Alzheimer’s Disease Drug Discovery from Herbs: Neuroprotectivity from β-Amyloid (1-42) Insult

DARRICK S.H.L. KIM, Ph.D.,1,2 JIN-YUNG KIM, Ph.D.,1–3 and YE SUN HAN, Ph.D.4

ABSTRACT

Objective: To comparatively evaluate selected herbs for their ability to protect neuronal cells from direct βA(1-42) insult.

Design: Twenty-seven (27) herbs were selected, extracted with aqueous methanol (90%) and chloroform, and the extracts were evaluated for their ability to protect PC12 rat pheochromocytoma and primary neuronal cells from βA(1-42) insult using both 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide reduction assay and lactate dehydrogenase efflux assay.

Results: Curcuma aromatia (ul-keum) and Zingiber officinale (ginger) extracts effectively protected cells from βA(1-42) insult, followed by Ginkgo biloba (ginkgo), Polygonatum sp. (King Solomon’s seal), Cinnamomum cassia (Chinese cinnamon), Rheum coreanum (Korean rhubarb), Gastrodia elata (gastrodia), and Scutellaria baicalensis (skullcap). Several extracts showed cytotoxicity at high concentration (~150 µg/mL), whereas other extracts did not at all protect cells from βA(1-42) insult.

Conclusion: Selective herbs may be potentially important resources to discover drug candidates against the onset of Alzheimer’s disease.

INTRODUCTION

Alzheimer’s disease (AD) is the most common cause of progressive cognitive dysfunction that affects approximately 4 million Americans, causing more than 100,000 deaths each year with a total annual cost approaching $100 billion in the United States.1 One of the principal pathologic characteristics of AD is an extracellular deposition of β-amyloid (βA) as senile plaques. βA(1-42) has been shown to exert direct toxic effects on neurons and inhibit the neurite outgrowth in vitro in a dose-dependent manner.2 Although βA does not appear to cause cell death at low concentration (1–5 µg/mL), it was found to cause profound cell damage, such that cell viability is undermined.3,4 Because βA insult to neuronal cells has been hypothesized as one of the major causes of AD pathology,5,6 neuronal cell protection from βA insult has been speculated to be an important therapeutic approach to control the onset of AD.

There are large numbers of herbs readily available on the market to treat various ailments. From these herbs, several potential drug candidates to treat AD have been discovered: for example, curcuminoids from Curcuma longa (tumeric), and3,4 shogaols from Zingiber officinale (ginger),7,8 huperzine-A from herbal moss Huperzia serrata (toothed clubmoss),9 and galanthamine from Ungernia sp. (daffodil bulbs).10 However, these plant extracts represent only a small fraction of herbs used in the world. Thus, it was of interest to identify herbs that could be used as resources for discovering potential drug candidates to treat AD. Because βA-induced neuronal cell death is one of the suspected etiologies

1CurXceL Corporation, The Business and Technology Center, West Lafayette, IN.
2Program for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL.
3Natural Science Research Institute, Seoul Woman’s University, Seoul, Korea.
4Department of Technology Fusion, Konkuk University, Seoul, Korea.
of the onset of AD, it was reasonable to postulate that select herbs may meet the criteria of potential drug candidate leads to treat AD by protecting neuronal cells from \( \beta A(1-42) \) insult. To our knowledge, there has been no published comparative evaluation of herbal extracts based on their efficacy toward protecting neuronal cells from \( \beta A(1-42) \) insult.

Herein, we report results from a pilot study on selected herbs as part of our ongoing effort to discover potential drug candidates to treat AD. Methanolic and chloroform extracts of the following herbs were evaluated for their neuroprotectivity against \( \beta A(1-42) \) insult: Aconitum carmichaelii Debs. (Ranunculaceae), Angelica gigas Nakai (Umbelliferae) (Korean angelica), Aralia continentalis Kitagawa (Araliaceae) (Manchurian spikenard), Astragalus membranaceus (Fisch.) Bunge. (Leguminosae) (astragalus), Atractylodes japonica (Koidz) Kitagawa (Compositae), Bupleurum falcatum (Bupleurum) (Umbelliferae), Cinnamomum cassia (Nees) Blume (Lauraceae) (Chinese cinnamon), Curcuma aromatica Salisbur (Zingiberaceae) (ul-keum), Dioscorea batatas Decaisne (Dioscoreaceae) (yam), Eleutherococcus senticosus Maxim (Araliaceae), (Siberian ginseng), Gastrodia elata Blume (Orchidaceae) (gastrodia), Ginkgo biloba Linn. (Ginkgoaceae) (ginkgo), Glycyrrhiza glabra Linn. (Leguminosae) (licorice), Inula helenium Linn. (Compositae) (elecampane inula), Ligusticum officinale Kitagawa (Umbelliferae) (common lavage), Macrobiotum officinale Sieb. Et Zucc (Cornaceae), Ophiopogon japonicus Ker-Gugler (Liliaceae) (dwarf lilyturf), Ostericum koreanum Kitagawa (Umbelliferae), Panax ginseng Nees (Araliaceae) (Korean ginseng), Platycodon grandiflorum A. DC. (Campanulaceae) (balloon flower), Polygonatum spp. (Liliaceae) (King Solomon’s seal), Poria cocos (Schw.) Wolf. (Polyporaceae) (cocos), Rehmannia glutinosa Liboschitz (Scrophulariaceae) (Chinese foxglove), Rheum coreanum Nakai (Polygonaceae) (Korean rhubarb), Schisandra chinensis Bailon (Schisandraceae) (schisandra), Scutellaria baicalensis Georgi. (Labiatae) (skullcap), and Z. officinale Roscoe (Zingiberaceae). PC12 rat pheochromocytoma and primary neuronal cells were primary used to evaluate the neuroprotectivity of the herbal extracts by means of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) reduction assay\(^3\) and lactate dehydrogenase (LDH) efflux assay\(^1\)\(^,\)\(^2\).

**MATERIALS AND METHODS**

**Materials**

PC12 cells were obtained from the American Type Culture Collection (Rockville, MD). Cells were routinely cultured on a poly-D-lysine-coated Corning tissue culture plate (Invitrogen-Gibco, Carlsbad, CA). Culture media and supplements were obtained from Life Technologies (Grand Island, NY). \( \beta A(1-42) \) was purchased from Bachem California (Torrance, CA). MTT and other chemicals were purchased from Sigma/Aldrich (St. Louis, MO) and Invitrogen-Gibco (Carlsbad, CA). The herbs were purchased from Korean Kyung-Dong herb market in Seoul, Korea and the voucher samples were labeled and deposited in PCRPS (Program for Collaborative Research in Pharmaceutical Science) storage, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago.

**Extraction of herbs**

Dried herb (100 g) was ground or chopped (average size \( \sim 0.5 \text{ cm} \), and extracted with 90% aqueous methanol (150 mL \( \times 3 \)) by percolation. The methanol extracts were combined, filtered, and the solvent was removed under vacuum at 35°C. The residue was dissolved in 10 mL of methanol, water (100 mL) was added, and partitioned with chloroform (pretreated with NaHCO\(_3\) overnight, 50 mL \( \times 4 \)). The chloroform layers were combined and washed twice with \( \text{H}_2\text{O} (100 \text{ mL } \times 2) \), dried (MgSO\(_4\)), filtered, and evaporated under vacuum to afford a residue.

**Primary neuronal cell culture**

Dissociated primary neuronal cell cultures were established from embryonic 18-day-old Sprague-Dawley rat fetuses using a previously described method.\(^1\) Pups were delivered by cesarean section while the dam was anesthetized with ether. Hippocampal tissue from embryonic day 18 Sprague-Dawley rat pups was dissected in ice-cold Ca\(^2+\)/Mg\(^2+\)-free Hank’s balanced salt solution (HBSS) containing 100 \( \mu \text{g/mL} \) each of streptomycin and penicillin. After gentle trituration of the tissue in a conical tube with a sterile pipette in cold buffer, it was centrifuged for 2 minutes at 2000 rpm. The resulting supernatant was discarded and replaced with 3 mL of 0.25% trypsin and 0.05% deoxyribonuclease (DNAased, Typell). Enzymatic digestion was continued for 10 minutes at 37°C with gentle agitation of the tissue. Proteolysis was terminated by adding 3 mL of HBSS containing 0.5% soybean trypsin inhibitor. Remaining tissue fragments were dissociated by repeated trituration using a sterile pipette. The resulting cell suspension was centrifuged at 2000 rpm for 5 minutes. The neurons were seeded into poly-D-lysine-coated 96-well tissue culture plates at a final density of 1.5 \( \times 10^5 \) cells/cm\(^2\). The cultures were maintained in Eagle’s Minimum Essential Medium supplemented with 10% fetal bovine serum, 10% horse serum, 35 \( \mu \text{mol/L} \) d-glucose and 2 \( \mu \text{mol/L} \) l-glutamine, and 100 \( \mu \text{g/mL} \) each of penicillin and streptomycin in a 5% CO\(_2\) and 95% humidified atmosphere at 37°C. After 3 days of incubation, the cultures were treated with medium containing 35 \( \mu \text{g/mL} \) uridine and 15 \( \mu \text{g/mL} \) fluoro-deoxyuridine, as well as N\(_2\) supplement to replace the serum and inhibit glial cell proliferation. The medium was changed every 2–3 days.

\( \beta A(1-42) \) effect on PC12 and primary neuronal cells using an MTT reduction assay

PC12 cells were maintained in high glucose Dulbecco’s Modified Eagle medium, 10% horse serum, 5% fetal calf

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serum, and 1% penicillin/streptomycin. Exponentially growing cells (2000 cells/mL) were placed (90 μL) in 96-well poly-L-lysine-coated tissue culture plates and incubated for 24 hours prior to the assay. For primary neuronal cells, cells were plated (1.5 × 10^5 cells/mL) in 96-well poly-L-lysine-coated tissue culture plates and incubated for 5 days prior to the assay. βA(1-42) stock solution was prepared in dimethyl sulfoxide (DMSO) at 10 mg/mL concentration and aged for 3 days before partition. Cells were incubated with various concentrations of βA(1-42) (100, 25, 6.25, 1.56, 0.39, 0.098, and 0.024 μg/mL) for 24 hours at humidified atmosphere containing 5% CO2 at 37°C. Cell viability was determined using an MTT reduction assay. Briefly, after incubation of cells in MTT solution (25 μL per well, 1 mg/mL stock solution) for 1 hour at 37°C, lysing buffer (50% aqueous dimethylformamide (DMF) and 20% sodium dodecyl sulfate (SDS) at pH 4.7) were added and incubated overnight at 37°C. The optical densities of the resulting solutions were colorimetrically determined at 570 nm using a microplate reader. Dose–response curves were obtained and results expressed as EC50 values in μg/mL.

**Cell viability assay using MTT**

The herbal extracts’ ability to protect PC12 cells and primary cortical neurons from βA(1-42) insult was investigated according to the published procedure. Cells were plated and prepared as described above and incubated with βA(1-42) ([1.0 μg/mL for PC12 cells and 3.0 μg/mL for primary neuronal cells], prepared from a stock solution [1.0 mg/mL in DMSO]) and herbal extracts at various concentrations (150, 100, 50, 25, 10, and 5 μg/mL) for 24 hours. The final DMSO concentration for all experiments was less than 1%. The herbal extracts’ ability to protect cells from βA(1-42) insult was determined by measuring the cell’s potential to reduce MTT with respect to the treatment of cells with 1% DMSO only and the treatment of cells with 2.0 μg/mL βA(1-42) and with or without the presence of herbal extract. MTT reduction assay was performed as described above. Dose–response curves were obtained and results were expressed as EC50 values in μg/mL concentration.

**Comparative analyses of cell viability using LDH release assay**

A comparative evaluation of herbal extracts’ ability to protect PC12 and primary neuronal cells from βA(1-42) insult was performed using an LDH release assay. Measurement of LDH released to the extracellular media by nonviable and stressed cells has been a quantitative method for assessing cell viability in cell culture. LDH activity in the cell medium was measured using a method described elsewhere. Cells were prepared in 96-well tissue culture plates as described above. Cells were incubated with βA(1-42) (5.0 μg/mL, prepared from a stock solution [1.0 mg/mL in DMSO]) and the herbal extracts (20 μg/mL) for 24 hours. The final DMSO concentration was less than 1%. MTT was dissolved in phosphate-buffered saline (PBS) (pH 7.4) at a concentration of 2.5 mg/mL (6 mmol/L) and stored at 4°C. 1-Methoxyphenazine methosulfate (MPMS) was dissolved in PBS at a concentration of 100 mmol/L and stored at 4°C. LDH substrate mixture (10 mL) was prepared immediately prior to the experiment by dissolving 25 mg of lithium L-lactate and 25 mg of nicotinamide adenine dinucleotide (NAD) in 9.0 mL of 0.2 mol/L Tris-HCl buffer (pH 8.2) containing 0.1% (v/v) Triton X-100, 1.0 mL of MTT stock solution, and 10 μL of MPMS stock solution. The culture supernatant (50 μL) was transferred to 96-well culture plates and mixed with 50 μL of the LDH substrate mixture for 30 minutes. The reaction was stopped by adding 100 μL of lysing buffer and was incubated overnight. The optical density of the resulting solutions was colorimetrically determined at 570 nm using a microplate reader.
RESULTS

βA(1-42) effect on neuronal cells

Figure 1 shows the effect of βA(1-42) on PC12 and primary neuronal cells’ viability as determined using MTT reduction assay. From the slope of the graphs, EC50 values were obtained. βA(1-42) attenuated the viability of PC12 cells at EC50 = 1.0 μg/mL and the viability of primary neuronal cells at EC50 = 3.0 μg/mL.

Efficacy of herbal extracts on neuronal cells

MTT is a monothetrazolium salt that can be intracellularly reduced to purple-colored formazan and is widely used for measuring cell viability. Table 1 shows that chloroform extracts of C. aromatia and Z. officinale extracts effectively protected cells from βA(1-42) insult at EC50 = 18–24 μg/mL under MTT reduction assay condition, followed by G. biloba extract (EC50 = 69–78 μg/mL). Methanol extracts of C. aromatia and Z. officinale required higher concentration to achieve EC50 = 38–54 μg/mL; and G. biloba, EC50 = 98–109 μg/mL. Chloroform extracts of C. cassia, Polygonatum spp., R. coreanum, I. helenium, G. elata, and S. baicalensis extracts only showed a marginal cell protection from βA(1-42) insult (EC50 = 102–137 μg/mL). The methanol extracts of Aconitum carmichaelis, A. continentalis, G. elata, I. helenium, O. coreanum, and S. baicalensis showed cytotoxicity at 150 μg/mL concentration. Chloroform extracts of A. continentalis, B. falcatus, E. senticosus, G. elata, O. japonicus, and R. coreanum showed cell protection against βA(1-42) insult, whereas their corresponding methanol extracts did not. The appearance of neuroprotectivity in the chloroform extracts from cytotoxic or nonactive methanol extracts of A. continentalis, B. falcatus, E. senticosus, G. elata, I. helenium, O. japon-

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Common name</th>
<th>PC12</th>
<th>Primary neuron</th>
<th>Methanol extract</th>
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<tr>
<td>Aconitum carmichaelis</td>
<td>Carmichael’s aconite</td>
<td>—</td>
<td>CT</td>
<td>CT</td>
</tr>
<tr>
<td>Angelica gigas</td>
<td>Korean angelica</td>
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<td>Manchurian spikenard</td>
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<td>—</td>
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<td>Astragalus membranaceus</td>
<td>Astragalus</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<td>Atractylodes japonica</td>
<td>Atractylodes</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Bupleurum falcatum</td>
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<td>&gt;150</td>
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<td>—</td>
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<tr>
<td>Cinnamomum cassia</td>
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<td>104 ± 17</td>
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<td>Yam</td>
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<td>—</td>
<td>—</td>
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<tr>
<td>Eleutherococcus senticosus</td>
<td>Siberian ginseng</td>
<td>&gt;150</td>
<td>&gt;150</td>
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<td>Gastrodia elata</td>
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<td>126 ± 22CT</td>
<td>130 ± 26CT</td>
<td>—</td>
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<tr>
<td>Ginkgo biloba</td>
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<td>78 ± 15CT</td>
<td>68 ± 11CT</td>
<td>112 ± 23</td>
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<tr>
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<td>—</td>
<td>—</td>
<td>97 ± 21</td>
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<tr>
<td>Inula helenium</td>
<td>Elecampane inula</td>
<td>125 ± 17</td>
<td>136 ± 16</td>
<td>&gt;150CT</td>
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<tr>
<td>Ligusticum officiale</td>
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<td>—</td>
<td>&gt;150</td>
<td>&gt;150</td>
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<tr>
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<td>—</td>
<td>&gt;150</td>
<td>—</td>
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<tr>
<td>Ophiopogon japonicus</td>
<td>Dwarf lilyturf</td>
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<tr>
<td>Platycodon grandiflorum</td>
<td>Balloon flower</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Polygonyss sp.</td>
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<td>112 ± 28</td>
<td>120 ± 27</td>
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<td>—</td>
<td>—</td>
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<td>Rehmannia glutinosa</td>
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<td>—</td>
<td>—</td>
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<tr>
<td>Rheum coreanum</td>
<td>Korean rhubarb</td>
<td>114 ± 17</td>
<td>110 ± 19</td>
<td>—</td>
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<tr>
<td>Schisandra chinensis</td>
<td>Schisandra</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Scutellaria baicalensis</td>
<td>Skullcap</td>
<td>132 ± 20CT</td>
<td>140 ± 19CT</td>
<td>—</td>
</tr>
<tr>
<td>Zingiber officinale</td>
<td>Ginger</td>
<td>18 ± 12</td>
<td>19 ± 11</td>
<td>34 ± 10</td>
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EC50 represents the sample concentration that is required to achieve 50% cell viability, a midpoint between the positive control values and the negative control values. — represents no activity given to those extracts that gave <20% cell viability at 150 μg/mL conc. CT: cytotoxic at 150 μg/mL concentration.

The tests were performed in triplets on three different dates. Data were subject to one-way analysis of variance (OriginPro 7.5, OriginLab, Northampton, MA). p values of <0.05 were considered significant.
icus, R. coreanum, and S. baicalensis was noteworthy. In general, chloroform extracts showed higher protectivity than methanol extracts did.

Comparative analysis of selected herbs’ ability to protect neuronal cells from βA insult using MTT reduction assay

Figure 2 shows a comparative analysis of chloroform extracts of selected herb extracts’ ability to protect PC12 and primary neuronal cells from βA(1-42) insult at various concentrations as determined by an MTT reduction assay. The data represent the relative efficacy of the chloroform extracts of selected herbs’ ability to protect cells from βA(1-42) insult with respect to each other. Effective cell protection from βA(1-42) insult was clearly demonstrated from C. aromatia and Z. officinale extracts while other herbal extracts’ cell protectivity from βA(1-42) insult lagged behind.

Comparative analysis of herbal extracts’ ability to protect neuronal cells from βA insult using the LDH efflux assay

In addition to the MTT reduction assay, βA(1-42)-induced cell viability reduction can be determined by observing the amount of LDH release in cells whose viability is undermined.11 Good correlation between the results from the MTT reduction assay and the LDH efflux assay has been reported.11,12 Figure 3 shows results from a comparative analysis on selected herbal extracts’ ability to protect PC12 cells and primary neuronal cells in the presence of βA(1-42) at 5.0 μg/mL concentration using an LDH efflux assay. The results showed that βA(1-42) and treated cells (negative control) release the most amount of LDH as expected, whereas 1% DMSO-only treated cells and C. aromatia and Z. officinale extracts-treated cells showed near background level of LDH release. Gastrodia elata, Polygonatum sp. and S. baicalensis showed only a marginal inhibition of LDH release, which is consistent with the result of MTT reduction assay. Herbal extracts that did not show positive results on MTT reduction assay did not inhibit the LDH release.

DISCUSSION

In cancer research, there has been a long history of medicinal plant drug discovery that yielded several clinically effective anticancer agents such as vinca alkaloids, taxanes, and camptothecins.14 Therefore, it was reasoned that such an approach may be effectively applied in the drug discovery against AD. One of the important criteria for selecting the plant resources was reasoned to be, contrary to anticancer research, their nontoxicity toward cells because protecting neuronal cells from the βA(1-42) insult is one of the important keys to discovering AD treatment. One of the reasonable medicinal plant resources for our study was herbs with a long history of safe use, and the herbs were chosen based on their ready availability in the market. Sparked by our effort, we hope scientists will come together to investigate all available herbs to one day discover the treatment against AD.

βA(1-42) was shown to exert direct toxic effects on neurons to inhibit the neurite outgrowth in vitro in a dose-dependent manner.2 Although βA does not appear to cause cell death at low concentration (1–5 μg/mL), it was found to
cause profound cell damage, such that cell viability is undermined.\(^3\)
\(^4\) Because \(\beta A\) insult to neuronal cells has been hypothesized as one of the major causes of AD pathology,\(^5\)
\(^6\) neuronal cell protection from \(\beta A\) insult is a potentially important therapeutic approach to control the onset of AD. As a result of strong interest in finding herbal resources with \(\beta A\)-toxicity-modulating property as a chemotherapeutic measure to discover potential drug candidates, we comparatively evaluated 27 selected herbs using PC12 cells and primary cortical neurons against \(\beta A(1-42)\) insult by means of an MTT reduction assay and LDH release assay.

![Graph](image.png)

**FIG. 3.** Comparative analysis of selected herbs’ ability to protect neuronal cells from \(\beta A\) insult using a lactate dehydrogenase (LDH) efflux assay. Chloroform extracts of 10 selected herbs were comparatively evaluated for PC12 and primary neuronal cell protection from \(\beta A(1-42)\) insult. “+ control” represents cells without \(\beta A(1-42)\) and any herbal extracts, whereas “– control” represents cells with \(\beta A(1-42)\) alone. Cells were incubated with \(\beta A(1-42)\) \(5.0 \mu\)g/mL and herbal extracts \(20 \mu\)g/mL for 24 hours. Cell viability was determined using an LDH release assay. The data points were obtained in triplets on three different dates. Data were subject to a one-way analysis of variance. \(p\) values of < 0.05 were considered to be significant. \(A. gigas, Angelica gigas\) (Korean angelica); \(C. aromatica, Curcuma aromatica\) (ul-keum); \(E. senticosus, Eleatherococcus senticosus\) (Siberian ginseng); \(G. elata, Gastrodia elata\) (gastrodia); \(P. ginseng, Panax ginseng\) (Korean ginseng); \(P. greggii, Polygonum sp.\) (King Solomon’s seal); \(R. glutinosa, Rehmannia glutinosa\) (Chinese foxglove); \(S. chinensis, Schisandra chinensis\) (schisandra); \(S. baicalensis, Scutellaria baicalensis\) (skullcap); \(Z. officinale, Zingiber officinale\) (ginger).

Recently, there have been an increasing number of drug discovery efforts on medicinal herbs to find potential drug candidates to treat neurologic dysfunctions such as AD. \(A. gigas, Saururus chinensis,\) and \(S. chinensis\) in an 8:1:1 ratio extract was shown to improve scopolamine-induced memory impairment in mice.\(^15\) \(A. gigas\) roots have been used traditionally in Korean herbal medicine for the treatment of anemia and as a sedative, anodyne, and tonic agent. \(Schizandra chinensis\) fruit has been used as a tonic in traditional medicine and possesses hepatoprotective activity.\(^16\) \(S. chinensis\) has been used in traditional medicine for edema, beriberi, jaundice, turbid urine, and gonorrhea in Korea.\(^17\) The ethyl ether fraction of \(Gastrodia elata,\) a traditional herb that has been in use as an anticonvulsant, and in treating general paralysis and tetanus,\(^18\) was shown to protect \(\beta A\)-induced cell death.\(^19\) Shogaols from \(Z. officinale\) have both been identified to protect neuronal cells from \(\beta A\) insult.\(^7\)\(^8\) Baicalein and baicalin from \(S. baicalensis\) demonstrated protection of PC12 cells from \(\beta A\) insult.\(^20\) Most notably, curcuminoids from \(Curcuma\) sp. have been recognized for their neuroprotectivity against \(\beta A(1-42)\) insult.\(^3\)\(^4\) In particular, curcumin, a potential drug candidate to treat AD, has demonstrated inhibition of the formation of \(\beta A\) oligomers and fibrils, binds \(\beta A\) plaques, and reduces \(\beta A\) load in vivo.\(^21\)

Thus, it is reasonable to anticipate that rigorous analyses of herbal extracts with neuroprotectivity against \(\beta A\) insult may yield potential drug candidates to treat AD.

Aqueous extracts from \(Verbena officinalis,\)\(^22\) \(Bambuseae concretio Salicea,\)\(^23\) and \(Rhizoma acori graminei\)\(^24\) have shown protection of neuronal cells from \(\beta A\) insult. One of the criteria for constituents affecting neuronal cells is their ability to cross the blood–brain barrier. Lipophilicity or hydrophobicity is one of the important properties that assist drug molecules to cross the blood–brain barrier to affect neuronal cells.\(^25\)\(^26\) Consequently, hydrophilic, water-soluble compounds from an aqueous layer could be considered unattractive drug candidates without certain structural modifications or formulations to assist the molecules in crossing the blood–brain barrier to influence neuronal cell survival from \(\beta A(1-42)\) insult. Interestingly, our results suggest increased neuroprotectivity from chloroform extracts over methanol extracts. Thus, analyses of chloroform extracts may be more important in determining potential drug candidate resources for protection of neuronal cells from \(\beta A(1-42)\) insult than those of methanol extracts. The results from investigation using the aqueous portion from chloroform partitioning did not reflect the neuroprotection observed from those of methanol or chloroform extracts. Some of the extracts from the aqueous portions were cytotoxic whereas others were nonconclusive and/or noteworthy (unpublished results).
Both methanol and chloroform extracts of *G. biloba* protected neuronal cells from βA(1-42) insult (EC₅₀ = 69–109 μg/mL). The currently marketed ginkgo product, EGb761, contains ginkgo-terpenoids, in particular ginkgolides, along with bioflavonoids such as quercetin as the active constituents.²⁷,²⁸ However, it is noteworthy that ginkgolides are highly water-soluble polar compounds, and quercetin concentration is nonsignificant in nonprocessed methanol and chloroform extracts. In addition, quercetin is a commonly found flavonoid from various plants without any remarkable report on their neuroprotectivity from βA insult. Therefore, our results clearly demonstrate that constituents other than the water-soluble ginkgolides may be responsible for the neuroprotectivity of ginkgo extracts. Efficacy of EGb761 as a preventive measure against the onset of AD has been controversial.²⁹ As a result, we are currently investigating the source of the neuroprotectivity of *G. biloba*.

CONCLUSION

It is reasonable to argue that the window of observation for neuroprotectivity of herbal extracts against βA(1-42) insult may have been too restricted to yield unbiased results. Because herbal extracts contain a large number of both active and non-active compounds, the presence of potent drug candidates in small quantity could have been unaccounted for. Our investigation was designed to provide a reference point in selecting potentially important herbal resources for natural product-based drug discovery effort against AD. Because this investigation only addresses neuroprotectivity from βA(1-42) insult, other potentially important drug candidates whose mechanism of neuroprotection are different from those of this study are not detected. Nevertheless, our results provide comparative and comprehensive analyses on extracts of 27 herbs’ ability to protect cells from βA(1-42) insult, which is one of the major recognized causes for the onset of AD.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the bioassay facility of PCPRPs in the College of Pharmacy, UIC, for generous usage of the equipment. The authors are grateful for a partial support by the National Research Laboratory Program (M10400000046-04J0000-04610) and the Real Time Molecular Imaging Project of the Korean Ministry of Science and Technology.

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Address reprint requests to:
Darrick S.H.L. Kim, Ph.D.
CurXcel Corporation
The Business and Technology Center
1291 Cumberland Avenue
West Lafayette, IN 47906
E-mail: curxcel@gmail.com