Neuroprotective Effect of Corydalis ternata Extract and Its Phytochemical Quantitative Analysis

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The tubers of Corydalis ternata have been used to treat cardiovascular diseases such as hypertension and cardiac arrhythmia. Its active components have anticholinesterase, antiinflammatory activities, and analgesic effects. In the present study, we performed quantitative analyses of the two components of C. ternata, coptisine and berberine, using HPLC. A 70% ethanol extract of C. ternata was prepared and the two components were separated using a C-18 analytical column on a gradient solvent system of acetonitrile and 0.1% (v/v) aqueous trifluoroacetic acid. Recordings were performed at a UV wavelength of 265 nm for two standard components. The established analytical method showed high linearity (correlation coefficient (r)=1.0000) and proper precision (0.49–3.88%), accuracy (97.88–102.7%), and recovery (95.12–103.79%) for two standard components. The amount of the coptisine and berberine was 4.968±0.089 mg/g and 3.73±0.075 mg/g, respectively. In addition, we investigated the effects of coptisine and berberine on acetylcholinesterase activity and amyloid-β aggregation, which are major biomarkers of dementia. Coptisine and berberine decreased acetylcholinesterase activity in a dose-dependent manner (IC50=0.74 and 0.48 μM, respectively). The C. ternata extract exerted an antioxidant activity by stimulating the radical scavenging activity of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), but not 2,2-diphenyl-1-picrylhydrazyl (DPPH). Furthermore, the C. ternata extract reversed the hydrogen peroxide-induced death of HT22 hippocampal cells, indicating its neuroprotective effect. Our results suggest the potential of C. ternata as a therapeutic agent against dementia via the inhibition of acetylcholinesterase activity and neuronal cell death.

Key words Corydalis ternata; coptisine; berberine; quantitative analysis; neuroprotection

Corydalis ternata NAKAI (Papaveraceae), also called Corydalis tuber for treating cardiovascular diseases such as hypertension. Although C. ternata has been used in Korea, China and Japan, scientific name and standard components of each herb has the difference depending on their source. According to the Korean Pharmacopoeia, coptisine and berberine have been suggested as the standard compounds of C. ternata. In contrast, the Chinese Pharmacopoeia regulate tetrahydropalmatine as a standard compound of C. yanhusui W. T. Wang. In the Japanese Pharmacopoeia, dehydrocorydalin is regulated as a standard compound of C. turtschaninovii Besser forma Yanhusui Y. H. CHOU et C. C. Hsu. Previous articles reported the biological activities of the major phytochemicals from C. ternata, such as antiinflammation, antineuroinflammation, and antiinflammatory activities. Although those reports suggest that the activities of several of those components are associated with neuronal diseases, there is no report of an anti-Alzheimer’s effect of C. ternata or its components.

Alzheimer’s disease is a type of neurodegenerative disorder in which the death of neuronal cells leads to memory loss and cognitive impairment. Although several risk factors are known, such as aging, family history, diabetes, or its related diseases, the pathological mechanisms underlying this condition remain unknown. To date, the U.S. Food and Drug Administration (FDA) has approved four acetylcholinesterase (AChE) inhibitors, i.e., donepezil, galantamine, rivastigmine, and tacrine, for the treatment of Alzheimer’s disease. However, these drugs can reduce symptoms but have no curative activities. Therefore, the development of new therapeutic drugs for Alzheimer’s disease is a pressing issue worldwide.

In the present study, we investigated whether C. ternata and its standard components, coptisine and berberine, affect Alzheimer’s or its related diseases by measuring its anti-AChE and antioxidant activity and its protective effect in neuronal cells. In addition, HPLC was conducted to assess the quality of C. ternata via the quantitative analyses of its two standard components.

Results and Discussion

C. ternata, commonly called three-leaf corydalis, is a medicinal herb found in East Asia, including Korea and China. Traditionally, C. ternata was thought to be useful as a painrelieving medication for abdominal pain, hernia-caused pain, headache, and traumatic injury pain. The biological actions of the chemical compounds of C. ternata extract have been reported in previous reports. Benzylisoquinoline alkaloids exhibited cytotoxic effects against various cancer cells, indicating its anticancer activity. Coryternic acid 3-O-β-D-glucuronopyranoside-6′-O-methyl ester, a triterpenoid, had anti-cancer and anti-inflammatory activities. 1-Tetrahydropalmatine inhibited methamphetamine-induced locomotor activity via the regulation of 5-hydroxytryptamine (5-HT) neuronal activity and dopamine D3 receptor expression.
topine had an antiamnesic effect by inhibiting cholinesterase activity. In addition, an alkalized C. ternata extract regulated glutamate levels in rat brains via the activation of glutamate dehydrogenase. In the present study, we established a quantitative analytical method for the two standard components of C. ternata, coptisine and berberine, and investigated their anti-Alzheimer’s effect by evaluating AChE activity, free radical scavenging activity, and neuroprotective effects.

**Optimization of HPLC Separation** To establish an efficient HPLC method for the separation of the two standard components coptisine (Fig. 1A) and berberine (Fig. 1B) from the 70% ethanol extract of C. ternata, we evaluated various mobile phases (trifluoroacetic acid (TFA), acetic acid, and phosphoric acid) in water. We obtained good separation chromatograms using mobile phases consisting of 0.1% (v/v) aqueous TFA (A) and acetonitrile (B). At a photo diode array (PDA) detector wavelength ranging from 190 to 400 nm, UV recordings were performed at a wavelength of 265 nm. Using this HPLC method, the two standard components were resolved within 20 min. The retention times of coptisine and berberine were 13.76 and 19.56 min, respectively. HPLC chromatograms of the 70% ethanol extract of C. ternata and the standard mixture are shown in Fig. 2.

**Linearity, Range, Limits of Detection (LOD) and Quantification (LOQ)** The linear relationships between the peak areas (y) and concentrations (x, µg/mL) of the components were expressed by the regression equations (y=ax+b) given in Table 1. The analytical method established here showed high linearity with a correlation coefficient (r²)=1.0000. The calibration curves showed good linearity over the concentration range of 6.25–400 µg/mL for both coptisine and berberine.

![Fig. 1. Chemical Structures of the Two Standard Compounds of C. ternata: (A) Coptisine and (B) Berberine](image1)

![Fig. 2. HPLC Chromatograms of the 70% Ethanol Extract of C. ternata (20 mg/mL) (A) and a Standard Mixture (100 µg/mL) (B) at 265 nm; Coptisine (1); Berberine (2)](image2)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Linear range (µg/mL)</th>
<th>Regression equation (y=ax+b)</th>
<th>Correlation coefficient (r²)</th>
<th>LOD (µg/mL)</th>
<th>LOQ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coptisine</td>
<td>6.25–400</td>
<td>29930</td>
<td>44156</td>
<td>0.473</td>
<td>1.433</td>
</tr>
<tr>
<td>Berberine</td>
<td>6.25–400</td>
<td>35962</td>
<td>71479</td>
<td>0.381</td>
<td>1.155</td>
</tr>
</tbody>
</table>

a) y=ax+b, y means peak area and x means concentration (µg/mL). b) LOD (Limit of detection): 3.3×(S.D. of the response/slope of the calibration curve). c) LOQ (Limit of quantitation): 10×(S.D. of the response/slope of the calibration curve).
range of 6.25–400 μg/mL for the two standard components. The LOD and LOQ for coptisine and berberine were 0.473 and 0.381 μg/mL, and 1.433 and 1.155 μg/mL, respectively.

**Determination of the Two Standard Components in C. ternata** The HPLC analytical method developed here was applied to the simultaneous determination of the two components in the C. ternata extract. The amount of the two standard components, coptisine and berberine, was 4.968 and 3.73 mg/g, respectively (Table 2). Of the two components, coptisine was the most abundant in the tubers of C. ternata.

**Precision, Accuracy, and Recovery** The precision was presented as relative standard deviation (RSD) of concentrations for standard components in C. ternata extract samples repeated five times at each concentration level. The results of the intra- and interday precision, and accuracy were shown in Table 3. The intra- and interday precisions of two standard components in the samples were less than 3.88% with accuracies ranging from 97.88 to 102.7%. The recovery tests of two standard components were carried out by analyzing the C. ternata extract samples at the original concentration and samples spiked with 20, 50, and 100% of the original concentration. The recoveries of coptisine and berberine were in the range of 95.12–103.79% with RSD ≤2.24% (Table 4). These results indicated that the established HPLC method was satisfied precision, accuracy, and recovery for quantitative analysis of coptisine and berberine.

**Inhibitory Effects of the C. ternata Extract and Its Standard Components on AChE Activity** Next, we investigated the biological activity of C. ternata on Alzheimer's disease by evaluating AChE activity and amyloid-β aggregation, which are key therapeutic targets for Alzheimer’s disease. As shown in Table 5, the C. ternata extract inhibited AChE activity by 75.62% at 100 μg/mL, while showing no significant effect on amyloid-β aggregation (at 100 μg/mL). To confirm the inhibitory effect of the C. ternata extract on AChE activity, the assay was performed at various concentrations, ranging from 12.5 to 100 μg/mL. The AChE activity was decreased by the C. ternata extract in a dose-dependent manner (Fig. 3B, C).

Recently, many articles reported the potential of natural products as therapies for Alzheimer’s disease via the inhibition of AChE activity. These findings suggest that natural products, including herbal medicines, may be complementary and alternative approaches to Western medicine for the treatment of Alzheimer’s disease. Although recent studies of Alzheimer’s disease therapy have been shifting to other targets, such as amyloid-β, Tau, reactive oxygen species, and the blood–brain barrier, AChE inhibitors are still the first choice for treating patients with AD.

![Table 2](image-url)  
Table 2. The Content of Standard Compounds in C. ternata

<table>
<thead>
<tr>
<th>Compound</th>
<th>Content (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coptisine</td>
<td>4.968±0.089</td>
</tr>
<tr>
<td>Berberine</td>
<td>3.73±0.075</td>
</tr>
</tbody>
</table>

![Table 3](image-url)  
Table 3. Precision and Accuracy of Two Standard Compounds in C. ternata

<table>
<thead>
<tr>
<th>Compound</th>
<th>Fortified conc. (μg/mL)</th>
<th>Intraday (n=5)</th>
<th>Interday (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed conc. (μg/mL)</td>
<td>Precision (%)</td>
<td>Accuracy (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coptisine</td>
<td>10</td>
<td>10.22</td>
<td>3.88</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>25.14</td>
<td>2.45</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>49.88</td>
<td>0.66</td>
</tr>
<tr>
<td>Berberine</td>
<td>8</td>
<td>8.00</td>
<td>2.97</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>20.18</td>
<td>1.65</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>39.91</td>
<td>0.49</td>
</tr>
</tbody>
</table>

*a) Precision is expressed as RSD (%)=(S.D./Mean)×100. b) Accuracy (%)=(Observed concentration/Fortified concentration)×100.
ternata was determined by assessing the release of lactate dehydrogenase (LDH) from the cells. Damage of HT22 cells was induced by exposing the cells to H$_2$O$_2$ in the absence or presence of the C. ternata extract. H$_2$O$_2$ stimulation significantly increased the release of LDH compared with the untreated control. In contrast, the C. ternata extract significantly decreased the H$_2$O$_2$-mediated LDH release compared with the H$_2$O$_2$ treatment alone (Fig. 5B), indicating the protective effect of C. ternata in neuronal cells.

**Conclusion**

C. ternata inhibited AChE activity and enhanced free radical scavenging activity. C. ternata also protected against neuronal cell death. Overall, these data indicate that C. ternata has potential as a candidate drug for the treatment of Alzheimer’s disease. In addition, we established an HPLC analysis method for the quantification of the two standard components of C. ternata, coptisine and berberine, which may be helpful for the control of the quality of C. ternata. Further studies are necessary to confirm the anti-Alzheimer’s activity and the regulatory mechanisms of C. ternata using an Alzheimer’s disease animal model.

**Experimental**

**Plant Material** The tubers of C. ternata (lot no. K1562201510) used in this study were purchased from the Kwangmyungdang herbal market (Ulsan, South Korea) and identified by Dr. Goya Choi, K-herb Research Center, Korea Institute of Oriental Medicine, Korea. A voucher specimen (Cot5-LE) has been deposited at the Herbal Medicine Research Division, Korea Institute of Oriental Medicine.

**Chemicals and Reagents** The standard components coptisine and berberine were purchased from ChemFaces Biochemical Co., Ltd. (Wuhan, China). The chemical structures of the standard components are shown in Fig. 1. The purity of these standard components was ≥98.0%, as assessed using HPLC analysis. The HPLC-grade solvents, acetonitrile and water, were purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ, U.S.A.), and the analytical-grade reagent TFA was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.).

**Table 4. Recovery of Two Standard Compounds in C. ternata**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Original conc. (µg/mL)</th>
<th>Spiked conc. (µg/mL)</th>
<th>Found conc. (µg/mL)</th>
<th>Recovery±S.D. (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coptisine</td>
<td>46.41</td>
<td>10</td>
<td>56.34</td>
<td>99.38±1.79</td>
<td>1.80</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>70.49</td>
<td>96.33±2.16</td>
<td>2.24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>93.97</td>
<td>95.12±1.98</td>
<td>2.08</td>
<td></td>
</tr>
<tr>
<td>Berberine</td>
<td>34.68</td>
<td>8</td>
<td>42.97</td>
<td>103.60±1.15</td>
<td>1.11</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>55.44</td>
<td>103.79±0.96</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>75.65</td>
<td>102.43±1.89</td>
<td>1.85</td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Inhibitory Activity of C. ternata Extract on in Vitro AChE Activity and Amyloid-β Aggregation (at 100 µg/mL)

<table>
<thead>
<tr>
<th></th>
<th>Inhibition of AChE activity (%)</th>
<th>Inhibition of amyloid-β aggregation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>75.62±0.09</td>
<td>−32.33±0.96</td>
</tr>
</tbody>
</table>

Fig. 3. Inhibitory Effects of the Standard Compounds of C. ternata on AChE Activity

**Apparatus and Chromatographic Conditions** A Waters Alliance e2695 HPLC system (Waters Corp., Milford, MA, U.S.A.) equipped with a pump, degasser, column oven, auto sample injector, and photodiode array detector (#2998; Waters Corp.) was used in this study. The data were acquired and processed using Empower software (version 3; Waters Corp). The chromatographic separation of the two standard components was performed at room temperature using a Luna C$_{18}$ analytical column (250×4.6 mm, 5 µm), supplied by Phe-
The gradient elution with two mobile phases consisting of 0.1% (v/v) aqueous TFA (A) and acetonitrile (B) was as follows: 25–35% B for 0–30 min, 35–100% B for 30–40 min, and 100% B for 40–47 min. A flow rate of 1.0 mL/min and a volume injection of 10 µL were kept constant in all cases. The detector wavelengths were monitored at 265 nm for two standard components.

**Preparation of Standard Solutions** The two components were weighed accurately, dissolved in methanol at 1.0 mg/mL, and stored at <4°C. The stock solutions were diluted to yield a series of standard solutions with different concentrations for quantitative analysis.

**Preparation of Sample Solutions** The dried tubers of *C. ternata* (50 g) were extracted twice with 70% ethanol (300 mL) by refluxing for 2 h. The extracted solution was filtered through a filter paper (5 µm) and evaporated using a rotary evaporator (EYELA N-1000; Rikakikai Co., Tokyo, Japan) under vacuum, to dryness (4.79 g). The yield of *C. ternata* extract was 9.58%. The 70% ethanol extract of the *C. ternata* was weighed accurately and dissolved in methanol at 20 mg/mL for simultaneous determination. The sample solution was filtered through a syringe filter (0.45 µm) before HPLC analysis.

**Calibration Curves, LOD, and LOQ** The calibration curves of components were obtained by assessment of the peak areas of the standard solutions at seven different concentrations. The tested concentration ranges were 6.25–400 µg/mL for the two standard components. The LOD and LOQ for the two standard components were calculated using the slope of the calibration curve and the standard deviation (S.D.) of the intercept, as follows:

$$\text{LOD} = 3.3 \times \frac{\text{S.D. of the response}}{\text{Slope of the calibration curve}}$$

and

$$\text{LOQ} = 10 \times \frac{\text{S.D. of the response}}{\text{Slope of the calibration curve}}$$

**Precision, Accuracy, and Recovery** To assess the precision and accuracy of the established HPLC conditions, intra- and interday precisions, and accuracy were tested with the 70% ethanol extract of the *C. ternata* samples spiked at the three concentration levels (low, medium, and high) of standard solutions. Precision was expressed as intra- and interday RSD (%) and accuracy was presented as the percentage of observed concentration for fortified concentration. The recoveries of two components were determined by adding standard solutions of three different concentrations levels (low, medium, and high) to 70% ethanol extract of the *C. ternata* samples (100 mg) and the total volume of each sample solution was monitored at 265 nm for two standard components.
10 mL (90% aqueous methanol). The recovery was carried out in five times at each level and calculated according to the following equation:

\[
\text{Recovery (\%)} = \left( \frac{\text{Found concentration} - \text{Original concentration}}{\text{Spiked concentration}} \right) \times 100
\]

**In Vitro AChE Activity Assay**  
In vitro AChE activity was measured using a spectrophotometric method (modification of Ellman’s method). The 70% ethanol extract of the *C. ternata*, the two standard components coptisine and berberine, was dissolved in 0.1 M sodium phosphate buffer (pH 8.0) for assay. The AChE was dissolved in 0.1% bovine serum albumin/H₂O at 25 U/mL, and these aliquots were stored at −80°C. Before the assay, the sample was dissolved in 0.1 M sodium phosphate buffer (pH 7.3, assay buffer), to a final concentration of 35.2 nM/mL. The substrates acetylthiocholine iodide and 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) were dissolved in H₂O and assay buffer at 100 mM, respectively, and kept at −80°C. Both solutions were mixed to a final concentration of 0.5 mM and used as the reaction mixture. To perform the enzymatic reaction in a 96-well plate, 50 μL of the sample solution and reaction mixture were mixed and preincubated for 10 min. The AChE solution was then added to the initial mixture, to start the reaction, which was carried out for 1 h at intervals of 10 min. The absorbance was measured at 412 nm using an Epoch microplate spectrophotometer (Bio-Tek Instruments). The reaction mixture was incubated for 30 min in the dark at room temperature. The absorbance of the resulting solution was measured at 734 nm using an Epoch microplate spectrophotometer (Bio-Tek Instruments). The radical scavenging capacity of the *C. ternata* extract-treated samples was calculated using the following equation.

\[
\text{Scavenging activity (\%)} = \left( \frac{1 - \text{Absorbance of } C. \text{ternata extract-treated sample}}{\text{Absorbance of untreated sample}} \right) \times 100
\]

**ABTS Radical Scavenging Activity**  
ABTS radical cations were produced by reacting a 7 mM ABTS solution with 2.45 mM potassium persulfate in the dark at room temperature for 16 h. The absorbance of the reactant was later adjusted to 0.7 at a wavelength of 734 nm. Aliquots of *C. ternata* extract solution (100 μL) at various concentrations were mixed with 100 μL of ABTS + solution. The reaction mixture was incubated for 5 min in the dark at room temperature. The absorbance of the resulting solution was measured at 734 nm using an Epoch microplate spectrophotometer (Bio-Tek Instruments). The radical scavenging capacity of the *C. ternata* extract-treated samples was calculated using the following equation.

\[
\text{Scavenging activity (\%)} = \left( \frac{1 - \text{Absorbance of } C. \text{ternata extract-treated sample}}{\text{Absorbance of untreated sample}} \right) \times 100
\]

**DPPH Radical Scavenging Activity**  
To determine the DPPH radical scavenging activity of *C. ternata* extract, 100 μL aliquots of the *C. ternata* extract sample solution at various concentrations were mixed with 100 μL of DPPH solution (0.15 mM in methanol). The reaction mixture was incubated for 30 min in the dark at room temperature. The absorbance of the resulting solution was measured at 517 nm using an Epoch microplate spectrophotometer (Bio-Tek Instruments). The radical scavenging capacity of the tested samples was calculated using the formula provided above.

**Cytotoxicity Assay**  
HT22 cells were maintained in Dulbecco’s modified Eagle’s medium (Hyclone/Thermo, Rockford, IL, U.S.A.) supplemented with 10% fetal bovine serum (Hyclone/Thermo) and penicillin/streptomycin in 5% CO₂ at 37°C.

To determine the nontoxic concentration of *C. ternata* extract in HT22 cells, a CCK assay was performed using the CCK-8 assay kit (Dojindo, Kumamoto, Japan). Cells were plated on 96-well microplates at a density of 5×10³ cells/well and treated with various concentrations (0, 12.5, 25, 50, and 100 μg/mL) of *C. ternata* extract for 24 h. The CCK-8 solution was added and the cells were incubated for 4 h. The absorbance was read at 450 nm using an Epoch microplate spectrophotometer (Bio-Tek Instruments). The cell viability was calculated using the following equation:

\[
\text{Cell viability (\%)} = \left( \frac{\text{Mean OD in } C. \text{ternata extract-treated cells}}{\text{Mean OD in untreated cells}} \right) \times 100
\]

**LDH Release Assay**  
To determine the neuroprotective effect of the *C. ternata* extract, the release of LDH was mea-
sured in the H$_2$O$_2$-damaged HT22 cells using the CytoTox 96 nonradioactive cytotoxicity assay kit (Promega, Madison, WI, U.S.A.). HT22 cells were cotreated with H$_2$O$_2$ and various concentrations of $C$. ternata extract for 6h. Cells were lysed to induce maximal LDH release and supernatants were collected to measure experimental LDH release. Cell lysates or supernatants were reacted with the substrate mixture at room temperature for 30 min in the dark. After adding stop solution, absorbance at 490 nm was measured on an Epoch microplate spectrophotometer (Bio-Tek Instruments). The cytotoxicity of the $C$. ternata extract was calculated using the following formula:

Cytotoxicity(%) = \frac{\text{Experimental LDH release}}{\text{Maximum LDH release}} \times 100

**Statistical Analysis** The data are expressed as the mean±standard error of the mean (S.E.M.) Data were analyzed using one-way ANOVA and Dunnett’s multiple comparisons test. $p<0.05$ was considered significant.

**Acknowledgment** This study was supported by a research Grant (K17293) from the Korea Institute of Oriental Medicine (KIOM).

**Conflict of Interest** The authors declare no conflict of interest.

**References**