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Screening of a Potent Antidementia Acetylcholinesterase Inhibitor-containing Fruits and Optimal Extraction Conditions

Eun-Na Lee, Jung-Hwa Song and †Jong-Soo Lee

Dept. of Life Science and Genetic Engineering, Paichai University, Daejeon 302-735, Korea

항치매성 아세틸콜린에스터라제(Acetylcholinesterase) 저해 물질 함유 과일의 선발 및 저해 물질의 추출 최적 조건

이은나 · 송정화 · †이종수

배재대학교 생명유전공학과

Abstract

아세틸콜린에스터라아제(AChE) 저해제에 의한 아세틸콜린 분해 억제는 알츠하이머 질병의 가장 확실한 치료 방법 중의 하나로 알려져 있다. 본 연구는 최근 웰빙 건강 소재로 각광을 받고 있는 과일과 채소로부터 새로운 AChE 저해제를 개발하여 항치매 식품이나 대체 의약품 생산에 응용하기 위해 과일과 채소로부터 AChE 저해 활성이 우수한 시료를 선별하고, AChE 저해 물질의 추출조건을 최적화하였다. AChE 저해 활성은 호두의 메탄올 추출물에서 72.6% (IC_{50}= 14.2 μg)로 가장 높았고, 호두의 AChE 저해 물질은 80% 메탄올로 40℃에서 12시간 동안 처리하였을 때 가장 많이 추출되었다.

Key words: acetylcholinesterase inhibitor, *Juglans regia* L, methanol extract, functional food.

INTRODUCTION

Recently, dementia disease including Alzheimer's disease (AD) has been increased from 339.5 per ten thousand people in 2000 to 535.7 per ten thousand people in 2010 in aged peoples over 65 years old of Korea. Acetylcholine (ACh) is one of neurotransmitter in both the peripheral nervous system and central nervous system in many organisms including humans and also it is converted normally, into the inactive metabolites choline and acetate by acetylcholinesterase (E.C. 3.1.1.7, AChE) (Dugue et al. 2003; Richer et al. 1980). Therefore, shortage of ACh in the brain has been associated with AD.

Some drugs that inhibit AChE are used in the treatment of AD. Several AChE inhibitors have been isolated and characterized from various natural sources including green tea (Kwak et al. 2009; Jeong et al. 2009), *Umbilicaria esculenta* (Lee et al. 2009), *Securinega suffruticosa* (Jang et al. 2003), *Onosma hispida* (Ijaz et al. 2003) and Chinese herb, *Huperzia serrata* (Xi & Yi 1999), etc. However, only some AChE inhibitors such as Rivastigmine, Galantamine, Huperzine A, Donepezil, Tacrine and Memantine were approved by FDA as drug therapy of dementia (Lahiri et al. 2002). But, they also have cholinergic side effects such as nausea, anorexia, vomiting and diarrhea. Therefore, it is necessary to develop new drug without side effects and high efficiency.

This study describes screening of a potent AChE inhibitor-containing fruit or vegetable, and optimization of conditions for AChE inhibitor extraction for development of new antidementia agent from fruits and vegetables and application into functional food industry.

† Corresponding author: Jong-Soo Lee, Dept. of Life Science and Genetic Engineering, Paichai University, Daejeon 302-735, Korea. Tel: +82-42-520-5388, Fax: +82-42-520-5388, E-mail: biotech8@pcu.ac.kr

MATERIALS AND METHODS

1. Plants and Chemicals

92 kinds of commercial fruits and vegetables were purchased at local market, that were cultivated in Korea at 2008. AChE (recombinant human acetylcholinesterase), acetylthiocholine chloride and 5,5'-dithiobis (2-nitrobenzoic acid) were purchased from the Sigma Chemical Co. (St, Louis, MO, U.S.A). VERSAmax microplate reader (Molecular Devices, sunnyvale, CA, U.S.A) was used in the assay of AChE activity. Unless otherwise specified, all chemicals and solvents were of analytical grade.

2. Extraction of Fruits and Vegetables

Powders of 92 kinds of fruits and vegetables were added in each water, 80% methanol and 70% ethanol as 1:10 w/v ratio and then shaked for 24 h at 40℃. Each extracts were filtered by Whatman 0.45 ㎛ membrane filter (No 7404-004) and lyophilized.

3. Assay of the AChE Inhibitory Activity

AChE inhibitory activity was measured spectrophotometrically by the method of Ellman et al. (1961; Seo et al. 2009). The mixture of 110 ㎕ of assay buffer (0.1 M sodium phosphate, pH 7.3), 30 ㎕ of AChE (0.8 unit/㎖ AChE), 30 ㎕ of substrate (0.1 M acetylthiocholine chloride), 20 ㎕ of 5,5'-Dithiobis (2-nitrobenzoic acid) (0.1 M DTNB) and 10 ㎕ of sample (1 ㎎/㎖) dissolved in the assay buffer (1 ㎎/㎖) was reacted for 60 min at 37℃ in 96 well plate. The reaction product, 5-thio-2-nitrobenzoate produced enzymatically was measured at 415 ㎚. The inhibition rate was obtained by 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 60 min of activation, C_0 was the radiation of control at zero time, S was the radiation of tested samples (enzyme, sample solution, DTNB, and substrate) after 60 min of activation, and S_0 was the radiation of the tested samples at zero time. All data are the mean of duplicated experiments.

To check the quenching effect of the samples, the sample solution was added to the reaction mixture C (control), and any reduction in radiation by the sample was then investigated. The IC_{50} value was defined as a concentration of the AChE inhibitor that is required to inhibit 50% of the AChE inhibitory activity.

RESULTS AND DISCUSSION

1. Screening of the AChE Inhibitor-containing Fruit and Vegetable

To select potent AChE inhibitor-containing fruit or vegetable, water extracts, 70% ethanol and 80% methanol extracts from 92 kinds fruits and vegetables were determined for their AChE inhibitory activities (Table 1, 2).

Ethanol extracts and methanol extracts from grape (skin), wild grape, plum and walnut were showed relatively high AChE inhibitory activity. However, AChE inhibitory activities of most vegetables extracts were weak or not detected. Among them, methanol extracts of walnut (*Juglans regia* L.) showed the highest AChE inhibitory activity of 72.6% (IC_{50}=14.2 ㎍). Finally, walnut was selected as a excellent AChE inhibitor-containing fruit, and this inhibitory activity was higher than that of Job's tears (55.1%) (Seo et al. 2009).

The walnut (*Juglans regia* L.) is the original walnut tree of the Old World. It is native in a region stretching from the Balkans eastward to the Himalayas and southwest China. Recently, the walnut is cultivated commercially throughout southern Europe, northern Africa, the USA, western South America and eastern Asia. In Korea, it is cultivated from Yeongdong, Cheonan, Muju and Yecheon, etc. The walnut is excellent alkaline food which is contained many protein with good quality and unsaturated fatty acid and vitamin A, B, C, E. Recently, walnut have been the subject of considerable attention because of its health-stimulating properties and medicinal effects such as antioxidant activity (Li et al. 2006), Superoxide dimutase (SOD)-like activity (Fukuda et al. 2003), antimicrobial activity (Mehrabian et al. 2000), anticancer activity (Hardman & Ion 2008) and antiallegic activity (Wallowitz et al. 2007). However, few studies have been done on the antidementia agents from walnut.

Meanwhile, proximate analysis of the selected walnut was performed by the methods of AOAC (1995). It contained 15.4% of crude protein, 66.7% crude lipid, 12.6% of carbohydrate and moisture and ash contents were 3.5% and 1.8% respectively (data not shown).

2. Optimal Conditions for Extraction of the AChE Inhibitor

Effects of methanol concentration, extraction time and temperature on the AChE inhibitory activity of the walnut were determined (Fig. 1).

Table 1. Acetylcholinesterase inhibitory activities of various extracts from fruits (Unit=%)

Scientific name	Korean name	Water extract[1)]	80% Methanol extract	70% Ethanol extract
Anacardium occidentale	캐슈넛	n.d[2)]	n.d	n.d
Ananas comosus	파인애플	4.3±1.0	n.d	n.d
Apteryx australis	키위	n.d	n.d	n.d
Arachis hypogaea	땅콩	n.d	n.d	n.d
Arecaceae palmae	야자	n.d	n.d	n.d
Citrullus vulgaris	수박	n.d	2.1±1.3	n.d
Citrus limon	레몬	n.d	n.d	n.d
Citrus nobilis	귤	n.d	n.d	n.d
Citrus parsdisi	자몽	n.d	19.0±1.6	1.0±0.7
Citrus sinensis	오렌지	n.d	n.d	n.d
Cocos nucifera L.	코코넛	n.d	n.d	5.3±1.4
Cucumis melo L.	멜론	n.d	n.d	n.d
Cucumis melo var. *makuwa*	참외	n.d	n.d	n.d
Cucurbita spp.	호박씨	0.9±2.0	33.1±1.1	10.8±1.2
Dimocarpus longan	롱간	n.d	1.0±3.8	n.d
Durio zibethinus	두리안	n.d	n.d	n.d
Garcinia mangostana	망고스틴	1.3±1.7	26.0±2.2	29.0±1.4
Helianthus annus	해바라기씨	16.0±1.5	13.9±2.3	n.d
Juglans regia L.	호두	n.d	72.6±0.9	60.9±2.3
Litchi chinensis	리치	n.d	4.6±2.6	7.7±2.9
Lycopersicon esculentum	토마토	n.d[1)]	n.d	n.d
Malus domestica Borkh.	사과	5.3±1.5	21.7±2.7	13.2±1.3
Mangifera indica	망고	n.d	14.3±0.8	14.4±2.8
Musa sapientum	바나나	3.4±2.1	n.d	n.d
Nephelium lappaceum	람부탄	n.d	n.d	5.3±2.6
Persea americana	아보카도	n.d	n.d	n.d
Pistachia vera	피스타치오	n.d	n.d	n.d
Prunus avium	체리	2.4±2.2	n.d	4.2±2.1
Prunus mume	매실	11.1±1.8	57.2±3.4	48.6±2.8
Prunus persica	복숭아	n.d	14.9±2.8	12.2±1.1
Prunus persica	천도복숭아	n.d	5.4±1.2	n.d
Prunus salicina	자두	1.9±3.9	24.7±2.1	33.0±1.9
Pyrus pyrifolia	배	n.d	28.0±3.1	n.d
Rubus coreanus	복분자	1.4±1.2	5.6±1.6	2.4±2.4
Vitis coignetiae	머루	12.2±2.2	46.9±2.5	45.8±1.7
Vitis vinifera	포도과피	31.7±1.7	53.7±1.5	51.7±2.7
Vitis vinifera	포도과육	n.d	7.6±0.2	18.0±0.4
Zizyphus jujuba	대추	n.d	3.6±1.8	1.5±2.5

[1)] Concentration ; 1 ㎎/㎖, [2)] n.d; not detected.

Table 2. Acetylcholinesterase inhibitory activities of various extracts from vegetables (Unit=%)

Scientific name	Korean name	Water extract[1)]	80% Methanol extract	70% Ethanol extract
Alium cepa L.	양파	n.d[2)]	n.d	n.d
Allium fistulosum	쪽파	n.d	n.d	n.d
Allium fistulosum L.	대파	n.d	n.d	n.d
Allium sativum	마늘	3.1±3.8	n.d	n.d
Allium tuberosum	부추	n.d	n.d	n.d
Aloe vera L.	알로에	n.d	13.3±2.6	0.6±4.6
Amaranthus mangostanus L.	비듬나물	n.d	n.d	n.d
Angelica gigas	당귀	n.d	n.d	n.d
Angelica makino	신선초	4.0±2.5	n.d	n.d
Aralia continentalis Kitagawa	땅두릅	n.d	17.6±2.5	5.0±3.1
Asparagus officinalis L.	아스파라거스	4.6±2.1	n.d	n.d
Bata valgaris	비트	n.d	n.d	n.d
Beta vulgaris var. *cicla* L.	근대	n.d	5.8±2.7	n.d
Brassica oleracea var. *acephala*	케일	n.d	n.d	n.d
Brassica alba Boiss	겨자	2.1±2.5	n.d	n.d
Brassica campestris	다청채	n.d	n.d	n.d
Brassica campestris	배추	0.2±3.8	n.d	n.d
Brassica campestris var. *chinensis*	청경채	n.d	n.d	n.d
Brassica deracea L.	적채	n.d	n.d	n.d
Brassica deracea L.	적채	n.d[2)]	n.d	n.d
Brassica oleracea var. *botrytis*	콜리플라	n.d	n.d	n.d
Brassica oleracea var. *italica*	브로콜리	2.0±3.4	n.d	n.d
Brassica oleracea	양배추	n.d	n.d	n.d
Cannabis sativa	마	3.3±3.8	n.d	n.d
Capsicum annuum L.	트레비소	n.d	8.8±2.1	10.4±2.3
Capsicum annuum	청피망	n.d	n.d	n.d
Capsicum annuum	풋고추	n.d	n.d	n.d
Capsicum annuum	홍고추	n.d	n.d	n.d
Capsicum annuum	청양고추	n.d	n.d	n.d
Capsicum annuum var. *angulosum* (Yellow)	황색 파프리카	4.2±3.7	n.d	n.d
Capsicum annuum var. *angulosum* (Red)	적색 파프리카	10.9±2.6	n.d	n.d
Chrysanthemum frutescens	쑥갓	n.d	n.d	3.4±1.5
Cichorium intybus	치커리	n.d	n.d	n.d
Cucumis sativus L.	오이	n.d	n.d	n.d
Cucurbita spp.	애호박	n.d	n.d	6.0±2.6
Cucurbita maxima	단호박	1.8±2.8	n.d	n.d
Daucus carota L.	당근	n.d	n.d	n.d
Ipomoea batatas	고구마	6.2±2.8	n.d	n.d
Lactuca sativa L.	청상추	n.d[2)]	3.0±2.1	n.d
Lactuca sativa L.	적코스	n.d	3.5±2.6	13.1±2.7
Lactuca sativa L.	청오크립	n.d	3.0±3.6	n.d
Ledebouriella seseloides	방풍나물	4.8±2.6	n.d	n.d

Table 2. Continued

Scientific name	Korean name	Water extract[1)]	80% Methanol extract	70% Ethanol extract
Ligularia fischeri Turcz	곰취나물	5.4±2.1	13.1±2.7	n.d
Malva verticillata L.	아욱	n.d	2.4±2.6	n.d
Nelumbo nucifera Gaertn	연근	5.2±3.6	4.8±1.7	12.5±2.9
Oenanthe javanica Dc.	미나리	n.d	n.d	2.2±3.1
Petasites japonicus	머우대	9.4±2.9	n.d	10.4±3.5
Petroselinum crispum	파슬리	8.1±2.9	2.7±2.5	3.3±2.9
Raphanus sativus L.	무	0.8±3.4	n.d	n.d
Solanum melongena L.	가지	n.d	2.0±2.6	1.1±2.7
Solanum tuberosum	감자	n.d	1.7±3.6	n.d
Spinacia oleracea L.	시금치	n.d	n.d	n.d
Staphylea bumalda	고추잎	n.d	n.d	n.d
Zea mays L.	옥수수	n.d	n.d	n.d

[1)] Concentration ; 1 ㎎/㎖, [2)] n.d ; not detected.

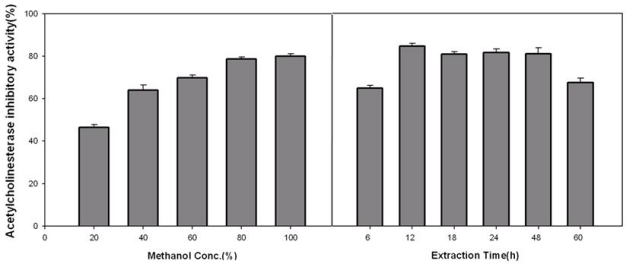

Fig. 1. Effect of methanol concentration and extraction time on the extraction of acetylcholinesterase inhibitor from walnut (*Juglans regia* L.).

As increased methanol concentration, increased its inhibitory activity and optimal methanol concentration for extraction of the AChE inhibitor was 80%. Optimal extraction time was 12 h and maximally extracted AChE inhibitor of walnut when it was treated for 12 h by 80% methanol. Furthermore, optimal extraction temperature was 40℃ (inhibitory activity: 78.8%, IC_{50}-13.5 ㎍) and extracts from 60℃ extraction was also showed high AChE inhibitory activity (data not shown). Meanwhile, Seo et al. (2009) reported AChE inhibitor of Job's tears was maximally extracted when its powder was treated with 60% methanol at 40℃ for 6 h.

CONCLUSION

To develop potent antidementia acetylcholinseterase (AChE) inhibitor from fruits and vegetables, AChE inhibitory activities of various extracts from fruits and vegetables were determined. Methanol extracts of walnut (*Juglans regia* L.) showed the highest AChE inhibitory activity of 72.6% (IC_{50}: 14.2 ㎍). AChE inhibitor of walnut was maximally extracted when it was treated for 12 h by 80% methanol at 40℃.

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