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# Nutritional and Physicochemical Characteristics of the Antidementia Acetylcholinesterase-Inhibiting Methanol Extracts from *Umbilicaria esculenta*

**Ji-Su Lee[1], Gyung-Hun Min[2] and Jong-Soo Lee[1,2]***

[1]*Department of Life Science and Genetic Engineering, Paichai University, Daejeon 302-735, Korea*
[2]*Department of Emergency Medical Technology, Woosong University, Daejeon 300-718, Korea*

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**To develop new antidementia nutraceuticals, a potent acetylcholinesterase (AChE)-inhibiting extract was screened from various extracts of nutritional mushrooms and lichens nutritional and its physicochemical properties were investigated. Among the several extracts tested, methanol extracts of *Umbilicaria esculenta* fruiting body showed the highest AChE inhibitory activity of 22.4%. *U. esculenta* AChE inhibitor was maximally extracted when fruiting bodies were treated with 80% methanol at 40°C for 18 h. The methanol extracts contained 18.9% crude lipid, 18.8% crude protein, and 11.6% total sugar. In addition, they contained 444 mg/g glutamic acid, 44 mg/g histidine, and 41 mg/g aspartic acid. The methanol extracts were soluble in a solution of methanol and 20% dimethylsulfoxide, insoluble in n-hexane, chloroform, and water, and were stable at 20~60°C and pH 1.0~5.0 for 1 h.**

**KEYWORDS : Antidementia, Acetylcholinesterase inhibitor, *Umbilicaria esculenta***

Alzheimer's disease is characterized by acetylcholine depletion, amyloid $\beta$ protein aggregation, and neurofibrillary tangle (Duyckaerts *et al.*, 1999). The neurotransmitter acetylcholine has important recognition functions such as memory (Richter *et al.*, 1980), and it is synthesized in certain neurons by the enzyme choline acetyltransferase from choline and acetyl-CoA.

Acetylcholinesterase (AChE) [EC 3.1.1.7] is an enzyme that hydrolyses acetylcholine into choline and acetate. Therefore, it promotes dementia by loss of neurotransmitter in the brain. Many AChE inhibitors have been developed from natural products and ultimately commercialized. However, commercial AChE inhibitors often have negative effects that include toxicity (including to the liver) and vomiting. Therefore, their use in the treatment of dementia is limited (Dubios and Albert, 2004; Muramoto *et al.*, 1979).

Recently, lichen have attracted attention because they contain compounds that can be used as medicinal agents or as neutraceuticals in functional foods. *Umbilicaria esculenta* is a lichen living symbiotically with ascomycetes and green algae or cyanobacteria (Hawksworth, 1988). It is generally found at high altitude on mountain rocks in East Asia. *U. esculenta* has been used in traditional preventatives and remedies for bloody vomit and diarrhea, skin disease, epilepsy, and yellow jaundice (Jang *et al.*, 2003).

Reported physiological functions of *U. esculenta* have included $\beta$-glucan-mediated anti-cancer effect, lecanoric acid- and vulpinic acid-mediated anti-inflammatory effects and histidine-decarboxylase inhibition, fibrinolytic effect, skin anti-aging effect, parietin- and emodin-mediated inhibition of Gram-positive bacteria, and antihyperglycemic effect (Kim *et al.*, 1995; Lee and Kim, 1999; Choi *et al.*, 2000; Jang *et al.*, 2003; Kim and Lee, 2006). *U. esculenta* also produces plant growth inhibitors such as evernic acid, usnic acid, atranorin, vulpinic acid, and cell division repressors including protolichesterinic acid and nephrosterinic acid (Hawksworth *et al.*, 1984). However, few studies have addressed the anti-dementia potential of *U. esculenta* AChE inhibitors.

To develop new anti-dementia agents from mushrooms and lichens, screening of potent anti-dementia AChE-inhibiting extracts from lichen and optimal extraction conditions of the AChE inhibiting-extract were investigated. Furthermore, the nutritional and physicochemical properties of AChE-inhibiting methanol extracts were also determined.

## Materials and Methods

**Mushrooms and chemicals.** Mushrooms were obtained from the National Institute of Agricultural Science and Technology in Suwon, Korea. Lichens were obtained from the Korea Lichen Research Institute of Suncheon National University in Suncheon, Korea. Unless otherwise specified, all chemicals and solvents were of analytical grade. AChE (recombinant human AChE; rAChE), acetylthiocholine chloride, and 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from the Sigma-Ald-

*Corresponding author <E-mail : biotech8@pcu.ac.kr>

rich (St. Louis, MO, USA). The VERSAmax microplate reader (Molecular Devices, Sunnyvale, CA, USA) was used in AChE activity measurements.

**Extraction of mushrooms and lichen.** Mushrooms and lichen powders were separately added to water and methanol (1 : 50, v/v) and shaken for 24 h at 50°C and 40°C, respectively. Each extract was filtered through a Whatman 0.45 $\mu$m membrane filter and lyophilized.

**Assay of AChE inhibitory activity.** AChE inhibitory activity was measured spectrophotometrically as previously described (Ellman *et al.*, 1961). A mixture of 110 $\mu l$ assay buffer (0.1 M sodium phosphate, pH 7.3), 30 $\mu l$ AChE (0.8 U/*ml*), 30 $\mu l$ substrate (acetylthiocholine chloride), 20 $\mu l$ of DTNB, and 10 $\mu l$ sample dissolved in the assay buffer was incubated for 60 min at 37°C. The reaction product, 5-thio-2-nitrobenzoate, which was produced enzymatically, was measured at 415 nm. The inhibition (%) was calculated using the equation:

$$\text{inhibition (\%)} = [1 - \{(S - S0) \div (C - C0)\}] \times 100\%$$

where C is the absorbance of a control (enzyme, assay buffer, DTNB, and substrate) after 60 min of incubation, C0 is the absorbance of a control at time zero, S is the absorbance of the tested samples (enzyme, sample solution, DTNB, and substrate) after 60 min of incubation, and S0 is the absorbance of the tested samples at time zero. All data represent the mean of duplicate experiments.

To check the quenching effect of the samples, we added the sample solution to reaction mixture C and investigated any reduction in the absorbance of the sample. The $IC_{50}$ value is defined as the concentration of the AChE inhibitor that is required to inhibit 50% of the AChE activity.

**Characterization of AChE-inhibiting methanol extracts from *U. esculenta*.** Protein and total sugar content of the AChE-inhibiting methanol extracts from *U. esculenta* were determined by the Lowry and phenol-sulfuric acid methods, respectively (Kim *et al.*, 2008). Crude lipid content was determined by the Soxhlet extraction method and amino acid content was determined using high-pressure liquid chromatography (Kim *et al.*, 2008).

## Results and Discussion

**Screening of AChE-inhibiting extracts from mushroom and lichen.** AChE inhibitory activities of water and methanol extracts from mushrooms and lichens are summarized in Table 1. Methanol extracts of *U. esculenta* showed the highest AChE inhibitory activity of 22.4%, with activities of the other extracts being lower or undetectable. Even though the AChE inhibitory activity of methanol extracts from *U. esculenta* was lower than that of *Sorghum bicolor* (63.4%) (Song, 2008), this is the first report concerning the presence of an AChE inhibitor from *U. esculenta*.

**Table 1.** AChE inhibitory activities of various extracts from secondary-screened mushrooms and lichens

| Scientific names | Distilled water extract[a)] | Methanol extract |
|---|---|---|
| *Pleurotus ostreatus* | N.D[b)] | 1.5 ± 0.2% [c)] |
| *P. eryngii* | 0.8 ± 0.3% | N.D |
| *Agaricus bisporus* | 1.5 ± 0.4% | N.D |
| *Lentinula edodes* | N.D | 2.3 ± 0.3% |
| *Flammulina velutipes* | N.D | 3.2 ± 0.5% |
| *Auricularia auricula-judae* | 4.9 ± 0.2% | N.D |
| *U. esculenta* | N.D | 22.4 ± 0.5% |

[a)]Extract concentration = 1 mg/*ml*, [b)]N.D: not detected, [c)]Values show mean ± SE from three experiments performed in triplicate

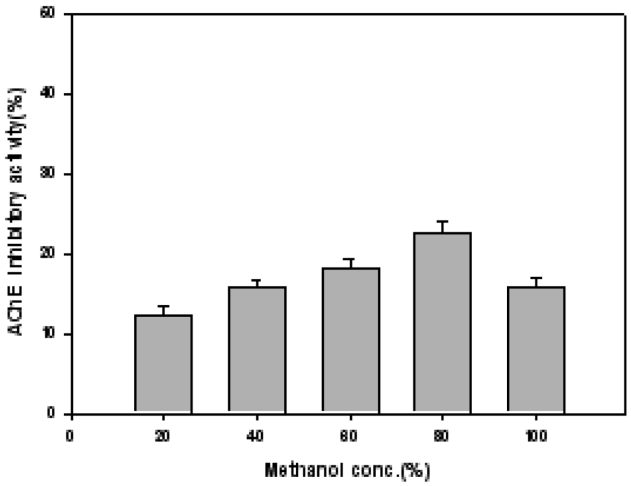


**Fig. 1.** Effects of methanol concentration on extraction of AChE inhibitor from *U. esculenta*.

**Optimal conditions for AChE inhibitor extraction.** Effects of methanol concentration, extraction temperature, and time on the AChE inhibitory activity of the methanol extracts were determined (Figs. 1 and 2). The AChE inhibitor was maximally extracted when *U. esculenta* was treated by 80% methanol at 40°C for 18 h.

**Nutritional characteristics of AChE-inhibiting methanol extracts.** The AChE-inhibiting methanol extracts from *U. esculenta* contained 18.8% of crude protein, 11.6% of total sugar, and 18.9% crude lipid per gram. Amino acid content of the methanol extracts was determined (Table 2), and included aspartic acid (41 mg/g), histidine (44 mg/g), alanine (34 mg/g), and, very notably, glutamic acid (444 mg/g). These amino acid contents were 4.0~30 times higher than those of the corresponding con-

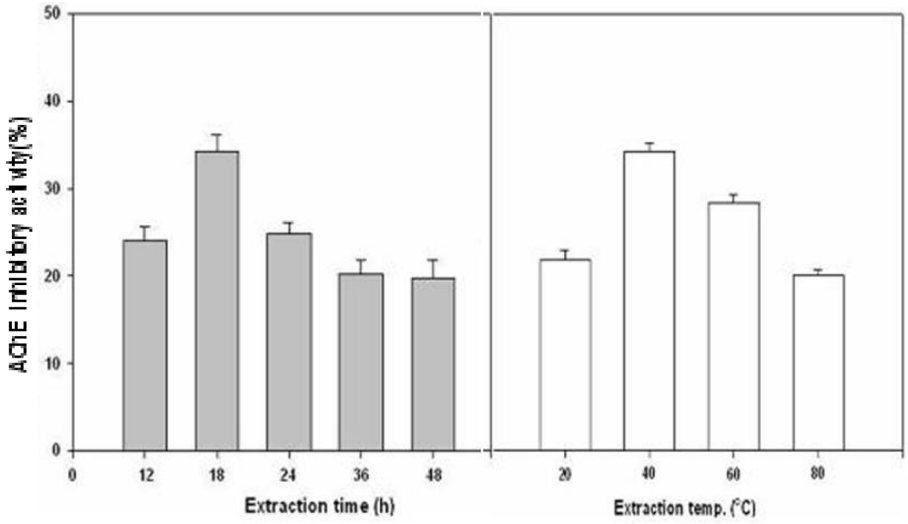


**Fig. 2.** Effects of extraction time and temperature on extraction of AChE inhibitor from *U. esculenta*.

**Table 2.** Amino acid contents of the methanol extracts from *U. esculenta*

| Amino acid | Contents[a)] | Amino acid | Contents[a)] |
|---|---|---|---|
| Aspartic acid | 41 | Leucine | 9 |
| Threonine | 6 | Tyrosine | 3 |
| Serine | 14 | Phenylalanine | 3 |
| Glutamic acid | 444 | Histidine | 44 |
| Proline | 4 | Lysine | 5 |
| Glycine | 11 | Arginine | 4 |
| Alanine | 34 | Cystine | 11 |
| Valine | 7 | Methionine | 1 |
| Isoleucine | 6 | Tryptophan | 0.6 |

[a)]Unit = mg/g solid

**Table 3.** Physicochemical properties of the AChE-inhibiting methanol extracts from *U. esculenta*

| | | |
|---|---|---|
| Appearance | | Dark green powder |
| Solubility | - soluble | methanol, DMSO (20%) |
| | - insoluble | n-hexane, chloroform ethyl acetate, water |
| Maximum ultraviolet absorbance (nm) | | 310 |

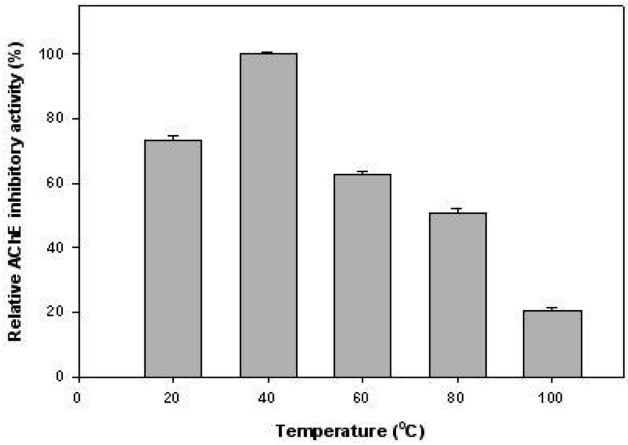


**Fig. 3.** Thermal stability of the methanol extracts from *U. esculenta*.

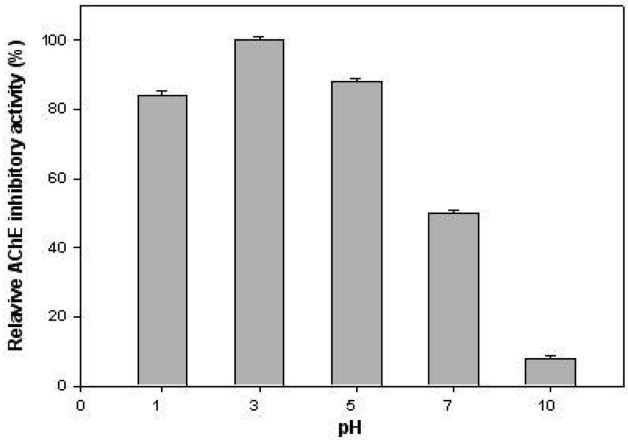


**Fig. 4.** pH stability of the methanol extracts from *U. esculenta*.

tents of aspartic acid (10.66 mg/g solid), histidine (7.95 mg/g solid), alanine (10.27 mg/g solid), and glutamic acid (15.84 mg/g solid) in *Lentinus edodes* (Korean Food Components Index, 2006). Furthermore, besides the AChE inhibitory activity of the methanol extracts, the high glutamic acid content evident in *U. esculenta* extracts is probably related to the function of glutamic acid in the brain as an energy source for neurotransmitter stimulation and excitation.

**Physicochemical properties of AChE-inhibiting methanol extracts.** The methanol extracts from *U. esculenta* were soluble in methanol and 20% dimethyl sulfoxide (DMSO), and were insoluble in n-hexane, chloroform,

ethyl acetate, and water (Table 3). Thermal and pH stability of the methanol extracts were investigated (Figs. 3 and 4). The methanol extracts were stable in the range of 20~60°C and pH 1.0~5.0 for 1 h.

In conclusion, we obtained a highly valuable *U. esculenta* extracts which were contained various nutritional compounds and antidementia AChE inhibitor. We are confident that the methanol extracts of *U. esculenta* from this study will become a good nutraceutical for preparation of functional foods. Further studies should purify and characterize the AChE inhibitor from *U. esculenta*.

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