Antioxidant Activity of Crude Extract and Pure Compounds of *Acer ginnala* Max.

Seong Soo Han, Seog Cho Lo, Yong wa Choi,† Jin Ho Kim,† and Seung Hwa Baek‡,*

Department of Agricultural Chemistry, College of Life Science and
†Department of Plant Resources, Sangju National University, Kyungbuk, Sangju 742-711, Korea
‡Department of Herbal Resources, Professional Graduate School of Oriental Medicine and
Institute of Basic Natural Sciences, Wonkwang University, Iksan 570-749, Korea
Received August 20, 2003

Key Words : Antioxidant activity, *Acer ginnala* Max., DPPH radical scavenging activity

Natural antioxidants such as α-tocopherol and L-ascorbic acid are widely used because they are seen as being safer and causing fewer adverse reactions, but their antioxidant activities are, however, lower than those of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Hence, the need exists for safe, economic antioxidants with high activity from natural sources to replace these synthetic chemicals. The antioxidant compounds present in edible plants have recently been promoted as food additives because they display little or no toxic side effects. During our search for antioxidant compounds from natural products, we carried out antioxidative assays on forty-five medicinal plants. To evaluate the antioxidant activity, the present study was initiated to compare the radical scavenging effect of plants extracts and two phenolic constituents of *Acer ginnala* Max. (*A. ginnala* Max.) with the simple and stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, which shows a strong absorption band at 517 nm.[1] Of the 45 plants tested, the methanol extract of the leaves of *A. ginnala* Max. showed the strongest antioxidant activity. The aim of our present work is to screen forty-five medicinal plants for antioxidant activity, and further identify two active compounds from *A. ginnala* Max. that show the strongest free radical scavenging activity.

Experimental Section

Plant Material. Forty-five plants were collected from Garden in Wonkwang University, in April 1997 and identified by one of the authors (S. S. Han). Voucher specimens were deposited in the herbatorium of the Division of Plant Resources, Wonkwang University, Korea. Ground dried leaves of each plant (700.0 g) were exhaustively extracted with methanol (2×7.0 L) under reflux (4 hrs). The extracts were concentrated to dryness in vacuo at 40 °C to produce the methanol extract.

Analytical Methods. Column chromatography SiO_2, 70-230 mesh and ODS gel, 70-230 mesh and Sephadex LH-20 75-150 mesh were adopted. High performance liquid chromatography was performed on an YMC instrument using a μBondapak C18 reverse phase column (20 mm × 250 mm) (Gilson-France, Villiers-Le-Bel, France). ^{1}H-NMR and ^{13}C-NMR spectra were obtained using CD_3OD as solvent with a Bruker DRX 300 spectrometer (GMBH, Germany). FAB-MS spectra were recorded with a Kratos Concept-1S mass spectrometer (Kratos, England) and EI-MS spectra were recorded with a Hewlett Packard MS-engine 5989A mass spectrometer (Hewlett Packard, USA). Ultraviolet and visible absorption spectra were measured with a Shimadzu Double Beam Spectrophotometer (Varian DMS 200, Shimadzu, Japan). DPPH and α-tocopherol (vitamin E) were purchased from Sigma Chemical Co (St. Louis, Mo.). BHA was purchased from Kanto Chemical Co. (Tokyo).

Measurement of Antioxidant Activity (RC_{50}). Four mL of methanol solution of each test extracts at various concentrations (2.5-120 μg/mL) were added to a 1 mL solution of DPPH (1.5×10^{-4} M) in methanol, and the reaction mixture was shaken vigorously.[1] After storage at room temperature for 30 min in air, the absorbance of DPPH was determined by spectrophotometer at 517 nm, and the radical scavenging activity of each sample was expressed by the ratio the of lowering of the absorption of DPPH (%), relative to the absorption (100%) of DPPH solution in the absence of test sample (control). The mean values were obtained from triplicate experiments.

Isolation, Purification and Identification of Aertannin (1) and Methyl Gallate (2). The methanol extract (185.0 g) of *A. ginnala* Max. showed the strongest antioxidant activity. It was dissolved in H_2O and further partitioned with *n*-hexane, chloroform, ethyl acetate, *n*-butanol and H_2O, successively. The ethyl acetate soluble fraction (57.5 g) showed antioxidative activity (RC_{50} values of 10.9 μg/mL). Therefore, the ethyl acetate soluble fraction was subjected to Si gel column chromatography using a gradient $CHCl_3$-MeOH solvent system. The active fraction (40% MeOH/$CHCl_3$, 5.58 g) was successively separated by ODS and Sephadex LH-20 column chromatography using a gradient MeOH-H_2O solvent system and HPLC using a reverse-phase column and a MeOH-H_2O solvent system to yield compound **1** (11.2 mg, 0.019%) and compound **2** (30 mg, 0.052%). Acertannin thus obtained was identified by comparison of their spectral data (TLC, MS, ^{1}H and ^{13}C-NMR, IR) with those published.[2-5] Methyl gallate thus obtained was identified by comparison of their spectral data (TLC, MS, ^{1}H and

*Author for Correspondence: Tel: +82-63-850-6225; Fax: +82-63-841-4893; e-mail: shbaek@wonkwang.ac.kr

^{13}C-NMR, IR) with those published or by direct comparison with an authentic sample.[2-5]

Table 1. Antioxidant activities of plant extracts on DPPH free radical

Scientific name	Part used	IC_{50} (μg/mL)[a]
Abeloiphyllum distichum	Leaves	60
Acanthopanax sieboldianum	Leaves	>100
Acer ginnala	Leaves	15
Aucuba japonica	Leaves	–
Aucuba japonica for. *variegata*	Leaves	–
Allium tuberosum	Leaves	–
Allium monanthum	Leaves	–
Aster scanver	Leaves	–
Berberis poiretii	Leaves	–
Betula davurica	Leaves	–
Bidens parviflora	Leaves	–
Brassica junceae	Flower	–
Brassica junceae	Root	37
Brassica junceae	Leaves	42
Callicarpa japonica	Leaves	57
Carpinus laxiflora	Leaves	33
Celastrus orbiculatus	Leaves	–
Corylus heterophylla var. *thunbergii*	Leaves	90
Daphniphyllum macropodum	Leaves	–
Elaeagnus umbellata var. *coreana*	Leaves	–
Eucommia ulmoides	Leaves	–
Fraxinus mandshurica	Leaves	–
Fraxinus rhychophlla	Leaves	70
Grewia biloba var. *parviflora*	Leaves	–
Ilex cretana	Leaves	–
Kalopanax pictus	Leaves	–
Koelreateria paniculata	Leaves	–
Ligustrum floilsum	Leaves	–
Lindera erythrocarpa	Leaves	70
Lonicera praeflorens	Leaves	–
Meliosma myriantha	Leaves	–
Nandina domestica	Leaves	–
Phellodendron amurense	Leaves	–
Phodotypos scandens	Leaves	–
Pyrus pyrifolia	Leaves	–
Rhamnas darvrica	Leaves	–
Rubus coreanus	Stem	–
Rubus coreanus	Root	–
Sambucus canadensis	Leaves	–
Sambucus nigra	Leaves	–
Sambucus williamsii	Leaves	100
Sorphora japonica	Leaves	–
Sorbaria sorbifolia var. *stellipila*	Leaves	–
Stewartia koreana	Leaves	28
Styrax japonica	Leaves	–
Syringa dilatana	Leaves	–
Viburnum saragentii for. *sterile*	Leaves	–

[a]Amount required for 50% reduction of DPPH free radical after 30 min. – ; >100.

Results and Discussion

A methanol solution of DPPH free radical was found to be stable for more than 60 min by spectrophotometry at 517 nm of an 80 μg/mL solution. The radical scavenging effects of 45 medicinal plant extracts and fractions of *A. ginnala* were then measured spectrophotometrically for DPPH free radical. The control intensity (absorbance of extracts, fractions and pure compounds) was taken as 100%, and the percentage intensity was calculated. The concentration for 50% inhibition is shown in Tables 1 and 2. As shown in Table 1, twelve plant extracts examined in this study exhibited scavenging effects on DPPH free radical.[1] The radical scavenging effects of 12 species was higher than all others in plants. *A. ginnala* Max. showed the strongest effects. The RC_{50} of this crude methanol extract was almost equivalent to BHA. The methanol extract of *A. ginnala* Max. was further partitioned with *n*-hexane, chloroform, ethyl acetate, *n*-butanol and water, successively and the ethyl acetate fraction had the strongest radical scavenging effect against DPPH free radical. This fraction was further purified to obtain two main

Table 2. Antioxidant activities of crude extract and compounds isolated from the leaves parts of *A. ginnala* Max. on DPPH free radical

Tested material	IC_{50} (μg/mL)[a]
MeOH extract	15.0
Hexane fr.	46.3
$CHCl_3$ fr.	15.0
EtOAc fr.	10.9
BuOH fr.	16.3
H_2O fr.	50.0
Methyl gallate (**2**)	2.8
Acertannin (**1**)	3.5
BHA	14.0
α-Tocopherol	12.0

[a]Amount required for 50% reduction of DPPH free radical after 30 min.

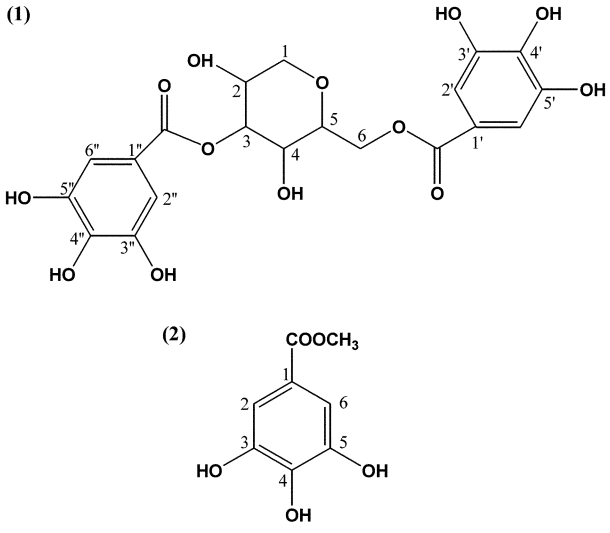

Figure 1. The structures of acertannin (**1**) and methyl gallate (**2**).

bioactive compounds, acertannin (**1**) and methyl gallate (**2**) (Fig. 1). Methyl gallate (**2**) and acertannin (**1**) thus obtained were identified by comparison of their spectral data (TLC, MS, ^{1}H and ^{13}C-NMR, IR) with those published or by direct comparison with an authentic sample.[2-5] Figure 1 showed the chemical structure of acertannin (**1**) and methyl gallate (**2**). Table 2 shows these two bioactive compounds, methyl gallate (**2**) and acertannin (**1**) are more effective than α-tocopherol or BHA.

Free radicals and other reactive oxygen species are considered to be important causative factors in the development of diseases of aging such as neurodegenerative diseases cancer, and cardiovascular diseases. Phytochemicals have long been recognized to possess many properties including antioxidant, antiallergic, anti-inflammatory, antiviral, antiproliferative and anticarcinogenic.[6] The sap of *A. ginnala* Max. has been used for treatment of stomachic and diarrhea.[7] The plant, *A. ginnala* Max. (family Aceraceae) grows as a perennial herb and is widely distributed in Korea. Among the plants examined, *Acer ginnala, lllicium* and *Cornus macrophylla* exerted the most strong inhibitory activity on aldose reductase.[8] Phytochemical studies of this plant have so far yielded poligalitol,[9] acertannin,[3,10] and polygagallin.[2] Two bioactive compounds have been isolated from related Aceraceae species;[11] for example, methyl gallate (**2**) has been isolated from *A. saccharium*.[11] Bailey *et al.*[11] reported that methyl gallate (**2**) from *A. saccharinum* showed antitumor activity. This study indicate that the methanolic extract of *A. ginnala* Max. and its components may be useful an antioxidant. Potential clinical applications for treatment of tumor warrant the herb worthy of further study.

Acknowledgments. This research was supported by Wonkwang University in 2003.

References

1. Choi, J. S.; Lee, J. H.; Park, H. J.; Kim, H. G.; Young, H. S.; Mun, S. I. *Kor. J. Pharmacogn.* **1993**, *24*, 299.
2. Han, K. D. *J. Pharm. Soc. Korea* **1962**, *6*, 1.
3. Bock, K. N.; Faurschou, L. C.; Jensen, S. R. *Phytochemistry* **1980**, *19*, 2031.
4. Park, W. Y. *Ph.D. Thesis*; Chungbuk National University: Korea, 1993; p 45.
5. Kuroyanagi, M.; Yamamoto, Y.; Fukushima, S.; Ueno, A.; Noro, T.; Miyase, T. *Chem. Pharm. Bull.* **1982**, *30*(5), 1602.
6. Eastwood, M. A. *Quarterly J. Med.* **1999**, *92*, 527.
7. Choi, O. J. *Components and Application of Herbal Medicine*; Ilwealseogak: Seoul, 1994; p 378.
8. Kim, H. Y.; Oh, J. H. *Biosci. Biotechnol. Biochem.* **1999**, *63*, 184.
9. Kim, J. H. *Kor. J. Pharmacogn.* **1983**, *14*, 4.
10. Woo, L. K. *J. Pharm. Soc. Korea* **1962**, *6*, 11.
11. Bailey, A. E.; Asplund, R. O.; Ali, M. S. *J. Nat. Prod.* **1986**, *49*, 1149.