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

Masato Watanabe¹ and Hari Prasad Devkota¹,²*

¹School of Pharmacy, ²Program for Leading Graduate School, HIGO Program, Kumamoto University, 5-1 Oe-honmachi, Chuo-ku, Kumamoto, 862-0973, Japan.

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 Japan¹. 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 wetlands². It is widely distributed in eastern parts of Japan but comparatively rare in western parts including Kyushu Island³. It was thought to be extinct for last 30 years in Kumamoto Prefecture, Japan until we recently reported it from Kumagun, Asagiri Town⁴.

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 food⁵. The stem and leaves of Acer palmatum are used in treatment of stomach ache and joint pain⁶. The sap of A. ginnala is used as stomachic and for the treatment of diarrhea⁷. The tea made from young leaves of A. ginnala, known as Gao-Cha is used for the prevention of hypertension and inflammation⁸ and also used in treatment of eye problems and headache⁹. 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. ¹H-, ¹³C- and 2D-NMR spectra were measured on a JEOL α-500 (¹H-NMR: 500 MHz and...
Antioxidant Phenolic Constituents from the Leaves of *Acer ginnala* var. *aidzuense*

13C-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 F254 (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; 1H-NMR (D2O) δH: 7.17 (2H, s, H-2, H-6); 13C-NMR (D2O) δC: 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; 1H-NMR (D2O-acetone-d6=1:1) δH: 6.98 (2H, s, H-2, H-6), 3.71 (3H, s, OCH3); 13C-NMR (methanol-d4) δC: 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 (OCH3).

2.7 Acertannin (3)

A brown amorphous powder; [α]D20 = +17.0° (c = 1.0, acetone); 1H-NMR (acetone-d6) δH: 6.98 (2H, s, H-2, H-6), 3.71 (3H, s, OCH3); 13C-NMR (methanol-d4) δC: 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 (OCH3).

2.8 Free Radical Scavenging Activity

The DPPH radical-scavenging activity of extract and isolated compounds was examined using the method reported previously10 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 mM 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 (EC50) 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
was to identify the chemical constituents in the leaves of title plant and evaluate the antioxidant activity. Three phenolic compounds namely, gallic acid (1)\textsuperscript{11}, methyl gallate (2)\textsuperscript{11} and acetannin (3)\textsuperscript{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 \textit{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\textsubscript{50} values) are given in Table 1. The results were compared with Trolox as positive control. The 70% MeOH extract (EC\textsubscript{50}, 7.0 µg) showed more potent activity than Trolox (EC\textsubscript{50}, 12.2 µg). Among the isolated compounds, the activity of acetannin (3) was the strongest (EC\textsubscript{50}, 8.6 µM) followed by gallic acid (1) (EC\textsubscript{50}, 12.9 µM) and methyl gallate (2) (EC\textsubscript{50}, 13.0 µM), and all of these compounds were more potent as compared to Trolox (EC\textsubscript{50}, 48.8 µM). These findings are similar to previous studies on Acer plants and their bioactivities\textsuperscript{8,12}.

\textbf{Table 1:} EC\textsubscript{50} values of extract, compounds and Trolox

<table>
<thead>
<tr>
<th>Samples</th>
<th>EC\textsubscript{50} (µg/ml)</th>
<th>EC\textsubscript{50} (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70% MeOH extract</td>
<td>7.0</td>
<td>-</td>
</tr>
<tr>
<td>gallic acid (1)</td>
<td>2.2</td>
<td>12.9</td>
</tr>
<tr>
<td>methyl gallate (2)</td>
<td>2.4</td>
<td>13.0</td>
</tr>
<tr>
<td>acetannin (3)</td>
<td>4.0</td>
<td>8.6</td>
</tr>
<tr>
<td>Trolox</td>
<td>12.2</td>
<td>48.8</td>
</tr>
</tbody>
</table>

Thus, these results suggest that the leaves of \textit{Acer ginnala} var. \textit{aidzuense} contain highly antioxidant phenolic compounds including gallic acid, methyl gallate and acetannin. 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 \textit{in vivo} biological activities of extracts and individual compounds.

\section{4. Acknowledgements}

We are grateful to Ms. Teruo Tanaka of Institute of Resource Development and Analysis, Kumamoto University for measurement of NMR measurements. This work was supported in part by Program for Leading Graduate Schools, Health life science: Interdisciplinary and Glocal Oriented (HIGO) Program, MEXT, Japan.

\section{5. References}


