Screening of Potent Anti-dementia Acetylcholinesterase Inhibitor-containing Edible Mushroom *Pholiota adiposa* and the Optimal Extraction Conditions for the Acetylcholinesterase Inhibitor

Do-Yeon Kim, Sang-Min Bae, Sang-Min Han and Jong-Soo Lee*

Department of Biomedicinal Science and Biotechnology, Paichai University, Daejeon 35345, Korea

**ABSTRACT:** To develop a new anti-dementia acetylcholinesterase (AChE) inhibitor from edible mushrooms, AChE inhibitory activities were determined on water and ethanol extracts of various edible mushrooms from oriental medicine markets and agriculture markets. As a result, the 70% ethanol extract from *Pholiota adiposa* fruiting body had the highest AChE inhibitory activity of 30.6, and its water extract also had an AChE inhibitory activity of 23.8%. Therefore, we finally selected *P. adiposa* as a potent anti-dementia AChE inhibitor-containing mushroom. The AChE inhibitor of *P. adiposa* was maximally extracted when its fruiting body was treated with water for 3hr at 70°C and 70% ethanol for 12 hr at 70°C, respectively.

**KEYWORDS:** Acetylcholinesterase Inhibitor, Anti-dementia, Ethanol extract, *Pholiota adiposa*

**Introduction**

Because of the significant increase in life expectancy over sixty-five years of age in Korea, dementia diseases have also increased. Some forms of dementia are caused by a lack of neurotransmitters. Acetylcholine is one of the neurotransmitters in the peripheral nervous system and central nerve system, and it is converted into choline and acetate by acetylcholinesterase (EC.3.1.1.7, AChE) [1, 2]. Therefore, AChE is a key enzyme in the pathophysiology of dementia.

Several AChE inhibitors as anti-dementia agents have been extracted and characterized from various plants or microorganisms including *Umbilicaria esculenta* [3], green tea [4, 5], *Securinega suffruticosa* [6], *Onosma hispida* [7], *Juglans regia* [2], the Chinese herb *Huperzia serrata* [8], etc. However, AChE inhibitors such as Galantamine, Rivastigmine, Donepezil, Tacrine and Memantine have been only approved by the FDA as drug therapy for dementia [9]. They also have some side effects including nausea and anorexia. Therefore, research on development of new anti-dementia agents with high efficacy and no side effects is necessary.

Meanwhile, bioactive compounds from mushrooms have been reported for their health-stimulating effects [10]. One of the edible mushroom *Pholiota adiposa* is classified under the genus *Pholiota* of the family *Strophariaceae*. This mushroom is cultivated in Asia including Korea, Europe, and North America. The pharmaceutical effects of *P. adiposa* have been reported its antihypertension [11], cholesterol-lowering [12], antibiotic, and antitumor activities [11]. This study describes the screening of a potent AChE inhibitor found in *P. adiposa* and the optimization of the extraction conditions to develop a new anti-dementia agent from edible mushrooms for application in the medicinal food industry.

**Materials and Methods**

**Mushrooms and chemicals**

Nine kinds of commercial edible mushrooms were purchased at local oriental medicinal markets and agriculture...
markets which were cultivated in Korea between 2014~2015. Acetylcholinesterase (AChE from *Electrophorus electricus*), acetylcholine chloride and 5,5'-dithiobis (2-nitrobenzonic acid) were purchased from the Sigma Chemical Co. (St, Louis, MO, USA). A VERSAmax microplate reader (Molecular Devices, Sunnyvale, CA, USA) was used to assay the AChE inhibitory activity.

**Water and ethanol extraction**

Air-dried (45°C for 48 hr) fruiting bodies were finely pulverized. The sample powders were added to water and 70% ethanol each at a 1:30 w/v ratio and then kept in a shaker for 24 hr at 30°C. Each extract was filtered with Whatman 0.45 μm membrane filter (NO 7404-004; Whatman, Piscataway, NJ, USA) and lyophilized.

**Acetylcholinesterase inhibitory activity assay**

The AChE inhibitory activity was measured spectrophotometrically as follows [2, 13, 14]. A mixture of 110 μL of assay buffer (0.1 M sodium phosphate, pH 7.3), 30 μL of AChE (0.8 unit/AChE), 30 μL of substrate (2 mM acetylthiocholine chloride), 20 μL of 5,5'-Dithiobis (2-nitrobenzonic acid, 2 mM DTNB) and 10 μL of sample (1 mg/mL) dissolved in the assay buffer (1 mg/mL) in a 96 well plate was kept at 37°C for 6 min. The reaction product 5-thio-2-nitrobenzate produced was measured at 415 nm. The inhibition rate was obtained with the following equation:

\[
\text{Inhibition} (\%) = \left[1 - \frac{(S - S_0)}{(C - C_0)} \right] \times 100,
\]

where C was the radiation of a control (enzyme, assay buffer, DTNB, and substrate) after an activation for 6 min; C₀ was the radiation of the control at time zero; S was the radiation of the tested samples (enzyme, assay buffer, DTNB, and substrate) after an activation of 6 min, and S₀ was the radiation of the tested samples at time zero.

To check the quenching effect of the samples, the sample was added to the reaction mixture C (control), and any reduction in radiation by the sample was investigated.

**Results and Discussion**

**Screening of potent acetylcholinesterase inhibitor-containing mushrooms**

To select a potent anti-dementia AChE inhibitor-containing mushroom, the acetylcholinesterase inhibitory activity of the extracts of various market mushrooms was assayed.

**Table 1.** Yield and acetylcholinesterase inhibitory activity of water and 70% ethanol extracts from various market mushrooms

| Scientific name         | Yield (%) | Acetylcholinesterase inhibitory activity (%) |
|-------------------------|-----------|---------------------------------------------|
|                         | Water extracts | 70% Ethanol extracts | Water extracts | 70% Ethanol extracts |
| Sparassis crispa        | 3.5        | 14.4                         | 12.9 (± 0.0) | 11.0 (± 0.5) |
| Auricularia auricula-judae | 1.1       | 3.0                          | 18.3 (± 0.2) | 19.2 (± 0.5) |
| Pholiota adiposa        | 10.5       | 22.7                         | 23.8 (± 0.0) | 30.6 (± 0.0) |
| Pleurotus ostreatus     | 43.8       | 21.8                         | 11.6 (± 0.1) | n.d         |
| Lentinula edodes        | 48.7       | 37.5                         | 2.8 (± 0.0)  | n.d         |
| Agaricus bisporus       | 45.2       | 44.6                         | 3.2 (± 0.3)  | 7.4 (± 0.4) |
| Pleurotus eringi        | 38.4       | 36.2                         | 5.9 (± 0.4)  | n.d         |
| Flammulina velutipes    | 35.7       | 39.3                         | 3.0 (± 0.0)  | n.d         |

Extraction condition: 1:30, 30°C, 24 hr.

n.d, not detected.

**Table 2.** Effect of extraction temperature on the yield of water and 70% ethanol extracts from *Pholiota adiposa*

| Extraction temperature (°C) | Water extracts (%) | 70% Ethanol extracts (%) |
|-----------------------------|--------------------|--------------------------|
|                             | Yield              | AChE inhibitory activity | Yield              | AChE inhibitory activity |
| 20                          | 10.3               | 23.6 (± 0.0)             | 20.4               | 26.7 (± 0.0)             |
| 30                          | 10.5               | 23.8 (± 0.0)             | 22.7               | 30.6 (± 0.0)             |
| 50                          | 11.7               | 24.7 (± 0.7)             | 23.5               | 33.8 (± 0.2)             |
| 70                          | 12.7               | 30.9 (± 0.2)             | 26.6               | 35.0 (± 0.0)             |

AChE, acetylcholinesterase.

*Ratio of sample and solvents, 1:30.

*Extraction time 24 hr at 20°C, 30°C and 50°C, 12 hr at 70°C.
ning mushroom, water and 70% ethanol extracts from nine kinds of edible mushrooms were prepared, and their yields and AChE inhibitory activities were determined. As shown in Table 1, the water extract from *Lentinula edodes* fruiting body had the highest yield of 48.7%, and the water and 70% ethanol extracts of *Agaricus bisporus* and the water extract of *Pleurotus ostreatus* also had yields over 40%.

However, the AChE inhibitory activity was the highest at 30.6% for the 70% ethanol extract of *P. adiposa*, and its water extract also had an AChE inhibitory activity of 23.8%. Finally, *P. adiposa* was selected as a good AChE inhibitor-containing edible mushroom. This inhibitory activity was lower than those from plants and fruits such as walnut (72.6%) and job's tears (55.1%) [2, 15, 16].

**Optimal conditions for the extraction of the acetylcholinesterase inhibitor**

The effects of the extraction temperature on the AChE inhibitory activity and yields from *Pholiota adiposa* fruiting body were determined (Table 2). The 70% ethanol extract had about twice higher yield than that of the water extract.
extract, and their yields were slightly increased as the extraction temperature was increased to 70°C.

The AChE inhibitory activity of the 70% ethanol extract from the extraction at 70°C had the highest activity at 35.0%. The water extract from the extraction at 70°C also had an inhibitory activity of 30.9%. However, water extract from extraction of 100°C for 6 hr showed inhibitory activity less than 10% (data not shown).

The effect of the extraction time on the AChE inhibitory activity and yield was investigated. As seen in Figs. 1 and 2, the yields of the water and 70% ethanol extracts increased when the extraction time was increased to 3 and 6 hr, respectively. The AChE inhibitory activities also increased when the extraction time was increased. The maximum inhibitory activity was 33.7% for the water extract at 3 hr and 35.0% for the 70% ethanol extract at 12 hr.

Lee et al. [2] reported AChE inhibitor of walnut (Juglans regia L.) was maximally obtained from extraction at 40°C for 12 hr by 80% methanol but Seo et al. [13] reported AChE inhibitor of job's tears (Coix lachrymajobi L.) was maximally extracted at 40°C for 6 hr with 60% methanol.

Meanwhile, the 95% ethanol extract had lower yield and AChE inhibitory activity than that of the 70% ethanol extract (data not shown).

REFERENCES

1. Dugu M, Neugroschl J, Sewell M, Marin D. Review of dementia. Mount Sinai J Med 2003;70:45-53.
2. Lee EN, Song JH, Lee JS. Screening of a potent antidementia acetylcholinesterase inhibitor-containing fruits and optimal extraction conditions. Korean J Food Nutr 2010;23:318-23.
3. Lee JS, Min GH, Lee JS. Nutritional and physicochemical characteristics of the antidementia Acetylcholinesterase-inhibiting methanol extracts from Umbilicaria esculenta. Mycobiology 2009;37:203-6.
4. Kwak JH, Jeong CH, Kim JH, Choi GN, Shin YH, Lee SC, Cho SH, Choi SG, Heo HJ. Acetylcholinesterase inhibitory effect of green tea extracts according to storage condition. Korean J Food Sci Technol 2009;41:435-40.
5. Jang CH, Eun JS, Park HW, Seo SM, Yang JH, Leem KH, Oh SH, Oh CH, Baek NI, Kim DK. An acetylcholinesterase inhibitor from the leaves of Securinega suffruticosa. Korean J Pharmacogn 2003;34:14-7.
6. Ahmad I, Anis I, Malik A, Nawaz SA, Choudhary MI. Cholinesterase inhibitory constituents from Onosma hispida. Chem Pharm Bull (Tokyo) 2003;51:412-4.
7. Tang XC, Han YF. Pharmacological profile of huperzine A, a novel acetylcholinesterase inhibitor from Chinese herb. CNS Drug Rev 1999;5:281-300.
8. Li L, Tsao R, Yang R, Liu C, Zhu H, Young JC. Polyphenolic profiles and antioxidant activities of heart nuts (Juglans ailanthifolia var. coriforium) and persian walnut (Juglans regia L.). J Agric Food Chem 2006;54:8033-40.
9. Zanabaatar B, Song JH, Seo GS, Noh HJ, Yoo YB, Lee JS. Screening of anti-gout Xanthine oxidase inhibitor from mushrooms. Kor J Mycol 2010;38:85-7.
10. Koo KY, Lee DH, Kim JH, Yu HE, Park JS, Lee JS. Production and characterization of antihypertensive angiotensin I-converting enzyme inhibitor from Pholiota adiposa. J Microbiol Biotechnol 2006;16:757-63.
11. Yu HE, Lee DH, Seo GS, Cho SM, Lee JS. Characterization of a novel β-hydroxy-β-methyl glutaryl coenzyme A reductase-inhibitor from the mushroom, Pholiota adiposa. Biotechnol Bioprocess Eng 2007;12:618-24.
12. Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88-95.
13. Lee JS, Jang JH, Min GH, Lee JS. Nutritional and physicochemical characteristics of the antidementia Acetylcholinesterase-inhibiting methanol extracts from Umbilicaria esculenta. Mycobiology 2009;37:203-6.