Biological Activity of Bioactive Components from *Acer ginnala* Max.

Yong Hwa Choi,[†] Sung Soo Han, Hyun Ok Lee,[‡] and Seung Hwa Baek[*]

Department of Agricultural Chemistry, College of Life Sciences and Department of Herbal Resources, Professional Graduate School of Oriental Medicine and Institute of Basic Natural Science, Wonkwang University, Iksan 570-749, Korea
[*]E-mail: shbaek@wonkwang.ac.kr
[†]Department of Plant Resources, Sangju National University, Sangju 742-711, Korea
[‡]Department of Dental Hygiene, Wonkwang Health Science College, Iksan 570-750, Korea
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The sap of *A. ginnala* Max. has been used for treatment of stomachic and diarrhea.[1] 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.[2] Phytochemical studies of this plant have so far yielded poligalitol,[3] acertannin,[4,5] and polygagallin.[6] 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 twelve species was higher than all others in plants. *A. ginnala* Max. showed the strongest effects. The IC_{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 bioactive compounds, acertannin (**1**) and methyl gallate (**2**) (Fig. 1). Acertannin (**1**) and methyl gallate (**2**) 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.[2-5,7] Figure 1 showed the chemical structure of acertannin (**1**) and methyl gallate (**2**). Table 2 shows these two bioactive compounds, acertannin (**1**) and methyl gallate (**2**) are more effective than α-tocopherol or BHA. Bioactive compounds (**1** and **2**) were found to have effective *in vitro* cytotoxic activity with IC_{50} values in the range 6.7-69.8 μM when evaluated against tumor cell lines. Methyl gallate (**2**) was the main cytotoxic component in the extract with an IC_{50} of 6.70 μM against skin melanoma cell lines (SK-MEL-2). Acertannin (**1**) was also cytotoxic with an IC_{50} of 22.12 μM (Table 3). These compounds were found to have effective *in vitro* antimicrobial activity with MIC values in the range 50-1,600 μg/mL when evaluated against microorganisms.

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 biological 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 (10-20 g) were exhaustively extracted with methanol (200 mL) 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 (IC_{50}, μg/mL). 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

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. *thunb.*	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]Concentration required for 50% reduction of DPPH free radical after 30 min. – ; > 100

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.

Table 2. Antioxidant activities of crude extract and phenol 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
Acertannin (**1**)	3.5
Methyl gallate (**2**)	2.8
BHA	14.0
α-Tocopherol	12.0

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

Table 3. Cytotoxic activity of acertannin (**1**) and methyl gallate (**2**) on tumor cell lines by the SRB assay

Tumor cells	IC_{50} (μM)[a]	
	1	**2**
A549	69.80	36.86
SK-OV-3	44.90	13.29
SK-MEL-2	22.12	6.70
XF498	29.24	30.14
HCT15	59.29	38.23

[a]IC_{50} represents the concentration of a compound required for 50% inhibition of cell growth.

Isolation, purification and identification of acertannin (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 (IC_{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 (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100% MeOH in $CHCl_3$, 1,000 mL each other). The active fraction (40% MeOH/$CHCl_3$, 5.58 g) was successively separated by ODS column chromatography with a gradient MeOH-H_2O (0, 10, 20, 30, 40% MeOH in H_2O, 500 mL each other) as eluents. The active subfraction (20% MeOH/H_2O, 2.36 g) was further seperated with Sephadex LH-20 column chromatography using a MeOH-H_2O solvent (4 : 1, v/v) and HPLC using a reverse-phase column with a eluent (45% MeOH in H_2O) 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.[2,4,8,9]

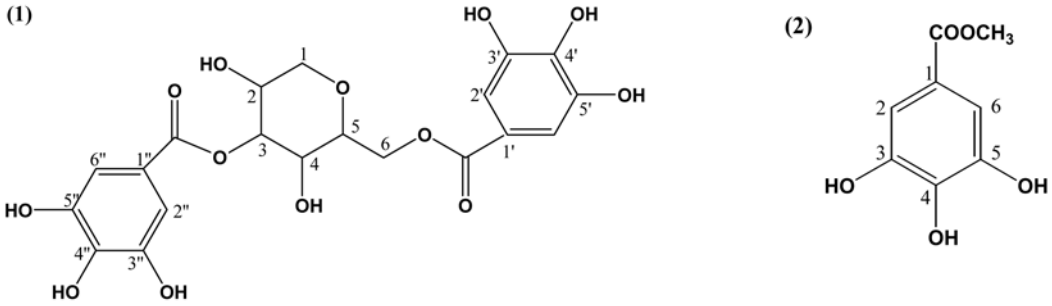

Figure 1. The structures of acertannin (**1**) and methyl gallate (**2**).

Table 4. Antimicrobial activity of acertannin (**1**) and methyl gallate (**2**)[a]

Tested microoganism	MIC (μg/mL)			
	1	**2**	KT	AM
Gram positive bacteria				
Streptococcus mutans JC-2	>1,600	>200	50	3.125
Staphylococcus epidermidis ATCC 12228	800	>200	50	50
Streptococcus aureus ATCC 201213	1,600	>200	>200	3.125
Gram negative bacteria				
Pseudomonas putida KCTC 8729	>1,600	200	50	>200
Salmonella typhimurium KCTC 1925	>1,600	100	25	>200
Pseudomonas aeruginosa KCTC 1636	–	>200	100	50
Fungus				
Candida albicans ATCC 1940	–	50	50	>200

[a]Acertannin; **1**, methyl gallate; **2**, ketoconazole; KT, ampicillin; AM, –, not determined. [b]Data are the average of three experiments.

Methyl gallate thus obtained was 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.[2,4,8,9]

Microorganisms. The microorganisms used included : *Streptococcus aureus* (ATCC 29213), *Streptococcus mutans* (JC-2), *Staphylococcus epidermidis* (ATCC 12228), *Pseudomonas aeruginosa* (KCTC1636). *Pseudomonas putida* (KCTC 8729), *Candida albicans* (KCTC 1940).

Screening for antimicrobial activity. The dried plant extracts were dissolved in 10% aqueous dimethyl sulfoxide (DMSO) to a final concentration 2,000 μg/mL and sterilized by filtration through a 0.45 μm membrane filter. Antimicrobial tests were then carried out by the agar serial dilution method.[10,11] Each of several concentrations of a tube of molten agar, which is then mixed, poured into a the petri plates, and allowed to solidify. The organisms containing 10^6 bacterial cells/mL or 10^8 yeast cells/mL were inoculated in to the petri plates. After the plates have been incubated for 24 hrs at 37 °C for bacteria and for five-seven days at 22 °C for fungi, the lowest concentration of methanolic extracts that inhibits grows of the organisms, was determined as the MIC of the antimicrobial agents. Ampicillin served as positive controls for *S. aureus*, *S. mutans*, and *S. epidermidis*, consequently, whereas, ketoconazole served as a negative control for *P. aeruginosa*. and *P. putida*. Each test was carried out in triplicate experiments.

Measurement of cytotoxicity assay. Tumor cells were seeded at 1.0×10^4 cells/well in a 96-well plate and incubated with test samples at doses of 6.25-100 μg/mL for 96 hrs, and the viability was determined by the SRB assay.[12,13]

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