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RESEARCH NOTE

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Hypoglycemic and Angiotension Converting Enzyme Inhibitory Effect of Water and Ethanol Extracts from *Haesongi* Mushroom (*Hypsizigus marmoreus*)

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Abstract Water and ethanol extracts were prepared from the *haesongi* mushroom (*Hypsizigus marmoreus*) to measure functional components. The ability of the extracts to inhibit angiotensin-converting enzyme (ACE) and their hypoglycemic effects were also determined; the latter was measured by α-amylase and glucosidase inhibition. Extraction yield, protein content, total phenol, and β-glucan in the water extracts were 55.86, 17.71, 1.89, and 21.93%, respectively. The respective values for the ethanol extracts were lower than those for water extracts. Both water and ethanol extracts showed dose-dependent ACE inhibition, the effect of the former being greater. The water extract inhibited ACE activity by 95.34% at 40 mg/mL. The IC_{50} values of the water extracts were 63.32 and 0.41 mg/mL for α-amylase and glucosidase, respectively. Thus, the water extracts had a greater hypoglycemic effect than the ethanol extracts. From these results, water is a better solvent than ethanol to extract from the *haesongi* mushroom functional components that show ACE inhibition and have hypoglycemic effects.

Keywords: *Hypsizigus marmoreus*, mushroom, glucose control, angiotensin converting enzyme inhibition, β-glucan

Introduction

'Food as medicine' underlies the concept of functional foods. Functional foods do not claim to cure diseases, but increasing evidence supports the role of some of these foods in disease prevention (1).

Since earliest recorded history, humans have favored mushrooms as food, savoring their delicious flavors and recognizing the nutritive value of this special group of fungi (2). Mushroom proteins contain all the essential amino acids and are particularly rich in lysine and leucine, which most staple cereal foods lack. Fresh mushrooms contain relatively large amounts of carbohydrate and fiber (3). Mushrooms are therapeutic as well as nutritional; they are used to prevent diseases such as hypertension, hypercholesterolemia, and cancer (3,4). Some mushroom components recently isolated and identified have other significant medical properties such as immunomodulatory, cardiovascular, liver protective, antifibrotic, antiinflammatory, antidiabetic, antiviral, and antimicrobial activities (5-9).

Recently, cultivation of the *haesongi* mushroom (*Hypsizigus marmoreus*) has increased, and it is now commercially available in Korea. The mushroom, also known as *bunshimeji* or *honshimeji*, is one of the most popular edible mushrooms in Japan (10). It possesses antitumor, antifungal, and anti-proliferative activities and scavenges free radicals. The nutritive value and taste components of *haesongi* mushroom have been studied (11-16). However, the functional components of the mushroom have not been extracted and their anti-hypertensive and anti-diabetic properties have not been explored. Therefore, this study aimed to determine the concentrations of β-glucan and total phenols in the extracts of *haesongi* mushroom and investigate the potential of these components to inhibit angiotensin-converting enzyme (ACE) and cause hypoglycemia. The study was conducted using water and ethanol extracts prepared from the fruiting body of *haesongi* mushroom.

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Materials and Methods

Materials *Haesongi* mushroom (*Hypsizigus marmoreus*) was provided by Haesong Bio Co., Ltd. (Gangreung, Korea). The mushroom was dried at 35°C for 24 hr and pulverized. All reagents used in this study were of analytical grade.

Proximate analysis Moisture content (oven-drying procedure), crude protein (N×6.25), lipid (ether extraction), and ash content were estimated by the AOAC Official Method (17).

Preparation of mushroom extracts For water extraction, the mushroom powder (50 g) was soaked in 1,000 mL of distilled water with shaking (120 rpm) at 100°C for 4 hr and filtered through Advantec 5C filter paper (Advantec Toyo, Tokyo, Japan). This extraction was repeated 2 times with the residue by using additional 1,000 mL of distilled water. The water extracts were concentrated by rotary evaporation at 50°C. The concentrated extract was then dried with lyophilizer. A similar method was used for ethanol extraction, except that the extraction medium was 95% ethanol.

Determination of total sugar and protein Total sugar content of the mushroom extracts was determined by the phenol-sulfuric acid method (18). In this procedure, 1 mL of 5% phenol solution and 2.5 mL of concentrated sulfuric acid were added to 1 mL of the sample solution, vortex mixed, and left to cool for 30 min. The optical density at 490 nm was measured using a spectrophotometer (Jasco V-570; Jasco, Tokyo, Japan). The sugar content in samples was quantified using a glucose standard curve (0 to 100 mg/mL). Protein content was determined by the method of Lowry *et al.* (19) and measured with a spectrophotometer at 750 nm using bovine serum albumin as a standard.

Determination of β-glucan content β-Glucan content was determined using the Megazyme Mushroom and Yeast Beta-glucan Assay kit (Megazyme International, Wicklow, Ireland), which uses purified enzymes, according to the manufacturer's protocol. β-Glucan was measured as the difference between the total glucan content and the α-glucan content.

Determination of total phenolic compound content The total phenolic compound content was measured using the Folin-Ciocalteu method with some modification (20). For the analysis, 50 μL appropriately diluted sample and standard solution at various concentrations were mixed with 100 μL Folin-Ciocalteu reagent. Distilled water was used as the control and diluent. The solution was diluted to a total volume of 1,150 μL with distilled water and mixed thoroughly. After incubation for 10 min at room temperature, 500 μL of 20% $NaCO_3$ solution was added, mixed, and further incubated at room temperature for 2 hr. The absorbance of the mixture was recorded at 765 nm against a catechin standard.

ACE inhibition The method of Cushman and Cheung (21) was used to measure ACE inhibition. The assay mixture contained 100 mM potassium phosphate buffer (pH 8.3), 300 mM sodium chloride, 5 mM hippuryl-L-histidyl-L-leucine (HHL), and 0-10 mU/0.25 mL of assay volume of an enzyme prepared from rabbit lung acetone powder (Sigma-Aldrich, St. Louis, MO, USA). An appropriate amount of extract was added to the assay mixture and incubated for 30 min at 37°C. The reaction was terminated by adding 1 N HCl (0.25 mL). The liberated hippuric acid was extracted with 1.5 mL of ethyl acetate, and 1.0 mL of the extract was evaporated by heating at 120°C for 30 min in an oil bath. The residue was then dissolved in 1.0 mL of distilled water. The absorbance was measured at 228 nm.

α-Amylase inhibition assay α-Amylase inhibition by the mushroom extracts was measured according to the method of Colowick and Kaplan (22). Porcine pancreatic α-amylase (EC 3.2.1.1) was purchased from Sigma-Aldrich. A total of 500 μL of 0.02 M sodium phosphate buffer (pH 6.9 adjusted with 0.006 μM sodium chloride) was incubated with α-amylase solution (0.5 mg/mL) at 25°C for 10 min. After this pre-incubation, 500 μL of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 adjusted with 0.006 M sodium chloride) was added to each tube at timed intervals. The reaction mixtures were incubated at 25°C for 10 min. The reaction was stopped with 1.0 mL of dinitrosalicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. Finally, the reaction mixture was diluted in 10 mL distilled water, and absorbance was measured at 540 nm. The reduction in α-amylase activity was expressed as percent inhibition and was calculated as follows:

$$\% \text{ Inhibition} = [(\Delta A_{blank} - \Delta A_{extract})/\Delta A_{blank}] \times 100$$

The IC_{50} of the extracts on α-amylase activity was defined as the concentration of extract that reduced absorbance at 540 nm to 50% of the maximum. Acarbose (Glucobay®; Bayer Ltd., Monheim, Germany) was used as a positive control.

α-Glucosidase inhibition assay The potential of the mushroom extracts to inhibit α-glucosidase was determined using the method of Watanabe *et al.* (23). α-Glucosidase (EC 3.2.1.20) was purchased from Sigma-Aldrich. Sample solution, 50 μL, and 0.1 M phosphate buffer (pH 6.9), 100 μL, were incubated with α-glucosidase solution (1.0 U/mL) in 96 well plates at 25°C for 10 min. After pre-incubation, 50 μL of 5 mM γ-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25°C for 5 min. Before and after incubation, absorbance readings were recorded with a microarray reader (Thermomax Molecular Device Co., Blacksburg, VA, USA) at 405 nm and compared to a control containing 50 μL of buffer solution instead of the extract. The degree of α-glucosidase inhibition was expressed as a percent as follows:

$$\% \text{ Inhibition} = [(\Delta A_{blank} - \Delta A_{extract})/\Delta A_{blank}] \times 100$$

The IC_{50} of the extracts on α-glucosidase activity was defined as the concentration of extract that reduced absorbance at 405 nm to 50% of the maximum. Acarbose was used as the positive control.

Statistical analysis The results of this study were averaged and analysis of variance (ANOVA) analysis with the SAS system was used to compare the experimental groups. The significance level was computed using Duncan's multiple-range tests at $p<0.05$.

Results and Discussion

Proximate composition Proximate composition of the mushroom powder was 6.66% moisture, 4.17% crude lipid, 8.12% crude ash, 20.21% protein, and 60.84% carbohydrate.

Extraction yields and sugar and protein contents In this study, *haesongi* mushroom extracts were prepared using 2 solvents. Table 1 shows the total sugar, protein, total polyphenol, and β-glucan contents as well as the extraction yield of each sample. Sugar and protein contents in the water extracts were 39.93 and 17.71%, respectively, which were higher than those in ethanol extracts. We suggest that water extracts contain more protein-linked glucan than ethanol extracts and that filtration had removed precipitates of proteins, sugars, and high molecular weight compounds from the *haesongi* mushroom ethanol extraction solution.

Table 1. Extraction, contents of sugar, protein, total polyphenol, and β-glucan from *haesongi* mushroom extracts

Extraction medium[1)]		Water	Ethanol
Content (%)	Sugar	39.93±0.55	36.80±0.38
	Protein	17.71±1.05	5.23±0.05
	Total phenol	1.89±0.03	1.22±0.02
	β-Glucan	21.93±0.35	18.70±0.83
Extraction yield (%)		55.86±4.17	21.29±2.04

[1)]Mean±SE (n=3).

The extraction yield using water was 55.86%, which was higher than that obtained with ethanol. This higher yield might be because *haesongi* mushroom contains more water-soluble substances (10). Therefore, yields are greater with water, and it is a more efficient solvent than ethanol for extracting crude polysaccharides and proteins. Epidemiological studies indicate that the consumption of polyphenol-rich foods significantly reduces the risk of developing a variety of non-communicable diseases, including diabetes (24,25). Polyphenols in plants are considered important components of a healthy diet. These compounds have been reported to exert various biological effects, such as inhibition of carbohydrate hydrolyzing enzymes (26).

β-Glucan contains repeats of glucose units joined together in linear chains by beta bonds. There are many types of β-glucans, and they have been isolated from almost every species of yeast, grain, and fungi. β-Glucans have been extensively studied for their pharmacology, which includes cholesterol-lowering effects and immunological activities (27).

A previous study had reported that the yields of total polyphenols were influenced by the extraction solvent (28). From a toxicological point of view, ethanol and water are safer than acetone, methanol, and other organic solvents (29). Therefore, in this study we used water and ethanol as extraction solvents.

The mean total phenolic content of the water extracts was 1.89%, which was higher than that achieved with ethanol (1.22%). Other studies have had similar results. Kim *et al.* (30-32) had reported that water extracts contained more phenolic compounds than did ethanol extracts in their studies of several mushrooms, including *Pleurotus eryngii, Flammulina velutipes*, and *Lyophyllum ulmarium*. We also found that water extracts contained more β-glucan, 21.93%, than did ethanol extracts, 18.80%.

ACE inhibition Hypertension, a risk factor for many cardiovascular diseases, is also associated with chronic diabetes. Control of hypertension by dietary antihypertensives that inhibit ACE could be an effective strategy for reducing this risk (33). Figure 1 shows the inhibitory effect of both *haesongi* mushroom extracts on ACE. The water and ethanol extracts inhibited ACE activity by 53.87 and 28.71%, respectively, at 10 mg/mL. Inhibition increased with concentration: 73.97 and 42.07% at 20 mg/mL for water and ethanol, respectively, and 85.56 and 59.83% at 30 mg/mL for water and ethanol, respectively. Lee *et al.* (34) reported similar results; they found that water extracts of mushrooms showed greater ACE inhibition.

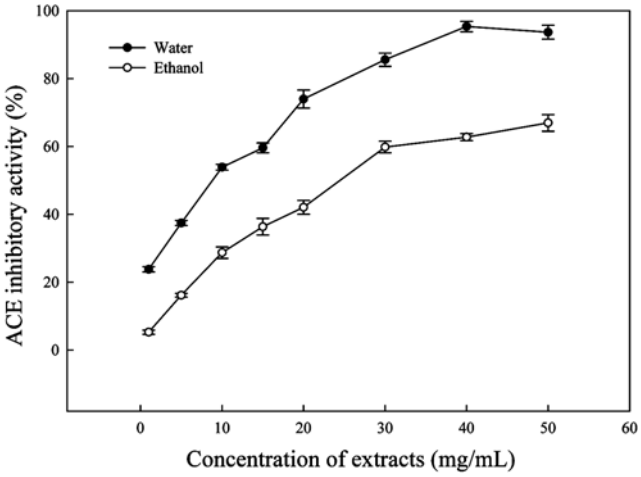

Fig. 1. ACE inhibitory activity of *haesongi* mushroom extracts.

Do *et al.* (35) showed that peptides and polyphenol compounds inhibit ACE. Water extracts contain more protein and polyphenols than do ethanol extracts, and therefore water would be expected to show greater enzyme inhibition. In our study, the composition of amino acids or peptides and the polyphenol content was dependent on solvent, and this likely influenced ACE inhibition.

α-Amylase and α-glucosidase inhibition α-Amylase and α-glucosidase inhibition was analyzed to determine whether *haesongi* mushroom has potential as a management strategy for type 2 diabetes. Several concentrations of water and ethanol extract were analyzed for their ability to inhibit the enzymes.

α-Amylase inhibition was measured using 10, 40, 80, and 160 μg/mL samples (Fig. 2A). α-Amylase acts on chemical bonds within large polysaccharides. Natural α-amylase inhibitors offer an attractive therapeutic approach to the treatment of postprandial hyperglycemia because they decrease glucose release from starch.

Water extracts inhibited α-amylase activity by 67.6 and 90.42% at 80 and 160 μg/mL, respectively. This inhibition was significantly greater than that seen with the respective ethanol extract concentrations.

Apostolidis *et al.* (36) reported that α-amylase inhibition by cranberry and oregano water extracts were 50 and 15%, respectively, at 100 μg/mL. The water extracts of *haesongi* mushroom in our study produced greater inhibition of α-amylase than did cranberry and oregano water extracts. Moreover, the 160 μg/mL water extract in our study showed similar α-amylase inhibition as that seen for 160 μg/mL acarbose, a popular drug used to inhibit α-amylase and α-glucosidase.

α-Glucosidase inhibition was measured using 0.5, 1.0, 1.5, and 2.0 mg/mL samples (Fig. 2B). α-Glucosidase catalyzes the final step in glucose absorption from the intestine during carbohydrate digestion. Hence, α-glucosidase inhibitors could retard the rapid utilization of dietary carbohydrates and suppress postprandial hyperglycemia.

Water extracts inhibited α-glucosidase to a greater extent than did ethanol extracts at all concentrations. They inhibited α-glucosidase activity by 88.3 and 91.6% at 1.5 and 2.0

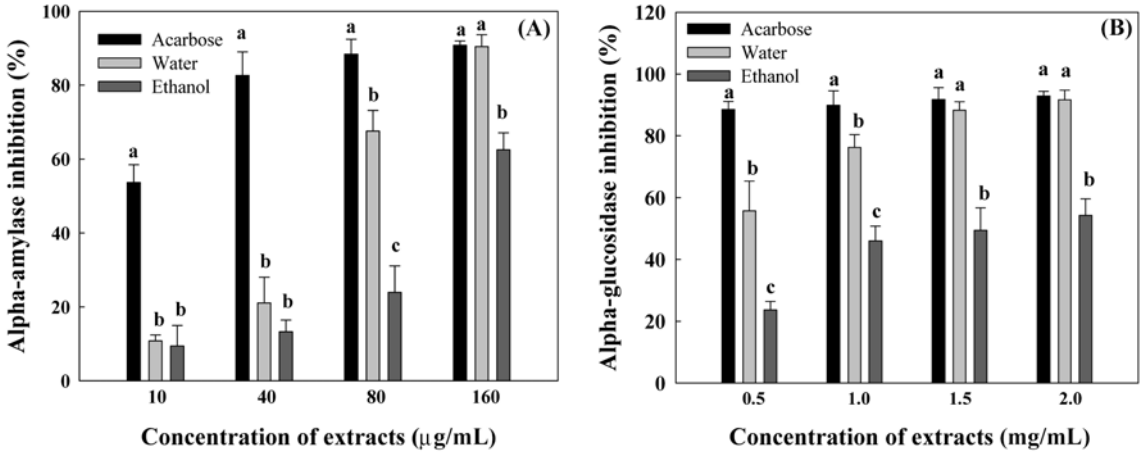

Fig. 2. α-Amylase (A) and α-glucosidase (B) inhibitory activity of *haesongi* mushroom extracts. All values are mean±SD, n=3. Different letters are significantly different by Duncan's multiple-range test after an ANOVA analysis (p<0.05).

Table 2. IC_{50} values of *haesongi* mushroom extracts for α-amylase and β-glucosidase inhibitory activity

	IC_{50} value of *haesongi* mushroom extracts		
Concentration of ethanol	Acarbose	Water	Ethanol
α-Amylase (μg/mL)	9.14	63.32	108.13
α-Glucosidase (mg/mL)	0.13	0.41	1.51

mg/mL, respectively. This was similar to the inhibition seen with acarbose at 1.5 and 2.0 mg/mL. Ethanol extracts reduced α-glucosidase activity by less than 60% at all concentrations.

The IC_{50} of water extracts against α-amylase and α-glucosidase activities was 63.32 and 0.41 mg/mL, respectively (Table 2). Acarbose was more potent than water, but the potency of the latter was greater than for the ethanol extracts.

Ganoderan A and B, glucans from *Garnoderma lucidum* fruiting bodies (37), and a β-glucan-protein complex obtained from submerged grown *Trametes versicolor* biomass (38) also have hypoglycemic effects. Apostolidis *et al.* (36) reported that α-amylase inhibition increased with increasing water soluble phenolic content. Maii *et al.* (39) reported a positive relationship between α-glucosidase inhibition and polyphenol content of edible plant extracts. In the present study, water extracts had more polyphenol, β-glucan, and protein, and showed greater inhibition of α-amylase and α-glucosidase activities than did ethanol extracts.

References

1. Steinmetz KA, Potter JD. Vegetables, fruit, and cancer. 1. Epidemiology. Cancer Cause Control 2: 325-357 (1991)
2. Chang ST, Buswell JA. Mushroom nutriceuticals. World J. Microb. Biot. 12: 473-476 (1996)
3. Bobek P, Galbavy S. Hypocholesterolemic and anti-atherogenic effect of oyster mushroom (*Pleurotus ostreatus*) in rabbit. Nahrung 43: 339-342 (1999)
4. Bobek P, Ozdyn L, Kuniak L. The effect of oyster (*Pleurotus ostreatus*) its ethanolic extracts and extraction residues on cholesterol levels in serum lipoproteins and liver of rat. Hypocholesterolemic and anti-atherogenic effect of oyster mushroom (*Pleurotus ostreatus*) in rabbit. Nahrung 39: 98-99 (1995)
5. Gunde-Cimerman N. Medicinal value of the genus pleurotus (Fr) P. Karst. (*Agaricales* S.L. *Basidimycetes*). Int. J. Med. Mushr. 1: 69-70 (1999)
6. Ooi VEC. Medicinally important fungi. pp. 41-51. In: Science and Cultivation of Edible Fungi. Balkema Publisher. Rotterdam, Netherland (2000)
7. Ooi VEC, Liu F. A review of pharmacological activities of mushroom polysaccharides. Int. J. Med. Mushr. 1: 195-206 (1999)
8. Wasser SP, Weis AL. Medicinal properties of substances occurring in higher asidiomycete mushrooms: Current perspective. Int. J. Med. Mushr. 1: 31-62 (1999)
9. Wasser SP, Weis AL. Therapeutic properties of substances occurring in higher basidiomycete mushrooms: A modern perspective. Int. J. Med. Mushr. 19: 65-96 (1999)
10. Lee YL, Yen MT, Mau JL. Antioxidant properies of various extracts from *Hypsizigus marmoreus*. Food Chem. 104: 1-9 (2007)
11. Ikekawa T, Saitoh H, Feng W, Zhang H, Li L, Matsuzawa T. Antitumor activity of *Hypsizigus marmoreus*. I. Antitumor activity of extracts and polysaccharides. Chem. Pharm. Bull. 40: 1954-1957 (1992)
12. Ikekawa T. Bunshimeji *Hypsizigus marmoreus*: Antitumor activity of extracts and polysaccharides. Food Rev. Int. 11: 207-209 (1995)
13. Tsuchida K, Aoyagi Y, Odani S, Mita T, Isemura M. Isolation of a novel of a novel collagen-binding protein from the mushroom, *Hypsizigus marmoreus*, which inhibits the Lewis lung carcinoma cell adhesion to type collagen. J. Biol. Chem. 270: 1481-1484 (1995)
14. Lam SK, Ng TB. Hypsin, a novel thermostable ribosome-inactivating protein with antifungal and antiproliferative activities from fruiting bodies of the edible mushroom *Hypsizigus marmoreus*. Biochem. Bioph. Res. Co. 285: 1071-1075 (2001)
15. Liu F, Ooi VEC, Chang ST. Free radical scavenging activities of mushroom polysaccharide extracts. Life Sci. 60: 763-771 (1997)
16. Lee YL. Taste quality, antioxidant, and antimutagenic properties of *Hypsizigus marmoreus* fruit bodies. MS thesis, University of National Chung-Hsing, Taichung, Taiwan (2003)
17. AOAC. Official Methods of Analysis of AOAC Int. 15th ed. Method 925.10, 920.53, 920.39, 923.03. Association of Official Analytical Chemists, Washington, DC, USA (1990)
18. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colormetric method for determination of sugars and related substances. Anal. Chem. 28: 350-356 (1956)
19. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol ragent. J. Biol. Chem. 193: 265-275 (1951)
20. Singleton VL, Rossi J Jr. Colorimetry of total phenolics with phosphomolybdic-phosphtungstic acid reagents. Am. J. Enol.

Viticult. 16: 144-158 (1965)
21. Chushman DW, Cheung HS. Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. Biochem. Pharmacol. 20: 1637-1648 (1971)
22. Colowick SP, Kaplan NO. Methods in Enzymology. Vol. 1. Academic Press, New York, NY, USA. pp. 149-150 (1995)
23. Watanabe J, Kawabata J, Kurihara H, Niki R. Isolation and identification of α-glucosidase inhibitors from *tochucha* (*Eucommia ulmoides*). Biosci. Biotech. Bioch. 61: 177-178 (1997)
24. Craig W, Beck L. Phytochemicals: Health protective effects. Can. J. Diet. Pract. Res. 60: 8-84 (1999)
25. Arts IC, Hollman PC. Polyphenols and disease risk in epidemiologic studies. Am. J. Clin. Nutr. 81: 317-325 (2005)
26. McDougall GJ, Shapiro F, Dobson P, Smith P, Blake A, Stewart D. Different polyphenolic components of soft fruits inhibited α-amylase and α-glucosidase. J. Agr. Food Chem. 53: 2760-2766 (2005)
27. Novak M, Vetvicka V. Beta-glucans, history, and present: Immuno-modulatory aspects and mechanisms of action. J. Immunotoxicol. 5: 47-57 (2008)
28. Sun T, Ho CT. Antioxidant activities of buckwheat extracts. Food Chem. 90: 743-749 (2005)
29. Oktay M, Gulcin I, Kufrevioglu OI. Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. Lebensm. -Wiss. Technol. 36: 263-217 (2003)
30. Kim, HK, Choi YJ, Kim KH. Functional activities of microwave-assisted extracts from *Flammulina velutipes*. Korean J. Food Sci. Technol. 34: 1013-1017 (2002)
31. Kim HK, Choi YJ, Jeoung SW, Kim KH. Functional activities of microwave-assisted extracts from *Lyophyllum ulmarium*. Korean J. Food Preserv. 9: 385-390 (2002)
32. Kim HK, Han HS, Lee GD, Kim KH. Physiological activities of fresh *Pleurotus eryngii* extracts. J. Korean Soc. Food Sci. Nutr. 34: 439-445 (2005)
33. Kwan YI, Jang HD, Shetty K. Evaluation of *Phodiola crenulata* and *Rhodiola rosea* for management of type diabetes and hypertension. Asia Pac. J. Clin. Nutr. 15: 425-432 (2006)
34. Lee DH, Kim JH, Cheong JC, Gong WS, Yoo YB, Park JS, Yoo CH, Lee JS. Screening of mushroom having angiotensin I-converting enzyme inhibitor. Korea J. Mycol. 31: 148-154 (2003)
35. Do JR, Heo IS, Back SY, Yoon HS, Jo JH, Kim YM, Kim KJ, Kim SK. Antihypertensive, antimicrobial, and antifungal activities of buckwheat hydroysate Korean J. Food Sci. Technol. 38: 268-272 (2006)
36. Apostolidis E, Kwon YI, Shetty K. Potential of cranberry-based herbal synergies for diabetes and hypertension management. Asia Pac. J. Clin. Nutr. 15: 433-441 (2006)
37. Hikino H, Konno C, Mirin Y, Hayashi T. Isolation and hypoglycaemic activities of ganoderan A and B, glucans of *Garnoderma lucidum* fruit bodies. Planta Med. 51: 339-340 (1985)
38. Ikuzawa M, Oguchi Y, Matsunaga K, Toyoda N, Furusho T, Fujii T, Yoshikumi C. Pharmaceutical preparation containing a glycoprotein. German patent DE 3,429,551 (1985)
39. Maii TT, Thu NN, Tien PG, Chuyen NV. Alpha-glucosidase inhibitory and antioxidant activities of Vietnamese edible plants and their relationships with polyphenol contents. J. Nutr. Sci. Vitaminol. 53: 267-276 (2007)