

Antioxidant Activities of Methanol Extracts from Root Parts of Korean Salad Plants

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Abstract - Phenolics level, total flavonoid content, and antioxidant were determined from the methanol extracts of the eight medicinal plants using roots. Total phenolics were found as the highest levels in the methanol extracts of *Arctium lappa*, and followed by *Youngia sonchifolia* and *Cirsium japonicum*. Total amount of the each phenol compounds were detected in *C. japonicum* extracts (319.2 mg kg⁻¹) as the greatest component, and followed by *A. lappa* (96.3 mg kg⁻¹) and *Y. sonchifolia* (22.9 mg kg⁻¹). Total flavonoid content showed the highest amount in methanol extracts from *A. lappa* (68.1 mg 100 g⁻¹), and followed by *Y. sonchifolia* (11.2 mg 100 g⁻¹). *Lycoris radiata* extracts (87.2%) had the highest nitrite scavenging activity and followed by *A. lappa* (81.5%) and *Y. sonchifolia* (77.5%). Methanol extracts of *A. lappa* at 25 mg 100 g⁻¹ exhibited the highest DPPH radical scavenging activity by 90.8%, even though less activity than synthetic antioxidants Vitamin C or butylated hydroxytoluene (BHT). Level of polyphenols was highly correlated with antioxidative activity ($r^2 = 0.85$). The results suggest that several medicinal plants selected had the potent biological activities, and that their activities were differential depending on plant species.

Key words - Korean medicinal plants, methanol extracts, total phenolics, antioxidant activity

Introduction

Pharmaceutical foods from plant resources have received great attention as functional agents that improve modulation or biologically-active functions of human body. Plants have many phytochemicals with various bioactivities, including antioxidant, anti-inflammatory, anticancer, and antidiabetic activities. Recently, there has been a worldwide trend towards the use of the phytochemicals from wild plants. Phenolic compounds are considered as secondary metabolites that are synthesized by plants during normal development and in response to stress conditions such as infection, wounding, and UV radiation. These compounds occur ubiquitously in plants and are diversified group of phytochemicals derived from phenylalanine and tyrosine (Harborne and Turner, 1984; Shahidi and Maczk, 2004).

Free radical scavenging is the generally accepted mechanism for antioxidants inhibiting lipid oxidation. Antioxidants, inhibitors of lipid peroxidation, are important not only for

food protection but also for the defense of living cells against oxidative damage. The model of scavenging stable DPPH free radicals can be used to evaluate the antioxidative activities in a relatively short time (Brand-Williams et al., 1995) compared to other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen-donating ability (Blois, 1958). The toxic and otherwise unfavorable effects of synthesized food antioxidants have been widely noted. Phenolic compounds, such as butylated hydroxyanisole (BHA), BHT, and *tert*-butylhydroquinone (TBHQ), have been widely used as synthetic antioxidants in food lipid. Although those antioxidants are considered as safe natural antioxidants, they do not always provide effective protection against in vitro oxidation (Frankle, 1980). Nevertheless, the phenolic antioxidants are still used extensively as food antioxidants because of their excellent results and low cost. When slightly larger doses (50 mg/kg/day) of these phenolic antioxidants are administered to rodents and monkeys, however, certain pathological, enzyme and lipid alterations as well as carcinogenic effects have been observed (Branen, 1975). Therefore, research on other natural antioxidants

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has gained momentum as they are considered, rightly or wrongly, to pose no health risk to consumers (Wanasundara and Shahidi, 1994; Wanasundara *et al.*, 1997). The development of alternative natural antioxidants has, therefore assumed as increased importance. Many investigators have found different types of antioxidants in various sources of plants (Larson, 1988).

The antioxidants present in fruits and vegetables limit the free radical initiation in the body and lower the incidence of various cancers. Vegetables, fruits and whole grains contain a wide variety of phytochemicals that have the potential to interfere with the development of cancer. A number of studies have suggested that regular consumption of tea decreased the risk of various types of cancers (Yang *et al.*, 2000; Kathiyar and Mukthar, 1996). The Korean medicinal plants in this study have long been used as traditional seasoned salads, and were screened for biologically-active effects of functional foods (Cho, 2005). Earlier studies showed that extracts from *Areca catechu* var. *dulcissima* possess antidepressant properties (Dar and Khatoon, 2000). Au *et al.* (2001) suggested that the methanol extracts of *Paeonia suffruticosa* potentially inhibit human immunodeficiency virus (HIV)-1 integrase. Lee *et al.* (1997) reported that silymarin and silybin purified from *Silybum marianum* have potential inhibiting activities against oxidation of ¹²⁵I-LDL by macrophages and endothelial cells.

Therefore, the phytochemicals present in various Korean medicinal plants, may act as preventative or therapeutic agents similar to prescription drugs. The objective of this research was to determine total phenolic level, antioxidant activity, and cytotoxicity of methanol extracts from root parts of the 8 Korean medicinal plants.

Materials and Methods

Preparation of methanol extracts

Root parts of the eight Korean medicinal salad plants, *Lycoris radiata*, *Allium victorialis*, *Cirsium japonicum*, *Youngia sonchifolia*, *Arctium lappa*, *Lycoris aurea*, *Codonopsis lanceolata*, and *Lilium hansonii*, were harvested in a mountain area of the Suncheon City, Korea, at a vegetative stage on June, 2005. The samples were directly freeze-dried at -40°C for 5 days, ground with a Wiley mill to pass a 1-mm

screen, and stored in a refrigerator at 2°C until used. The samples were extracted with 95% methanol at room temperature. The extract was then filtered through a Whatman No. 1 filter paper. The collected filtrate was evaporated to dryness under vacuum at 40°C using a rotary evaporator (N-1000V-W, Eyela, Japan). After evaporation, the yield of dried extracts (methanol extract) was about 10% of the original plant sample. The methanol extracts from each plant were used for measuring DPPH radical scavenging activity, total phenolic content, and cytotoxicity.

Total phenol analysis

The concentration of total phenolics (TP) was measured using the Folin-Ciocalteu assay (Singleton and Rossi, 1965). Briefly, 5 mL of Nanopure water, 0.5 - 1.0 mL of sample, and 1.0 mL of Folin-Ciocalteu reagent were added to a 25 mL volumetric flask. The contents were mixed and allowed to stand for 5-8 min at room temperature. Next, 10 mL of a 7% sodium carbonate solution was added, and followed by the addition of Nanopure water filled to volume. Solutions were mixed and allowed to stand at room temperature for 2 h. Sample aliquots were filtered through a Whatman 0.45 µm poly (tetrafluoroethylene) filter prior to the determination of TP concentration using a UV-1650 spectrophotometer (Shimadzu, Japan) monitoring 640 nm. TP content was standardized against ferulic acid and expressed as mg kg⁻¹ of ferulic acid equivalents (FAE). The linearity range for this assay was determined as 0.5-5.0 mg/L FAE (R² = 0.9990), giving an absorbance range of 0.050-0.555 AU.

Identification and quantification of phenolic acids

The dried methanol extracts were redissolved in HPLC grade MeOH to give 1,000 ppm for HPLC analysis. The standard phenol compounds used for HPLC analysis were coumarine, 3-hydrocinnamic acid, *p*-coumaric acid, salicylic acid, ferulic acid, caffeic acid, chlorogenic acid, and syringic acid (Aldrich Co., USA). The chemicals were purchased as high purity standards and the used solvents were HPLC spectral grade. The compounds were identified by a high-performance liquid (HPLC) using SPP 10AVP (Shimadzu, Tokyo, Japan) with a flow rate of 1 mL min⁻¹, the column was CAPCELL PAK C18 SG120 (4.6 × 250 mm) and an autoinjector with a 10 µL sample loop was employed. The

mobile phase consisted of water, methanol and acetic acid in the ratio of 12:15:1 volume, respectively. The UV detector wavelength was set at 275 nm. Standard compounds were chromatographed alone and as mixtures. Retention times for the standard compounds and the major peaks in the extract were recorded. The compounds were identified by retention times or standard addition, and amounts were calculated by comparing peak area with those of standards.

Total flavonoid analysis

The total flavonoid content of methanol extracts from root parts of 8 Korean medicinal plants was determined according to colorimetric method as described by Zou *et al* (2004). In brief, 0.5 ml of sample solution was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5% NaNO₂ solution. After 6 min of incubation, 0.15 ml of 10% AlCl₃ solution was added and then allowed to stand for 6 min, followed by adding 2 ml of 4% NaOH solution to the mixture. Immediately after water was added to the sample to bring the final volume to 5 ml, the mixture was thoroughly mixed and allowed to stand for another 15 min. The mixture absorbance was determined at wavelength 510 nm. The total flavonoid content was expressed in milligrams of rutin equivalents per gram of plant extracts.

Nitrate scavenging activity

This assay was carried out as described by Saha, Lajis, and Israf (2004) and Zhang, Nie, Tao, and Ye (2002) with some modifications. The pigment extract was diluted with the distilled water to a suitable concentration for analysis. Three ml of pigment sample was per in the tube (10 ml), and then 2 ml of citric acid buffer (pH 3.0) and 0.1 ml of 200 µg/ml NaNO₂ were added, respectively. Finally, water was added up to 10 ml. The mixture was immediately incubated for 60 min in the water bath at 37°C. Then equal volume of Griess reagent (1% sulfanilamide, 0.1% N-(1-naphthyl)-ethylene diamine hydrochloride, 2.5% H₃PO₄) was added to the above mixture. The absorbance was determined by UV/Vis spectrophotometer (Dojin, Japan) after 10 min at 538 nm. At the same time the control (without NaNO₂) and standard (without NaNO₂ and without pigment sample) were also measure. Vc and butylated hydroxyanisole (BHA) were used

as the positive control compounds. NaNO₂ scavenging activity (Sa) was calculated using the following equation: $Sa (\%) = \{OD_s - (OD_p - OD_c)\} / OD_s \times 100\%$. Where Sa is the NaNO₂ scavenging rate of tested sample (%), OD_s is the OD value of standard, OP_p is the OD value in the presence of tested sample, OD_c is the OD value of control.

DPPH radical scavenging activity

DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay was carried out according to the procedure described by Blois (1958). Each methanol extract at various concentrations (3.1, 6.3, 12.5, 25, and 50 mg 100 g⁻¹) was added to a 1.5 x 10⁻⁴ M solution of DPPH in methanol and the reaction mixture was shaken vigorously. The amount of DPPH remaining was determined at 520 nm, and the radical scavenging activity was obtained from the following equation: $Radical\ scavenging\ activity (\%) = \{(OD_{control} - OD_{sample}) / OD_{control}\} \times 100$. The antioxidant activity of plant extracts was partially expressed as IC₅₀, which was defined as the concentration (in mg 100 g⁻¹) of extract required to inhibit the formation of DPPH radicals by 50%.

Results and Discussion

Total phenolics content

Total phenolic content showed the highest amount in methanol extracts from *A. lappa* (11.6 mg 100 g⁻¹), and followed by *Y. sonchifolia* (8.1 mg 100 g⁻¹) and *C. japonicum* (5.5 mg 100 g⁻¹). However, *L. radiata*, *C. lanceolata*, and *L. hansonii* extracts were very low (Fig. 1). The result was highly consistent with the finding of DPPH radical scavenging activity (Velioglu *et al.*, 1998). Zhou and Yu (2006) also reported that total phenolic content of the tested vegetable extracts was correlated with the DPPH radical scavenging activity, suggesting that total phenolics can play a major role in the antioxidant activity of plant materials.

Identification and quantification of phenolic acids

The major antioxidant substances, coumarine, 3-hydrocinnamic acid, *p*-coumaric acid, salicylic acid, ferulic acid, caffeic acid, chlorogenic acid, and syringic acid present in the 8 Korean medicinal plant species were analyzed by HPLC using

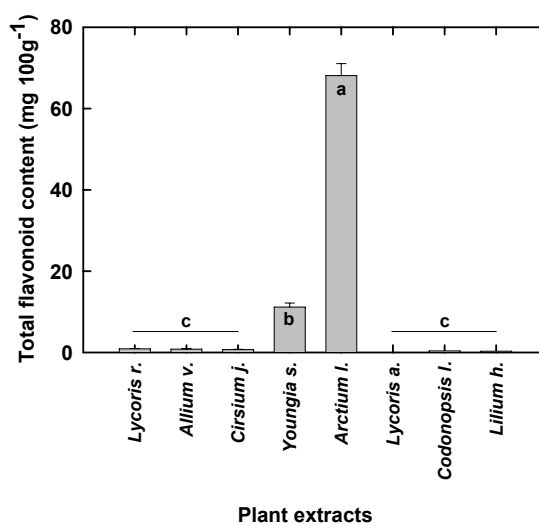
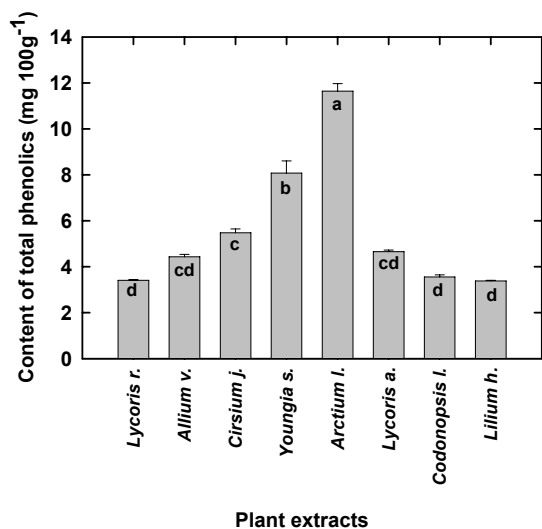


Fig. 1. Total phenolic content of methanol extracts from the root parts of the eight Korean medicinal plants. Means with the same letter in a graph are not significantly different at $p < 0.05$. Bars represent SE.

Fig. 2. Total flavonoid content of methanol extracts from the root parts of the eight Korean medicinal plants. Means with the same letter in a graph are not significantly different at $p < 0.05$. Bars represent SE.

standard compound. Total amount of the compounds were detected in *C. japonicum* extracts (319.2 mg kg⁻¹) as the greatest component, and followed by *A. lappa* (96.3 mg kg⁻¹) and *Y. sonchifolia* (22.9 mg kg⁻¹). Chlorogenic acid was detected in *C. japonicum* extracts (38.4 mg kg⁻¹) as the greatest amount. Especially, *C. japonicum* have the highest amount of salicylic acid (Table 1). Radical scavenging effect of phenolic compounds isolated from natural sources has been widely studied (Yoshida *et al.*, 1989). The antioxidative potency and phenolic acids are generally inter-related. These

phenolic compounds react with the free radicals formed during autoxidation, and generate a new radical which is stabilized by the resonance effect of the aromatic nucleus (Cuvelier *et al.*, 1992).

Total flavonoid analysis

Total flavonoid content showed the highest amount in methanol extracts from *A. lappa* (68.1 mg 100 g⁻¹), and followed by *Y. sonchifolia* (11.2 mg 100 g⁻¹). However, in other plant extracts the content was very low (Fig. 2). Plant

Table 1. Contents of phenolic acids in the methanol extracts from the eight Korean medicinal plants using roots.

Plant species (Extracts)	Phenolic acids (mg kg ⁻¹)								
	COU*	3HC	PCO	SAL	FER	CAF	CHL	SYR	Total
<i>Lycoris radiata</i>			4.7			4.3	3.2		12.3
<i>Allium victorialis</i>		2.5	3.2				13.3		19.1
<i>Cirsium japonicum</i>		3.1		293.9	7.4		14.9		319.2
<i>Youngia sonchifolia</i>		7.8			2.1		13.1		22.9
<i>Arctium lappa</i>	4.9	14.2	3.4	20.9	3.5	11.1	38.4		96.3
<i>Lycoris aurea</i>	4.5								4.5
<i>Codonopsis lanceolata</i>	4.6							9.7	14.3
<i>Lilium hansonii</i>	4.1					5.3			9.4

* COU: coumarine, 3HC: 3-hydrocinnamic acid, PCO, p-coumaric acid, SAL: salicylic acid, FER: ferulic acid, CAF: caffeic acid, CHL: chlorogenic acid, and SYR: syringic acid.

tissues contain a wide variety of compounds with antioxidant activity. Phenolic compounds (flavonoids and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids and amines), carotenoids, lignans and terpenes were reported to possess antioxidative activity in suppressing the initiation or propagation of the chain reactions (Hall and Cuppett, 1997). Flavonoids and phenolic compounds are the main antioxidative compounds of fruits and vegetables (Huang *et al.* 1998). In this study, however, the magnitude of antioxidative potency varies with the type of extracts. This could be due to the different in concentration and type of antioxidative compounds present in these extracts.

Nitrite scavenging activity

The plant extracts exhibited a plant species-dependent radical scavenging activity by inhibiting nitrite. *L. radiata* extracts (87.2%) was the highest nitrite scavenging activity and followed by *A. lappa* (81.5%) and *Y. sonchifolia* (77.5%). However, *L. radiata*, *C. lanceolata*, and *L. hansonii* extracts showed very low activities (Fig. 3).

DPPH radical scavenging activity

Methanol extracts of *A. lappa* had the highest DPPH radical scavenging activity, and followed by *C. japonicum*, and *Y. sonchifolia*, indicating IC₅₀ values of 6.1, 35.1, and 56.0 mg 100 g⁻¹, respectively (Table 2). These values were

lower than those of synthetic antioxidants Vitamin C and BHT, with IC₅₀ values of < 3.1 and 11.3 mg 100 g⁻¹, respectively. Methanol extracts of *A. lappa* at 25 mg 100 g⁻¹ exhibited the highest DPPH radical scavenging activity by 90.8%. However, other extracts showed lower values in activity. All samples of plant species showed DPPH radical scavenging activity in a dose-dependent manner. The present results noted that various compounds that cause antioxidant

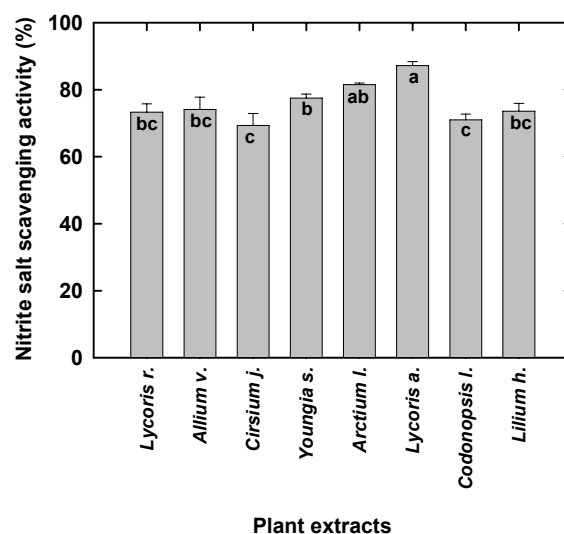


Fig. 3. Nitrite scavenging ability of methanol extract from the root parts of the eight Korean medicinal plants using roots. Means with the same letter in a graph are not significantly different at $p < 0.05$. Bars represent SE.

Table 2. DPPH radical scavenging activity of methanol extracts from the eight Korean traditional salad plants using roots. Their activities were compared with synthetic antioxidants, Vitamin C and BHT.

Scientific name (Extracts)	Extract concentration, mg 100 g ⁻¹					IC ₅₀ [†] value
	3.1	6.3	12.5	25	50	
<i>Lycoris radiata</i>	0.0	0.0	1.2	3.1	7.2	>50
<i>Allium victorialis</i>	9.5	12.4	14.3	20.6	31.0	>50
<i>Cirsium japonicum</i>	7.5	12.9	23.1	40.9	71.1	35.1
<i>Youngia sonchifolia</i>	13.3	13.3	20.8	37.5	61.3	56.0
<i>Arctium lappa</i>	35.5	52.7	85.5	90.8	91.1	6.1
<i>Lycoris aurea</i>	9.9	12.9	17.2	20.6	27.0	>50
<i>Codonopsis lanceolata</i>	2.8	4.3	7.4	10.6	16.3	>50
<i>Lilium hansonii</i>	4.0	4.1	7.3	12.6	21.4	>50
Vitamin C	81.8	96.1	96.1	96.7	96.9	< 3.1
BHT	15.6	33.5	55.2	81.3	92.4	11.3

[†] Extract concentrations, which show 50% activity of DPPH radical scavenging, were determined by interpolation.

activity could be produced with different amount from plant species. Lee *et al.* (2003) reported that the methanol extracts of nine medicinal plants traditionally used in Chinese medicine were screened for antioxidant activity versus resveratrol, and that relatively high levels of DPPH radical scavenging activity were detected in extracts of *Areca catechu* var. *dulcissima*, *Paeonia suffruticosa* and *Cinnamomun cassia* ($IC_{50} < 6.0 \mu g mL^{-1}$). Especially, they reported that the extracts of *Areca catechu* var. *dulcissima* showed higher antioxidant activity than resveratrol in all experiments.

Correlation coefficient between the extracted polyphenols with methanol and antioxidative activity was about 0.85 (Fig. 4). Relatively high correlation between above mentioned variables in the studied extracts was compared with tea, propolis and *U. davidiana* extracts (Jastrzebski *et al.*, 2007; Turkmen *et al.*, 2006; Jung *et al.*, 2008).

In conclusion, methanol extracts from several plants among the eight Korean traditional salad plants using roots showed high antioxidant activity, through measurement of DPPH free radical scavenging activity. *A. lappa* showed the highest DPPH free radical scavenging activity with high levels of total phenolics and flavonoid. The medicinal plants dose-dependently increased the biological activities, *in vitro*. Results also showed that total phenolics level was highly correlated with the free radical scavenging activity. Compounds that cause the DPPH free radical scavenging activities could be produced with different amounts and types of phytochemicals depending on plant species. Such differences might be related to specific compounds being produced in larger quantities in certain plant species. Therefore, the eight Korean medicinal plants using root parts had the potent

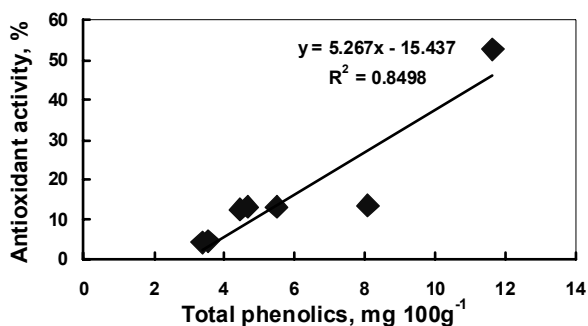


Fig. 4. Correlation between the polyphenols amount and antioxidant activity (%) in the eight Korean medicinal plants.

antioxidant activity with important values for an alternative based on natural plant extracts.

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