INTRODUCTION

Neurodegenerative diseases (NDs) include a variety of conditions arising from the chronic breakdown and deterioration of neurons in the central nervous system; Alzheimer’s disease (AD) and Parkinson’s disease (PD) are the two best-known NDs (Houghton and Howes, 2005). Although the etiology of AD and PD is still not fully understood, it is clear that neurodegeneration in these diseases is multifactorial; several mechanisms have been implicated in a cascade of events involving many biochemical and signaling pathways, such as oxidative stress, neuroinflammation, dysregulation of protein aggregation (e.g., amyloid-β (Aβ)) in AD and Lewy bodies in PD, metabolic impairment, mitochondrial instability, reduced clearance of toxins, DNA damage, and apoptosis (Mandel et al., 2008; Kim and Oh, 2012). Present clinical treatments for AD and PD only improve the symptoms and cause side effects during or after long-term administration, because the therapeutic approaches merely address a single mechanism (Houghton and Howes, 2005; Mandel et al., 2008). Thus, there is a continuing need to develop new compounds that simultaneously act on two or more pharmacological targets as disease-modifying therapies for AD and PD.

Natural products have a special role in modern drug development, evidenced by the fact that roughly half of the drugs currently in clinical use were derived from them (Decker, 2011). Especially, natural products have a high potential to be developed into optimum pharmaceuticals and nutraceuticals for NDs because their multiple properties can effectively target multifactorial diseases such as AD and PD (Decker, 2011). For

Identification of Neuroactive Constituents of the Ethyl Acetate Fraction from Cyperi Rhizoma Using Bioactivity-Guided Fractionation

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Abstract

Cyperi Rhizoma (CR), the rhizome of Cyperus rotundus L., exhibits neuroprotective effects in in vitro and in vivo models of neurological diseases. Nevertheless, no study has aimed at finding the neuroactive constituent(s) of CR. In this study, we identified active compounds in a CR extract (CRE) using bioactivity-guided fractionation. We first compared the anti-oxidative and neuroprotective activities of four fractions and the CRE total extract. Only the ethyl acetate (EA) fraction revealed strong activity, and further isolation from the bioactive EA fraction yielded nine constituents: scirpusin A (1), scirpusin B (2), luteolin (3), 6'-acetyl-3,6-diferuloylsucrose (4), 4',6' diacetyl-3,6-diferuloylsucrose (5), p-coumaric acid (6), ferulic acid (7), pinelllic acid (8), and fulgidic acid (9). The activities of constituents 1-9 were assessed in terms of anti-oxidative, neuroprotective, anti-inflammatory, and anti-amloid-β activities. Constituents 1, 2, and 3 exhibited strong positive; constituents 1 and 2 were characterized for the first time in this study. These results provide evidence for the value of CRE as a source of multi-functional neuroprotectants, and constituents 1 and 2 may represent new candidates for further development in therapeutic use against neurodegenerative diseases.

Key Words: Cyperi Rhizoma, Scirpusin A, Scirpusin B, Neuroprotection, Bioactivity-guided fractionation
example, major advances in the treatment of AD have included the use of acetylcholinesterase inhibitors, such as galantamine from *Galanthus nivalis* L., huperzine A from *Huperzia serrata* (Thunb. ex Murray) Trevis., and physostigmine from *Physostigma venenosum* Baill. (Houghton and Howes, 2005). Likewise, bromocriptine, pergolide, cabergoline, and lisuride from ergot, *Claviceps purpurea* (Fr.) Tul., have dopaminergic receptor-stimulating effects and are now used clinically for PD patients (Houghton and Howes, 2005).

Cyperi Rhizoma (CR) is the rhizome of *Cyperus rotundus* L., a sedge of the Cyperaceae family that grows naturally in tropical and temperate regions, and has been used for the treatment of several diseases, including stomach disorders and menstrual or emotional disturbances in women in Korea, China, Japan, and other Asian countries (Kim and Park, 1997; Jung et al., 2013). Several studies have investigated the pharmacological effects of CR, including its anti-diabetic, antibacterial, anti-apoptotic, anti-inflammatory, and anti-oxidative activities (Natarajan et al., 2006; Kilani et al., 2008). Previously, we demonstrated that a CR extract (CRE) exhibited neuroprotective effects in *in vitro* and *in vivo* PD models (Lee et al., 2010; Kim et al., 2013). CRE attenuated the neuronal damage induced by 6-hydroxydopamine (6-OHDA) in both PC12 and primary dopaminergic cells by inhibiting reactive oxygen species (ROS) and nitric oxide (NO) generation, mitochondrial membrane reduction, and caspase-3 activity, suggesting that the neuroprotective effects of CRE involve its anti-oxidative and anti-apoptotic activities (Lee et al., 2010). Additionally, CRE protected dopaminergic neurons from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine stress in estrogen-deprived mice via inhibition of mitochondrial Bcl-2 reduction and Bax elevation, cytosolic cytochrome c elevation, and caspase-3 activity (Kim et al., 2013). These results demonstrate that CRE has neuroprotective effects due to its phytochemical constituents. Although previous studies on the neuroprotective effects of CRE, specifically its anti-oxidative and anti-apoptotic activities, have been reported, no study has aimed at finding its neuroactive constituents. In this study, we sought to find the active constituents of CRE using bioactivity-guided fractionation. To explore the active constituents of CRE, we first compared the anti-oxidative activities of four fractions and the CRE total extract using radical-scavenging assays and their protective effects against 6-OHDA-induced neurotoxicity in PC12 cells using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays. We then compared the anti-oxidative, neuroprotective, anti-inflammatory, and anti-Aβ activities of the constituents isolated from the bioactive fraction.

**MATERIALS AND METHODS**

**Materials**

Dulbecco’s modified Eagle’s medium (DMEM), Roswell Park Memorial Institute (RPMI) 1640 medium, fetal bovine serum (FBS), and penicillin-streptomycin (P/S) were purchased from Hyclone Laboratories Inc. (Logan, UT, USA). Horse serum was purchased from Gibco Industries Inc. (Auckland, NZ). MTT, 6-OHDA, collagen, Griess reagent, corticosterone, 2,2-azinobis-(3-ethyl-benzthiazoline-6-sulphonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), thioflavin T (ThT), dimethyl sulfoxide (DMSO), lipopolysaccharide (LPS), and phosphate buffered saline (PBS) were purchased from Sigma-Aldrich (St Louis, MO, USA). Aβ1-42 peptide was purchased from American Peptide (Sunnyvale, CA, USA).

**Plant material**

The rhizomes of *Cyperus rotundus* L. (Cyperaceae) were obtained from a domestic Korean market (Kyungdong Drug Market, Seoul, Republic of Korea), in June 2011. The origin of the herbal material was identified by Prof. Dae Sik Jang and a voucher specimen (CYRO1-2011) has been deposited in the Lab. of Natural Product Medicine, College of Pharmacy, Kyung Hee University, Republic of Korea.

**Extraction and isolation**

The dried and milled plant material (2.8 kg) was extracted with 10 L of 80% EtOH three times by maceration. The extracts were combined and concentrated in vacuo at 40°C to give the 80% EtOH extract (399 g). A portion of the 80% EtOH extract (392 g) was suspended in H2O (2 L) and successively extracted with n-hexane (3×2 L), ethyl acetate (EA) (3×2 L) and BuOH (3×2 L) to give n-hexane- (45.8 g), EA- (23.5 g), BuOH- (52.4 g) and water-soluble extracts (270.3 g), respectively. Based on the initial biological testing, we chose the EA-soluble extract for detailed phytochemical investigation. A portion of the EA-soluble extract (15.0 g) was chromatographed over silica gel (6.5×41 cm, 70-230 mesh) as stationary phase with a CHCl3-MeOH gradient [99:1 (11.5 L), 49:1 (3.5 L), 19:1 (5.0 L), 9:1 (11.0 L), 4:1 (4.0 L), 1:1 (2.0 L), 0:1 (2.0 L)] as mobile phase to afford 17 pooled fractions (E01~E17). Fraction E10 [eluted with CHCl3-MeOH (19:1 v/v)]; 1.19 g was subjected to a Sephadex LH-20 column (3.5×58.5 cm) eluting with CHCl3-MeOH mixture (1:1, 600 mL) to produce 6 subfractions (E10-1~E10-6). Constituent 7 (8.3 mg) was purified from the subfraction E10-5 (35.0 mg) by using a flash chromatographic system (26 g C18 flash column, RediSep®Rf, Teledyne Isco Inc. (Lincoln, NE, USA) with MeOH-H2O (30:70 to 33:67 v/v, 7 mL/min). Fraction E11 [eluted with CHCl3-MeOH (9:1 v/v)]; 1.04 g was subjected to a silica gel column (3.8×28 cm, 230-400 mesh) with CHCl3-MeOH-H2O mixture (9:1:0.1, 1.0 L) to produce 8 subfractions (E11-1~E11-8). Fraction E11-6 (225.7 mg) was fractionated using a Sephadex LH-20 column (3.5×58.5 cm) with CHCl3-MeOH mixture (1:1, 500 mL) to afford constituent 5 (7.2 mg). Fraction E12 [eluted with CHCl3-MeOH (9:1 v/v)]; 690 mg was subjected to a silica gel column (3.8×28 cm, 230-400 mesh) with CHCl3-MeOH-H2O mixture (9:1:0.1, 1.5 L) to produce 7 subfractions (E12-1~E12-7). Constituents 6 (6.9 mg) and 4 (8.2 mg) were purified by preparative HPLC (YM-C Pack ODS A, MeOH-H2O, 40:60 to 75:25 v/v, 7.5 mL/min) from the subfraction E12-6 (99.7 mg). Fraction E13 [eluted with CHCl3-MeOH (9:1 v/v)]; 1.09 g was subjected to a silica gel column (3.8×28 cm, 230-400 mesh) with CHCl3-MeOH-H2O mixture (9:1:0.1, 1.5 L) to produce 7 subfractions (E13-1~E13-7). Constituents 3 (5.0 mg) and 1 (9.4 mg) were isolated from the subfractions E13-2 (238.8 mg) and E13-4 (90.5 mg), respectively, by repeated chromatography. Fraction E14 [eluted with CHCl3-MeOH (9:1 v/v)]; 444 mg was subjected to a silica gel column (3.8×28 cm, 230-400 mesh) with CHCl3-MeOH-H2O mixture (8:5:1.5:0.1, 1.5 L) to produce 7 subfractions (E14-1~E14-7). Constituents 7 (9.6 mg) and 8 (9.5 mg) were purified by preparative HPLC (YM-C Pack ODS A, MeOH-H2O, 60:40 to 75:25 v/v, 7.5 mL/min) from the subfraction E14-2 (99.7 mg). Fraction E15 [eluted with CHCl3-MeOH (4:1 v/v)]; 590 mg was subjected to a reversed phase column chromatography (3.6×24 cm, YM gel) with MeOH-H2O gradient (1:1, 3:2, 4:1, 1:0, 500 mL each eluent) to purify constituent 8 (10.4 mg).
Measurement of ABTS radical cation scavenging activity

ABTS solution of 7.4 mM was added to 2.6 mM potassium persulfate for 1 day before starting the experiment in the dark. Fractions and total extract of CRE at various concentrations of 2-500 μg/mL and constituents 1-9 at various concentrations of 0.1-100 μM were mixed with 0.2 mM DPPH ethanol solution (1:1). After incubation at room temperature in the dark for 30 min; the absorbance of the mixture was determined at 732 nm using a spectrophotometer (VersaMax microplate reader; Molecular Device, Sunnyvale, CA, USA). Also, the activity was expressed as half maximal inhibiting concentration (IC50) which is defined as the concentration of fractions and total extract of CRE and constituents 1-9 required to scavenge 50% of DPPH radical cations.

Measurement of DPPH radical scavenging activity

Fractions and total extract of CRE at various concentrations of 2-500 μg/mL and constituents 1-9 at various concentrations of 0.1-100 μM were mixed with 2.0 mM DPPH solution (1:1). After incubation at room temperature in the dark for 30 min; the absorbance of the mixture was determined at 517 nm using a spectrophotometer (VersaMax microplate reader; Molecular Device, Sunnyvale, CA, USA). Also, the activity was expressed as IC50 which is defined as the concentration of fractions and total extract of CRE and constituents 1-9 required to scavenge 50% of DPPH radicals.

Measurement of cell viability

All statistical parameters were calculated using GraphPad Prism 5.01 software (GraphPad Software Inc., San Diego, USA). Values were expressed as the mean ± standard error of the mean (SEM). The results were analyzed by one-way analysis of variance followed by the Tukey’s post hoc test. Differences with a p-value less than 0.05 were considered statistically significant.

RESULTS

EA fraction of CRE showed the highest radical scavenging activities among the fractions and total extract of CRE

Firstly, we compared the anti-oxidative activities of four
fractions and CRE total extract to find the bioactive fraction of CRE by performing ABTS and DPPH free radical scavenging activity assays. The water extract of Scutellariae Radix (SBE) was used as a positive control due to its remarkable anti-oxidative and neuroprotective activities (Gao et al., 1999). In both assays, the EA fraction of CRE exhibited the most potent radical scavenging activities, showing higher activity than SBE (Table 1).

EA fraction of CRE exhibited the protective effect against 6-OHDA neurotoxicity in PC12 cells

To compare the activities of fractions and CRE total extract in PC12 cells stressed with 6-OHDA, we performed an MTT assay. 6-OHDA, a hydroxylated analog of the neurotransmitter dopamine, generates ROS and is one of the most common neurotoxins used in degenerative models of catecholaminergic neurons and dopaminergic neurons (Schober, 2004). Treatment of the fractions and CRE total extract (10 μg/mL) for 4 h had no influence on cell proliferation and caused no apparent cell toxicity. Incubation with 6-OHDA (100 μM) reduced cell viability by 37.77 ± 5.87% compared with the control group, whereas only pretreatment with the EA fraction of CRE showed significant protective effects, increasing cell viability to 87.28 ± 9.20% (Fig. 1). These results are consistent with the anti-oxidative activities of the EA fraction of CRE. Taken together, the EA fraction (among the four fractions and CRE total extract) showed strong anti-oxidative and neuroprotective activities.

Constituents 1-9 isolated from EA fraction of CRE were identified

Here, we considered that the EA fraction may contain the most active constituent(s). To identify the active constituent(s) from the EA fraction of CRE, we isolated nine compounds. Repeated chromatography of the EA fraction resulted in the isolation and characterization of two stilbenes (1 and 2), one flavonoid (3), two phenolic glycosides (4 and 5), two phenylpropanoids (6 and 7), and two fatty acids (8 and 9). The structures of these known compounds were identified as scirpusin A (1) (Lam et al., 2008), scirpusin B (2) (Kobayashi et al., 2006), luteolin (3) (Han et al., 2007), 6′-acetyl-3,6-diferuloylsucrose (4) (Nakano et al., 1986), 4′,6′-diacetyl-3,6-diferuloylsucrose (5) R=H, R=Ac, 6′-coumaric acid (6) R=H, R=OCH3, 7′-ferulic acid (7) R=H, R=OCH3, 8′-p-coumaric acid (8) R=H, R=OCH3, 9′-furalidic acid (9) R=H, R=OCH3.

Fig. 1. Protective effects of fractions and total extract of CRE against 6-OHDA in PC12 cells. Cells were treated with fractions and total extract of CRE (10 μg/mL) for 1 h and incubated without or with 6-OHDA (100 μM) for a further 3 h. Cell viabilities are expressed as a percentage of the controls (cells treated with vehicle for 4 h). Values are indicated as the mean ± SEM. ***p<0.001; mean values were significantly different from the control group.###p<0.001; mean values were significantly different from the 6-OHDA only treated group.

Fig. 2. Chemical structures of constituents 1-9 as followings; (1) scirpusin A, (2) scirpusin B, (3) luteolin, (4) 6′-acetyl-3,6-diferuloylsucrose, (5) 4′,6′-diacetyl-3,6-diferuloylsucrose, (6) p-coumaric acid, (7) ferulic acid, (8) pinellin acid, and (9) fulgidiacid.
in vitro against neurotoxins in Cre.

Constituents 1-3 significantly protected neuronal cells against neurotoxins in in vitro.

To compare the neuroprotective effects of constituents 1-9 against 6-OHDA toxicity in PC12 cells, we performed an MTT assay. Among the isolated constituents, constituents 2 and 3 showed significant neuroprotective effects against 6-OHDA in PC12 cells (Fig. 3A). We also investigated whether constituents 1-9 protects HT22 hippocampal cells against corticosterone exposure. Stress is another factor in NDS and affects various brain areas, including the hippocampus (Sharvit et al., 2015). When stressful conditions occur, corticosterone, a steroid hormone synthesized in rodents, is typically elevated and induces decreased cell viability and distinct morphological changes in the HT22 cell line, a mouse hippocampal neuronal precursor cell (Xu et al., 2011). Among the nine isolated constituents, constituents 1 and 2 (10 μM) significantly protected hippocampal neurons against chronic corticosterone exposure in HT22 cells (Fig. 3B).

Constituents 2 and 3 showed the higher radical scavenging activities than other constituents.

We compared the anti-oxidative activities of constituents 1-9 using ABTS and DPPH free radical scavenging activity assays. As shown in Table 2, constituent 2 and 3 exhibited the most potent radical-scavenging activity compared with curcumin, the positive control (Ak and Gülçin, 2008). In Table 2, constituent 6 showed a very low activity in DPPH assay, compared with ABTS assay. This gap of anti-oxidative activities depending on assays are similarly shown in other reports (Yang et al., 2011; Badanai et al., 2015). It is reported that compound 6, a hydroxyl cinnamic acid derivative, has only one hydroxyl group and has no reaction with DPPH regardless of incubation time or concentrations, thus, this phenolic compound shows a slow kinetic reaction with DPPH (Brand-Williams et al., 1995; Von Gadow et al., 1997; Roginsky and Lissi, 2005).

Constituents 1, 3, 4, 5, 6, and 7 significantly reduced LPS-stimulated NO production, and constituent 3 had the most potent anti-inflammatory activity (Fig. 4B).

Table 2. Radical scavenging activities of constituents 1-9

| Sample         | ABTS IC50 (μM) | DPPH IC50 (μM) |
|----------------|----------------|----------------|
| Curcumin*      | 4.34           | 17.26          |
| Constituent 1  | 10.20          | 20.33          |
| Constituent 2  | 6.88           | 8.40           |
| Constituent 3  | 18.80          | 11.05          |
| Constituent 4  | 9.91           | 29.08          |
| Constituent 5  | 9.42           | 28.99          |
| Constituent 6  | 20.52          | > 500          |
| Constituent 7  | 19.05          | 30.10          |
| Constituent 8  | > 500          | > 500          |
| Constituent 9  | > 500          | > 500          |

*Curcumin: a positive control.
Constituents 1, 2, 3, and 9 revealed the significant anti-Aβ aggregation effect

Aβ acts as a neurotoxin to cells in culture via multiple pathways and its toxicity is correlated with the degree of peptide aggregation (Rivière et al., 2010). Inhibition of Aβ fibril formation is an attractive therapeutic and preventive strategy in the development of disease-modifying drugs for AD (Fujwara et al., 2009). We compared the inhibitory effects of constituents 1-9 on Aβ aggregation using fluorescence spectroscopy with ThT, a cationic benzothiazole dye, which is used widely for the identification and quantification of Aβ fibrils in vitro (Vassar and Culling, 1959). Treatment with curcumin and constituents 1, 2, 3, and 9 (100 μM) significantly inhibited the aggregation of monomeric Aβ1-42 (100 μM), as demonstrated by reduced ThT fluorescence intensity compared with the control group (Fig. 5).

DISCUSSION

In this study, we found that EA fraction is the most bio-active fraction of CRE by comparing the anti-oxidative effects and protective activities against 60HDA-induced neurotoxicity in PC12 cells. And then we isolated and identified the nine constituents (constituents 1-9) from EA fraction of CRE. We compared the anti-oxidative, neuroprotective, anti-inflammatory, and anti-Aβ activities of the constituents 1-9 and found the neuroactive constituents.

As shown in the present study, constituents 1-3 (among constituents 1-9 isolated from EA fraction of CRE) exhibited strong anti-oxidative, neuroprotective, anti-inflammatory, and anti-Aβ activities. On the basis of their chemical structures, constituents 1-3 are natural polyphenolic compounds. Numerous studies have reported that polyphenols in natural products prevent NDs through different mechanisms, including oxidative stress prevention and modulation of enzymes and receptors (Bastianetto et al., 2009; Essa et al., 2012; Ferreres et al., 2013). Thus, the multiple activities of constituents 1-3 might be due to their chemical structures. More experimental evidences on the multiple activities of them have been reported. Previous studies demonstrated that constituent 1 protects against singlet oxygen-induced DNA strand breakage (Kong et al., 2010). Furthermore, constituent 1 has β-secretase and Aβ inhibitory activities (Jeon et al., 2007; Rivière et al., 2010; Richard et al., 2011), but the direct neuroprotective effects of constituent 1 were demonstrated for the first time in the present study. Several studies have reported that constituent 2 exhibits anti-oxidative, anti-diabetic, and anti-photoaging activities, as well as vasorelaxing effects (Kobayashi et al., 2006; Sano et al., 2011; Maruki-Uchida et al., 2013; Tran et al., 2014), while no study on the neuroprotective effects of constituent 2 has been reported. However, further investigation is necessary to confirm these effects in animal models and to determine the molecular mechanisms underlying the neuroprotection of constituents 1 and 2. Moreover, further optimization is necessary before use of these constituents as lead compounds for the treatment of NDs. Regarding constituent 3, recent studies have shown that constituent 3 has neuroprotective effects. Constituent 3 protects dopaminergic neurons in in vitro and in vivo PD models (Yoo et al., 2013; Zhu et al., 2014). Moreover, constituent 3 ameliorates scopolamine-induced amnesia (Patil et al., 2014). The present study demonstrated that constituents 1-3 have neuroprotective effects by reducing oxidative damage, neuro-inflammation, and Aβ protein aggregation. Due to the multifactorial nature of NDs, they are considered to be among the most enigmatic diseases and place the me-
necinal chemist working in this field in a challenging situation (Decker, 2011). The present one-target paradigm for anti-ND treatment appears to be clinically unsuccessful (Kim and Oh, 2012). Future trends could involve the use of multi-functional drugs that act in different ways by mechanisms such as anti-oxidative and anti-inflammatory activities and inhibition of the formation of fibillary tangles and Aβ plaques (Houghton and Howes, 2005). The current data show that constituents 1 and 2 are novel multi-functional compounds that exert a variety of activities, including anti-oxidative, neuroprotective, anti-inflammatory, and anti-Aβ activities.

In conclusion, our study identified the neuroactive constituents of CRE using bioactivity-guided fractionation. We compared the anti-oxidative and neuroprotective activities of four fractions and CRE total extract, and found that only the EA fraction of CRE exhibits strong anti-oxidative and neuroprotective activities. We then isolated nine constituents from the EA fraction and compared their anti-oxidative, neuroprotective, and anti-Aβ activities. Constituents 1-3 from the EA fraction showed the most potential as multi-functional neuroprotectants through their anti-oxidative, anti-inflammatory, and anti-Aβ activities. Moreover, constituents 1 and 2 may represent new candidates for further development as therapeutics against NDs.

ACKNOWLEDGMENTS

This work was supported by the National Research Foundation of Korea grant funded by the Korea government (MSIP) (NRF-2015R1A2A2A01004341) and the Bio-Synergy Research Project (NRF-2012M3A9C4048795) of the Ministry of Science, ICT and Future Planning through the National Research Foundation.

REFERENCES

Ak, T. and Gülcin, I. (2008) Antioxidant and radical scavenging properties of curcumin. Chem. Biol. Interact. 174, 27-37.

Badanai, J., Silva, C., Martins, D., Antunes, D. and Miguel, M. G. (2015) Ability of scavenging free radicals and preventing lipid per-oxidation of some phenols and ascorbic acid. J. Pharm. Sci. 5, 34-41.

Bastianetto, S., Dumont, Y., Han, Y. and Quirion, R. (2009) Comparative neuroprotective properties of stilbene and catechin analogs: action via a plasma membrane receptor site? CNS Neurosci. Ther. 15, 76-83.

Brand-Williams, W., Cuvelier, M. E. and Berset, C. (1995) Use of a free radical method to evaluate antioxidant activity. LWT Food Sci. Technol. 28, 25-30.

Decker, M. (2011) Hybrid molecules incorporating natural products: applications in cancer therapy, neurodegenerative disorders and beyond. Curr. Med. Chem. 18, 1464-1475.

Essa, M. M., Vijayan, R. K., Castellano-Gonzalez, G., Memon, M. A., Braidy, N. and Guillen, G. J. (2012) Neuroprotective effect of natural products against Alzheimer’s disease. Neurochem. Res. 37, 1829-1842.

Ferreiras, F., Grosso, C., Gil-Izquierdo, A., Valentão, P. and Andrade, P. B. (2013) Ellagic acid and derivatives from Cochlospermum angolensis Welw. Extracts: HPLC-DAD-ESI/MS(n) profiling, quantification and in vitro anti-depressant, anti-cholinesterase and anti-oxidative activities. Phytochem. Anal. 24, 534-540.

Fujiiwa, H., Tabuchi, M., Yamaguchi, T., Iwasaki, K., Furukawa, K., Sekiguchi, K., Ikarashi, Y., Kudo, Y., Higuchi, M., Saito, T. C., Mae- da, S., Takashima, A., Hara, M., Yagasaki, N., Kase, Y. and Arai, H. A. (2009) Traditional medicinal herb Paeonia suffruticosa and its active constituent 1,2,3,4,6-penta-O-galloyl-beta-D-glucopyranoside have potent anti-aggregation effects on Alzheimer’s amyloid beta proteins in vitro and in vivo. J. Neurochem. 109, 1648-1657.

Gao, Z., Huang, K., Yang, X. and Xu, H. (1999) Free radical scavenging and antioxidative activities of flavonoids extracted from the radix of Scutellaria baicalensis Georgi. Biochim. Biophys. Acta 1472, 643-650.

Ha, S. K., Moon, E., Ju, M. S., Kim, D. H., Ryu, J. H., Oh, M. S. and Kim, S. Y. (2012a) 6-Shogaol, a ginger product, modulates neuroinflammation: a new approach to neuroprotection. Neuropharmacol. 63, 211-223.

Ha, S. K., Moon, E., Lee, P., Ryu, J. H., Oh, M. S. and Kim, S. Y. (2012b) Acacetin attenuates neuroinflammation via regulation of the response to LPS stimuli in vitro and in vivo. Neurochem. Res. 37, 1560-1567.

Hong, S. H., Hong, S. S., Hwang, J. S., Lee, M. K., Hwang, B. Y. and Ro, J. S. (2007) Monoamine oxidase inhibitory components from Caryatia japonica. Arch. Pharm. Res. 30, 13-17.

Hong, S. S. and Oh, J. S. (2012) Inhibitors of antigen-induced degranulation of RBL-2H3 cells isolated from wheat bran. J. Korean Soc. Appl. Biol. Chem. 55, 69-74.

Houghton, P. J. and Howes, M. J. (2005) Natural products and deriva-tives affecting neurotransmission relevant to Alzheimer’s and Parkinson’s disease. Neurosignals 14, 6-22.

Jeon, S. Y., Kwon, S. H., Seong, Y. H., Bae, K., Hur, J. M., Lee, Y. Y., Suh, D. Y. and Song, K. S. (2007) Beta-secretase (BACE1)-inhibiting stilbenoids from Smlax Rhizoma. Phytotherapy 24, 403-408.

Jin, C. Y., Lee, J. D., Park, C., Choi, Y. H. and Kim, G. Y. (2007) Cur- cumin attenuates the release of pro-inflammatory cytokines in lipopolysaccharide-stimulated BV2 microglia. Acta Pharmacol. Sin. 28, 1645-1651.

Jung, S. H., Kim, S. J., Jun, B. G., Lee, K. T., Hong, S. P., Oh, M. S., Jang, D. S. and Choi, J. H. (2013) α-Cyperone, isolated from the rhizomes of Cyperus rotundus, inhibits LPS-induced COX-2 expression and PGE2 production through the negative regulation of NFκB signalling in RAW 264.7 cells. J. Ethnopharmacol. 147, 208-214.

Kilani, S., Sghanier, M. B., Limen, I., Bouhlel, I., Boubaker, J., Bhouri, W., Skandranli, I., Neffati, A., Ammar, R. B., Dijoux-Franca, M. G., Ghedira, K. and Chekir-Ghedira, L. (2008) In vitro evaluation of antibacterial, antioxidant, cytotoxic and apoptotic activities of the tubers infusion and extracts of Cyperus rotundus. Biore source. Technol. 99, 9004-9008.

Kim, H. G. and Oh, M. S. (2012) Herbal medicines for the prevention and treatment of Alzheimer’s disease. Curr. Pharm. Des. 18, 57-75.

Kim, H. G., Hong, J., Huh, Y., Park, C., Hwang, D. S., Choi, J. H. and Oh, M. S. (2013) Cyperi Rhizoma inhibits the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced reduction in nigrostriatal dopa-minergic neurons in estrogen-deprived mice. J. Ethnopharma-col. 148, 322-329.

Kim, T. H. and Park, J. Y. (1997) Effect of Cyperi rhizoma on CC14 induced hepatotoxicity and lipid peroxidation. Kor. J. Pharmacogn. 28, 185-191.

Kobayashi, K., Ishihara, T., Khono, E., Miyase, T. and Yoshizaki, F. (2006) Constituents of stem bark of Callistemon rigidus showing inhibitory effects on mouse alpha-amylase activity. Biol. Pharm. Bull. 29, 1275-1277.

Kong, Q., Ren, X., Jiang, L., Pan, Y. and Sun, C. (2010) Scirpusin A, a hydroxystilbene dimer from Xinjiang wine grape, acts as an effec-tive singlet oxygen quencher and DNA damage protector. J. Sci. Food. Agric. 90, 823-828.

Kurashina, Y., Miura, A., Enomoto, M. and Kuwahara, S. (2011) Stereoselective synthesis of malyngic acid and fulgidic acid. Tetrahedron 67, 1649-1653.

Lam, S. H., Chen, J. M., Kang, C. J., Chen, C. H. and Lee, S. S. (2008) Alpha-Glucosidase inhibitors from the seeds of Syagrus romanzof-fiana. Phytochemistry 69, 1173-1178.

Lee, C. H., Hu, X., Kong, D. S., Kim, H. G., Oh, H., Park, H., Cho, J. H., Lee, J. M., Jang, J. B., Lee, K. S. and Oh, M. S. (2010) Protective effect of Cyperi rhizoma against 6-hydroxydopamine-induced neuronal damage. J. Med. Food. 13, 564-571.

http://dx.doi.org/10.4062/biomolther.2016.091
Liu, B. and Hong, J. S. (2003) Role of microglia in inflammation-mediated neurodegenerative diseases: mechanisms and strategies for therapeutic intervention. *J. Pharmacol. Exp. Ther.* **304**, 1-7.

Mandel, S. A., Amit, T., Weinreb, O., Reznichenko, L. and Youdim, M. B. (2008) Simultaneous manipulation of multiple brain targets by green tea catechins: a potential neuroprotective strategy for Alzheimer and Parkinson diseases. *CNS Neurosci. Ther.* **14**, 352-365.

Maruki-Uchida, H., Kurita, I., Sugiyama, K., Sai, M., Maeda, K. and Ito, T. (2013) The protective effects of piceatannol from passion fruit (Passiflora edulis) seeds in UBV-irradiated keratinocytes. *Biol. Pharm. Bull.* **36**, 845-849.

Miyase, T. and Ueno, A. (1993) Sucrose derivatives from the roots of Polygala tenuifolia. *Shoyakugaku Zasshi* **47**, 267-278.

Nakano, K., Murakami, K., Takaishi, Y. and Tomimatsu, T. (1986) Feruloyl sucrose derivatives from Heloniopsis orientalis. *Chem. Pharm. Bull.* **34**, 5005-5010.

Natarajan, K. S., Narasimhan, M., Shanmugasundaram, K. R. and Shanmugasundaram, E. R. B. (2006) Antioxidant activity of a salt-spice-herbal mixture against free radical induction. *J. Ethnopharmacol.* **105**, 76-83.

Patil, S. P., Jain, P. D., Sancheti, J. S., Ghumatkar, P. J., Tambe, R. and Sathaye, S. (2014) Neuroprotective and neurotrophic effects of Apigenin and Luteolin in MPTP induced parkinsonism in mice. *Neuropharmacology* **86**, 192-202.

Richard, T., Pawlus, A. D., Igłęsiass, M. L., Pedrot, E., Waffo-Teguo, P., Mériton, J. M. and Monti, J. P. (2011) Neuroprotective properties of resveratrol and derivatives. *Ann. N. Y. Acad. Sci.* **1215**, 103-108.

Rivièere, C., Papastamoulis, Y., Fortin, P. Y., Delchier, N., Andriamanerivo, S., Waffo-Teguo, P., Kapche, G. D., Amira-Guebala, H., Deaumay, J. C., Mériton, J. M., Richard, T. and Monti, J. P. (2010) New stilbene dimers against amyloid fibril formation. *Bioorg. Med. Chem. Lett.* **20**, 3441-3443.

Roginsky, V. and Lissi E. A. (2005) Review of methods to determine chain-breaking antioxidant activity in food. *Food Chem.* **92**, 235-254.

Sano, S., Sugiyama, K., Ito, T., Katano, Y. and Ishihata, A. (2011) Identification of the strong vasorelaxing substance scirpusin B, a dimer of piceatannol, from passion fruit (Passiflora edulis) seeds. *J. Agric. Food Chem.* **59**, 6209-6213.

Schober, A. (2004) Classic toxin-induced animal models of Parkinson’s disease: 6-OHDA and MPTP. *Cell Tissue Res.* **318**, 215-224.

Sharvit, A., Segal, M., Kehat, O., Stork, O. and Richter-Levin, G. (2015) Differential modulation of synaptic plasticity and local circuit activity in the dentate gyrus and CA1 regions of the rat hippocampus by corticosterone. *Stress* **18**, 319-327.

Swiślocka, R., Kowczyk-Sadowy, M., Kalinowska, M. and Lewandowski, W. (2012) Spectroscopic (FT-IR, FT-Raman, 1H and 13C NMR) and theoretical studies of p-coumaric acid and alkali metal p-coumarates. *Spectroscopy* **27**, 35-48.

Tran, H. H., Nguyen, M. C., Le, H. T., Nguyen, T. L., Pham, T. B., Chau, V. M., Nguyen, H. N. and Nguyen, T. D. (2014) Inhibitors of α-glucosidase and α-amylase from Cyperus rotundus. *Pharm. Biol.* **52**, 74-77.

Vassar, P. S. and Culling, C. F. (1959) Fluorescent stains, with special reference to amyloid and connective tissues. *Arch. Pathol.* **68**, 487-488.

Von Gadow, A., Joubert, E. and Hansmann C. F. (1997) Comparison of the antioxidant activity of aspalathin with that of other plant phenols of rooibos tea (Aspalathus linearis), α-tocopherol, BHT, and BHA. *J. Agric. Food Chem.* **45**, 632-638.

Wysz-Coray, T. and Mucke, L. (2002) Inflammation in neurodegenerative disease—a double-edged sword. *Neuron* **35**, 419-432.

Xu, Y., Zhang, C., Wang, R., Govindarajan, S. S., Barish, P. A., Vernon, M. M., Fu, C., Acharya, A. P., Chen, L., Boykin, E., Yu, J., Pan, J., O’Donnell, J. M. and Ogle, W. O. (2011) Corticosterone induced morphological changes of hippocampal and amygdaloid cell lines are dependent on 5-HT7 receptor related signal pathway. *Neuroscience* **182**, 71-81.

Yang, H., Dong, Y., Du, H., Shi, H., Peng, Y. and Li, X. (2011) Anti-oxidant compounds from propolis collected in Anhui, China. *Molecules* **16**, 3444-3455.

Yoo, D. Y., Choi, J. H., Kim, W., Nam, S. M., Jung, H. Y., Kim, J. H., Won, M. H., Yoon, Y. S. and Hwang, I. K. (2013) Effects of luteolin on spatial memory, cell proliferation, and neuroblast differentiation in the hippocampal dentate gyrus in a scopalamine-induced amnesia model. *Neuro. Res.* **35**, 813-820.

Yoshioka, T., Inokuchi, T., Fujoka, S. and Kimura, Y. Z. (2004) Phenolic compounds and flavonoids as plant growth regulators from fruit and leaf of Vitex rotundifolia. *Z. Naturforsch., C, J. Biosci.* **59**, 509-514.

Zhu, L., Bi, W., Lu, D., Zhang, C., Shu, X. and Lu, D. (2014) Luteolin inhibits SH-SY5Y cell apoptosis through suppression of the nuclear transcription factor-κB, mitogen-activated protein kinase and protein kinase B pathways in lippopolysaccharide-stimulated cocultured BV2 cells. *Exp. Ther. Med.* **7**, 1065-1070.