A methanolic extract of *Agrimonia eupatoria* (Rosaceae) significantly attenuated glutamate-induced oxidative stress in HT22 hippocampal cells. A new flavonoid, characterized as kaempferol 3-O-β-D-(2′-O-acetyl-6′-(E)-p-coumaroyl)-glucopyranoside (2′-acetyl-tilliroside (1), was isolated from the methanolic extract of *A. eupatoria* stems together with nine known flavonoids. Compounds 4, 7, 8 and 9 all showed a neuroprotective effect on glutamate-induced toxicity in HT22 cells.

**Key words:** *Agrimonia eupatoria*; Lauraceae; HT22 hippocampal cell; neuroprotective; flavonoid

The production of reactive oxygen species (ROS) and their detoxification are normal physiological processes; however, it is well established that an imbalance between the production and removal of ROS induces oxidative stress and contributes to the pathogenesis of many neurodegenerative diseases, including Alzheimer’s disease and Parkinson’s disease.\(^1\)–\(^3\) A variety of antioxidants have been investigated as potential therapeutic agents for treating oxidative stress-related diseases. We have therefore tried to find neuroprotective compounds from natural products using glutamate-injured immortalized mouse hippocampal HT22 cells. A high concentration of glutamate inhibits the cystine uptake by HT22 cells through the cystine/glutamate antiporter, leading to a depletion of glutathione, an increase in ROS level, and ultimately cell death.\(^4\)–\(^7\) Our screening system found that the methanolic extract of *Agrimonia eupatoria* exhibited significant neuroprotective activity.

*A. eupatoria* L. (agrimony, Rosaceae) is a medicinal plant extensively used in traditional medicine for treating several disorders. There are several reports in the literature indicating that agrimony has pharmacological properties as an antioxidant, antibacterial, anti-hepatitis B virus, and anti-diabetic.\(^8\)–\(^13\) Studies on the chemical constituents of *A. eupatoria* have been confined to the flavonoids, phenolic acids, triterpenes, and tannins.\(^9,12\) The EtOAc and n-BuOH fractions of the methanolic extract of *A. eupatoria* in the present study led to the isolation of a new flavonoid (1), together with nine known flavonoids (Fig. 1). We describe here the isolation and identification of this new compound and evaluation of the neuroprotective activity of all ten flavonoids toward glutamate-injured HT22 cells.

Herbs of *A. eupatoria* (1 kg, provided from Biokorea Co., Seoul, Korea) were extracted three times with 80% MeOH for 2 h by ultrasonic apparatus. Removal of the solvent under vacuum enabled 100 g of the methanolic extract to be obtained. The methanolic extract was suspended in H\(_2\)O and successively partitioned with n-hexane (6 g), EtOAc (22 g), and n-BuOH (22 g). The neuroprotective activity of the extract and fractions was evaluated by the method previously reported.\(^14\) The respective neuroprotective activities of the methanolic extract, and n-hexane, EtOAc, n-BuOH and H\(_2\)O fractions were 76.2 ± 7.2\%, 18.9 ± 6.1\%, 64.5 ± 5.5\%, 37.1 ± 6.8\% and 10.9 ± 2.7\% (at 75 μg/ml). Among these fractions, the EtOAc and n-BuOH fractions which showed the significant neuroprotective activity were used for isolating the active compounds. The EtOAc (22 g) fraction was subjected to CC over silica gel (300 g, 6 x 60 cm), eluting with n-hexane-EtOAc-MeOH mixtures (50:1:0, 20:1:0, 10:1:0, 5:1:0, 3:1:0, 2:1:0, 1:1:0, 0:1:0, 0:20:1, 0:10:1, 0:5:1, 0:3:1, 0:2:1, 0:1:1, and 0:1:0, with 1 liter of each solvent) to afford 13 fractions (AEE1–AEE13). Among these fractions, AEE6 was subjected to CC over silica gel, Lauraceae; HT22 hippocampal cell; neuroprotective; flavonoid

**Note**

**Effect of Neuroprotective Flavonoids of *Agrimonia eupatoria* on Glutamate-Induced Oxidative Injury to HT22 Hippocampal Cells**

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glucopyranoside (2"-acetyl-tiliroside). The nine known compounds were identified as kaempferol 3-O-β-D-(-2"
O-acetyl)glucopyranoside (2), tiliroside (3), astragalin (4), apigenin 7-O-β-D-glucuronide (5), rutin (6), isoquercitrin (7), quercitrin (8), luteolin 7-O-β-D-glucuronide (9), and luteolin 7-O-β-D-glycosidicpyranoside (10) by comparing the physicochemical and spectroscopic data with previously reported data.15-19 Among the ten isolated flavonoids, compound 1 was newly characterized as an acetyl derivative of tiliroside.

Compounds 1-10 were evaluated for their neuroprotective activity toward glutamate-injured HT22 cells using MTT assay. As shown in Table 1, compounds 4, 7, and 8 significantly attenuated the glutamate-induced oxidative neurotoxicity at a concentration of 100 μM (p < 0.001). The high content of isoquercitrin (7) shown in Fig. 2 and the potent neuroprotective activity of this compound could explain the neuroprotective effect of the methanolic extract of A. eupatoria at least in part. The antioxidative and free radical scavenging activities of flavonoids have been extensively studied in various experimental systems, including gluta-

tamate-intoxicated mouse hippocampal cell line HT-22.20 Several structural determinants have been suggested as a requirement for the antioxidative activity of flavonoids. According to Bors et al., the presence of the hydroxyl group at C3 and the 3',4'-diol groups on B ring are required for the antioxidative activity at a concentration of 100 μM (p < 0.001). The high content of isoquercitrin (7) shown in Fig. 2 and the potent neuroprotective activity of this compound could explain the neuroprotective effect of the methanolic extract of A. eupatoria at least in part. The antioxidative and free radical scavenging activities of flavonoids have been extensively studied in various experimental systems, including gluta-

Table 1. Neuroprotective Activities of Compounds 1-10 toward HT22 Cells Injured by Glutamate

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cell viability (%)</th>
<th>Controla,b</th>
<th>Glutamate-injuredd,e</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 μM</td>
<td>1000 μM</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.3 ± 1.8</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>15.5 ± 3.5</td>
<td>2.1 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>17.9 ± 1.2</td>
<td>6.2 ± 4.4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>27.6 ± 6.2</td>
<td>82.9 ± 4.7</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>11.2 ± 1.0</td>
<td>10.7 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>30.4 ± 1.2</td>
<td>34.7 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>61.9 ± 3.8</td>
<td>88.8 ± 11.1</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>12.0 ± 4.1</td>
<td>65.7 ± 3.9</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>15.6 ± 8.4</td>
<td>52.2 ± 2.9</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>12.3 ± 2.1</td>
<td>4.1 ± 2.5</td>
<td></td>
</tr>
</tbody>
</table>

*a HT22 cells were pretreated with each test compound for 1 h. The cultures were then exposed to 5 μM glutamate for 8 h, after which they were assessed for the extent of neuronal damage.

*b Cell viability was measured by an MTT assay. Data are expressed as the percentage protection relative to the vehicle-treated control culture: 100 x [optical density (OD) of i-glutamate + sample-treated culture - OD of i-glutamate-treated culture]/OD of control culture - OD of i-glutamate-treated culture.

c ODs of the control and glutamate-injured cultures were 0.62 ± 0.07 and 0.30 ± 0.06, respectively.

*d The values shown are the mean ± SD of at least three experiments. Asterisks indicate significance vs. the glutamate-injured group (a one-way analysis of variance Tukey’s test: **p < 0.01; *p < 0.001.

The glutamate-injured value differed significantly from the control at the level of p < 0.001. The cell viability of ascorbic acid (Sigma, >99% purity), which was used as a positive control, was 45.8 ± 5.4% at 10 μM.

Fig. 1. Chemical Structures of Compounds 1-10 Isolated from A. eupatoria (A) and Important HMBC Correlations for Compound 1 (B).

Ac, acetyl; Cunn, coumaroyl; Glucu, glucuronic acid 5'), 7.39 (1H, d, J = 16.4 Hz, H-7'), 7.41 (2H, d, J = 8.6 Hz, H-2", 6"), 7.96 (2H, d, J = 8.8 Hz, H-2', 6'), 12.59 (1H, s, 5-OH). 13C-NMR (DMSO-d6, 125 MHz) δ: 156.3 (C-2), 132.7 (C-3), 177.1 (C-4), 161.2 (C-5), 98.7 (C-6), 164.2 (C-7), 93.7 (C-8), 156.6 (C-9), 103.9 (C-10), 120.6 (C-1'), 130.8 (C-2', 6'), 115.1 (C-3', 5'), 160.1 (C-4'), 98.5 (C-1"), 74.2 (C-2"), 73.7 (C-3"), 70.0 (C-4"), 74.1 (C-5"), 62.8 (C-6"), 124.9 (C-1"), 130.2 (C-2"), 66.1 (C-3"), 115.8 (C-3"), 59.6 (C-4"), 144.7 (C-7"), 113.6 (C-8"), 166.1 (C-9"), 21.0 (−COCH3), 169.6 (−COOH).
against glutamate-induced oxidative stress suggested the effect of the sugar moiety. Voltammetric rotating-ring disk studies have shown that the oxidizability of flavonoid glycosides decreased as the substituent at C3 became a poorer leaving group. Thus, the lower oxidizability of rutin than of quercetin monoglycosides could be explained by the poorer leaving disaccharide moiety. Between two quercetin monoglycosides, glucose-containing isoquercitrin (7) showed markedly higher activity than rhamnose-containing quercitrin (8) which can be interpreted by the lower polarity of rhamnose due to its methyl group positioned at C5. The hydrophobicity of a flavonoid has also been reported as an important determinant of its antioxidative activity. However, our results did not show a clear correlation between the hydrophobicity and neuroprotective activity of flavonoids. Both the solubility and oxidizability, as well as the uptake behavior of flavonoids might have affected the neuroprotective activity of the tested compounds in our experimental system.

In conclusion, the methanolic extract of A. eupatoria and compounds 4, 7, 8 and 9 isolated from the extract exhibited significant neuroprotective activity against glutamate-induced toxicity in HT22 cells, partly due to their antioxidative effect. The isolated flavonoids showed different protective activity according to their structural variation of hydroxyl groups and sugar moieties.

Acknowledgments

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References