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Antioxidant Phenolic Constituents from the Leaves of *Acer ginnala* var. *aidzuense*

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Abstract

Acer ginnala Maxim. var. *aidzuense* (Franch.) Pax was reported recently from Kumamoto Prefecture, Japan after many years. Thus, the main objective of present study was to identify the chemical constituents in the leaves of title plant and evaluate the antioxidant activity of the extract and isolated compounds. Gallic acid (1), methyl gallate (2) and acertannin (3) were isolated from the 70% MeOH extract of the leaves. The extract and all isolated compounds showed potent antioxidant activity.

Keywords: *Acer ginnala* var. *aidzuense*, Acertannin, Gallic Acid, Karakogi-Kaede

1. Introduction

The genus *Acer*, belonging to family Aceraceae has about 200 species worldwide and among them, 26 species are distributed in Japan[1]. *Acer ginnala* Maxim. var. *aidzuense* (Franch) Pax (syn. *Acer aidzuense* (Franch.) Nakai), commonly known as 'Karakogi-kaede' is a deciduous tree about 2-5 m mainly found in wetlands[2]. It is widely distributed in eastern parts of Japan but comparatively rare in western parts including Kyushu Island[3]. It was thought to be extinct for last 30 years in Kumamoto Prefecture, Japan until we recently reported it from Kumagun, Asagiri Town[4].

Many species of *Acer* are widely used as traditional medicines in Japan and other countries. For example, the stem bark of *Acer nikoense* is used for the treatment of hepatic disorders and eye diseases and also as health food[5]. The stem and leaves of *Acer palmatum* are used in treatment of stomach ache and joint pain[6]. The sap of *A. ginnala* is used as stomachic and for the treatment of diarrhea[7]. The tea made from young leaves of *A. ginnala*, known as Gao-Cha is used for the prevention of hypertension and inflammation[8] and also used in treatment of eye problems and headache[9]. These traditional uses suggested that the different plant parts *Acer ginnala* var. *aidzuense* may also have some health beneficial effects. In this paper, we report the chemical constituents in the leaves of *Acer ginnala* var. *aidzuense* and the antioxidant activity of the extract and isolated compounds.

2. Materials and Methods

2.1 General Experimental Procedures

Optical rotations were measured with a JASCO DIP-1000KUY polarimeter. ^{1}H-, ^{13}C- and 2D-NMR spectra were measured on a JEOL *a*-500 (^{1}H-NMR: 500 MHz and

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^{13}C-NMR: 125 MHz). Chemical shifts are given in ppm with reference to tetramethyl silane (TMS). Absorbance was recorded on Infinite 200 PRO® (Tecan Austria GmBH, Grodig, Austria). Column chromatography was carried out with MCI gel CHP20P (75 ~ 150 µm, Mitsubishi Chemical Industries Co., Ltd., Tokyo, Japan), Sephadex LH-20 (Amersham Pharmacia Biotech, Tokyo, Japan) and Chromatorex ODS (30 ~ 50 µm, Fuji Silysia Chemical Co., Ltd., Aichi, Japan). TLC was performed on a precoated silica gel 60 F_{254} (Aluminum sheet, Merck KGaA, Darmstadt, Germany).

2.2 Chemicals

1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) and Trolox were purchased from Wako Pure Chemicals, Osaka, Japan, and MES buffer was purchased from Dojindo Chemical Research, Kumamoto, Japan.

2.3 Plant Materials

The leaves of *Acer ginnala* var. *aidzuense* were collected from Asagiri Town, Kumamoto, Japan in May 2014 and shade dried for one month. The voucher specimens are deposited at the Museum of Traditional Medicines, School of Pharmacy, Kumamoto University.

2.4 Extraction and Isolation

The dried leaves (1800 g) were extracted two times with 70% MeOH (8 L) for 48 hours at room temperature. The combined extract was evaporated under reduced pressure to give 406.0 g extract. A part of the extract (167.0 g) was then subjected on MCI gel CHP20P CC and eluted successively with water, 40%, 60%, 80% and 100% MeOH to give nine fractions (1~9). Fraction 2 (2.6 g, 40% eluate) was subjected to Sephadex LH-20 CC (50% acetone) and to ODS CC (water, 10%, 20% and 25% MeOH) to afford compounds **1** (84.5 mg), **2** (390.0 mg) and **3** (140.5 mg). Fraction 4 (5.4 g, 60% eluate) was obtained as **3** (5400.0 mg).

2.5 Gallic acid (1)

A white amorphous powder; ^{1}H-NMR (D_2O) δ_H: 7.17 (2H, s, H-2, H-6); ^{13}C-NMR (D_2O) δ_C: 174.9 (C-7), 145.2 (C-3, C-5), 136.9 (C-4), 127.9 (C-1), 110.4 (C-2, C-6).

2.6 Methyl gallate (2)

A white amorphous powder; ^{1}H-NMR (D_2O:acetone-d_6=1:1) δ_H: 6.98 (2H, s, H-2, H-6), 3.71 (3H, s, OCH_3); ^{13}C-NMR (methanol-d_4) δ_C: 168.9 (C-7), 146.2 (C-3, C-5), 139.5 (C-4), 121.3 (C-1), 110.0 (C-2, C-6), 52.2 (OCH_3).

2.7 Acertannin (3)

A brown amorphous powder; $[\alpha]_D^{20}$ = +17.0° (c = 1.0, acetone); ^{1}H-NMR (acetone-d_6) δ_H: 7.16, 7.18 (2H each, H-2', H-6', H-2", H-6"), 4.92 (1H, dt, J = 10.0, 5.2 Hz, H-2), 4.64 (1H, d, J = 10.8 Hz, H-6), 4.38 (1H, dd, J = 10.8, 5.2 Hz, H-6), 4.09 (1H, m, H-1), 3.86 (1H, t, J = 8.9 Hz, H-3), 3.63 (2H, m, H-4 and H-5), 3.40 (1H, t, J = 9.8 Hz, H-1); ^{13}C-NMR (acetone-d_6) δ_C: 166.8, 167.2 (C-7', C-7"), 145.8, 145.7 (2C each, C-3', C-5', C-3", C-5"), 138.9, 139.0 (C-4', C-4"), 120.7, 121.0 (C-1', C-1"), 109.7, 109.8 (2C each, C-2', C-6', C-2", C-6"), 79.2 (C-5), 75.9 (C-3), 72.7 (C-2), 71.2 (C-4), 67.1 (C-1), 64.5 (C-6).

2.8 Free Radical Scavenging Activity

The DPPH radical-scavenging activity of extract and isolated compounds was examined using the method reported previously[10] with slight modifications. Briefly, 50 µL of 200 mM MES [2-(*N*-morpholino) ethanesulphonic acid] buffer (pH 6.0), 100 µL of samples with different concentrations (in DMSO:Ethanol = 1:1) and 50 µL of 800 *m*M DPPH in ethanol solution were mixed in a 96-well plate and kept in dark at room temperature for 20 minutes. The anti-oxidative activity corresponding to the scavenging of DPPH radicals was measured at 510 nm with UV spectrophotometer using following formula: Radical scavenging activity (%) = 100×(A−B)/A. Where, A is the control absorbance of DPPH radicals without samples and B is the absorbance after reacting with samples. Trolox was used as the positive control. The result is expressed as mean of three experiments. From these data, curve was plotted and concentration ((µg/mL or µM)) of the sample required for 50% reduction of the DPPH radical absorbance (EC_{50}) was calculated.

3. Results and Discussion

Since, we have reported *Acer ginnala* var. *aidzuense* from Kumamoto after many years, the main aim of this study

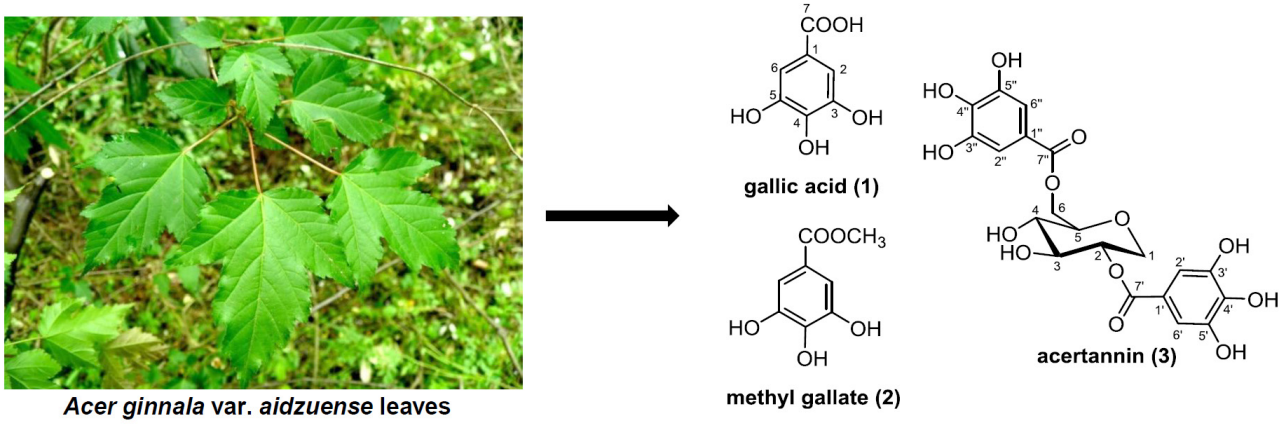

Fig. 1. Compounds isolated from the leaves of *Acer ginnala* var. *aidzuense*.

was to identify the chemical constituents in the leaves of title plant and evaluate the antioxidant activity. Three phenolic compounds namely, gallic acid (**1**)[11], methyl gallate (**2**)[11] and acertannin (**3**)[12] (Figure 1) were isolated from the 70% MeOH extract of the leaves. Structures of these compounds were elucidated on the basis of NMR spectra and comparison with literature data.

The 70% MeOH extract and all of the isolated compounds were tested for their *in vitro* antioxidant activity towards DPPH free radical scavenging assay. The concentrations of the sample required for 50% reduction of the DPPH radical absorbance (EC_{50} values) are given in Table 1. The results were compared with Trolox as positive control. The 70% MeOH extract (EC_{50}, 7.0 µg) showed more potent activity than Trolox (EC_{50}, 12.2 µg). Among the isolated compounds, the activity of acertannin (**3**) was the strongest (EC_{50}, 8.6 μM) followed by gallic acid (**1**) (EC_{50}, 12.9 μM) and methyl gallate (**2**) (EC_{50}, 13.0 μM), and all of these compounds were more potent as compared to Trolox (EC_{50}, 48.8 μM). These findings are similar to previous studies on *Acer* plants and their bioactivities[8, 12].

Table 1: EC_{50} values of extract, compounds and Trolox

Samples	EC_{50} (μg/ml)	EC_{50} (μM)
70% MeOH extract	7.0	-
gallic acid (1)	2.2	12.9
methyl gallate (2)	2.4	13.0
acertanin (3)	4.0	8.6
Trolox	12.2	48.8

Thus, these results suggest that the leaves of *Acer ginnala* var. *aidzuense* contain highly antioxidant phenolic compounds including gallic acid, methyl gallate and acertannin. The consumption of the tea made from the leaves may have preventive effects against damage caused by oxidative stress. However, more studies are required for the quantitative estimation of these compounds and *in vivo* biological activities of extracts and individual compounds.

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