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Nutritional Characteristics and Physiological Functionality of Antidementia Acetylcholinesterase Inhibitor-containing Methanol Extract from *Sorghum bicolor*

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항치매성 아세틸콜린에스터레이즈 저해 물질을 함유한 수수 (*Sorghum bicolor*) 메탄올 추출물의 영양학적 특성과 생리 기능성

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Abstract

새로운 항치매성 건강식품을 개발하기 위해 아세틸콜린에스터레이즈(AChE)를 강력하게 저해하는 추출물을 곡류와 두류의 다양한 추출물로부터 선발한 후 최적 추출 조건을 조사하였다. 추출물 중 수수 메탄올 추출물이 63.4%의 가장 높은 AChE 저해 활성을 보였다. 수수를 80% 메탄올로 40℃에서 12시간 추출했을 때 최대의 AChE 저해 활성을 나타내었다. AChE를 저해하는 수수 메탄올 추출물의 영양성과 생리 기능성을 조사하였다. 메탄올 추출물은 유리당으로 고형물 100 g당 4.78 g의 포도당과 4.13 g의 자당 및 0.97 g의 과당을 함유하였다. 또한, 수수 메탄올 추출물의 고형물 100 g에 linoleic acid 273.82 ㎎, oleic acid 215.65 ㎎, palmitic acid 122.03 ㎎, stearic acid 4.96 ㎎을 함유하고 있었고, 주요 유기산으로 주석산 27.45 ㎎, 말론산 15.43 ㎎, 사과산 9.94 ㎎이 메탄올 추출물 1 g에 함유되어 있었다. 수수 메탄올 추출물은 항치매 활성 외에도 콜레스테롤 합성을 저해하는 HMG-CoA reductase 저해 활성이 50.2%이었고, 항산화 활성은 56.1%를 보였다.

Key words: acetylcholinesterase inhibitor, *Sorghum bicolor*, methanol extract, nutraceuticals.

INTRODUCTION

Acetylcholine(ACh) is one of many neurotransmitters in the autonomic nervous system and the only neurotransmitter used in the somatic nervous system(Dale HH 1914). Acetylcholine can be found in the brain, neuromuscular junctions, and spinal cord. Acetylcholine is particularly important in the stimulation of muscle tissue. The transmission of an impulse to the end of the nerve causes it to release neurotransmitter molecules onto the surface of the next cell, stimulating it. After such release, the acetylcholine is quickly broken into acetate and choline, which pass back to the first cell to be recycled into acetylcholine again.

Acetylcholinesterase(E.C. 3.1.1.7., AChE) hydrolyzes the neurotransmitter acetylcholine at neuromuscular junctions and brain cholinergic synapses and thus terminates signal transmission. The major form of acetylcholinesterase found in brain, muscle and other tissues is the hydrophilic species, which forms disulfide-linked oligomers with collagenous or lipid-containing structural subunits(Sussman et al. 1991).

Some AChE inhibitors have been isolated and characterized from various natural sources including Amaryllidaceae(Rhee et al. 2001), several plants(Pul et al. 2007), *Securinega suffruticosa*

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(Jang et al. 2003), *Onosma hispida*(Ijaz et al. 2003), *Corydalie soeciosa*(Kim et al. 2004) and the Chinese herb, *Huperzia serrata* (Xi & Yi 1999), etc. However, these inhibitors have low yield and some side effects. Therefore, the only some AChE inhibitors such as Tacrine, Cognex, Aricept, Donepezil, Rivastigmine and Galantamine were approved by FDA as antidementia drug(Lahiri et al. 2002). These FDA approved drugs also have cholinergic side effects such as nausea, anorexia, vomiting, and diarrhea. Therefore, it is clear that better drugs with high efficiency and no harmful side effects are required(Vincent & Delagarza 2003).

For development of a new antidementia agent from cereals and legumes and application it into manufacturing of functional foods, screening of antidementia AChE-inhibiting cereals and legumes and optimization of extraction conditions for the AChE inhibitor were investigated. Further, nutraceutical properties and physiological functionalities of the AChE inhibiting methanol extracts of *Sorghum bicolor* were determined.

MATERIALS AND METHODS

1. Materials and Chemicals

Forty kinds of commercial cereals and legumes cultivated in Korea in 2006 were purchased at a local market. Unless otherwise specified, all chemicals and solvents were of analytical grade. Acetylcholinesterase(recombinant human acetylcholinesterase, E.C. 3.1.1.7), butyrylcholinesterase(horse serum butyrylcholinesterase, E.C. 3.1.1.8), acetylthiocholine chloride and 5,5'-dithiobis(2-nitrobenzoic acid) were purchased from the Sigma Chemical Co.(St, Louis, MO, U.S.A.). A VERSAmax microplate reader(Molecular Devices, sunnyvale, CA, U.S.A.) was used in acetylcholinesterase activity. A β-Secretase(recombinant human BACE1) assay kit was purchased from the PanVera Co.(Madison, WI, U.S.A.).

2. Extraction of Cereals and Legumes

Forty kinds of cereals and legumes were freeze-dried and pulverized. Each powders was separately added to water, methanol and ethanol in 1:10 w/v ratios and then shaken for 24 hr at 40℃. Each extract was filtered using a Whatman 0.45 ㎛ membrane filter(No 7404-004) and lyophilized.

3. Assay of the Acetylcholinesterase Inhibitory Activity

AChE inhibitory activity was measured spectrophotometrically applying the technique of Ellman et al.(1961). The mixture of 110 ㎕ of assay buffer(0.1 M sodium phosphate, pH 7.3), 30 ㎕ of AChE(0.8 U/㎖), 30 ㎕ of substrate(acetylthiocholine chloride), 20 ㎕ of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) and 10 ㎕ of sample(1 ㎎ of the freeze-dried extract dissolved in 1 ㎖ of assay buffer) was incubated for 60 min at 37℃. The reaction product 5-thio-2-nitrobenzoate produced enzymatically was measured at 415 ㎚.

The inhibition ratio was obtained by the following equation: inhibition(%)=[1−{(S−S_0)/(C−C_0)}]×100, where C is the radiation of a control(enzyme, assay buffer, DTNB and substrate) after 60 min of incubation, C_0 is the radiation of control at zero time, S is the radiation of tested samples(enzyme, sample solution, DTNB and substrate) after 60 min of incubation and S_0 is 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, each sample solution was added to the reaction mixture C 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.

4. Nutritional Characteristics

Protein content of the AChE-inhibiting methanol extracts from *Sorghum bicolor* was determined by Lowry method and total sugar and reducing sugar content were determined by the Phenol-sulfuric acid method and DNS method, respectively. Crude lipid content was also determined by the Soxhlet extraction method and ash content was determined by the Calcification method in a muffle furnace at 550℃. Mineral content was determined by ion-chromatography(Pfaff JD 1993). Free amino acid content was determined using an amino acid analyzer. Organic acids (Jung & Roh 2004) and free sugars were determined by HPLC (Prodolliet et al. 1995) and fatty acid content was determined by GC(Metcalf et al. 1966).

5. Physiological Functionality

The assay of β-secretase inhibitory activity of the methanol extract from *Sorghum bicolor* was carried out according to the supplied manual with modifications(Lee et al. 2007). The mixture of 10 ㎕ of assay buffer(50 mM sodium acetate, pH 4.5), 10 ㎕ of β-secretase(BACE1)(1.0 U/㎖), 10 ㎕ of the substrate(750 nM Rh-EVNLDAEFK-Quencher in 50 mM ammonium bicarbonate) and 10 ㎕ of sample(1 ㎎ of the freeze-dried extracts dissolved in 1 ㎖ of assay buffer) was incubated for 60 min at 25℃ in dark conditions. The mixture was allowed for excitation at 530

nm and the emitted light of 590 ㎚ was collected. The inhibition ratio was obtained by the following equation: inhibition(%)=[1−{(S−S_0)/(C−C_0)}]×100, where C is the fluorescence of a control (enzyme, assay buffer, and substrate) after 60 min of incubation, C_0 is the fluorescence of control at zero time, S is the fluorescence of tested samples(enzyme, sample solution and substrate) after 60 min of incubation and S_0 is the fluorescence of the tested samples at zero time. All data represent the mean of duplicated experiments. To check the quenching effect of the samples, the sample solution was added to the reaction mixture C and any reduction in fluorescence by the sample was then investigated (Lee et al. 2007).

Butyrylcholinesterase inhibitory activity was determined by the same method as the AChE inhibitory activity assay. The only change in the procedure was that the butyrylcholinesterase was used as the enzyme and butyrylthiocholine was used as the substrate.

The HMG-CoA reductase inhibitory activity was assayed spectrophotometrically by measuring the rate of decrease in absorbance at 340 ㎚ due to the oxidation of NADPH(Kim et al. 2005). The reaction mixture in a volume of 0.5 ㎖ contained the following: potassium phosphate buffer, pH 7.0, 50 ㎛M dithiothiothreitol, 2 ㎛M HMG-CoA, 0.15 ㎛M enzyme and 100 ㎍ of protein. Two reaction mixtures were preincubated in a 2 ㎜ light path glass cuvette for 5 min at 37℃ without HMG-CoA or the extracts. The assay was performed by adding HMG-CoA into one reaction mixture and adding HMG-CoA with 10 ㎕ of sample(1 ㎎ of the freeze-dried extracts was dissolved in 100 ㎕ of 0.1 M potassium phosphate buffer, pH 7.0) into the other reaction mixture at 37℃ in a recording spectrophotometer. The initial velocity of the reaction was measured and the net rate of NADPH oxidation was determined by subtracting the rate of its oxidation in the absence of HMG-CoA from the rate observed with both substrates present.

Angiotensin I-converting enzyme(ACE) inhibitory activity was assayed by a modification of the method of Cushman and Cheung(Cushman & Cheung 1971). A mixture containing 100 mM sodium borate buffer(pH 8.3), 3 units of ACE and 50 ㎕ of sample(1 ㎎ of the freeze-dried extracts was dissolved in 50 ㎕ of 100 mM sodium borate buffer, pH 8.3) was preincubated for 10 min at 37℃. The reaction was initiated by adding 50 ㎕ of Hip-His-Leu at a final concentration of 5 mM and terminated after 30 min of incubation by adding 250 ㎕ of 1.0 N HCl. The hippuric acid liberated was extracted with 1 ㎖ of ethyl acetate and 0.8 ㎖ of the extracts was evaporated until dry using a Speed Vac Concentrator(Eyela Co., Tokyo). The residue was then dissolved in 1 ㎖ of sodium borate buffer. The absorbance at 228 ㎚ was measured to estimate the ACE inhibitory activity.

Superoxide dismutase-like activity was assayed by the method of Marklund & Marklund(1974). A 950 ㎕ of sample(5.28 ㎎ of the freeze-dried extracts was dissolved in 5 ㎖ of 55 mM Tris-cacodylic acid buffer, pH 8.2) was added to 20 ㎖ of 55 mM Tris-cacodylic acid buffer, pH 8.2, homogenized for 2 min and centrifuged at 4℃ for 30 min at 12,000×g. The supernatant was adjusted to 50 ㎖ by 55 mM Tris-cacodylic acid buffer, pH 8.2(sample extract). 50 ㎕ of 24 mM pyrogallol containing 10 mM HCl(substrate) was added to 0.95 ㎖ of the sample extracts and the increase in absorbance at 420 nm was determined for the initial 2 min.

Antioxidant activity was assayed by the method of Blois MS (1958) using 1,1-diphenyl-2-picrylhydrazyl(DPPH). A 0.8 ㎖ DPPH solution(12.5 ㎎ of DPPH dissolved in 100 ㎖ of ethanol) was added to 200 ㎕ of sample(1 ㎎ of the freeze-dried extracts was dissolved in 200 ㎕ of distilled water), shaken for 10 sec, left for 10 min and then the absorbance at 525 ㎚ was determined.

RESULTS AND DISCUSSION

1. Screening of the Acetylcholinesterase-inhibiting Cereals and Legumes

Acetylcholinesterase(AChE) inhibitory activities of water, methanol and ethanol extracts(50 ㎍/㎖) from cereals and legumes were investigated(Tables 1 and 2). Ethanol extracts of job's tears, sorghum and red rice as well as methanol extracts of millet, sorghum and glutinous rice showed high AChE inhibitory activity. However, the water extracts showed almost no AChE inhibitory activity. Among the efficacious extracts, methanol extracts(50 ㎍/㎖) of *Sorghum bicolor* showed the highest AChE inhibitory activity at 63.4%. Therefore, *Sorghum bicolor* was selected as a new source of AChE inhibitor.

Sorghum is the fifth most important cereal crop in the world after wheat, rice, corn and barley. Sorghum outperforms other cereals under various environmental stresses and is thus generally more economical to produce. More than 35% of sorghum is cultivated directly for human consumption. The balance is used primarily for animal feed, alcohol and industrial products(Joseph & Lloyd 2004). Furthermore, sorghum flavonoids and tannins are also known as important sources of ingredients for functional food and other applications(Hyun et al. 2003; Joseph & Lloyd

Table 1. Acetylcholinesterase inhibitory activity of various extracts from cereals (Unit : %)

Sample names	EtOH extracts[1)]	MeOH extracts	Water extracts
Rice	9.1	n.d[2)]	n.d
Embryonic rice	9.9	n.d	n.d
Glutinous rice	32.4	45.1	n.d
Glutinous black rice	21.4	n.d	n.d
Milled glutinous black rice	n.d	29.5	n.d
Black pigment rice	15.7	14.9	n.d
Sprouted black rice	22.6	9.0	12.7
Brown rice	17.5	n.d	n.d
Glutinous brown rice	17.7	15.3	n.d
Sprouted brown rice	n.d	n.d	n.d
Yellow glutinous millet	n.d	10.3	n.d
Job's tears	n.d	46.1	n.d
Sorghum	43.2	63.4	36.5
Red rice	38.8	39.6	n.d
Sproutedred rice	38.9	36.1	10.7
Green kerneled rice	n.d	16.8	n.d
Sprouted green kerneled rice	17.7	24.3	n.d
Barley	n.d	n.d	n.d
Glutinous barley	n.d	n.d	n.d
Cutted glutinous barley	n.d	n.d	n.d
Naked barley	n.d	n.d	n.d
Millet	n.d	48.7	n.d
Glutinous millet	n.d	10.1	n.d
Buckwheat	20.5	16.6	n.d
Cassia tora	22.4	n.d	15.5
Sesame	23.5	16.7	n.d

[1)] Concentration: 50 ㎍/㎖. [2)] n.d: not detected.

2004; Linda et al. 2006).

The effects of methanol concentration, extraction temperature and time on the AChE inhibitory activity of the methanol extracts were determined next. The AChE inhibitory activity increased with increases in extraction time and temperature. The maximal AChE inhibitory activity resulted when *Sorghum bicolor* was treated with methanol at 40℃ for 12 hr(Fig. 1).

Table 2. Acetylcholinesterase inhibitory activity of various extracts from legumes (Unit : %)

Sample names	EtOH extracts[1)]	MeOH extracts	Water extracts
Mungbean	25.3	3.5	n.d[2)]
Cracked mungbean	22.4	n.d	n.d
Jeokdu	29.5	9.4	5.9
Geodu	12.8	n.d	14.5
Yak-bean	24.9	n.d	n.d
Cheongyak-bean	18.9	9.6	n.d
Namul-bean	n.d	n.d	n.d
Sokseoritae	n.d	n.d	n.d
Baektae	n.d	n.d	n.d
Atae	6.2	n.d	n.d
Heugtae	14.7	n.d	n.d
Chugtae	7.0	n.d	n.d
Whitesoybean	10.5	n.d	n.d
Kidney bean	22.4	27.8	n.d

[1)] Concentration: 50 ㎍/㎖. [2)] n.d: not detected

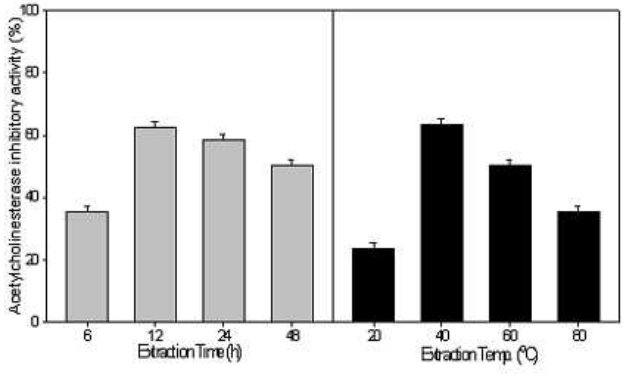

Fig. 1. Effects of extraction time and temperature on the preparation of acetylcholinesterase-inhibiting methanol extracts from *Sorghum bicolor*.

2. Characteristics of the Acetylcholinesterase-inhibiting Methanol Extracts

1) Nutritional Characteristics

Table 3 shows the general components and mineral contents

Table 3. General components and mineral contents of acetylcholinesterase-inhibiting methanol extracts from *Sorghum bicolor*

General components(g/100 g solid)				Minerals(㎎/100 g of solid)						
Protein	Total sugar(reducing sugar)	Crude lipid	Ash	Na	K	Mg	NH_4	F	Cl	SO_4
11.3	22.8(9.4)	46.1	18.4	464.3	4,360.6	736.8	163.3	134.2	1,299.0	657.3

of the methanol extracts from *Sorghum bicolor*. They contained 11.3 g of crude protein, 22.8 g of total sugar and 18.4 g of ash/100 g solid. They also contained about 4.3 g of potassium and 0.74 g of magnesium, as well as 1.30 g of chloride and 0.66 g of sulfate. Free amino acids, organic acids, free sugars, and fatty acids of the AChE inhibiting methanol extracts from *Sorghum bicolor* were also determined(Tables 4～6). The extracts contained 4.78 g of glucose/100 g solid and 4.13 g of sucrose/100 g solid as free sugars. The extracts was contained 117.66 ㎎ of aspartic acid/100 g solid and 90.71 ㎎ of gamma butyric acid/100 g solid as free amino acids. The methanol extracts contained 215.65 ㎎ of oleic acid and 273.82 ㎎ of linoleic acid/g solid as unsaturated fatty acids. These significant protein, sugar and essential fatty acid contents indicate that the methanol extracts are of high value for use in nutraceuticals.

Table 4. Free amino acids contents of the methanol extracts from *Sorghum bicolor*

Amino acids	Contents(㎎/100 g)
Aspartic acid	117.66
Threonine	16.57
Serine	29.17
Glutamine	70.85
Sarcosine	4.60
Proline	86.26
Glycine	29.30
Alanine	87.77
Citrulline	4.05
Valine	26.52
Cysteine	6.52
Cysthathionine	3.32
Isoleucine	6.84
Leucine	8.64
β-Alanine	9.18
Phenylalanine	10.19
Homocystein	1.84
γ-Aminobutyric acid	90.71
Ethanolamine	9.89
Hydroxylysine	0.71
Ornithine	2.08
Lysine	21.78
1-Methyl-histidine	3.18
Histidine	7.28
Tryptophan	11.02
Arginine	84.54
Tyrosine	16.46

Table 5. Free sugars and organic acids contents of the methanol extracts from *Sorghum bicolor*

Free sugars	Contents (g/100 g)	Organic acids	Contents (㎎/g)
Xylose	n.d[1)]	Oxalic acid	4.43
Fructose	0.97	Citric acid	2.73
Glucose	4.78	D-Tartaric acid	27.45
Galactose	n.d	D-Malic acid	9.94
Sucrose	4.13	Malonic acid	15.43
Maltose	n.d	Succinic acid	3.79
Lactose	n.d	Lactic acid	1.42
		Furmaric acid	3.26
		Acetic acid	1.31

[1)] n.d: not detected.

Table 6. Fatty acids contents of the methanol extracts from *Sorghum bicolor*

Fatty acids	Contents(㎎/100 g)
Lauric acid	n.d[1)]
11-Methyltridecanoic acid	n.d
Myristic acid	n.d
Myristoleic acid	n.d
Palmitic acid	122.03
Palmitoleic acid	n.d
Stearic acid	4.96
Oleic acid	215.65
Linoleic acid	273.82
Linolenic acid	11.76
Arachidic acid	n.d
Arachidonic acid	n.d
cis-5,8,11,14,17-Eicosapentaenoic acid	n.d
cis-4,7,10,13,16,19-Docosahexaenoic acid	n.d

[1)] n.d: not detected.

2) Physiological Functionalities

The AChE inhibiting methanol extracts also showed a 50.2% of HMG-CoA reductase inhibitory activity and a 56.1% antioxidant activity(Table 7). However, other functionalities such as SOD-like activity, ACE inhibitory activity, β-secretase inhibitory activity and butyrylcholinesterase inhibitory activity were weak or undetected. This HMG-CoA reductase inhibitory activity,

Table 7. Physiological functionalities of the methanol extracts from *Sorghum bicolor*

Functionalities	β-Secretase inhibitory activity (%)	Butyrylcholines-terase inhibitory activity (%)	HMG-CoA reductase inhibitory activity (%)	ACE[1] inhibitory activity (%)	SOD-like activity (%)	Antioxidant activity (%)
Contents	26.2	6.3	50.2	n.d[2]	n.d	56.1

[1] ACE: angiotensin I-converting enzyme. [2] n.d: not detected.

which is related to the inhibition of cholesterol biosynthesis was lower than those of ethanol extracts of *Pueraria thunbergiana* (77.0%)(Kim et al. 2005) and water extracts of perilla leaves (83.0%)(Hyun et al. 2003). However, it was higher than that of Gugija(*Lyciuum chinensis*)(Park et al. 2007). Furthermore, Cho et al.(2000) reported that sorghum and proso millet inhibit rat liver microsomal 3-hydroxy-3-methylglutaryl CoA(HMG-CoA) reductrase.

Meanwhile, it is known that the phenol contents of sorghums correlate highly with their antioxidant activity, as measured by their oxygen radical absorbance capacity(ORAC), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)(ABTS) and 2,2-diphenyl-1-picrylhydrazyl(DPPH)(Joseph et al. 2003). Therefore, the antioxidant functions of the methanol extracts in this study are presumed to result from the presence of phenolic compounds in the extracts.

From these results, it is concluded that methanol extracts of *Sorghum bicolor* has high anti-dementia AChE inhibitory activity and anti-hyperlipemia HMG-CoA reductase inhibitory activity as well as antioxidant activity. The methanol extracts also contained protein, sugar and essential fatty acids. Therefore, methanol extract from *Sorghum bicolor* is expected to be potentially useful in the preparation of functional foods without any accompanying side effects.

REFERENCES

Blois MS. 1958. Antioxidant determination by the use of stable free radical. *Nature* 191:1199-1202

Cho SH, Choi Y, Ha TY. 2000. *In vitro* and *in vivo* effects of prosomillet, buckwheat and sorghum on cholesterol metabolism. *FASEB J* 14:A249

Cushman DW, Cheung HS. 1971. Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. *Biochem Pharmacol* 20:375-382

Dale HH. 1914. The action of certain esters and ethers of choline and their relation to muscarine. *J Pharmacol* 6:147-190

Ellman GL, Courtney KD, Andres V, Featherstone RM. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* 7:68-75

Hyun KW, Kim JH, Song KJ, Lee JB, Jang JH, Kim YS, Lee JS. 2003. Physiological functionality in Geumsan perilla leaves from greenhouse and field cultivation. *Korean J Food Sci Technol* 35:975-979

Ijaz A, Itrat A, Abdul M, Sarfraz AN, Muhammad IC. 2003. Cholinesterase inhibitory constituents from *Onosmahispida*. *Chem Pharm Bull* 4:412-414

Jang CH, Eun JS, Park HW, Seo SM, Yang JH, Leem KH, Oh SH, Oh CH, Baek NI, Kim DK. 2003. An acetylcholinesterase inhibitor from the leaves of *Securinega suffruticosa*. *Kor J Pharmacogn* 1:14-17

Joseph MA, Lloyd WR, Xianli W, Ronald LP, Luis CZ. 2003. Screening methods to mesasure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products. *J Agric Food Chem* 51:6657-6662

Joseph MA, Lloyd WR. 2004. Sorghum phytochemicals and their potential impact on human health. *Phytochemistry* 65: 1199-1221

Jung BM, Roh SB. 2004. Physicochemical quality comparison of commercial doenjang and traditional green tea doenjang. *J Korean Soc Food Sci Nutr* 1:132-139

Kim DK, Lee KT, Baek NI, Kim SH, Park HW, Lim JP, Shin TY, Eom DO, Yang JH, Eun JS. 2004. Acetylcholinesterase inhibitors from the aerial parts of *Corydalis speciosa*. *Arch Pharm Res* 27:1127-1131

Kim HJ, Lee DH, Hwang YY, Lee KS, Lee JS. 2005. Characterization of β-hydroxy-β-methylglutaryl Coenzyme A reductase inhibitor from *Pueraria thunbergiana*. *J Agric Food Chem* 53:5882-5888

Lee DH, Lee DH, Lee JS. 2007. Characterization of a new antidementia β-secretase inhibitory peptide from *Saccharomyces cerevisiae*. *Enzyme and Microbial Technology* 42:83-88

Linda D, Lloyd W. Rooney. 2006. Sorghum and millet phenols and antioxidants. *J Cereal Sci* 44:236-251

Marklund S, Marklund G. 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem* 47:469-474

Metcalf LD, Schmit AA, Pelka JR. 1966 Rapid preparation of fatty acids ester from gas chromatographic analysis. *Anal Chem* 38:514

Park WJ, Lee BC, Lee JC, Lee EN, Song JE, Lee DH, Lee JS. 2007. Cardiovascular biofunctional activity and antioxidant activity of Gugija(*Lyciuum chinensis*) species and its hybrids. *Korean J Medicinal Crop* 15:391-397

Pfaff JD. 1993. Method 300.0, Determination of inorganic anions in water by ion chromatography. Cincinnati. OH. U.S. EPA. Environmental monitoring systems laboratory

Prodolliet J, Bruelhart M, Lador F, Martinez C, Obert L. 1995. Determination of free and total carbohydrate profile insoluble coffee. *J AOAC Int* 3:749-761

Prodolliet J, Bruelhart M, Lador F, Martinez C, Obert L. 1995. Determination of free and total carbohydrate profile insoluble coffee. *J AOAC Int* 3:749-761

Pul ok KM, Venkatesan K, Mainak M, Peter JH. 2007. Acetylcholinesterase inhibitors from plants. *Phytomedicine* 14:289-300

Rhee IK, Michiel van de M, Kornkanok I, Robert V. 2001. Screening for acetylcholinesterase inhibitors from amaryllidaceae using silica gel thin-layer chromatography in combination with bioactivity staining. *J Chromatography* 915:217-223

Sussman JL, Harel M, Frolow F, Oefner C, Goldman A, Toker L, Silman I. 1991. Atomic structure of acetylcholinesterase from *Torpedo californica*: a prototypic acetylcholine-binding protein. *Science* 253:872-879

Vincent We, Delagarza MD. 2003. Pharmacologic treatment of Alzheimer's disease: An Update. *Clinical Pharmacology* 68: 1365-1372

Xi CT, Yi FH. 1999. Pharmacological profile of huperzine A, a novel acetylcholinesterase inhibitor from Chinese herb. *CNS Drug Reviews* 3:281-300

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