Running title: α-glucosidase and Aldose Reductase activities from *Phellinus merrillii*

α-Glucosidase and Aldose Reductase Inhibitory activities from the Fruiting Body of *Phellinus merrillii*

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Abstract

The inhibitory activity from the isolated component of the fruiting body *Phellinus merrillii* (PM) was evaluated against α-glucosidase and lens aldose reductase from Sprague-Dawley male rats and compared to the quercetin as an aldose reductase inhibitor and acarbose as an α-glucosidase inhibitor. The ethanol extracts of PM (EPM) showed the strong α-glucosidase and aldose reductase activities. α-Glucosidase and aldose reductase inhibitors were identified as hispidin (**A**), hispolon (**B**) and inotilone (**C**) which were isolated from EtOAc soluble fractions of EPM. The above structures were elucidated by their spectral and comparison with the literatures. Among them, hispidin, hispolon and inotilone exhibited potent against α-glucosidase inhibitor activity with IC_{50} values of 297.06 ± 2.06, 12.38 ± 0.13 and 18.62 ± 0.23 μg/mL, respectively, and aldose reductase inhibitor activity with IC_{50} values of 48.26 ± 2.48, 9.47 ± 0.52, and 15.37 ± 0.32 μg/mL, respectively. These findings demonstrated that PM may be a good source for lead compounds as alternatives for antidiabetic agents currently used. The importance of finding effective antidiabetic therapeutics led us to further investigation of natural compounds.

Key words: Chinese herb; *Phellinus merrillii*; α-glucosidase; aldose reductase; hispidin derivatives; flavonoid;

INTRODUCTION

Diabetes mellitus is a common disease with many complications such as atherosclerosis, cardiac dysfunction, retinopathy, neuropathy, and nephropathy (*1*). α-Glucosidase (EC 3.2.1.20) catalyzes the final step in the digestive process of carbohydrates. Its inhibitors can retard the uptake of dietary carbohydrates and suppress postprandial hyperglycemia and could be useful for treating diabetic and/or obese patients (*2*). α-Glucosidase inhibitors such as acarbose, miglitol, and voglibose are known to reduce postprandial hyperglycemia primarily by interfering with the carbohydrate digestive enzymes and by delaying glucose absorption. Aldose reductase (AR) (E.C.1.1.1.21) is the first enzyme in the polyol pathway; it catalyzes the reduction of the aldehyde functionality of D-glucose to form D-sorbitol with concomitant conversion of NADPH to $NADP^+$ (*3*). It is generally accepted that this polyol pathway plays an important role in the development of some degenerative complications of diabetes. The elevated blood glucose levels, characteristic of diabetes mellitus cause a significant flux of glucose through the polyol pathway in tissues such as nerves, retina, lens, and kidney, where glucose uptake is independent of insulin (*4*). Thus, AR inhibitors have attracted attentions in diabetic complication treatment researches.

The inhibitory activities of plant phytochemicals, including polyphenols, against

carbohydrate hydrolyzing enzymes contribute to the lowering of postprandial hyperglycemia in diabetic management as observed *in vivo* (*5*). *In vivo* studies have shown the phenolic compounds in plant materials are capable of reducing oxidative stress by scavenging reactive oxygen species and preventing cell damage in diabetic rats (*6*). The phenolic compounds in edible plants are currently regarded as natural antioxidants, and their antioxidant activities are important for human health (*7*).

Mushrooms are nutritionally functional foods and important sources of physiologically beneficial medicines. They produce various classes of secondary metabolites with interesting biological activities and thus have the potential to be used as valuable chemical resources for drug discovery (*8*). Several mushrooms belonging to the genera *Inonotus* and *Phellinus* have been used as traditional medicines for the treatment of gastrointestinal cancer, cardiovascular disease, heart diseases, stomach ailments, and diabetes (*9*). Interestingly, these mushrooms commonly produce a number of yellow antioxidant pigments that comprise hispidin derivatives and phenols.

PM is a mushroom that belongs to the genus *Phellinus* and is commonly called "Sangwhang" in Taiwan. It is popular in oriental countries and has been traditionally used as food and medicine. Sangwhang contains many bioactive compounds, and is known for improving health, preventing and remedying various diseases, such as

gastroenteric disorders, lymphatic diseases, and cancer (*10~12*). However, it was the first time that phenolic compound including compounds **A, B,** and **C** were identified from the fruiting body of PM. Moreover, there have been little studies of the effect of mushroom's constituents on α-glucosidase and aldose reductase inhibitory activity. Therefore, we investigated the inhibitory effect of the fruiting body of PM on α-glucosidase and aldose reductase in order to evaluate its potential in treating diabetic complications. Active compounds isolated from the fruiting body of PM may be a good source for lead compounds as alternatives for antidiabetic agents currently used. Antidiabetic therapeutics is an important event; we are encouraged to study the active principles for researching the lead compounds.

Materials and Methods

Materials: DL-glyceraldehyde, PNP-glycoside, PIPES, NADPH, *N*-(1-naphthyl) ethylenediamine, and quercetin were purchased from Sigma Chemical (St. Louis, MO). *Phellinus merrillii* was purchased from the Ji Pin mushroom store (Nantou, Taiwan), and identified by Drs. Yu-Cheng Dai (Institute of Applied Ecology, Chinese Academy of Science, China), and Sheng-Hua Wu (Department of Botany, National Museum of Natural Science, Taiwan).

Isolation and Determination of the Active Compounds. The fruiting body of PM (about 1.5 kg, air dry weight) was powdered, and extracted with 95% EtOH 6 L at room temperature (3 times, 72h each). Extracts were filtered and combined together, and then evaporated at 40 ℃ (N-11, Eyela, Japan) to dryness under reduced pressure to give a dark brown residue (60 g). The yield obtained for PM is about 4 %. The crude extract was suspended in H_2O (1 L), and then partitioned with 1 L *n*-hexane (× 2), 1 L EtOAc (× 2) and 1 L *n*-butanol (× 2), successively. It yielded five fractions, *n*-hexane soluble fraction, EtOAc soluble fraction, *n*-butanol soluble fraction, suspended fraction, and water soluble fraction. The yield of every fraction was shown as in Fig. 1. The weights of every fraction are 3 g, 20 g, 23 g, 13 g, for *n*-hexane, ethyl acetate, *n*-butanol, and suspension between *n*-BuOH and H_2O fractions, respectively.

Active components were purified from the EtOAc soluble portion (10 g) by a bioassay-guided separation. A portion of the active EtOAc fraction (10 g) was subjected to silica gel chromatography using stepwise $CHCl_3$-MeOH (9:1, 8:2, 1:1 v/v) as eluent. Final purification was achieved by preparative HPLC (Spherisorb ODS-2 RP18, 5 μm (Promochem), 250×25 mm, acetonitrie-H_2O (83: 17 v/v), at a flow rate of 10 mL/min and UV detection at 375nm), Yields: 200 mg of hispidin (**A**), 150 mg of hispolon (**B**) and 100 mg of inotilone (**C**). The identification of three compounds A-C was performed by comparing their physical spectral data with literature values (*12-14*).

Inhibition Assay for Alpha-Glucosidase Activity. The alpha-glucosidase inhibitory effect of the fractions of PM was assayed according to the procedure described previously by Matsui *et al*. (*15*) with minor modifications. Briefly, the enzyme reaction was performed using PNP-glycoside as a substrate in 0.1M PIPES buffer, pH 6.8. The PNP-glycoside (2.0 mM) was premixed with samples at various concentrations, and the mixture was added to an enzyme solution (0.01 units) to make 0.5 ml of final volume. The reaction was terminated by adding 1 ml of 0.64% *N*-(1-naphthyl)ethylenediamine solution (pH 10.7). Enzymatic activity was quantified by measuring the *p*-nitrophenol released from PNP-glycoside at 400 nm wave length. All reactions were carried out at 37 ℃ for 30 min with three replications. Acarbose was used as a positive control. PIPES buffer was used in blank experiments and expressed as $\triangle A_{blank}$/min. The concentration of the extracts required to inhibit 50% of α-glucosidase activity under the assay conditions was defined as the IC_{50} value.

Measurement of Aldose Reductase Activity *in vitro*. Crude AR was prepared as in the following steps: lenses were removed from Sprague-Dawley (SD) rats weighing 250–280 g, and were kept frozen until use. A rat lens homogenate was prepared in accordance with the method described by Hayman and Kinoshita (*16*). A partially purified enzyme, with a specific activity of 6.5 U/mg, was routinely used in the

evaluations of enzyme inhibition. The partially purified material was separated into 1.0 mL aliquots, and stored at −40 ℃. The AR activity was spectrophotometrically assayed by measuring the decrease in NADPH absorption at 340 nm over a 4 min period, using DL-glyceraldehyde as a substrate. Each 1.0 mL cuvette contained equal units of enzyme, 0.10 M sodium phosphate buffer (pH 6.2) and 0.3 mM NADPH, both with and without 10 mM of the substrate and an inhibitor (*17*). Sodium phosphate buffer was used in blank experiments and expressed as $\triangle A_{blank}$/min. The concentration of the extracts required to inhibit 50% of AR activity under the assay conditions was defined as the IC_{50} value.

Statistical Analysis. Experimental results were presented as the mean ± standard error (S.E.) of three parallel measurements. The statistical analyses were performed by one-way ANOVA, followed by Dunnett's *t* test. The difference was considered to be statistically significant when the *p* value was less than 0.05.

RESULTS AND DISCUSSION

Many of bioactive chemicals from plants are largely free from adverse effects and have excellent pharmacological actions; they could lead to the development of new classes of possibly antidiabetic agents. Therefore, we investigated the inhibitory effect

of the fruiting body of PM on α-glucosidase and aldose reductase in order to evaluate its potential in treating diabetic complications. We evaluated that the EtOAc fraction showed the strongest potent of inhibiting α-glucosidase and aldose reductase activities (Table 1).

Inhibition Assay for alpha-Glucosidase Activity. The percentages of α-glucosidase inhibitory activity of the fraction of the EPM were shown in Table 1. The qualities of enzymatic inhibition in the fraction of the EPM were determined by calculating IC_{50}, with lower numbers indicating higher qualities of enzymatic inhibition. The IC_{50} of α-glucosidase inhibitory activity in the extracts of the fraction of the EPM ranged from 9.25 to 332.55 μg/mL, and increased as in the following order: *n*-BuOH soluble fraction (9.25 ± 0.41 μg/mL) > EtOAc soluble fraction (9.34 ± 0.23 μg/mL)> suspension between *n*-BuOH and H_2O soluble fraction (14.35 ± 0.42 μg/mL)> H_2O soluble fraction (231.59 ± 10.14 μg/mL) > *n*-hexane soluble fraction (332.55 ± 16.35 μg/mL). BuOH soluble fraction had the highest α-glucosidase inhibitory activity. The positive control against α-glucosidase were acarbose (IC_{50} = 637.04± 0.56 μg/mL) and quercetin (IC_{50} = 12.35 ± 0.35 μg/mL).

Compounds **A, B**, and **C** (Fig. 1) are highly oxygenated and functionalized aromatic compounds that possess the unique basic structural unit, namely, hispidin,

hispolon and inotilone respectively. Inhibitory activities against α-glucosidase of compounds **A, B,** and **C** were evaluated. For α-glucosidase, PNP-glycoside was used as substrate and cofactor, respectively. As shown in Table 2, Inhibitory activity of hispolon (compound **B)** (IC_{50} = 12.38 ± 0.13 μg/mL) against α-glucosidase was stronger than that of hispidin (compound **A)** (IC_{50} = 297.06 ± 2.06 μg/mL) and inotilone (compound **C)** (IC_{50} = 18.62 ± 0.23 μg/mL). Quercetin showed strong inhibition against α-glucosidase (IC_{50} = 12.35 ± 0.35 μg/mL) activity, while acarbose (IC_{50} = 637.04± 0.56 μg/mL) did not inhibit at all.

Phenolic compounds in plants have long been recognized to inhibit the activities of digestive enzymes because of their ability to bind with proteins (*18*). Various *in vitro* assays have shown that many plant phenols possess carbohydrate hydrolyzing enzyme inhibitory activities. These compounds include green tea polyphenols which inhibit the activities of α-glucosidase and sucrase (*19*), sweet potato polyphenols (*15*), and berry polyphenols which inhibit α-glucosidase and α-amylase activities (*20*).

α-Glucosidase inhibitors are currently the most commonly used oral agents for improving postprandial hyperglycemia due to the lack of a hypoglycemic threat, and, more importantly, the prospect of blood glucose control without hyperinsulinemia and body weight gain (*21*). Inhibition of α-glucosidase and α-amylase should result in

delayed carbohydrate digestion and glucose absorption with attenuation of postprandial hyperglycemic excursions. It has been reported that α-glucosidase inhibitors usually do not alter the total amount of carbohydrate absorbed and, therefore, do not cause any net nutritional caloric loss although they slow carbohydrate digestion. Quercetin is oral agents for good penetration of sorbitol through cellular membranes, fast metabolism of sorbitol by sorbitol dehydrogenase, and, more importantly, the therapeutic prospect of patient treatment associated with such diabetic complications as retinopathy, cataracts, neuropathy, and nephropathy (*22*). In this regard, hispolon and inotilone may be used as a lead compound for the development of antidiabetic therapeutics, the inhibitory activity of hispolon and inotilone was lower than that of acarbose and quercetin. The IC_{50} of positive control for alpha-glucosidase inhibitor (acarbose) is found much higher in the present assay which is similar to many previous literatures (*23, 24*). When compared to acarbose as the control, only mammalian enzyme was inhibited. This was expected since acarbose has been shown to be a potent inhibitor of mammalian sucrase and maltase and inactive against yeast and bacterial forms (*25*).

Measurement of aldose reductase activity *in vitro*. Aldose reductase, the principal enzyme of the polyol pathway, has been shown to play an important role in

the complications associated with diabetes. The percentages of AR inhibitory activities of the five fractions from EPM are shown in Table 1. The IC_{50} of the every fraction from EPM on AR inhibitory activities ranged from 7.57 μg/mL to 240.36 μg/mL, and increased as in the following order: EtOAc (7.57 ± 0.36 μg/mL) > n-BuOH (14.12 ± 1.53 μg/mL) > suspension between *n*-BuOH and H_2O (33.90 ± 5.57 μg/mL)> H_2O (104.53 ± 9.01 μg/mL) > n-hexane (240.36 ± 1.37 μg/mL). EtOAc had the highest AR inhibitory activity.

Compounds A-C with inhibitory activities against AR was evaluated (Fig. 1). For AR, DL-glyceraldehyde and NADPH were used as substrate and cofactor, respectively. As shown in Table 2, inhibitory activities against AR of compounds **A, B,** and **C** were evaluated. Inhibitory activity of hispolon (compound **B)** (IC_{50} = 9.47 ± 0.52 μg/mL) against AR was strongest than that of hispidin (compound **A)** (IC_{50} = 48.26 ± 2.48 μg/mL) and inotilone (compound **C)** (IC_{50} = 15.37 ± 0.32 μg/mL). Positive control of quercetin showed stronger inhibition against AR (IC_{50} = 8.54 ± 0.14 μg/mL) activity than hispolon (**B**). It has been previously reported that hispidin (**A**) also have AR inhibitory activity. The IC_{50} of AR inhibitory activity hispidin (**A**) was about 12.45 μM (*26*).

Many natural compounds have been tested for AR inhibitory activity. Medicinal plants are particularly likely to be non-toxic and may be useful for the prevention and

treatment of diabetes-related complications (*27*). In addition to its antioxidant properties, quercetin has an inhibitory effect on the formation of advanced glycation end products (*28*). Furthermore, quercetin has been shown to decrease blood glucose and glycated hemoglobin levels ($HbA1_C$) and increase the glucagon/insulin ratio in type 2 diabetic animals (*29*).

It has been well acknowledged that plant-derived extracts and phytochemicals are potential alternatives to synthetic inhibitors against aldose reductase (30-*32*). In this study, the components isolated from PM against aldose reductase were identified as the hispidin, hispolon, and inotilone, although the inhibitory responses varied with chemical and concentration tested. It has been reported that the PM-derived materials including phenols compounds have anti-oxidative (*33*), anti-tumour (*34*), and anti-inflammatory effects (*35*). It might be expected, then, that the active components isolated from PM have a range of pharmacological actions for antidiabetic principles.

Aldose reductase inhibitors are the most commonly used oral agents for good penetration of sorbitol through cellular membranes and fast metabolism of sorbitol by sorbitol dehydrogenase. More importantly, they are considered as prospective therapeutics for treatment of diabetic complications such as retinopathy, cataracts, neuropathy, and nephropathy (*22*). Hispolon and inotilone may be used as lead compounds for the development of antidiabetic therapeutics.

Plant-derived extracts and phytochemicals are potential alternatives to synthetic inhibitors against AR and α-glucosidase (*36*). Currently, α-glucosidase inhibitor and AR inhibitor compounds isolated from plants are classified as diterpene-, triterpene-, and flavonoid-related compounds (*21*). The EtOAc soluble fraction and BuOH soluble fraction contain great inhibitory activity against α-glucosidase and aldose reductase with different compounds (26). In this study, the active component isolated from PM against α-glucosidase inhibitor and AR was identified as hispidin, hispolon, and inotilone, although the inhibitory responses varied with concentrations tested.

In conclusion, the results from *in vitro* experiments, including α-glucosidase inhibition, AR inhibition (Table 1 and 2). These results indicate that PM materials have inhibitory effects in *vitro* against α-glucosidase and rat lens aldose reductase. Based upon our limited data and some earlier findings, the inhibitory action of PM-derived phenol compounds confirms their potential utility as antidiabetic agents, although their use in *vivo* and the clinical efficacies remain to be evaluated.

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LITERATURE CITED

(1) Yokozawa, T.; Kim, H. Y.; Cho, E. J. Erythritol attenuates the diabetic oxidative stress through modulating glucose metabolism and lipid peroxidation in streptozotocin-induced diabetic rats. *J. Agric. Food Chem.* **2002**, *50*, 5485–5489.

(2) Niwa, T.; Doi, U.; Osawa, T. Inhibitory activity of corn-derived bisamide compounds against α-glucosidase. *J. Agric. Food Chem.* **2003**, *51*, 90–94.

(3) Lee, H. S. Rat Lens Aldose Reductase Inhibitory activities of *Coptis japonica* root-derived isoquinoline alkaloids. *J. Agric. Food Chem.* **2002**, *50*, 7013–7016.

(4) Kato, A.; Minoshima, Y.; Yamamoto, J.; Adachi, I.; Watson, A. A.; Nash, R. J. Protective effects of dietary chamomile tea on diabetic complications. *J. Agric. Food Chem.* **2008**, *56*, 8206–8211.

(5) Griffiths, D.W.; Moseley, G. The effect of diets containing field beans of high or low polyphenolic content on the activity of digestive enzymes in the intestines of

rats. *J. Sci. Food Agric.* **1980**, *31*, 255–259.

(6) Fukuda, T.; Ito, H.; Yoshida, T. Effect of the walnut polyphenol fraction on oxidative stress in type 2 diabetes mice. *Biofactors.* **2004**, *21*, 251–253.

(7) Sabu, M. C.; Smitha, K.; Kuttan, R. Anti-diabetic activity of green tea polyphenols and their role in reducing oxidative stress in experimental diabetes. *J. Ethnopharmacol.* **2002**, *83*, 109–116.

(8) Zjawiony, J. K. Biologically active compounds from *Aphyllophorales* (Polypore) Fungi. *J. Nat. Prod.* **2004,** *67*, 300–310.

(9) Cho, J. H.; Cho, S. D.; Hu, H.; Kim, S. H.; Lee, S. K.; Lee, Y. S.; Kang, K. S. The roles of ERK1/2 and p38 MAP kinases in the preventive mechanisms of mushroom *Phellinus linteus* against the inhibition of gap junctional intercellular communication by hydrogen peroxide. *Carcinogenesis*. **2002**, *23*, 1163–1169.

(10) Chang, H. Y.; Ho, Y. L.; Sheu, M. J.; Pen, W. H.; Wu, S. H.; Huang, G. J.; Chang, Y. S. Antioxidants and free radical scavenging activity of *Phellinus merrillii* extracts. *Bot. Stud.* **2007**, *48*, 407–417.

(11) Sliva, D.; Jedinak, A.; Kawasaki, J.; Harvey, K.; Slivova, V. *Phellinus linteus* suppresses growth, angiogenesis and invasive behaviour of breast cancer cells through the inhibition of AKT signalling. *Br. J. Cancer*. **2008,** *98*, 1348–1356.

(12) Ali, N. A. A.; Pilgrim, H.; Liberra, K.; Lindequist, U.; Jansen, R. Hispolon, a

yellow pigment from *Inonotus hispidus*. *Phytochemistry*. **1996**, *41*, 927–929.

(13) Gonindard, C.; Bergonzi, C.; Denier, C.; Sergheraert, C.; Klaebe, A.; Chavant, L. Hollande, E. Synthetic hispidin, a PKC inhibitor, is more cytotoxic toward cancer cells than normal cells *in vitro*. *Cell Biol. Toxicol.* **1997**, *13*, 141-153.

(14) Wangun, H.V.; Härtl, A.; Tam Kiet, T.; Hertweck, C. Inotilone and related phenylpropanoid polyketides from *Inonotus sp.* and their identification as potent COX and XO inhibitors. *Org. Biomol. Chem.* **2006,** *4*, 2545-2548.

(15) Matsui, T.; Ueda, T.; Oki, T.; Sugita, K.; Terahara, N.; Matsumoto, K. Alpha-glucosidase inhibitors action of natural acylated anthocyanins. Survey of natural pigments with potent inhibitory activity. *J. Agric. Food Chem.* **2001**, *49*, 1948–1951.

(16) Hayman, S.; Kinoshita, J. H. Isolation and properties of lens aldose reductase. *J. Biol. Chem.* **1965**, *240*, 877–882.

(17) Lim, S.S.; Jung, Y. J.; Hyun, S. K.; Lee, Y. S.; Choi, J. S. Rat lens aldose reductase inhibitory constituents of Nelumbo nucifera stamens. *Phytother. Res.* **2006**, *20*, 825–830.

(18) Griffiths, D. W.; Moseley, G. The effect of diets containing field beans of high or low polyphenolic content on the activity of digestive enzymes in the intestines of rats. *J. Sci. Food Agric.* **1980**, *31*, 255–259.

(19) Hara, Y.; Honda, M.; Inhibition of rat small intestinal sucrase and alpha-glucosidase activities by tea polyphenol. *Biosci. Biotechnol. Biochem.* **1992,** *57*, 123–124.

(20) McDougall, G. J.; Stewart, D. The inhibitory effects of berry polyphenols on digestive enzymes. *Biofactors.* **2005**, *23*, 189–195.

(21) Lee, H. S. Cuminaldehyde: Aldose reductase and α-glucosidase inhibitor derived from *Cuminum cyminum* L. seeds. *J. Agric. Food Chem.* **2005**, *53*, 2446–2450.

(22) Williamson, J.; Kilo, C.; Tilton, R. G. Mechanism of glucose- and diabetes-induced vascular dysfunction. In *Hyperglycemia, Diabetes, and Vascular Disease*; Ruderman, N., Williamson, J., Brownlee, M., Eds.; American Physiol. Society: New York, **1992**, pp 107−132.

(23) Youn, J.Y.; Park, H.Y.; Cho, K.H. Anti-hyperglycemic activity of *Commelina communis* L. inhibition of α-glucosidase. *Diabetes Res. Clin. Pract.* **2004,** *66*, 149–155.

(24) Shinde, J.; Taldone, T.; Barletta, M.; Kunaparaju, N.; Hu, B.; Kumar, S.; Jplacido, J.; Zito, S.W. α-Glucosidase inhibitory activity of *Syzygium cumin*i (Linn.) Skeels seed kernel in vitro and in Goto–Kakizaki (GK) rats. *Carbohydr. Res.* **2008,** *343*, 1278–1281.

(25) Kim, Y.M.; Wang, M.H. Rhee, H.I. A novel α-glucosidase inhibitor from pine bark. *Carbohydr. Res.* **2004,** *339*, 715–717.

(26) Lee, H. S.; Kang, Y. H.; Jung, J. Y.; Kang, I. J.; Han, S. N.; Chung, J. S.; Shin, H. K.; Lim, S. S. Inhibitory constituents of aldose reductase in the fruiting body of *Phellinus linteus. Biol. Pharm. Bull.* **2008**, *31*, 765–768.

(27) Kim, H. Y.; Oh, J. H. Screening of Korean forest plant for rat lens aldose reductase inhibition, *Biosci. Biotechnol. Biochem.* **1999**, *63*, 184–188.

(28) Wu, C. H.; Yen, G. C. Inhibitory effect of naturally occurring flavonoids on the formation of advanced glycation end products. *J Agric Food Chem.* **2005**, *53*, 3167–3173.

(29) Iwai, K.; Kim, M. Y.; Onodera, A.; Matsue, H. α-Glucosidase inhibitory and antihyperglycemic effects of polyphenols in the fruit of *Viburnum dilatatum* Thunb. *J. Agric. Food Chem.* **2006**, *54*, 4588–4592.

(30) Kato, A.; Higuchi, Y.; Goto, H.; Kizu, H.; Okamoto, T.; Asano, N.; Hollinshead, J.; Nash, R. J.; Adachi, I. Inhibitory Effects of *Zingiber officinale* Roscoe derived components on aldose reductase activity *in vitro* and *in vivo*. *J. Agric. Food Chem.* **2006**, *54*, 6640–6644.

(31) Kerem, Z.; Bilkis, I.; Flaishman, M. A.; Sivan, L. Antioxidant activity and inhibition of α-glucosidase by *trans*-resveratrol, piceid, and a novel

trans-stilbene from the roots of Israeli *Rumex bucephalophorus* L. *J. Agric. Food Chem.* **2006**, *54*, 1243–1247.

(32) Leopoldini, M.; Russo, N.; Chiodo, S.; Toscano, M. Iron Chelation by the powerful antioxidant flavonoid quercetin. *J. Agric. Food Chem.* **2006**, *54*, 6343–6351.

(33) Kim, M. Y.; Seguin,P.; Ahn, J. K.; Kim, J. J.; Chun, S. C.; Kim, E.H.; Seo, S. H.; Kang, E. Y.; Kim, S. L.; Park, Y. J.; Ro, H. M.; Chung, I. M. Phenolic compound concentration and antioxidant activities of edible and medicinal mushrooms from Korea. *J. Agric. Food Chem.* **2008**, *56*, 7265–7270.

(34) Park, H. G.; Shim, Y. Y.; Choi, S. O.; Park, W. M. New method development for nanoparticle extraction of water-soluble β-(1→3)-D-Glucan from edible mushrooms, *Sparassis crispa* and *Phellinus linteus*. *J. Agric. Food Chem.* **2009**, *57*, 2147–2154.

(35) Shin, J. Y.; Lee, S.; Bae, I. Y.; Yoo, S. H.; Lee, H. G. Structural and biological study of carboxymethylated *Phellinus linteus* polysaccharides. *J. Agric. Food Chem.* **2007**, *55*, 3368–3372.

(36) Yoshida, K.; Hishida, A.; Iida, O.; Hosokawa, K.; Kawabata, J. Flavonol caffeoylglycosides as α-glucosidase inhibitors from *Spiraea cantoniensis* flower. *J. Agric. Food Chem.* **2008**, *56*, 4367–4371.