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Short Communication

The ingredients in *Saengshik*, a formulated health food, inhibited the activity of α-amylase and α-glucosidase as anti-diabetic function

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BACKGROUND/OBJECTIVES: We investigated total 26 ingredients of *Saengshik* which will be commercially produced as an anti-diabetic dietary supplement.
SUBJECTS/METHODS: Thirteen vegetables, nine cereals, three legumes and one seed were extracted with aqueous ethanol for 2 h at 60°C, and evaluated for their inhibitory effects against α-amylase and α-glucosidase and for total phenolic and flavonoid contents.
RESULTS: All ingredients inhibited α-amylase activity except cabbage. Strong inhibitory activity of α-amylase was observed in leek, black rice, angelica and barley compared with acarbose as a positive control. Stronger inhibition of α-glucosidase activity was found in small water dropwort, radish leaves, sorghum and cabbage than acarbose. All *Saengshik* ingredients suppressed α-glucosidase activity in the range of 0.3-60.5%. Most ingredients contained total phenols which were in the range of 1.2-229.4 mg gallic acid equivalent/g dried extract. But, total phenolic contents were not observed in carrot, pumpkin and radish. All ingredients contained flavonoid in the range of 11.6-380.7 mg catechin equivalent/g dried extract.
CONCLUSIONS: Our results demonstrate that *Saengshik* containing these ingredients would be an effective dietary supplement for diabetes.

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INTRODUCTION

Diabetes mellitus is a major endocrine disorder and chronic metabolic disease characterized by hyperglycemia. Type 1 diabetes is caused by the failed production of insulin by pancreatic islets of Langerhans, and type 2 diabetes is resulted from insulin resistance combined with inadequate insulin secretion [1]. Diabetes can lead to a number of cardiovascular and neurological complications. Globally, about 90% of diabetes patients have type 2 diabetes which can be prevented or recovered with appropriate diet [2]. The most effective diet or therapeutic approach to prevent and treat diabetes and its complications has been considered to decrease the post-prandial hyperglycemia by delaying the absorption of glucose in the small intestine. This is often done by the inhibition of α-glucosidase and α-amylase which are pivotal enzymes to hydrolyze carbohydrate. Such inhibitory effect can retard the digestion and absorption of carbohydrate and result in the suppression of post-prandial hyperglycemia [3]. According to Korean Food Standards Codex [4], *Saengshik* is "uncooked foods, referring to powder, granule, bar, paste, gel, liquid, or other forms of food made of animal or plant derived materials, usually processed by drying, to be used as it is or after mixing with water or others". Non-heat processing allows *Saengshik* to preserve digestive enzymes and heat labile nutrients such as vitamins, minerals and phytochemicals [5]. Thus, *Saengshik* has been consumed as a functional food for human health as well as a substitute for meal. Kim *et al*. [6] reported that *Saengshik* possessed the antioxidant defense system property by scavenging oxidants in an *in vivo* animal model and by stimulating total antioxidant activity in human. Also, *Saengshik* has been found to have beneficial effects on weight loss with decreased total- and LDL-cholesterol levels [7, 8] and on alleviated fatty liver damage [9]. Our previous study proved a positive effect of *Saengshik* on blood glucose concentrations in diabetic rats, which prolonged their survival [10]. However, there are still few studies on the anti-diabetic effects of *Saengshik*. Therefore, this study aimed to evaluate the inhibitory activity of twenty six ingredients in *Saengshik* against α-amylase and α-glucosidase.

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MATERIAL AND METHODS

Materials

Total 26 *Saengshik* ingredients were obtained from Erom Co., Ltd (Seongnam-si, Gyeonggi-do, Korea; Table 1). Each of the *Saengshik* ingredients was extracted with 80% ethanol for 2 h at 60℃, centrifuged at 3,800 x g for 10 min, evaporated at 60℃, and freeze-dried.

α-Amylase inhibitory activity

Ten microliter of α-amylase solution (1 mg/ml) from human saliva (Sigma, St. Louis, MO, USA) was mixed with 340 μl of 20 mM phosphate buffer (pH 7.0 with 6 mM NaCl) containing different concentration of each extract. After incubation of the reaction mixture at 37℃ for 10 min, 100 μl of the 1% starch solution was added and incubated for 1 h. After 0.1 ml of 1% iodine solution and 5 ml distilled water were added to the reaction mixture, its absorbance was measured at 565 nm using a spectrophotometer (Ultrospec 2100 pro, Biochrom Ltd., Cambridge, England). The inhibition of α-amylase was calculated as $(A_{test}-A_{control}) \times 100/(A_{blank}-A_{control})$.

α-Glucosidase inhibitory activity

The enzyme solution was prepared using 0.5 U of α-glucosidase originated from *Bacillus stearothermophilus* (Sigma, St. Louis, MO, USA). The equivalent contents (50 μl each) of α-glucosidase and 0.2 M potassium phosphate buffer, pH 6.8 were mixed with 50 μl of different concentration of the extract [11]. After incubating at 37℃ for 15 min, 100 μl of 3 mM *p*-nitrophenyl-α-D-glucopyranoside was added as a substrate. The reaction mixture was incubated at 37℃ for 10 min, and stopped by adding 750 μl of 0.1 M Na_2CO_3. Absorbance was measured at 405 nm using a spectrophotometer. Solution without substrate was used as the blank. The inhibition of α-glucosidase was calculated as $\{1-(A_{test}-A_{blank})/A_{control}\} \times 100$.

Table 1. Ingredients of *Saengshik*

Common name	Scientific name
Angelica	*Angelica keiskei* (Milq.) Koidzumi
Barley	*Hordeum vulgare* L.
Black rice	*Oryza sativa* L.
Black sesame	*Sesamum indicum* L.
Broccoli	*Brassica oleracea* L. var. *italica* Plenck
Brown rice	*Oryza sativa* L.
Burdock	*Arctium lappa* L.
Cabbage	*Brassica oleracea* var. *capitata* L.
Carrot	*Daucus carota subsp. sativa* (Hoffm.) Arcang.
Deodok	*Codonopsis lanceolata* (Siebold & Zucc.) Trautv.
Glutinous brown rice	*Oryza sativa* L.
Glutinous foxtail millet	*Setaria italica*
Job's tears	*Coix lachryma-jobi* L. var. *mayuen* Stapf
Kale	*Brassia oleracea* L. var. *acephala* D
Leek	*Allium tuberosum* Rottler
Lingshi mushroom rice	*Ganoderma lucidum* (Leyss. ex. Fr.) Karst rice
Millet	*Panicum miliaceum* L.
Potato	*Solanum tuberosum* L.
Pumpkin	*Cucurbita moschata* Duchesne
Radish leaves	*Raphanus sativus* L. leaves
Radish	*Raphanus sativus* L.
Red bean	*Phaseolus angularis* (Wild.) W.F.Wight
Small black soybean	*Rhynchosia molubilis*
Small water dropwort	*Oenanthe javanica* D.C
Sorghum	*Sorghum bicolor* (L.) Moench
Soybean	*Glycine max* (L.) Merr.

Total phenolic content assay

The total phenolic compounds were quantified by the modified method of Singleton *et al*. [12]. Briefly, 20 μl of samples (1 mg/ml) was mixed with 1.58 ml of distilled water and 100 μl of 50% Folin-Ciocalteu reagent. The mixture was allowed to react for 3 min and then added with 300 μl of 20% Na_2CO_3. The mixture was placed in a dark place for 2 h, and the absorbance was recorded at a wavelength of 765 nm using a spectrophotometer. The total phenolic contents were expressed as milligrams of gallic acid equivalent per gram of dried extract.

Total flavonoid content assay

Total flavonoid content was determined following the procedure by Dewanto *et al*. [13]. A 1.25 ml of sample (1 mg/ml) was mixed with 75 μl of 5% $NaNO_2$. The mixture was allowed to react for 6 min and then added with 150 μl of 10% $AlCl_3$. After 5 min, 0.5 ml of 1M NaOH was added and the volume was made up to 2.5 ml with distilled water. The absorbance was measured at 510 nm using a spectrophotometer. Total flavonoid amounts were expressed as milligrams of catechin equivalent per gram of dry matter of extract.

DPPH radical scavenging activity

The DPPH radical scavenging activity was evaluated for antioxidant activity of ethanol extract of *Saengshik* ingredients by a modified method of Brand-Williams *et al*. [14]. The ethanol extract (0.1 ml) was added to 3.9 ml of DPPH solution and incubated for 30 min at 25℃ in dark. Absorbance was measured spectrophotometrically at 517 nm. DPPH radical scavenging activity of ethanol extracts was calculated as (1-absorbance of sample/absorbance of control) x 100, and ascorbic acid was used as reference standard.

Statistical analysis

All tests were performed at least in triplicate. Statistically significant differences between groups were calculated by the one-way ANOVA. A value of $P < 0.05$ was considered significant.

RESULTS

Effects of Saengshik extracts on α-amylase inhibition

Total 26 extracts of *Saengshik* ingredients were evaluated for the α-amylase inhibitory activity (Fig. 1). Acarbose was used as a positive control at 0.001 mg/ml. Higher α-amylase inhibitory activity than acarbose was detected in leek (49.8%) at 10 mg/ml, black rice (34.0%) at 10 mg/ml, angelica (30.3%) at 0.1 mg/ml, barley (29.8%) at 10 mg/ml and black sesame (29.0%) at 10 mg/ml. All ethanol extracts showed α-amylase inhibitory activity except for cabbage, and that was dependent of concentration and ingredient.

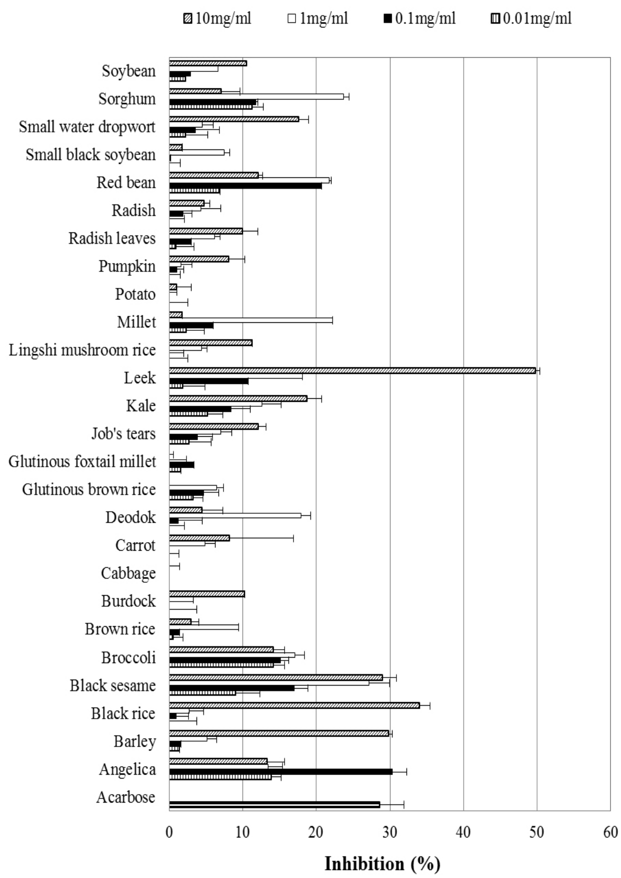

Fig. 1. Inhibition of α-amylase by various *Saengshik* ingredient extracts.

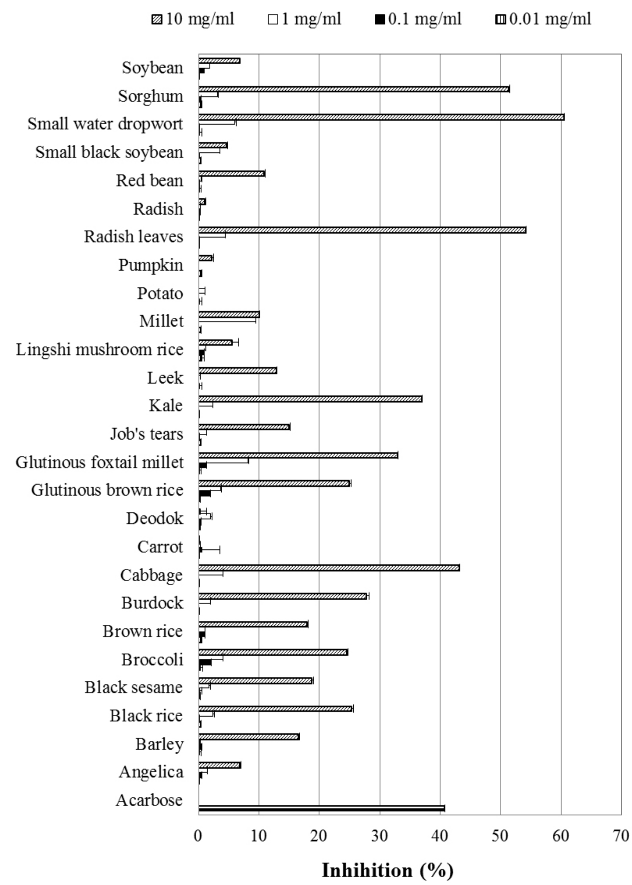

Fig. 2. Inhibition of α-glucosidase by various *Saengshik* ingredient extracts.

Effects of Saengshik extracts on α-glucosidase inhibition

All extracts of *Saengshik* ingredients showed α-glucosidase inhibitory activity (Fig. 2). Increased concentration of each ingredient from 0.001 to 10 mg/ml resulted in stronger α-glucosidase inhibitory activity. At 10 mg/ml, small water dropwort showed the highest inhibition of 60.5%, followed by radish leaves (54.2%), sorghum (51.4%), cabbage (43.2), kale (37%), glutinous foxtail millet (33%), burdock (27.8%), black rice (25.3%), glutinous brown rice (25%), broccoli (24.6%), black sesame (18.8%), barley (16.5%), Job's tears (15.0%), leek (12.9%), red bean (10.9%), millet (10.1%), angelica and soybean (6.9%), lingshi mushroom rice (5.6%), small black soybean (4.6%), pumpkin (2.2%), potato (1.1%), radish root (1.0%), carrot (0.6%), and deodok (0.3%).

DPPH radical scavenging activity

The DPPH radical scavenging activity of ethanol extract is present in Fig. 3. All ethanol extracts of *Saengshik* ingredients exhibited the DPPH radical scavenging activity. The complete scavenging activity was detected in brown rice. Red bean (94.9% scavenging activity) ranked below brown rice, followed by burdock (94.2%), black sesame (85.8%), radish leaves (70.4%), kale (62.7%), millet (58.9%), small black soybean (52.2%) and leek (50.2%), respectively.

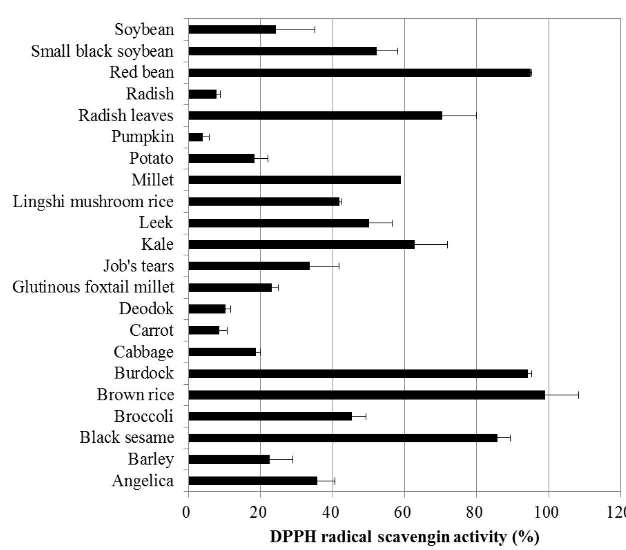

Fig. 3. DPPH radical scavenging activity of various *Saengshik* ingredient extracts.

Total phenolic and flavonoid contents

The total phenolic and flavonoid contents varied largely with the ingredient (Fig. 4). High phenolic contents (> 200 mg gallic acid eq./g dried extract) were detected in small water dropwort, burdock, and black rice. Small water dropwort and black rice

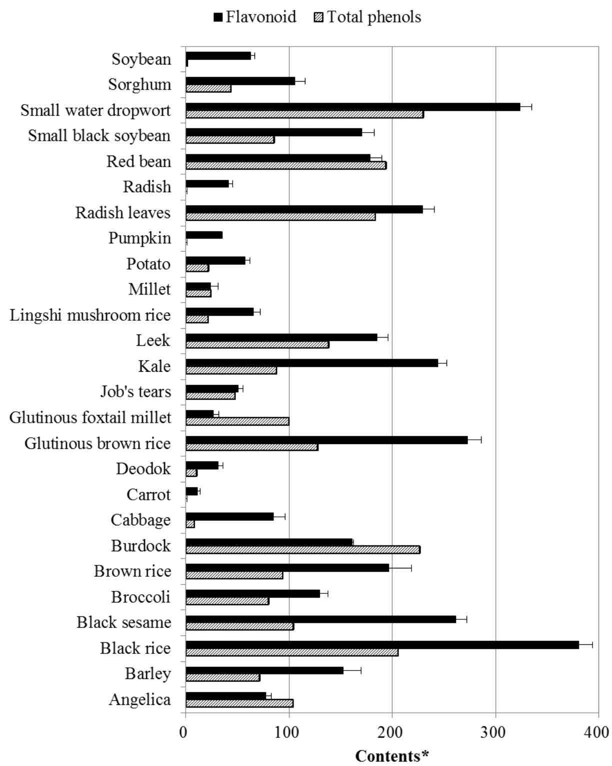

Fig. 4. Total phenolic and flavonoid contents of various *Saengshik* ingredient extracts. *Total phenolic content is milligram gallic acid equivalent, and flavonoid content is milligram catechin equivalent per gram of dried extract.

also ranked in the highest flavonoid contents. All extracts contained flavonoid compounds that were in the range of 11.6-380.7 mg catechin eq./g dried extract. High flavonoid contents (> 200 mg catechin eq./g dried extract) were observed in black rice, small water dropwort, glutinous brown rice, black sesame, kale and radish leaves whereas the lowest content was in carrot.

DISCUSSION

Both α-amylase and α-glucosidase are the essential enzyme catalyzing the degradation of starch to glucose. Thus, its manipulation is considered an effective strategy to maintain glucose homeostasis for diabetes since only glucose among starch-decomposed carbohydrates is readily absorbed by the intestinal lumen [15-17].

All ethanol extracts of *Saengshik* possessed the α-amylase inhibitory activity except for cabbage, although the inhibition degree of α-amylase displayed wide ranges between 1.0% and 49.8%. Higher α-amylase inhibitory activity than acarbose were exhibited in leek, black rice, angelica, barley and black sesame. Among these, barley, black sesame and angelica were reported as a good source of α-amylase inhibitors. According to Alu'datt *et al.* [18], phenolics from prolamin fraction and protein isolate in barley inhibited α-amylase activity up to 57-77%. Methanol extract from black sesame at 0.75 mg/ml showed mild α-amylase inhibition (17%). Enoki *et al.* [19] reported that two major chalcones of 4-hydroxyderricin (4-HD) and xanthoangelol from angelica possessed strong insulin-like activities by activating the peroxisome proliferator-activated receptor-γ ligands. Moreover, the supplementation of 4-HD prevented the progression of diabetes in genetically diabetic mice. According to de Sales *et al.* [20], α-amylase inhibitors naturally isolated from plant and cereals significantly diminished the peak of postprandial glucose. The α-amylase inhibitory activity was caused from a wide range of compounds such as alkaloids, glycosides, galactomannan, peptidoglycans, guanidine, steroids, glycopeptides and terpenoids [21].

All ethanol extracts of *Saengshik* possessed α-glucosidase inhibitory activity, ranged from 0.3% to 60.5%. On the whole, their α-glucosidase inhibitory activities were stronger compared to the α-amylase inhibition. Nine among total 26 ingredients possessed strong α-glucosidase inhibitory activity more than 25% whereas five ingredients revealed α-amylase inhibition above 25%. According to Krentz and Bailey [22], the effective management of diabetes can be achieved by combined mild inhibition of α-amylase and strong inhibition of α-glucosidase. All extracts possessed α-glucosidase inhibitory activity that had the potential to suppress glucose absorption in the intestinal lumen despite low inhibition of carrot and deodok less than 1%. Park *et al.* [15] screened 50 fruit and vegetables commonly consumed in Korea for α-glucosidase inhibitory activity and found only 17 samples possessing inhibitory activity. The α-glucosidase inhibitory activity of potato (73.9%), young radish (26.1%) and water dropwort (23.9%) at 50 mg/ml were somewhat inconsistent with our results. This may have been caused by the varying ethanol extract conditions such as extraction period and temperature, and tested concentrations. Food derived α-glucosidase and α-amylase inhibitors were mostly polyphenols and flavonoids, able to mimic the pyranosyl moiety of the α-glucosidase [20,23-30].

High phenolic contents (> 200 mg gallic acid eq./g dried extract) were detected in small water dropwort, burdock, and black rice. Small water dropwort and black rice also ranked in the highest flavonoid contents. Small water dropwort revealed the highest α-glucosidase inhibitory activity, and black rice showed the second strongest α-amylase inhibitory activity. Total phenolic compounds and flavonoids were also reported with their antioxidant capacities [28,29-31]. Diabetes is closely associated with oxidative stress because hyperglycemia causes oxidative stress and produces free radicals which can induce the diabetic complications such as endothelial dysfunction and atherosclerosis [27]. All *Saengshik* ingredients possessed DPPH radical scavenging effects, meaning antioxidant activities. Most ingredients showed strong scavenging effects compare to α-amylase and α-glucosidase inhibitory activities. Brown rice, burdock, red bean and black sesame showed more than 80% scavenging effects. Lee *et al.* [32] reported that leek supplementation to streptozotocin-induced diabetic rats inhibited lipid and protein oxidation in the skin and suppressed the accumulation of lipofuscin, a major risk factor in a degeneration of the eye. *Sangshik* ingredients containing high phenolic and flavonoid contents were related to the ability inhibiting carbohydrate-degrading enzymes or oxidative activity. No phenolic contents were observed in carrot, pumpkin and radish, which showed low α-amylase (< 8.5%) and α-glucosidase (< 2.5%) inhibitory activities and low antioxidant activity (< 8.6%). However, their strong correlation was not present. Because we used the crude extract from each *Saengshik* ingredient to evaluate α-amylase and α-glucosidase inhibitory activities and antioxidant activity, the synergistic or antagonistic effects among nutrients in the food matrix contributed to the inhibitory activity. It was not contributed by only total phenolic compounds and flavonoids as many studies reported strong correlation between total phenols, flavonoids, α-amylase, and α-glucosidase [15,23-26].

In conclusion, *Saengshik* has been shown to have a positive effect of anti-diabetic properties with phytochemicals. Continuous intake of *Saengshik* may help prevent occurrence of diabetes and its complications and alleviate its symptoms. Further studies are needed to prove anti-diabetic effects of *Saengshik in vivo*, and to conclude the optimized contents of each ingredient in order to meet both the anti-diabetic property and taste.

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