

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

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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 herbarium of the Division of Plant Resources, Wonkwang University, Korea. Ground dried leaves of each plant (700.0 g) were exhaustively extracted with methanol (2 \times 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 SiO₂, 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 μ Bondapak C18 reverse phase column (20 mm \times

250 mm) (Gilson-France, Villiers-Le-Bel, France). ¹H-NMR and ¹³C-NMR spectra were obtained using CD₃OD 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 (RC₅₀). Four mL of methanol solution of each test extracts at various concentrations (2.5-120 μ g/mL) were added to a 1 mL solution of DPPH (1.5 \times 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 H₂O and further partitioned with *n*-hexane, chloroform, ethyl acetate, *n*-butanol and H₂O, successively. The ethyl acetate soluble fraction (57.5 g) showed antioxidative activity (RC₅₀ values of 10.9 μ g/mL). Therefore, the ethyl acetate soluble fraction was subjected to Si gel column chromatography using a gradient CHCl₃-MeOH solvent system. The active fraction (40% MeOH/CHCl₃, 5.58 g) was successively separated by ODS and Sephadex LH-20 column chromatography using a gradient MeOH-H₂O solvent system and HPLC using a reverse-phase column and a MeOH-H₂O solvent system to yield compound 1 (11.2 mg, 0.019%) and compound 2 (30 mg, 0.052%). Aertannin thus obtained was identified by comparison of their spectral data (TLC, MS, ¹H and ¹³C-NMR, IR) with those published.²⁻⁵ Methyl gallate thus obtained was identified by comparison of their spectral data (TLC, MS, ¹H 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
<i>Abelophyllum distichum</i>	Leaves	60
<i>Acanthopanax sieboldianum</i>	Leaves	>100
<i>Acer ginnala</i>	Leaves	15
<i>Aucuba japonica</i>	Leaves	—
<i>Aucuba japonica</i> for. <i>variegata</i>	Leaves	—
<i>Allium tuberosum</i>	Leaves	—
<i>Allium monanthum</i>	Leaves	—
<i>Aster scamver</i>	Leaves	—
<i>Berberis poiretii</i>	Leaves	—
<i>Betula davurica</i>	Leaves	—
<i>Bidens parviflora</i>	Leaves	—
<i>Brassica juncea</i>	Flower	—
<i>Brassica juncea</i>	Root	37
<i>Brassica juncea</i>	Leaves	42
<i>Callicarpa japonica</i>	Leaves	57
<i>Carpinus laxiflora</i>	Leaves	33
<i>Celastrus orbiculatus</i>	Leaves	—
<i>Corylus heterophylla</i> var. <i>thunbergii</i>	Leaves	90
<i>Daphniphyllum macropodum</i>	Leaves	—
<i>Elaeagnis umbellata</i> var. <i>coreana</i>	Leaves	—
<i>Eucommia ulmoides</i>	Leaves	—
<i>Fraxinus mandshurica</i>	Leaves	—
<i>Fraxinus rhychophilla</i>	Leaves	70
<i>Grewia biloba</i> var. <i>parviflora</i>	Leaves	—
<i>Ilex cretana</i>	Leaves	—
<i>Katopanax pictus</i>	Leaves	—
<i>Koelreuteria paniculata</i>	Leaves	—
<i>Ligustrum floissun</i>	Leaves	—
<i>Lindera erythrocarpa</i>	Leaves	70
<i>Lonicera praeflorens</i>	Leaves	—
<i>Meliosma myriantha</i>	Leaves	—
<i>Nandina domestica</i>	Leaves	—
<i>Phellodendron amurense</i>	Leaves	—
<i>Phodotypos scandens</i>	Leaves	—
<i>Pyrus pyrifolia</i>	Leaves	—
<i>Rhamnus darvrica</i>	Leaves	—
<i>Rubus coreanus</i>	Stem	—
<i>Rubus coreanus</i>	Root	—
<i>Sambucus canadensis</i>	Leaves	—
<i>Sambucus nigra</i>	Leaves	—
<i>Sambucus williamsii</i>	Leaves	100
<i>Sorphora japonica</i>	Leaves	—
<i>Sorbaria sorbifolia</i> var. <i>stellipila</i>	Leaves	—
<i>Stewartia koreana</i>	Leaves	28
<i>Styrax japonica</i>	Leaves	—
<i>Syringa dilatana</i>	Leaves	—
<i>Viburnum saragentii</i> for. <i>sterile</i>	Leaves	—

^aAmount 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 RC₅₀ 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 ₂ O fr.	50.0
Methyl gallate (2)	2.8
Acertannin (1)	3.5
BHA	14.0
α-Tocopherol	12.0

^aAmount required for 50% reduction of DPPH free radical after 30 min.

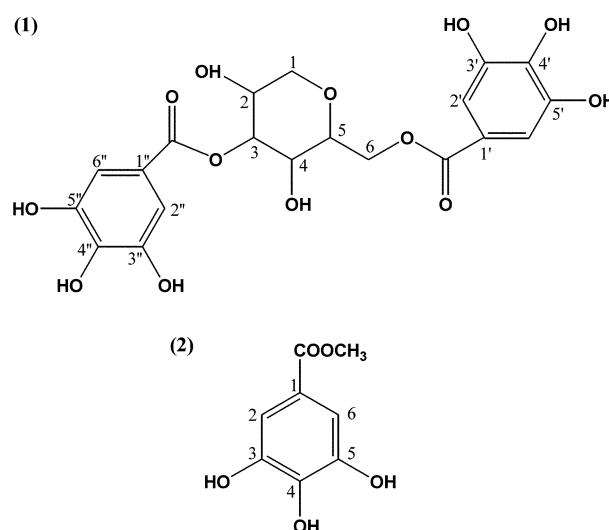


Figure 1. The structures of accertannin (1) and methyl gallate (2).

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, ^1H 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, anti-allergic, 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*, *Illicium* 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 polygagallicin.² Two bioactive compounds have been isolated from related Aceraceae species:¹¹ for example, methyl gallate (2) has been isolated from *A. saccharinum*.¹¹ Bailey *et al.*¹¹ reported

that methyl gallate (2) from *A. saccharinum* showed anti-tumor activity. This study indicates that the methanolic extract of *A. ginnala* Max. and its components may be useful as an antioxidant. Potential clinical applications for treatment of tumor warrant the herb worthy of further study.

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