한 국 균 학 회 지 The Korean Journal of Mycology Research Article

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 serrate* [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.

Kor. J. Mycol. 2016 December, **44**(4): 314-317
https://doi.org/10.4489/KJM.2016.44.4.314
pISSN 0253-651X • eISSN 2383-5249

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

Received December 5, 2016
Revised December 16, 2016
Accepted December 20, 2016

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:

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

where C was the radiation of a control (enzyme, assay buffer, DTNB, and substrate) after an activation for 6 min; C_0 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 S_0 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-contai-

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)[b]	Water extracts[a] (%)		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	27.4 (± 0.7)	23.5	33.8 (± 0.2)
70	12.7	30.9 (± 0.2)	26.6	35.0 (± 0.0)

AChE, acetylcholinesterase.
[a]Ratio of sample and solvents, 1:30.
[b]Extraction time 24 hr at 20°C, 30°C and 50°C, 12 hr at 70°C.

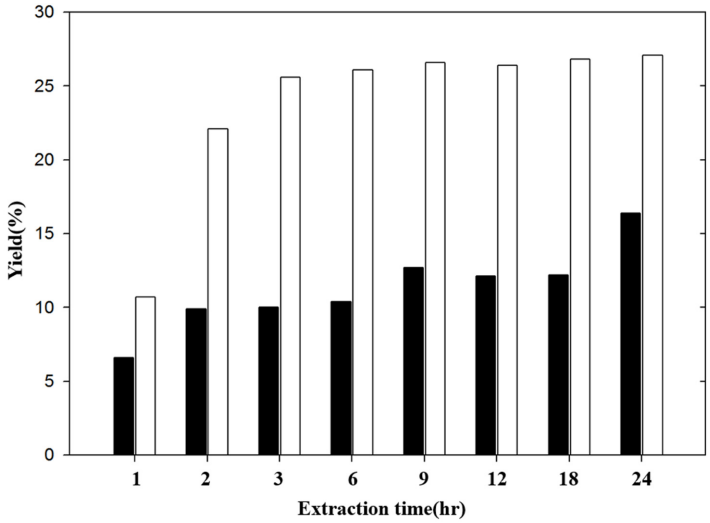

Fig. 1. Effect of extraction time at 70°C on the yield of water extracts (■) and 70% ethanol extracts (□) from *Pholiota adiposa*.

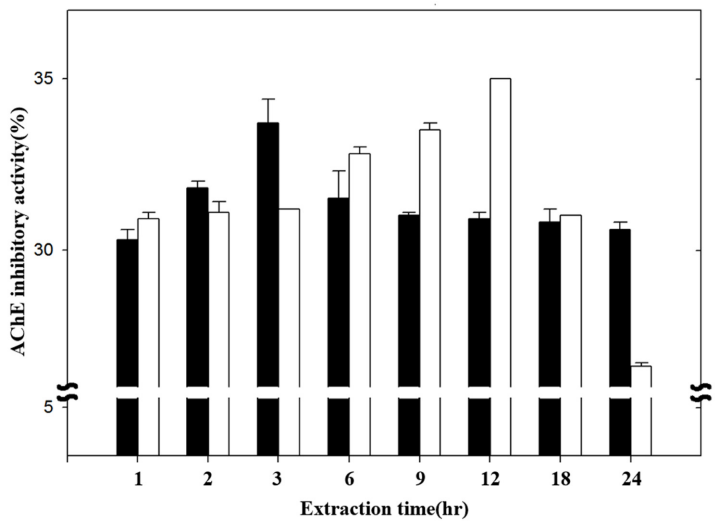

Fig. 2. Effect of extraction time at 70°C on acetylcholinesterase inhibitory activity of water extracts (■) and 70% ethanol extracts (□) from *Pholiota adiposa*.

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, 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] reperted 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 heartnut (*Juglans ailanthifolia* var. *cordiformis*) 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. Seo DS, Jang JH, Kim NM, Lee JS. Optimal extraction condition and characterization of antidementia acetylcholinesterase inhibitor from job's tears (*Coix lachrymajobi* L.). Korean J Med Crop Sci 2009;17:434-8.
14. Fukuda T, Ito H, Yoshida T. Antioxidative polyphenols from walnuts (*Juglans regia* L.). Phytochemistry 2003;63:795-801.
15. Hardman WE, Ion G. Suppression of implanted MDA-MB 231 human breast cancer growth in nude mice by dietary walnut. Nutr Cancer 2008;60:666-74.