.01 ± 0.2^{a}
α-Aminobutyric acid	1.97 ± 0.1^{b}	0.74 ± 0.04^{a}	5.44 ± 0.27^{c}	6.09 ± 0.3^{c}
Valine	10.14 ± 0.51^{a}	12.13 ± 0.61^{b}	13.16 ± 0.66^{b}	14.13 ± 0.71^{c}
Cystine	0.98 ± 0.05^{c}	0.57 ± 0.03^{a}	0.74 ± 0.04^{b}	0.91 ± 0.05^{c}
Methionine	3.17 ± 0.16^{a}	3.51 ± 0.18^{a}	4.23 ± 0.21^{a}	4.60 ± 0.23^{a}
Isoleucine	0.95 ± 0.05^{a}	1.78 ± 0.09^{b}	1.25 ± 0.06^{a}	1.06 ± 0.05^{a}
Leucine	5.1 ± 0.26^{a}	6.74 ± 0.34^{b}	7.23 ± 0.36^{b}	5.46 ± 0.27^{a}
Tyrosine	6.31 ± 0.32^{a}	6.49 ± 0.32^{a}	7.89 ± 0.39^{b}	8.17 ± 0.41^{b}
Phenylalanine	0.74 ± 0.04^{a}	0.75 ± 0.04^{a}	0.62 ± 0.03^{a}	0.61 ± 0.03^{a}
γ-Aminobutyric acid	2.30 ± 0.12^{a}	2.62 ± 013^{a}	3.23 ± 0.16^{b}	2.10 ± 0.1^{a}
Histidine	2.16 ± 0.11^{a}	1.34 ± 0.07^{a}	2.41 ± 0.12^{a}	1.66 ± 0.08^{a}
Glutamic acid	23.46 ± 1.17^{a}	25.26 ± 1.26^{a}	22.83 ± 1.14^{a}	23.48 ± 1.17^{a}
Alanine	15.47 ± 0.77^{a}	14.67 ± 0.73^{a}	14.33 ± 0.72^{a}	14.74 ± 0.74^{a}
Arginine	2.13 ± 0.11^{a}	2.71 ± 0.14^{a}	2.16 ± 0.11^{a}	2.41 ± 0.12^{a}
Total	150.47 ± 8.24^{a}	156.01 ± 8.47^{a}	166.23 ± 9.03^{a}	164.45 ± 9.06^{a}

Data are mean ± SD values of three measurements.
Values in rows with different superscript letters are significantly different ($P < .05$).

Amino acids

The total contents of the studied amino acids (Table 2) were higher in Garam and Chungyang cultivars (166.23 ± 9.03 and 164.45 ± 9.06 mg/g DW, respectively) than in Inchisa and Muan, but not significantly different ($P > .05$). Among the amino acids, glutamic and serine were the highest (23.46 ± 1.17, 25.26 ± 1.26, 22.83 ± 1.14, and 23.48 ± 1.17 mg/g DW and 20.13 ± 1.01, 21.41 ± 1.07, 23.35 ± 1.17, and 22.55 ± 1.13 mg/g DW for Inchisa, Muan, Garam, and Chungyang, respectively). We did not find any differences in the contents of 13 (phosphoserine, aspartic acid, hydroxyproline, threonine, serine, proline, citrulline, methionine, phenylalanine, histidine, glutamic acid, alanine, and arginine) out of the 22 amino acids in the cultivars.

Total phenolics

The wavenumbers of FTIR spectra for catechin at 827, 1,039, 1,115, 1,143, 1,286, 1,478, 1,511, and 1,610 cm^{-1} were assigned to C-H alkenes, -C-O alcohols, C-OH alcohols, -OH aromatic, C-O alcohols, C-H alkanes, C = C aromatic ring, and C = C alkenes. Gallic acid showed wavenumbers of 866, 1,026, 1,238, 1,450, 1,542, and 1,618 cm^{-1}. Lotus sample (lower line, Fig. 1) in the region of polyphenols showed slightly different bands than the standards. Common peaks for lotus were fixed at 1,141, 1,233, 1,454, 1,508, and 1,646 cm^{-1}, and as a comparison green pepper was used (upper line, Fig. 1).

Fluorimetric measurements (Fig. 2) showed the following peaks (nm) with the AU: lotus with a peak at 343.5 nm and 264.58 AU, green pepper with a peak at 343.5 nm and 310.47 AU, and catechin at 343.5 nm and 666.83 AU (Fig. 2A); for lotus at 336.5 nm and 264.58 AU, catechin, 286 nm and 9.72AU (Fig. 2B); and for lotus at 391.5 nm and 120.49 AU and 424 nm and 111.27AU, green pepper at 428 nm and 280.26 AU and 393 nm and 224.04 AU, and catechin at 390.5 nm and 35.09 AU (Fig. 2C).

Chungyang and Inchisa cultivars had the significant highest content of total phenolics (in mg of GAE/g DW, Fig. 3, $P < .05$). The cultivars Muan and Garam had the significantly lowest content of these bioactive compounds ($P < .05$).

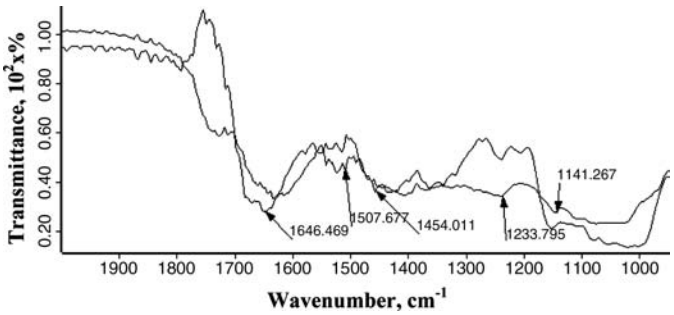

FIG. 1. FTIR spectra of Chungyang lotus cultivar (lower line) and green pepper (upper line), respectively.

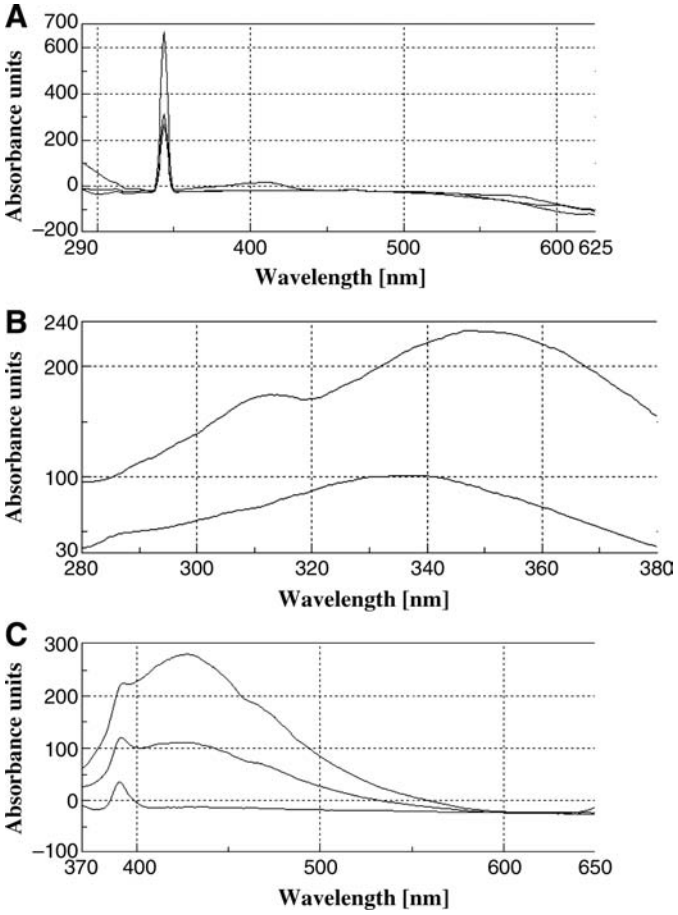

FIG. 2. Fluorimetric excitation spectra of extracts with emission wavelength at (**A**) 685 nm recorded over the frequency range from the excitation wavelength to a wavelength of 750 nm for lotus, green pepper, and catechin, (**B**) excitation at 450 nm and recording from 280 to 380 nm, and (**C**) excitation at 350 nm and recording from 370 to 650 nm. Methanol extracts of polyphenols from lotus and green pepper (0.25 mg/mL) and 0.001 *M* catechin were used.

Ascorbic acid

The Garam cultivar had the significantly highest content of ascorbic acid (in mg/g DW, Fig. 3, $P < .05$), following by Inchisa. The cultivars Muan and Chungyang had the significant lowest content of this vitamin ($P < .05$).

PPO activity and BP

All the above studied cultivars are white lotus roots, and these plants are used as edible ones in fresh and cooking forms and juice. PPO and BP are the most important indices for browning during storage of lotus. PPO activity of the Inchisa cultivar was significantly lower than those in the other three cultivars studied (Fig. 3). A significantly higher BP (Fig. 3) was recorded in the Garam cultivar ($P < .05$), followed by Muan.

Antioxidant activity

The antioxidant activity determined in the ABTS and DPPH assays was significantly higher in the Chungyang and

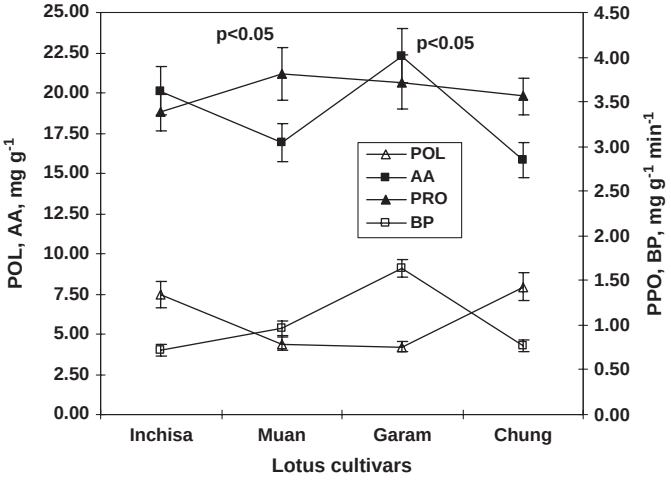

FIG. 3. Contents of total phenolics (POL, in mg of GAE/g DW) and ascorbic acid (AA, in mg/g of DW) (scale on the left) and PPO activity (in mg/g DW/minute) and BP (in mg/g DW/minute) (scale on the right) in the four cultivars of the Korean white lotus studied.

Inchisa cultivars ($P < .05$). A complete correlation (the highest contents of total phenolics and the highest antioxidant activity) between the total phenolics, ABTS, and DPPH data was recorded in the Chungyang and Inchisa cultivars (Figs. 3 and 4).

Antiproliferative activity

It was observed that the antiproliferative activity of Inchisa, Muan, Garam, and Chungyang Korean white lotus cultivars on two cell lines (Calu-6 for human pulmonary carcinoma and SMU-601 for human gastric carcinoma) was different (Fig. 5). The percentage cell viability at concentrations of 2,000 μg/mL for the Inchisa and Chungyang cultivars (Fig. 5A and D) for Calu-6 was $66.17 \pm 3.60\%$ and $59.75 \pm 3.99\%$.

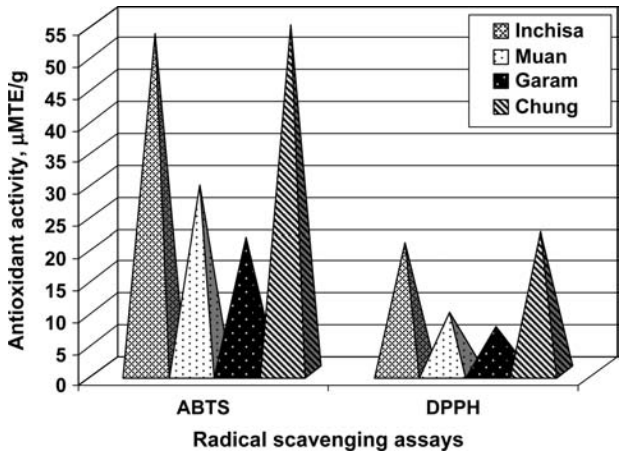

FIG. 4. Antioxidant activities (in μM Trolox equivalents [TE]/g DW) determined by ABTS and DPPH assays of the four cultivars of the Korean white lotus studied.

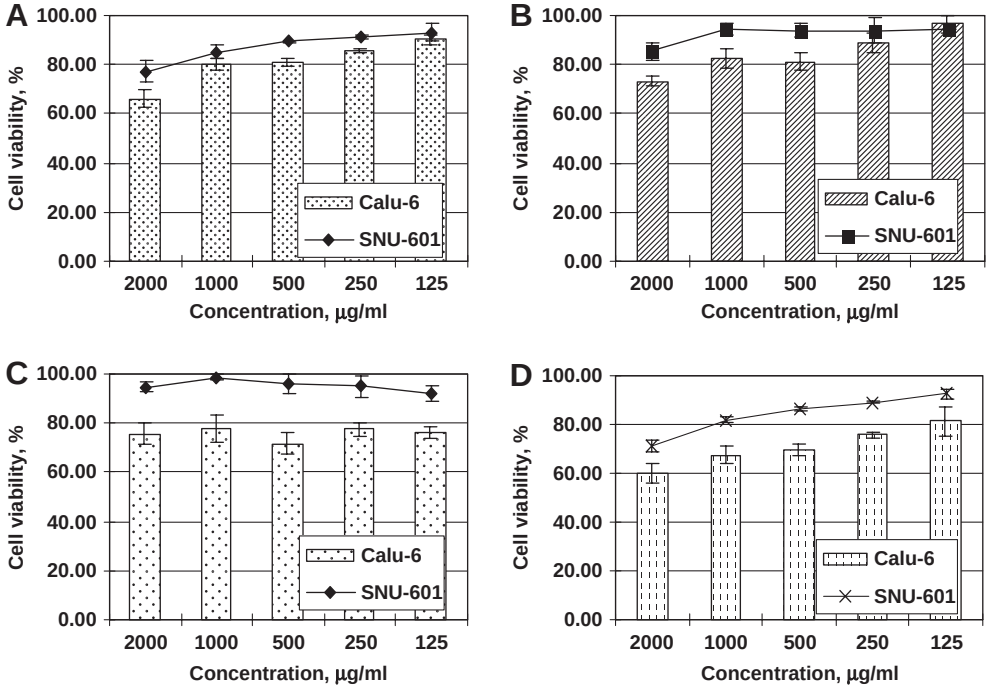

FIG. 5. Proliferation rate of Calu-6 (human pulmonary carcinoma) and SNU-601 (human gastric carcinoma) cells treated with the lotus extracts of the (**A**) Inchisa, (**B**) Muan, (**C**) Garam, and (**D**) Chungyang cultivars. Antiproliferative effects of the lotus cultivars were expressed as percentage cell viability after exposure to treatment for 24 hours.

The Muan and Garam cultivars (Fig. 5B and C) for Calu-6 showed exactly the same reaction of cells: $73.15 \pm 1.93\%$ and $75.75 \pm 4.64\%$. The antiproliferative activity for SNU-601 (percentage cell viability) was $77.32 \pm 4.26\%$ and $71.21 \pm 2.79\%$ for Inchisa and Chungyang cultivars (Fig. 5A and D) and $85.62 \pm 3.60\%$ and $94.58 \pm 1.89\%$ for Muan and Garam cultivars (Fig. 5B and C). The Chungyang cultivar showed significantly highest inhibition activity ($P < .05$) for the two cell lines.

DISCUSSION

Recently the consumption of medicinal foods of high nutritional value, including Korean white lotus cultivars, has increased rapidly.[1–3] Because of the lotus's hard and crispy texture, special aroma, and mouth feel, this fruit is popular among Asian peoples.[23]

It was shown that the elemental composition of lotus cultivars was about 13% protein, 2.3% crude fat, 66% carbohydrates, and 2.8% fiber (Table 1). Our data were slightly lower than those in other reports,[1,9,10] showing the range of protein content as 10.6–19.5%, crude fat as 1.93–2.80%, carbohydrates as 61.3–72.2%, and crude fiber as 2.1–2.7%, because these data were determined in some cases in lotus seeds.

The amount of potassium was about 30%, of calcium 27%, of phosphorus 4.4%, of sodium 36.2%, and magnesium of 28.7% (Table 1). Our data are in accordance with others, showing that potassium is present at 28.5% and calcium at 22.1%.[1,9,10] Magnesium and sodium were lower: 9.2% and 1%, respectively. The contents of Ca and Mg in lotus root starch were 212.2 μg/g and 399.6 μg/g, respectively.[3]

Lotus possesses a high content of different vitamins, and among them is ascorbic acid. We recorded that all cultivars contain high amounts of amino acids. Our results (Table 2) are in accordance with those of others,[2] who isolated alanine, tyrosine, phenylalanine, valine, threonine, arginine, leucine, isoleucine, serine, and aspartic acid from *Nymphaea lotus*. Other authors have reported that lotus seeds contain 18 different amino acids.[24] We found in the roots 22 amino acids. Wu *et al.*[24] reported that the total amino acid content was 19.4–20.0% DW, and our results were lower (15.05–16.62% DW). Content of neutral amino acids was higher (8.92–9.92% DW) than of acidic amino acids (7.28–7.29% DW) or basic amino acids (3.20–3.39% DW). We found that the content of neutral amino acids was about 9.85–11.01%. Glu was the most abundant amino acid (4.98–4.99% DW). The total essential amino acid content was 6.20–6.30% DW, and in our research it was 2.85–3.42% DW. The amount of Leu was the highest (1.40–1.41% DW) of the essential amino acids. Leu was lower in the investigated cultivars (0.51–0.72% DW). Also, we found that glutamic acid was the most abundant amino acid: $2.35 \pm 0.11\%$, $2.53 \pm 0.13\%$, $2.28 \pm 0.11\%$, and $2.35 \pm 0.12\%$ DW for Inchisa, Muan, Garam, and Chungyang white lotus cultivars, respectively. In lotus seeds[10] 16 amino acids were detected, and glutamic acid was the highest at 4.5% in dehulled kernel. Our data were about 2.28–2.53% DW, because they were determined not in seeds, but in roots.

The lotus samples were excited at 350 nm, and recordings of fluorescence were carried out in lotus extracts in the wavelength range from 370 to 650 nm. Then two other emission wavelengths were used (450 and 685 nm), and the spectra were recorded from 280 to 400 nm. The difference in

the recorded peaks of lotus and green pepper samples and their absorbance intensities can be explained by variation of the polyphenols.[16,18,19] Our results of fluorimetry and FTIR spectroscopy show the potential use of these methods for the rapid assessment of lotus pigments such as carotenoids, chlorophylls, and polyphenols. The results of our investigation showed that all four Korean white lotus cultivars studied are rich in total phenols. It can be compared with green pepper, which is one of the vegetables used widely in Europe as lotus is in Far Eastern countries.[25,26] The polyphenols in green pepper were about 2.4 μM catechin equivalents/g DW, and the antioxidant activity determined by DPPH was 2.1 μM Troxol equivalents/g DW,[25,26] which are similar to the data from lotus samples. Also, others reported that the content of total phenolics in lotus is high; the antioxidative capacity of extracts from rhizomes (lotus roots) knot and whole rhizomes exhibited high antioxidative capacity, and total phenol content in the plant extract correlated with the antioxidant capacity.[27] Rai *et al.*[28] reported that total phenolic content in hydroalcoholic extract of *N. nucifera* seeds was $7.61 \pm 0.04\%$ (wt/wt). The antioxidant activity shows that this index was high, but significantly different for the Korean white lotus cultivars studied (Fig. 4). Also, others confirmed our data.[29,30] We also recorded a complete correlation between the contents of total phenolics and radical scavenging and antioxidant activities as shown by ABTS and DPPH. The methanol extracts from various parts of lotus are preferable,[31] in comparison with 10 methanol extracts from various parts of seven medicinal plants. The extracts were evaluated for antioxidative radical scavenging activities by DPPH as in the present report. We showed previously that the methanolic extracts of plants that contain polyphenols possess antiproliferative properties.[21] These findings indicate that lotus extracts and their polyphenols could be considered as possible anticancer agents. Also, others found that extracts from *N. nucifera* suppress cell cycle progression, expression of cytokine genes, and cell proliferation in human peripheral blood mononuclear cells.[5] The results of their investigation indicate a mechanism by which lotus extracts and their polyphenols exert chemopreventive properties and therefore can be considered as possible anticancer agents. Also, Du *et al.*[30] studied the antiproliferative effect of procyanidins from lotus seedpod against human carcinoma cells. The MTT assay demonstrated that lotus seedpod procyanidins inhibited the growth of nasopharyngeal epidermoid carcinoma and rat basophilic leukemia cells rather than mouse fibrosarcoma and a human mast cell line. It was of great interest to learn which of the Korean white lotus cultivars studied exercised the highest antiproliferative activity. It was found that all Korean white lotus cultivars possess high levels of antiproliferative activity, and the significantly highest was recorded in the Chungyang cultivar ($P < .05$). As was already shown the highest antioxidant activity was recorded in this cultivar ($P < .05$). Other investigators have supported our findings.[20] Boivin *et al.*[20] studied inhibitory effects of extracts from 34 vegetables on the proliferation of eight different tumor cell lines. The extracts from cruciferous vegetables as well as those from vegetables of the genus *Allium* inhibited the proliferation of all cancer cell lines tested, whereas extracts from vegetables most commonly consumed in Western countries were less effective. The antiproliferative effect of vegetables was specific to cells of cancerous origin and was found to be largely independent of their antioxidant properties.

In conclusion, all Korean white lotus cultivars studied contain high quantities of basic nutritional compounds and exercise antioxidant and antiproliferative activities, which were different for each of them. Korean white lotus cultivars and in particular Chungyang are valuable as medicinal food. In order to receive the best results a combination of lotus cultivars has to be consumed.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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