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Short communication

Neuroprotective iridoid glycosides from *Cornus officinalis* fruits against glutamate-induced toxicity in HT22 hippocampal cells

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ABSTRACT

The methanolic extract of the fruits of *Cornus officinalis* S et Z. (Cornaceae) showed the significant neuroprotective activity against glutamate-induced toxicity in HT22 hippocampal cells. Chemical profile of *n*-BuOH fraction of the methanolic extract of *C. officinalis* fruits, which showed the most potent activity, was established using HPLC-diode array detector-electrospray-MS (HPLC-DAD-ESI-MS). Through bioactivity-guided isolation, five iridoid glycosides including one new compound, 7-*O*-butylmorroniside (**1**), loganin (**2**), morroniside (**3**), 7*R*-*O*-methylmorroniside (**4**), 7*S*-*O*-methylmorroniside (**5**) were isolated from the *n*-BuOH fraction. The protective activities of the isolated compounds, themselves, were not statistically significant. However, the hydrolyzed products of compounds **1**, **4** and **5** significantly protected glutamate-injured HT22 cells up to 78 ± 2.2%, 60 ± 3.2% and 59 ± 2.5% of non-treated control, respectively.

Introduction

Glutamate is known to cause neuronal cell death through both glutamate receptor-mediated excitotoxicity and reactive oxygen species (ROS)-mediated oxidative glutamate toxicity (Satoh et al. 2000). Oxidative stress, an imbalance between the production and destruction of ROS, has been involved in many neurodegenerative diseases (Coyle and Puttfarcken 1993; Teepker et al. 2007; Sultana and Butterfield in press). Therefore, diverse antioxidants especially isolated from natural products have received attention as potential therapeutic agents against oxidative stress-related diseases. Oxidative toxicity can be induced by glutamate insult in immortalized hippocampal cell lines, HT22, which lacks ionotropic glutamate receptors. In HT22 cells, excessive glutamate inhibits the glutamate/cystein antiporter and leads to the decrease in intracellular cysteine and the depletion of glutathione. Subsequent accumulation of ROS and increased influx of Ca^{2+} contribute to neuronal cell death (Davis and Maher 1994; Fukui and Zhu 2010; Sagara et al. 2002). In this screening system, the methanolic extract of *Cornus officinalis* S et Z. (Cornaceae) showed the significant neuroprotective activity against glutamate-induced toxicity in HT22 cells.

C. officinalis is widely distributed in Korea and the fruits of this plant have been used in traditional medicine for its tonic, analgesic and diuretic properties in Korea, Japan and China (Kim and Kwak 1998). Iridoid glycoside, secoiridoid glycoside, tannin and triterpenoid have been reported from this plant. In the previous study in our lab, the analytic method for the simultaneous determination of three active constituents of *C. officinalis* fruits including loganin, morroniside and gallic acid have been developed using HPLC-DAD system (Kim et al. 2009). The neuroprotective activity of iridoid glycosides isolated from *C. officinalis* has been reported through a variety of *in vitro* and *in vivo* studies. Morroniside protected SH-SY5Y neuroblastoma cells against hydrogen peroxide-induced cytotoxicity, and also protected rat brain from damage by focal cerebral ischemia through antioxidant and antiapoptotic properties (Wang et al. 2009, 2010). Loganin and cornel iridoid glycosides were revealed to improve cognitive impairment caused by scopolamine and fimbria-fornix lesions, respectively (Kwon et al. 2009; Lee et al. 2009; Zhao et al. 2010). However, there have been no reports on the protective activity of *C. officinalis* fruits against glutamate-induced neurotoxicity in HT22 hippocampal cells. In the present study, we tried to identify bio-active constituents of the fruits of *C. officinalis* representing neuroprotective activity against glutamate-injured HT22 hippocampal cells.

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Materials and methods

Chromatographic conditions

Finnigan Surveyor high performance liquid chromatography (HPLC) system with a pump, an autosampler, a PDA plus detector, and a Finnigan LCQ advantage MAX with Xcalibur software was used for HPLC electrospray ionization mass spectrometry (HPLC-ESI-MS). Separation was achieved at 25 °C on a Shisheido Capcell Pak C18 MG (5 μm, 4.6 mm × 150 mm). Ion polarity was negative. Molecular weight was determined on a JEOL JMS AX505 WA mass spectrometer (Tokyo, Japan) using the mobile phase composed of AcCN–MeOH–water with 0.03% formic acid (10:5:85, v/v) at a flow of 0.5 ml/min.

Plant material

The fruits of *C. officinalis* were purchased from Kyungdong Oriental Herbal Market, Seoul, Korea, in 2006 and identified by Dr. Jong Hee Park, professor of Pusan National University. A voucher specimen (SNU-204) has been deposited in Herbarium of the Medicinal Plant Garden, College of Pharmacy, Seoul National University, Koyang, Korea

Extraction and isolation

The air-dried fruits of *C. officinalis* (10 kg) were extracted three times with 80% MeOH in an ultrasonic apparatus. Removal of the solvent in vacuo yielded a methanolic extract (4 kg). The methanolic

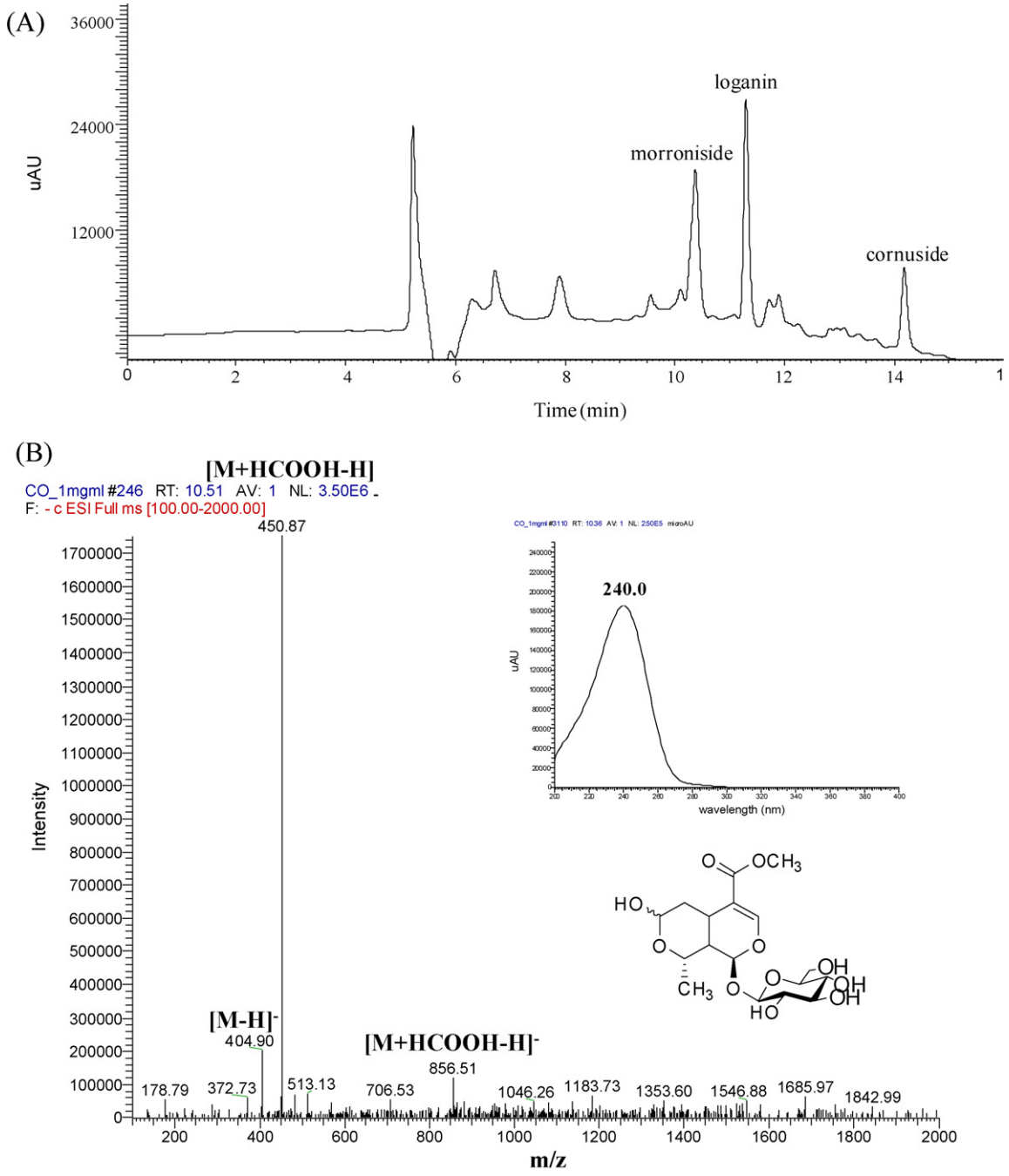

Fig. 1. HPLC chromatogram of the *n*-BuOH fraction of *C. officinalis* fruits (A), ESI-MS spectrum and UV absorption of morroniside (B), lognin (C) and cornuside (D) detected in HPLC chromatogram of *n*-BuOH fraction.