In vitro assessment of selected Korean plants for antioxidant and antiacetylcholinesterase activities

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ABSTRACT
Context: Antiacetylcholinesterase (AChE) drugs have been a main therapeutic treatment for Alzheimer’s disease because increased AChE levels play a key role in reducing neurotransmission.

Objectives: Extracts from 35 Korean plants were selected and screened for antioxidant and antiacetylcholinesterase activity to explore new sources derived from Korean natural resources that could be used as AD therapeutic agents.

Materials and methods: The antioxidant effect of extracts from 35 selected Korean plants was determined using two most common free radical scavenging assays using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2’-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS). Additionally, the effect of extracts, identified as antioxidants, on acetylcholinesterase inhibition was assessed by an acetylcholinesterase assay kit.

Results: Out of 36 extracts of 35 plants tested, Oenothera biennis L. (9.09 µg/mL), Saururus chinensis (Lour.) Bail. (9.52 µg/mL) and Betula platyphylia var. japonica (9.85 µg/mL) showed strong DPPH scavenging activity. Twelve other extracts also exerted moderate free radical scavenging activities with IC50 values ranging from 10 to 50 µg/mL. Antioxidant capacity detected by ABTS assay was only significant in O. biennis (23.40 µg/mL), while the other extracts were weak or unable to reduce the production of ABTS. Based on the antioxidant activities of these plant extracts, 19 extracts with IC50 values less than 100 µg/mL in DPPH assay were selected for further AChE inhibition assay. Among the extracts tested, the IC50 value for Prunella vulgaris var. ilicinca NAKAI (18.83 µg/mL) in AChE inhibitory activity was the lowest, followed by O. biennis (20.09 µg/mL) and Pharbitis nil Chosy (22.79 µg/mL).

Conclusions: Considering complex multifactorial etiology of AD, the extracts of P. vulgaris var. ilicinca (aerial part), O. biennis (seed) and P. nil (seed) may be safe and ideal candidates for future AD modifying therapies.

Introduction

Alzheimer’s disease (AD), a progressive age-related disease of the central nervous system (CNS), is characterized by deterioration in neurological function (Bartzokis 2004; Adewusi et al. 2011). AD is the most common type of dementia; 50–60% of dementia cases in the aging population are reported to be AD (Nordin et al. 1995). Although its exact cause remains uncertain, previous research has shown that lack of cholinergic neurotransmission and deposition of misfolded extracellular β-amyloid (Aβ) plaques and neurofibrillary tangles in the CNS are hallmarks of this disease (Ali et al. 2015). As the main pathological feature of AD, Aβ deposition prevents neurons from acquiring sufficient nutrition and causes increased levels of reactive oxygen metabolites (Ali et al. 2015; Haque and Nazir 2016; Wu et al. 2017).

Oxidative stress is known to be a key factor in the aging process. Since this is associated with Aβ plaque deposition which causes neuronal oxidative stress in AD patients, it is considered as a main pathogenesis cause of AD (Adewusi et al. 2011; Zhao et al. 2013). It was previously reported that reactive oxygen species (ROS) play an important role in neurodegenerative diseases (Zhao et al. 2013), and eventually contribute to neuronal death, ultimately causing impaired memory, cognitive ability and behavioral problems (Ali et al. 2015; Haque and Nazir 2016; Wu et al. 2017).

Recent studies have also elucidated the involvement of acetylcholinesterase (AChE) in AD cognitive deficits (Haque and Nazir 2016). The enzyme AChE hydrolyzes and breaks down acetylcholine (ACh) in the synaptic cleft. ACh is the neurotransmitter responsible for cholinergic transmission in the brain, and deposited within neurofibrillary tangles and Aβ plaques in the CNS (Dhanasekaran et al. 2015). The resulting lack of cholinergic neurotransmission due to reduced ACh levels eventually leads to cognitive deficits and in the worst cases, death (Adewusi et al. 2011; Ali et al. 2015). Thus, therapies that inhibit AChE and thereby increase ACh levels are promising temporary treatments for AD (Sallam et al. 2016).

AD’s multifactorial nature suggests that a multitargeted therapeutic approach might be more advantageous than single-target...
drugs and combination therapies. This has led to sustained searches by many research groups for natural drug candidates with antiamyloidogenic and antioxidant properties in addition to cholinesterase inhibitory activity (Mathew and Subramanian 2014). Natural products have been proven as antioxidant sources with antiamyloidogenic and antioxidant properties in addition to searches by many research groups for natural drug candidates such as anti-inflammatory and antioxidant activity, which are related to brain function. In this study, 35 different plants traditionally used in Korea for rejuvenation, anti-inflammation and/or improving memory and cognitive function were selected (Table 1) (Zee 2009). The current study is the first attempt to identify and compare potential antioxidant and AChE inhibition candidates from these plants.

Table 1. Details of the Korean plants used in the current study and their usage related to effects on the CNS/cognitive functions.

| Botanical name       | Family                   | Common name          | Usage (Zee 2009)            | Sampling location                  |
|----------------------|--------------------------|----------------------|-----------------------------|-----------------------------------|
| A. bidentata Blume   | Amaranthaceae            | Ox knee              | Anti-inflammation            | Suwon, Korea (July 2013)          |
| A. japonica (Michx.) Nakai | Amaranthaceae       | Chaff flower         | Antioxidant                 | Suwon, Korea (July 2013)          |
| A. rugosa            | Lamiaceae                | Korean mint          | Antioxidant                 | National Institute of Horticultural and Herbal Science (NIHHS) (May 2013) |
| A. tuberosum Roth.   | Liliaceae                | Garlic chives        | Antioxidant                 | Suwon, Korea (July 2012)          |
| A. continentalis KITAGAWA | Araliaceae            | Dok Hwai             | Neuroprotection Antioxidant  | NIHHS (May 2013)                  |
| A. capillaris Thunb. | Compositeae             | Redstem wormwood     | Anti-inflammation            | NIHHS (May 2013)                  |
| A. koraeensis NAKAI  | Compositeae             | Korean starwort       | Antioxidant                 | Suwon, Korea (July 2013)          |
| A. tartarica L.      | Compositeae             | Aster                | Antioxidant                 | Suwon, Korea (July 2013)          |
| A. japonica Koizd.   | Compositeae             | Atractylodes         | Anti-inflammation            | Suwon, Korea (July 2012)          |
| B. platyphyllo var. japonica | Betulaceae       | White birch          | Anti-inflammation            | Chungcheongbuk-do, Korea          |
| F. balsamum L.       | Apiaceae                 | Sickie hare’s ear    | Anti-inflammation            | Jeollanam-do, Korea (June 2012)   |
| B. koreana Nakai ex Chung & al. | Buxaceae    | Korean box tree      | Against neuralgia, rheumatism, | NIHHS (May 2013)                  |
| C. setidens NAKAI    | Compositeae             | Korean gondre thistle| Anti-inflammation            | Suwon, Korea (July 2012)          |
| C. pilosula (Fr.) NANNF | Campanulaceae         | Pilose asialbell      | Promotes growth of neurons   | Suwon, Korea (July 2012)          |
| E. ciliata (Thunb.Hyl.) | Lamiaceae             | Vietnamese balm      | Anti-inflammation            | Suwon, Korea (July 2013)          |
| L. japonica Houtt.   | Lamiaceae                | Vietnamese balm      | Anti-inflammation            | Suwon, Korea (July 2013)          |
| F. vulgare Mill.    | Umbelliferae (Apiaceae) | Fennel               | Sedative                    | NIHHS (May 2013)                  |
| G. scabra Bunge for. Scabra | Gentianaceae         | Korean gentian        | Anti-inflammation            | Suwon, Korea (July 2013)          |
| H. dulcis Thunb.     | Rhamnaceae              | Raisin tree          | Anti-inflammation            | Gangwon-do, Korea (May 2014)      |
| I. tinctoria var. yezoensis OHWI | Cruciferace       | Cruciferate          | Anti-inflammation            | Suwon, Korea (July 2013)          |
| L. indica var. lacinata | Compositeae           | Korean lettuce       | Sedative                    | Suwon, Korea (July 2012)          |
| L. japonicus Houtt.  | Lamiaceae                | Chinese motherwort   | Anti-inflammation            | NIHHS (May 2013)                  |
| M. sibiricus Linne   | Labiatae                 | Honeyweed            | Antioxidant                 | Suwon, Korea (July 2013)          |
| M. charantia L.      | Cucurbitaceae           | Bitter gourd         | Antioxidant                 | Suwon, Korea (July 2013)          |
| O. biennis L.        | Onagraceae               | Evening primrose     | Anti-inflammation            | Suwon, Korea (July 2013)          |
| O. japonicus KER-GAWLER | Liliaceae             | Liriope tuber        | Protects brain cells, promotes growth of neurons | Suwon, Korea (July 2012) |
| P. nil Chosy         | Convolvulaceae           | Morning glory        | Anti-inflammation            | Suwon, Korea (July 2012)          |
| P. amurense Rupr.    | Rutaceae                | Amur cork tree       | Improves memory             | Suwon, Korea (July 2012)          |
| P. vulgaris var. lilicina NAKAI | Labiatae       | Common self-heal     | Antiaging                   | Suwon, Korea (July 2013)          |
| R. glutinosa (GAERTNER) LIBOSCHITZ | Scrophulariaceae | Rehmnia root          | Protection of brain cells, improves memory | Suwon, Korea (July 2013) |
| S. chinensis (Lour.) Baill. | Saururaceae        | Lizard’s tail         | Protection of brain cells   | Suwon, Korea (July 2013)          |
| S. tenufolia (Benth.) Briq. | Lamiaceae             | Schizonepeta spike   | Anti-inflammation            | Suwon, Korea (July 2012)          |
| S. buegeriana Miq.   | Scrophulariaceae         | Scrophularia root     | Anti-inflammation            | Suwon, Korea (July 2013)          |
| Senna tora (L.) Roxb. | Leguminosae           | Cassia seed           | Antiaging                   | NIHHS (May 2013)                  |
| T. chinensis Maxim   | Cucurbitaceae           | Chinese cucumber     | Anti-inflammation            | Suwon, Korea (July 2013)          |

**Materials and methods**

**Chemicals**

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), ascorbic acid (vitamin C) and donepezil hydrochloride were purchased from Sigma Aldrich (Seoul, Korea). The other chemicals and reagents used were of high quality and obtained from commercial sources.

**Plant materials**

All plant materials were procured from the National Institute of Horticultural and Herbal Science (NIHHS), Eumseong-gun, Chungcheongbuk-do, Korea, and authenticated by Dr Seung-Eun Lee, Department of Herbal Crop Research, NIHHS, Eumseong-gun, Chungcheongbuk-do, Korea. Voucher specimens of the
Table 2. Antioxidant activities of 36 different extracts from 35 selected plants.

| Test samples       | Part | Extract solvent | IC_{50} (µg/mL)^a | IC_{50} (µg/mL)^a |
|--------------------|------|-----------------|--------------------|--------------------|
| A. bidentata       | RT   | 100% MeOH       | >200               | >200               |
| A. japonica        | RT   | 100% MeOH       | >200               | >200               |
| A. rugosa          | ST   | 100% H_2O       | >200               | >200               |
| A. tuberosum       | AP   | 100% MeOH       | 169.32             | >200               |
| A. continentalis   | ST   | 100% H_2O       | >200               | >200               |
| A. capillaris      | AP   | 100% EtOH       | 13.17              | 114.87             |
| A. koraiensis      | AP   | 100% MeOH       | 44.23              | 94.68              |
| A. tartaricus      | FL   | 100% EtOH (74°C)| 11.28              | >200               |
| A. japonica        | BU   | 100% MeOH       | 194.54             | >200               |
| B. platyphylla var. japonica | BA | 80% EtOH/H_2O | 9.85               | >200               |
| B. falcatum        | RT   | 100% EtOH (74°C)| >200               | >200               |
| B. koreana         | ST   | 100% H_2O       | 107.79             | >200               |
| C. setidens        | AP   | 100% MeOH       | 36.20              | >200               |
| C. pilosula        | AP   | 100% EtOH (85°C)| 32.44              | >200               |
| E. ciliata         | AP   | 100% MeOH       | 69.26              | >200               |
| F. japonica        | ST   | 100% EtOH       | 14.03              | >200               |
| F. vulgare         | ST   | 100% EtOH       | 123.02             | >200               |
| G. scabria         | RT   | 100% MeOH       | >200               | >200               |
| H. dulcis          | TW   | 100% MeOH       | 29.17              | 189.62             |
| I. tinctoria var. yezoensis | AP | 100% EtOH (85°C)| 32.14              | 133.46             |
| L. indica var. laciniata | RT | 100% MeOH | >200               | >200               |
| L. japonicus       | ST   | 100% EtOH       | 52.77              | >200               |
| L. sibiricus       | AP   | 100% MeOH       | 83.85              | 137.63             |
| M. charantia       | AP   | 100% MeOH       | >200               | >200               |
| O. biennis         | SE   | 100% MeOH       | 9.09               | 23.40              |
| O. japonicus       | WP   | 100% MeOH       | 131.80             | >200               |
| P. nil             | SE   | 100% MeOH       | 33.75              | >200               |
| P. amurensis       | BA   | 100% EtOH (74°C)| 124.58             | >200               |
| P. vulgaris var. lilacina | AP | 100% EtOH (85°C)| 18.13              | >200               |
| R. glutinosa       | RT   | 100% EtOH (74°C)| >200               | >200               |
| S. chinensis       | AP   | 100% MeOH       | 9.52               | >200               |
| S. tenuifolia      | AP   | 100% MeOH       | 43.87              | >200               |
| S. buergariiana    | AP   | 100% MeOH       | >200               | >200               |
| S. buergariiana    | RT   | 100% MeOH       | 48.10              | >200               |
| Senna tora         | SE   | 100% H_2O       | >200               | >200               |
| T. kirilowii       | AP   | 100% EtOH (74°C)| 61.19              | 79.39              |
| Ascorbic acid^b    |      |                 | 4.97               | 9.88               |

^aIC_{50} values were determined by curve-fitting the data points using nonlinear regression.
^bAscorbic acid was used as a positive control for DPPH and ABTS radical scavenging activities.

WP: whole plant; RT: root; FL: flower; AP: aerial part; ST: stem; SE: seed; BU: bulb; BA: bark; TW: twig.

**Plant extract preparation**

Freshly collected plant materials were dried in a hot air oven at 55°C and then pulverized. Plant parts used are presented in Table 2. To prepare the extracts, 5 g of each powdered plant was extracted using the indicated solvent under different conditions (Table 2) and each filtered extract was concentrated by complete evaporation in a vacuum centrifuge. The dried extract was then stored at −20°C. The entire study was conducted using a single batch of each plant extract to avoid batch-to-batch variation and maximize product consistency.

**Determination of antioxidant activity by scavenging effect on 2,2′-diphenyl-1-picryl hydrazyl radical (DPPH)**

In each well of 96-well microplate, 100 µL aqueous solution from the sample (control: 100 µL of distilled water) was added to an ethanolic solution of DPPH (100 µL, 60 µM) based on a previously reported method with minor modifications (Eom et al. 2016). The absorbance at 540 nm was measured using a microplate reader (Tecan SPECTRAFluor; Tecan UK, Goring-on-Thames, UK) after mixed gently and allowed to stand at room temperature for 30 min. Ascorbic acid was used as a DPPH scavenging positive control.
Determination of antioxidant activity by scavenging effect on 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS)

The ABTS activity was measured according to the previously reported method (Moreno-Montoro et al. 2017). After adding 0.5 mL of sample to 3 mL of the diluted ABTS solution, the absorbance was measured at 415 nm using a spectrophotometer. The ABTS radical scavenging (%) was calculated as (1 – A/A₀) × 100, where A₀ is the absorbance of the control, and A is the absorbance of the samples. The IC₅₀ value was calculated as the concentration of sample required to scavenge 50% of free radicals. Ascorbic acid was used as an ABTS scavenging positive control.

AChE enzyme activity assay

Acetylcholinesterase inhibition activity was determined using an acetylcholinesterase assay kit according to the manufacturer’s protocol (BioVision, Milpitas, CA). In brief, 50 μL sample and assay buffer was added to each well of a 96-well plate, followed by the addition of 50 μL of the reaction mixture. After incubation at 37°C for 20 min, the absorbance at 570 nm was measured using a microplate reader (PowerWave XS; Bio-Tek Instruments, Winooski, VT).

Statistical analysis

Statistical significance was determined with analysis of variance (ANOVA) followed by a multiple comparison test with Bonferroni’s adjustment. p Values less than 0.05 were considered statistically significant. Analyses were performed using SPSS ver. 19.0 (SPSS Inc., Chicago, IL).

Results

Free radical scavenging activity of the extracts using DPPH and ABTS assay

Multiple factors are involved in the development of neurodegenerative disease. Considering AD’s complex multifactorial etiology, phytochemicals that have antioxidant as well as AChE inhibitory activity have been considered to be safer and better therapeutic candidates for treating AD (Parodi et al. 2015). Antioxidant therapy has proven successful for improving cognitive function and behavioral deficits in patients with mild to moderate AD (Gutzmann and Hadler 1998).

The antioxidant activity of extracts from 35 selected Korean plants (Table 1) was determined using the free radical DPPH. IC₅₀ values for DPPH radical scavenging were determined based on the concentration of the extract required for approximately 50% of the original activity. IC₅₀ values for all extracts are presented in Table 2. Strong IC₅₀ values were obtained for O. biennis L. (9.09 μg/mL), S. chinesis (Lour.) Baill. (9.52 μg/mL) and B. platyphylla var. japonica (9.85 μg/mL), whereas the value for ascorbic acid was 4.97 μg/mL. F. japonica (Houtt.) Ronse Decr., A. capillaris Thunb., S. buergeriana Miq., S. tenuifolia (Benth.) Briq., A. koreiensis NAKAI, C. pilosula (FR.) NANNF., L. tinctoria var. yezeonis OHWI, A. tartaricus L., H. dulcis Thunb., P. vulgaris var. lilacina NAKAI, P. nil Chosy and C. setidens NAKAI also exerted moderate free radical scavenging activities with IC₅₀ values ranging from 10 to 50 μg/mL (Table 2). Interestingly, the MeOH extract from the roots of S. buergeriana Miq. exhibited good DPPH scavenging activity with a corresponding IC₅₀ value of 48.10 μg/mL, whereas the MeOH extract from its aerial parts was not active (IC₅₀ > 200 μg/mL). In addition, the extracts of 35 selected Korean plants were investigated for their antioxidant properties using ABTS radical scavenging capacity assay (Table 2). Antioxidant capacity detected by ABTS assay was only significant in O. biennis (23.40 μg/mL), while the other extracts were weak or unable to reduce the production of ABTS (Table 2).

Acetylcholinesterase (AChE) inhibitory activity of the selected extracts

Based on plant extract antioxidant activities, 19 extracts with IC₅₀ values less than 100 μg/mL in DPPH assay were selected for further AChE inhibition assays. The main pathological feature that characterizes AD is reduction in cholinergic acetylcholine (ACh) neurotransmission (Bae and Lee 2015; Li et al. 2016; Xu et al. 2016). Acetylcholinesterase (AChE) is responsible for ACh hydrolysis. Reduced levels of ACh eventually lead to cognitive dysfunction and even death. Thus, AChE inhibition is considered as the most valuable therapy for AD (Sim et al. 2014). Although this disease cannot be prevented from progressing, there are various AChE inhibitors currently available that can improve symptoms in mild to moderate AD patients (Schulz 2003; Mehta et al. 2012). However, intensive research is still needed to discover new candidates against AD since the current AChE inhibitors carry adverse effects (Wilcock et al. 2000; Mehta et al. 2012). 19 extracts selected based on their antioxidant activity were tested for AChE inhibitory activity and the results are shown in Table 3 represent % inhibition at 100 μg/mL and the IC₅₀ for tested extracts. Donepezil hydrochloride was used as the standard AChE inhibitor in this study which showed an IC₅₀ of 0.03 μg/mL. The IC₅₀ value for AChE inhibitory activity was the lowest for P. vulgaris var. lilacina (18.83 μg/mL) followed by O. biennis (20.09 μg/mL) and P. nil (22.79 μg/mL). These plant extracts also showed very high antioxidant activity in the DPPH assay. In addition, O. biennis exhibited significant activity in reducing the production of ABTS (Table 2).

Table 3. AChE inhibition assays for 19 extracts of selected plants with antioxidant activity.

| Test samplesa | % AChE inhibition at 100 μg/mL | IC₅₀ (μg/mL)b |
|---------------|-------------------------------|--------------|
| A. capillaris  | 101.39 ± 0.66                 | >200         |
| A. koraiensis | 97.06 ± 3.28                  | >200         |
| A. tartaricus | 93.34 ± 0.66                  | >200         |
| B. platyphylla var. japonica | 89.63 ± 5.90 | >200 |
| C. setidens   | 91.79 ± 1.09                  | >200         |
| C. pilosula   | 102.47 ± 4.81                 | >200         |
| E. ciliata    | 68.27 ± 1.09                  | >200         |
| F. japonica   | 64.09 ± 3.50                  | >200         |
| H. dulcis     | 96.44 ± 1.09                  | >200         |
| I. tinctoria var. yezeonis | 87.00 ± 3.06 | >200 |
| L. japonicus  | 95.67 ± 2.63                  | >200         |
| L. sibiricus  | 82.82 ± 1.97                  | >200         |
| O. biennis    | 25.41 ± 0.43                  | 20.09        |
| P. nil        | 39.16 ± 1.31                  | 22.79        |
| P. vulgaris var. lilacina | 39.03 ± 0.87 | 18.83 |
| S. chinesis   | 59.15 ± 2.65                  | >200         |
| S. tenuifolia | 92.42 ± 2.41                  | >200         |
| S. buergeriana| 109.90 ± 1.31                 | >200         |
| T. kirilowii  | 96.75 ± 0.66                  | >200         |
| Donepezil hydrochloride | 92.42 ± 3.72 | 0.03 |

aTested extracts were the same as those used for the antioxidant assay.

bIC₅₀ values were determined by curve-fitting the data points using nonlinear regression.


Out of 35 Korean plants screened, the extracts of dry plant parts from *P. vulgaris* var. *lilacina* (aerial part), *O. biennis* (seed) and *P. nil* (seed) were selected as candidate sources for potent AChE inhibitors as well as antioxidants. Due to AD’s multifactorial pathogenesis, multi-targeted drugs are preferred as an effective therapeutic strategy. These selected Korean plants exhibiting in vitro AChE inhibition and antioxidant activity act on multiple therapeutic AD targets and can be consumed daily in our diet to provide their neuroprotective effects.

*Oenothera biennis* (Onagraceae) is commonly known as evening primrose and its seeds are known for their high antioxidant activities (Budin et al. 1995). The seeds of *O. biennis* are also believed to have medicinal value mainly due to the presence of γ-linolenic acid, which is known to be an essential dietary supplementation for humans. γ-Linolenic acid improves many pathological conditions including dermatitis, platelet aggregation and high blood pressure (Corrigan et al. 1998; Barre 2001). γ-Linolenic acid is an omega-6 (n-6) fatty acid, which is known to have anti-inflammatory activity along with omega-3 (n-3) fatty acids and affects the pathogenesis of many diseases where inflammation plays a critical role including cancer, diabetes, heart disease and AD (Kapoor and Huang 2006). In one study, n-6 fatty acids were reported to contribute to the improvement in learning tasks and recovery from neurotoxins, which suggested the potential for *O. biennis* seeds to be utilized as a candidate therapeutic agent for AD (Yehuda et al. 1996). The crude extract from *O. biennis* was also reported to have antioxidant activities due to the involvement of phenolic constituents (Wettasinge et al. 2002). In this work, the MeOH extract from *O. biennis* seeds showed AChE inhibitory effects with an IC_{50} value of 20.09 μg/mL, which was the highest antioxidant activity among the tested extracts.

The aerial parts of *P. vulgaris* var. *lilacina* (Labiateae), which have been used in Chinese folk medicine to calm irritated skin and heal wounds, are rich in phenolic acids such as rosmarinic acid, caffeic acid and kaempferol. Rosmarinic acid is the main component in the aerial parts of *P. vulgaris* var. *lilacina*. It has been shown to exhibit antioxidant effects by suppressing lipoperoxidation and scavenging superoxide radicals (Skottová et al. 2004). In addition, it also inhibits the formation of β-amyloid (Aβ) plaques. Deposition of misfolded extracellular Aβ plaques is one of the main possible causes of AD. The inhibitory activity of rosmarinic acid eventually protects against memory impairment (Alkam et al. 2007). Caffeic acid, one of the main components of the aerial parts of *P. vulgaris* var. *lilacina*, is well-known for its antioxidant and anti-inflammatory activities and was also found to possess a significant protective effect against β-amyloid-induced neurotoxicity by inhibiting calcium influx and tau phosphorylation (Sul et al. 2009). Kaempferol, another major component of *P. vulgaris* var. *lilacina*, has been found to protect PC12 and T47D cells from β-amyloid-induced toxicity (Roth et al. 1999). In this regard, the aerial parts of *P. vulgaris* var. *lilacina*, with these phytochemicals as the major content, should be further assessed as an adjuvant therapeutic agent for AD. In this study, the MeOH extract from the aerial parts of *P. vulgaris* var. *lilacina* was tested for AChE inhibition as well as antioxidant activity and it showed the highest AChE inhibition with an IC_{50} value of 18.83 μg/mL along with high antioxidant activity.

*Pharbitis nil* (Convolvulaceae) is known as morning glory and the seeds of *P. nil* (Pharbitidis Semen) have been used as a purgative drug in folkloric medicine in Asian countries (Kim et al. 2009). The seeds of *P. nil* are reported to be a rich source of diverse phytochemicals, including resin glycosides, gibberellins, flavonoids, anthocyanins, diterpenoids, lignans, triterpene saponins and phenolic compounds (Kim et al. 2008, 2009, 2010, 2011, 2013, 2014; Park et al. 2016). The bioactivity of the constituents of *P. nil* is still underexplored; however, polysaccharides from *P. nil* seeds were recently reported to possess antioxidant activities (Wang et al. 2014). Although study on the possible medicinal usage of *P. nil* seeds is still limited, the properties of phytochemicals such as polysaccharides, flavonoids, diterpenoids and phenolic compounds suggest the potential use of *P. nil* seeds as a treatment for AD. Flavonoids are known to possess neuroprotective properties that improve cognitive function, and show protective effects against memory deficits associated with normal aging (Macready et al. 2009). There has also been a report on the potential AChE inhibitory activity of diterpenoids, which provides a theoretical basis for further research and utilization of *P. nil* seeds for cholinesterase inhibitory activity (Hung et al. 2011). In addition, some of the diterpenes, lignans and phenolic compounds isolated from *P. nil* seeds were found to show anti-neuroinflammatory activity by inhibiting nitric oxide (NO) production in lipopolysaccharide (LPS)-activated BV-2 microglia cells (Kim et al. 2011, 2013, 2014). Under pathological conditions, microglia cells, which are the immune resident cells of the brain, are over-activated and produce a variety of pro-inflammatory mediators including NO, which consequently leads to various neurodegenerative conditions of the CNS including Parkinson’s and Alzheimer’s disease (Kim et al. 2015; Suh et al. 2016). In the current study, the MeOH extract from the seeds of *P. nil* was tested for antioxidant activity and showed high activity in scavenging reactive oxygen species, which led to a subsequent assay to observe its AChE inhibitory effects. The extract showed AChE inhibition with an IC_{50} value of 22.79 μg/mL.

### Conclusions

The extracts from plants originating in Korea, which have been used in Korea for rejuvenation and anti-inflammatory and/or improving memory and cognitive function, were screened for AChE inhibition and antioxidant activity for the first time. Of the 35 plant materials tested, the extracts from the aerial part of *P. vulgaris* var. *lilacina*, the seeds of *O. biennis* and the seeds of *P. nil* were selected as promising candidates for sources of potent AChE inhibitors as well as antioxidants. Considering the complex multifactorial etiology of AD, these selected plant extracts may be safe and ideal candidates as therapies against AD. Further evaluation to identify active ingredients and assess safety and bioavailability using *in vivo* animal models is required.

### Disclosure statement

The authors declare no conflicts of interest.

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