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RESEARCH ARTICLE

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In Vitro α-Amylase, α-Glucosidase, Pancreatic Lipase, Xanthine Oxidase Inhibiting Activity of *Agaricus bisporus* Extracts

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ABSTRACT

In this study, the α-amylase inhibitory activity, α-glucosidase inhibitory activity, pancreatic lipase inhibitory activity, and Xanthine Oxidase inhibitory activity of the fruiting body extracts of 5 varieties of *Agaricus bisporus* (AB) were confirmed. First, the α-amylase inhibitory activity of AB12, AB13, AB18, AB34, and AB40 methanol extracts was lower than that of acarbose, a positive control, in all concentration ranges. The α-glucosidase inhibitory activity of the AB40, AB13, and AB12 methanol extracts at the extract concentration of 1.0 mg/mL was 80.5%, 81.3%, and 78.5%, respectively, similar to that of acarbose, a positive control. The pancreatic lipase inhibitory activity of the methanol extract of *Agaricus bisporus* fruiting body was significantly lower than that of the positive control orlistat in the concentration range of 50 ~ 1.000 (mg/mL). The Xanthine Oxidase inhibitory activity was 0.5 ~ 8.0 mg/mL of each extract, which was significantly lower than that of the positive control allopurinol in the same concentration range. However, the Xanthine Oxidase inhibitory activity of AB13 and AB40 at 8.0 mg/mL was about 70%, which was higher than that of other mushrooms. In conclusion, five kinds of *Agaricus bisporus* fruiting bodies seem to have inhibitory effects on enzymes such as α-amylase, α-glucosidase, pancreatic lipase, and Xanthine Oxidase that degrade starch and protein. In particular, it has an inhibitory effect and a reduction effect on xanthine oxidase that causes gout, so it is expected that it can be developed and used as a food or health supplement with health functional properties through future research.

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1. Introduction

Chronic metabolic diseases are increasing due to irregular diet, excessive nutrient intake, stress, and lack of physical exercise. While the mortality rate by infectious diseases, the major causes of death in the 1970s and before, has drastically declined since the 1980s, cancers, cerebrovascular diseases, cardiovascular diseases, diabetes, and hypertension have held the top ranks in the list of the causes of death in the Korean population since 2017 [1,2]. The prevalence of chronic diseases among Koreans has continuously increased with the increase of the life expectancy and the changes in the causes of death. According to the 2016 Korea National Health and Nutrition Examination Survey, the prevalence of obesity was 42.3% in males and 26.4% in females, and it has greatly increased each year, particularly among male citizens. The prevalence of hypercholesterolemia was 20.2% in males and 19.3% in females, that of hypertension was 35.0% in males and 22.9% in females, and that of diabetes was in 12.9 in males and 9.6% in females. These results show that the prevalence of chronic diseases has been continuously high over a number of years [3]. Metabolic syndrome resulting from metabolic abnormalities, including obesity, hypertension and dyslipidemia, may develop into chronic diseases such as type 2 diabetes and arteriosclerotic cardiovascular diseases [4].

Various factors are involved in the pathogenesis of cardiovascular diseases, but hyperlipidemia, caused by an increase of the total serum cholesterol, LDL-cholesterol, and triglyceride, has been reported to be the biggest factor [5]. Hyperlipidemia generally refers to the state where the plasma concentration of cholesterol or triglyceride-carrying lipoproteins is above the normal limit, wherein lipoproteins, deposited between the aorta and the arteries, limit the blood supply to the heart and thus causes atherosclerosis. Many attempts have been made to treat hyperlipidemia, and drugs having a high therapeutic effect, such as atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin, have been developed. However, due to concerns

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about the long-term administration of these drugs, a natural substance-based diet with a high hyperlipidemia improving effect and less side effects has become preferred [6].

Edible mushrooms, widely known as healthy food, have long been popular, because they are rich in general nutrients, including various carbohydrates, proteins, minerals, and vitamins, and have unique tastes, flavors, and textures. In addition, many studies have shown that mushrooms have various health functional components including glucan, dietary fiver, terpene, peptides, glycoproteins, minerals, unsaturated fatty acids, and phenolic acid [7]. The most representative functional active substance is polysaccharide, which is a cell wall component such as β-glucan. The mushroom β-glucan has anti-cancer, immunoregulatory, anti-cholesterol, anti-oxidant, and neuroprotective effects. β-glucan has the capacity to bind to cell membrane receptors to induce biological reactions.

Mushrooms are classified as fungi taxonomically. However, in contrast to common fungi, mushrooms produce large fruit bodies to form spores, and belong to mycomycetes such as Basidiomycota and Ascomycota. About 1,900 kinds of mushrooms grow naturally in Korea, but only about 100 kinds are used for food or pharmaceuticals. About 50 kinds are actually cultivated for food or have a high economic value [8]. Button mushroom (*Agaricus bisporus*) contains 91.5% water, 2.45% protein, 0.34% lipids, 0.93% ash, 4.73% carbohydrates, and 13.38% total dietary fiber [9]. A report has shown that button mushroom contains total polyphenol, free sugars, and β-glucan, and thus has a radical scavenging effect and α-glucoamylase inhibitory activity [10]. Other reports have shown that button mushroom has the effect of reducing oxidative stress and mutagenicity [9] and an anti-tumor effect [11].

This study was conducted to investigate the antioxidant effect of different button mushroom fungal species that helps to maintain homeostasis and the effect of reducing chronic diseases, such as hyperlipidemia, cardiovascular diseases, and obesity, caused by the excessive fat and cholesterol intake by people today.

2. Material and methods

2.1. Agaricus bisporus *strains*

The fungal varieties of *Agaricus bisporus* (AB) used in the experiments conducted in this study were KME59012 (AB12), KME59013 (AB13), KME59018 (AB18), KME59034 (AB34), and KME59040 (AB40) provided by the Mushroom Research Institution in Gyeonggi-do Agricutural Research & Extension Services.

2.2. Preparation of mushroom samples and extracts

The mushroom fruit body used in the experiments was dried by a hot-air dryer at 45 °C for 48 h and pulverized using a pulverizer. The mushroom extracts were prepared by using 80% methanol according to the method developed by Jang and Yoon [12]. 50 g of the fruit body power was immersed in 1 L of methanol (Ducksan Company, Korea) for 48 h to be extracted at room temperature twice. The extracts were filtered with filter paper (Advantec Toyo Co., No. 2., Japan), concentrated with a rotary evaporator, and freeze-dried (LP-10, Ilshin Bio Base Co., Korea) to obtain brown mushroom extract powder. The resulting mushroom extracts were kept at −70 °C to be used in the experiment.

2.3. Measurement α-amylase inhibitory activity

The α-amylase inhibitory activity of the mushroom extract was measured by modifying the method developed by Tadera et al. [13]. Each mushroom extract was dissolved in methanol at the concentration of 2.0 mg/mL, and diluted by factors of 2, 4, 6, and 8 to prepare mushroom extract solutions of different concentrations. The mushroom extract solution (125 μL) was mixed with 12 unit/mL pancreatine-derived α-amylase solution 62.5 μL and 200 mM potassium phosphate buffer (pH 6.8) 62.5 μL, and subject to culture at 37 °C for 10 min. Then, 1% starch 125 μL was added to the culture solution and subject to a reaction at 37 °C for 5 min. 48 mM 3,5-dinitrosalicylic acid (DNS, 30% sodium potassium tartrate in 0.5 M NaOH) 125 μL was added to the solution after the reaction for color development, and the resulting mixture was subjected to reaction at 100 °C for 10 min. After cooling, distilled water was added in a quantity of three times as much as the solution, and the absorbance at 540 nm was measured by using a spectrophotometer (UV-2550, Shimadzu Co., Japan) to calculate the α-amylase inhibitory activity by using the following equation. Acarbose, an α-amylase inhibitor, was used as a positive control.

$$\alpha - \text{amylase inhibitory effect } (\%) = \left[(A_{control} - A_{sample})/A_{control}\right] \times 100$$

$A_{control}$ denotes the absorbance of the control and A_{sample} the absorbance of the sample.

2.4. Measurement of α-glucosidase inhibitory activity

The α-glucosidase inhibitory activity of the mushroom extract was measured by modifying the method developed by Tadera et al. [14]. Each

mushroom extract was dissolved in methanol at a concentration of 1.0 mg/mL, and diluted by factors of 2, 4, 6, and 8 to prepare mushroom extract solutions of different concentrations. 0.1 M phosphate buffer (pH 6.8) 50 μL was mixed with the mushroom extract 20 μL and α-glucosidase (1 unit/mL) 10 μL, and the resulting mixture was subjected to reaction at 37 °C for 5 min. Then, 2 mM *p*-nitrophenyl-α-D-glucopyranoside (*p*-NPG) 20 μL was added and the resulting mixture was subjected to an enzymatic reaction at 37 °C for 10 min, and 1 M Na_2CO_3 was added to stop the reaction. The absorbance at 405 nm was measured by using a spectrophotometer (UV-2550, Shimadzu Co., Japan) to calculate the amount of the produced *p*-NPG and the α-glucosidase inhibitory activity by using the following equation. Acarbose, an α-glucosidase inhibitor, was used as a positive control.

$$\alpha - \text{glucisidase inhibitory effect } (\%) = \left[(A_{control} - A_{sample})/A_{control}\right] \times 100$$

$A_{control}$ denotes the absorbance of the control and A_{sample} the absorbance of the sample.

2.5. Measurement of pancreatic lipase inhibitory activity

The pancreatic lipase inhibitory activity of the mushroom extract was measured by modifying the method developed by Kwon et al. [15]. Each mushroom extract was dissolved in methanol at a concentration of 1.0 mg/mL, and diluted by factors of 2, 4, 6, and 8 to prepare mushroom extract solutions of different concentrations. The mushroom extract solution (50 μL), a lipase (1.0 U/mL) enzyme solution (50 μL), and 100 mM sodium phosphate buffer (pH 6.8) (50 μL) were mixed together and subjected to a preliminary reaction at 37 °C for 10 min. After the reaction, 100 μL of 5 mM *p*-nitrophenyl butyrate (*p*-NPB) solution in the sodium phosphate buffer was added and subjected to a reaction under the same conditions for 10 min. Then, 100 mM Na_2CO_3 0.75 mL was added to stop the reaction, and the absorbance at 420 nm was measured by using a spectrophotometer (UV-2550, Shimadzu Co., Japan). The pancreatic lipase inhibitory activity was calculated as the difference in the absorbance between the group with the mushroom sample and the group without the mushroom sample. Orlistat (Sigma-Aldrich Co., St Louis, MO, USA), a pancreatic lipase inhibitor, was used as a positive control.

$$\text{Inhibition effect } (\%) = \left[(A_{control} - A_{sample})/A_{control}\right] \times 100$$

$A_{control}$ denotes the absorbance of the control and A_{sample} the absorbance of the sample.

2.6. Measurement of xanthine oxidase inhibiting activity

The xanthine oxidase inhibitory activity of the mushroom extract was measured by the method developed by Stirpe and Corte Della [16]. Each mushroom methanol extract was dissolved in methanol at a concentration of 8.0 mg/mL, and diluted with distilled water by factors of 2, 4, 6, and 8 to prepare mushroom extract solutions of different concentrations. The mushroom extract (0.1 mL), 0.1 M potassium phosphate buffer (pH 7.5) (0.6 mL), and xanthine substrate solution (0.2 mL) were mixed, and xanthine oxidase (0.2 uint/mL) (0.1 mL) was added to the resulting mixture. The mixture was subjected to a reaction at 37 °C for 15 min, which was then stopped by adding 1 N HCl (1 mL). The absorbance at 290 nm was measured by using a spectrophotometer (UV-2550, Shimadzu Co., Japan) to calculate the amount of the produced uric acid in the solution. Allopurinol, a xanthine oxidase inhibitor used in pain treatment, was used as a positive control. The xanthine oxidase inhibitory activity (%) was calculated by using the following equation.

$$\text{Inhibition effect } (\%) = [1 - (A - B)/C)] \times 100$$

A: Absorbance after adding the extract sample and the enzyme solution to the xanthine solution (2 mM) and subjecting the resulting solution to a reaction.

B: Absorbance of the A solution above prepared by the same method except that distilled water (0.1 mL) was added instead of the enzyme solution.

C: Absorbance of the A solution above prepared by the same method except that distilled water (0.1 mL) was added instead of the extract sample.

3. Results and discussion

3.1. Measurement of α-amylase inhibition effect

Alpha-amylase is a hydrolase that breaks the α-D-(1,4)-glucan bond of polysaccharides, such as starch and glycogen, to convert them into glucose, maltose, and dextrin. In the intestines, the enzyme convert polysaccharides into maltose or glucose, which may be easily absorbed to the body and thus the sugars may be directly absorbed to the bowel wall to be immediately used as energy sources. However, an excessive increase of the amylase activity in the small intestines results in a rapid increase of the blood concentration of the sugars produced by the decomposition of starch and other polysaccharides, increasing the possibility of adult diseases such as diabetes and obesity. Therefore, the prevention and the treatment of these diseases require the regulation and control of the starch-decomposing enzymes, including α-amylase and α-glucosidase,

which play critical roles in the hydrolysis of starch and carbohydrates in the intestines [17].

Table 1 shows the experimental results for the α-amylase inhibitory activity of the five kinds of button mushroom fruit body methanol extracts. The α-amylase inhibitory activity of AB40, AB13, AB34, AB12, and AB18 at the extract concentration of 0.063∼1.0 mg/mL was 8.3∼83.5%, 37.2∼82.5%, 36.8∼78.5%, 34.3∼76.3%, and 32.2∼74.3%, respectively, and that of the positive control acarbose at the same concentration was 40.7 ∼ 89.9%. The α-amylase inhibitory activity of the five kinds of button mushroom fruit body methanol extracts used in the present experiment was lower than that of the positive control acarbose in all the concentration ranges applied to the experiment. However, as the α-amylase inhibitory activity of the samples was over 60% at the concentration of 0.5 mg/mL, the intake of the body fruit of the mushrooms may help to prevent early stage diabetes.

Acarbose, used as a positive control in the present experiment, is produced by liquid fermentation of *Actinoplanes* sp., which is an actinomyces in the form of a pharmaceutical, and lowers the activity of α-amylase, which is secreted from the salivary glands and pancreas, and α-glucosidase, which exists at the brush border membrane of the small intestines [18]. The oral administration of acarbose retards the digestion and absorption of carbohydrates as well as the rapid increase of the blood sugar level, thus relieving diabetes [13]. However, because continued administration of the medicine is highly likely to cause stomach-related side effects including abdominal distention and diarrhea, many studies have been conducted to identify new natural substances that can inhibit the activity of α-amylase and β-glucosidase with less side effects. Yoon et al. [19] reported that the α-amylase inhibitory effect of the *Agaricus blazei* fruit body extracts prepared by using methanol and hot water was 37.1% and 63.6% at concentrations of 1.0 mg/mL and 2.0 mg/mL, respectively, which were significantly lower than that of the positive control acarbose at the same concentrations (83.7% and 89.9%). Therefore, the α-amylase inhibitory effect was higher in the five kinds of button mushroom fruit body methanol extracts used in the present study than in the *Agaricus blazei* fruit body extracts prepared by using methanol and hot water.

3.2. Measurement of Alpha-Glucosidase inhibitory activity

The α-glucosidase enzyme, found on the microvilli of the small intestines, plays an essential role in the digestion of carbohydrates. To be completely absorbed to the small intestines, the carbohydrates should be decomposed into monosaccharides by α-glucosidase. Therefore, the inhibition of the α-glucosidase activity retards the digestion and absorption of carbohydrates and thus suppresses the increase of the blood sugar level after a meal, helping to improve type II noninsulin dependent diabetes and obesity [20]. Acarbose, currently used as a diabetes drug, is an orally administered medicine that is prescribed to type II diabetes patients and has a very high α-amylase and α-glucosidase inhibitory activity [21].

Table 2 shows the experimental results for the α-glucosidase inhibitory activity of the five kinds of button mushroom fruit body methanol extracts. The α-glucosidase inhibitory activity of the AB40, AB13, AB34, AB12, and AB18 methanol extracts was

Table 1. Alpha-amylase inhibitory activity of methanol extract from fruiting bodies of *Agaricus bisporus* varieties.

	Concentration (mg/mL)				
Samples	0.063	0.125	0.25	0.50	1.0
AB12	34.3 ± 2.41^{cd}	40.2 ± 2.25^{cd}	54.2 ± 2.36^{c}	61.7 ± 2.52^{c}	76.3 ± 1.53^{cd}
AB13	37.2 ± 1.76^{bc}	46.3 ± 1.53^{b}	57.5 ± 2.29^{b}	67.3 ± 1.53^{b}	82.5 ± 2.29^{b}
AB18	32.2 ± 1.26^{d}	38.3 ± 1.53^{d}	50.3 ± 1.53^{d}	57.3 ± 1.53^{d}	74.3 ± 1.53^{d}
AB34	36.8 ± 2.02^{bc}	42.2 ± 2.25^{c}	55.7 ± 1.53^{bc}	63.3 ± 2.08^{c}	78.5 ± 2.29^{c}
AB40	38.3 ± 1.53^{ab}	48.8 ± 1.76^{b}	58.3 ± 1.53^{b}	68.7 ± 1.53^{b}	83.5 ± 1.80^{b}
Acarbose	40.7 ± 1.53^{a}	62.5 ± 1.50^{a}	72.2 ± 1.53^{a}	83.7 ± 1.53^{a}	89.9 ± 1.68^{a}

The values are mean ± SD ($n = 3$). Different letters in the same column indicate significant differences among groups at $p < 0.05$ as determined by Duncan's multiple range tests. Acarbose was used as a positive control.

Table 2. Alpha-glucosidase inhibitory activity of methanol extract from fruiting bodies of 5 *Agaricus bisporus* varieties.

	Concentration (mg/mL)				
Samples	0.063	0.125	0.25	0.50	1.0
AB12	32.3 ± 2.08^{c}	36.2 ± 1.26^{cd}	40.7 ± 2.08^{bc}	47.7 ± 1.33^{bc}	78.5 ± 1.80^{ab}
AB13	33.8 ± 1.26^{bc}	37.2 ± 1.76^{bc}	42.8 ± 1.89^{ab}	48.3 ± 1.53^{ab}	76.7 ± 1.53^{b}
AB18	28.7 ± 1.53^{d}	33.2 ± 2.02^{d}	39.3 ± 1.53^{c}	44.7 ± 2.08^{c}	73.0 ± 2.00^{c}
AB34	36.2 ± 2.02^{ab}	39.8 ± 2.02^{ab}	43.5 ± 1.50^{ab}	51.2 ± 2.36^{ab}	81.3 ± 1.53^{a}
AB40	36.3 ± 1.53^{ab}	40.8 ± 2.25^{a}	44.3 ± 1.53^{a}	51.0 ± 1.95^{ab}	80.5 ± 2.18^{a}
Acarbose	37.3 ± 0.64^{a}	41.5 ± 1.50^{a}	44.1 ± 1.00^{a}	51.8 ± 1.33^{a}	81.8 ± 1.60^{a}

The values are mean ± SD ($n = 3$). Different letters in the same column indicate significant differences among groups at $p < 0.05$ as determined by Duncan's multiple range tests. Acarbose was used as a positive control.

respectively 36.3∼80.5%, 36.2∼81.3%, 33.8∼76.7%, 32.3∼78.5% and 28.7∼73.0% in the concentration range of 0.063∼1.0 mg/mL, while that of the positive control acarbose was 37.3 ∼ 81.8% in the same concentration range. At the extract concentration of 1.0 mg/mL, the α-glucosidase inhibitory activity of the AB40, AB13, and AB12 methanol extracts was respectively 80.5%, 81.3%, and 78.5%, which were similar to that of acarbose (81.8%). However, the α-glucosidase inhibitory activity of the AB34 and AB18 methanol extracts was significantly lower than that of acarbose at all concentrations. Lee et al. [22] measured the α-glucosidase inhibitory activity of the shiitake mushroom fruit body hot water extract and reported that the inhibitory activity at the concentration of 1.0 mg/mL was 8.9%, which was much lower than that of the *Agaricus bisporus* methanol fruit body extract of the present study (80.5%) at the same concentration. This may be because the content of the effective ingredient was dependent on the extraction solvent.

Therefore, various components contained in edible mushrooms may inhibit the action of α-glucosidase in the small intestines to inhibit the starch decomposition and the glucose absorption to the blood, allowing control of the blood sugar level after meals and helping to prevent and treat diabetes.

3.3. Measurement of pancreatic lipase inhibitory activity

Pancreatic lipase, a lipid hydrolase, hydrolyzes the ester bond of triglycerides to decompose one glycerol molecule into three fatty acid molecules [23]. The intake of triacylglycerol contained in food causes the decomposition of triacylglycerol into 2-monoacylglyceride and fatty acids in the small intestines by pancreatic lipase secreted from the pancreas. The bile salt emulsifies the fatty acids to form micelles to enhance the lipid decomposition and its absorption to the small intestines. The fatty acids absorbed to the intestines are again synthesized into triglyceride, which enters the blood and circulates in the body and is sent to the liver, adipose tissues, and muscles. The remaining triglyceride is accumulated in each tissue of the body, and excessive accumulation of the lipids results in obesity. Therefore, the inhibition of the activity of pancreatic lipase, a lipid decomposing enzyme essential to lipid digestion, prevents the decomposition of triglycerides to be excreted from the body, allowing for the prevention of obesity by suppressing the lipid absorption into the body and lipid accumulation in the body. Orlistat (trade name: xenical), a commercially available obesity drug, inhibits the decomposition of triglycerides and decreases the absorption of triglycerides into the body by 25 ∼ 30% by irreversibly binding to lipase, the lipid decomposing enzyme secreted from the pancreas, and has a mechanism to excrete the triglycerides that are not decomposed, but it has various side effects including abdominal distention, abdominal pain, and diarrhea [24]. Therefore, many studies are being conducted to develop natural anti-obesity materials without such side effects [25].

Table 3 shows the pancreatic lipase inhibitory activity of the five kinds of button mushroom fruit body methanol extracts. The pancreatic lipase inhibitory activity at the concentration of 50∼1.000 was AB40 6.1∼65.5%, AB13 5.9∼63.3%, AB34 4.7∼57.3%, AB12 3.4∼55.7%, and AB18 3.2∼52.2%, which were significantly lower than that of the positive control orlistat (93.9 ∼ 99.6%) in the same concentration range. Lee et al. [26] prepared *Phellinus linteus* fruit body extracts by using distilled water and ethanol and measured the pancreatic lipase inhibitory effect, and reported that the pancreatic lipase inhibitory effect of the water-based extract was 52.7% under the extraction conditions of 80 °C, 72 h and extraction concentration of 4.0 mg/mL and that of the 80% ethanol-based extract was 73.2% under the extraction condition of 100 °C and extraction concentration of 4.0 mg/mL. Therefore, considering that the pancreatic lipase inhibitory activity of the five kinds of button mushroom fruit body methanol extracts used in the present study was about 52.2 ∼ 65.5% at a concentration of 1.0 mg/mL, the pancreatic lipase inhibitory activity of the mushroom extracts used in the present study was similar to or slightly higher than that of the *Phellinus linteus* mushroom.

Table 3. Pancreatic lipase inhibitory activity of methanol extract from fruiting bodies of 5 *Agaricus bisporus* varieties.

	Concentration (mg/mL)				
Samples	0.05	0.1	0.2	0.5	1.0
AB12	3.4 ± 0.15^{d}	12.3 ± 0.58^{c}	30.2 ± 0.76^{c}	42.2 ± 0.76^{d}	55.7 ± 1.53^{d}
AB13	5.9 ± 0.32^{bc}	16.3 ± 1.53^{b}	31.5 ± 1.32^{c}	50.2 ± 0.76^{b}	63.3 ± 1.53^{c}
AB18	3.2 ± 0.58^{d}	11.7 ± 0.58^{c}	27.3 ± 1.15^{d}	41.5 ± 1.32^{d}	52.2 ± 0.76^{e}
AB34	6.1 ± 0.85^{b}	17.2 ± 0.76^{b}	33.4 ± 0.53^{b}	51.7 ± 1.53^{b}	65.5 ± 1.32^{b}
AB40	4.7 ± 0.58^{c}	12.5 ± 0.92^{c}	31.2 ± 0.76^{c}	47.7 ± 1.15^{c}	57.3 ± 0.58^{d}
Orlistat	93.9 ± 1.15^{a}	99.2 ± 1.04^{a}	98.9 ± 1.21^{a}	99.4 ± 0.55^{a}	99.6 ± 0.53^{a}

The values are mean ± SD ($n = 3$). Different letters in the same column indicate significant differences among groups at $p < 0.05$ as determined by Duncan's multiple range tests. Orlistat was used as a positive control.

Table 4. Xanthine oxidase (XO) inhibitory activity of methanol extract from fruiting bodies of 5 *Agaricus bisporus* varieties.

	Concentration (mg/mL)				
Samples	0.5	1.0	2.0	4.0	8.0
AB12	37.0 ± 2.00^{d}	43.1 ± 1.21^{d}	49.3 ± 1.53^{cd}	53.2 ± 1.37^{e}	58.7 ± 2.08^{d}
AB13	38.7 ± 1.53^{b}	45.4 ± 2.03^{b}	52.5 ± 1.80^{b}	58.2 ± 1.26^{b}	63.3 ± 1.53^{b}
AB18	39.1 ± 2.15^{cd}	43.4 ± 2.68^{d}	48.5 ± 2.29^{d}	52.7 ± 2.08^{e}	57.3 ± 1.53^{d}
AB34	42.7 ± 1.53^{cd}	50.7 ± 2.08^{cd}	58.7 ± 1.53^{c}	65.2 ± 1.26^{d}	70.0 ± 2.00^{c}
AB40	41.7 ± 1.10^{bc}	48.5 ± 1.86^{bc}	56.0 ± 2.00^{b}	61.7 ± 1.53^{c}	69.8 ± 1.89^{b}
Allopurinol	89.7 ± 1.54^{a}	91.5 ± 1.45^{a}	92.4 ± 1.91^{a}	94.1 ± 1.90^{a}	97.2 ± 1.66^{a}

The values are mean ± SD ($n = 3$). Different letters in the same column indicate significant differences among groups at $p < 0.05$ as determined by Duncan's multiple range tests. Allopurinol was used as a positive control.

3.4. Measurement of xanthine oxidase inhibitory activity

Xanthine oxidase (XO) is an enzyme involved in the purine metabolism in the body and produces uric acid from xanthine or hypoxanthine [27]. The excessive production of uric acid due to abnormal purine metabolism increases the uric acid concentration in the blood, and deposits the uric acid to joints and tissues adjacent to joints or kidneys, causing inflammation and pain. In addition, the uric acid produces superoxide radicals that may cause various diseases by causing inflammation, arteriosclerosis, cancers, and aging through oxidative damage of tissues. Therefore, the inhibition of XO may suppress the onset of gout by decreasing the uric acid production. Allopurinol, commercially available at present, is widely used as a drug for treating gout and inhibits the action of XO, but has hypersensitivity reaction problems including Stevens–Johnson syndrome, which is a skin allergic symptom, kidney toxicity, and fatal hepatonecrosis [28]. Therefore, many studies are being conducted to find a natural XO inhibitor from plants or mushrooms to developed a highly effective drug for treating gout with less side effects.

Table 4 shows the experimental results for the XO inhibitory activity of the five kinds of button mushroom fruit body methanol extracts used in the present study. The XO inhibitory activity at the concentration of 0.5∼8.0 mg/mL was AB13 42.7.∼70.0%, AB40 41.7∼69.8%, AB34 38.7∼63.3%, AB12 37.0∼58.7%, and AB18 39.1∼57.3%; these values are significantly lower than that of the positive control allopurinol in the same concentration range. However, the XO inhibitory activity of AB13 and AB40 at 8.0 mg/mL was about 70%, which was higher than that of the other mushrooms. The XO inhibitory activity of the five kinds of button mushroom fruit body methanol extracts increased as the concentration was increased in a concentration-dependent manner.

Zanabaatar et al. [29] measured the XO inhibitory activity of the fruit body extracts of four edible mushrooms, including button mushroom, *Pleurotus eryngii*, yellow oysters mushroom, and pink mushroom, prepared by using water, and reported that their XO inhibitory activity was 56.7%, 37.0%, 30.1%, and 60.1%, respectively, at a concentration of 5 mg/mL. Alam et al. [30] reported that the XO inhibitory activity of the *Pleurotus ferulae* fruit body hot water extract was 3.2 ∼ 45.6% at a concentration of 0.5 ∼ 8.0 mg/mL. The XO inhibitory activity reported above was lower than that of the five kinds of edible button mushroom fruit body methanol extracts used in the present study. The XO inhibitory activity of the edible button mushroom extracts in the present study was high, likely because the polyphenol and flavonoids content in the fruit body of the edible mushrooms used in the present study was relatively high in comparison with the mushrooms used in the reports mentioned above.

Disclosure statement

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