

Suppressive Effect of Polyphenols from the Seed Coat of Scarlet Runner Beans on Blood Glucose Levels

Zhaohong Ci^{1,2}, Chengyu Jiang^{1,2}, Michiyuki Kojima^{2,3,*}

¹Department of Food Production Science, Obihiro University of Agriculture and Veterinary Medicine, 11, Nishi-2-sen, Inada-machi, Obihiro, Hokkaido 080-8555, Japan

²United Graduate School of Agricultural Sciences, Iwate University, 3-18-8, Ueda, Morioka, Iwate 020-8550, Japan

³Department of Human Sciences, Obihiro University of Agriculture and Veterinary Medicine, 11, Nishi-2-sen, Inada-machi, Obihiro, Hokkaido 080-8555, Japan

*Corresponding author: kojima@obihiro.ac.jp

Abstract Scarlet runner beans (SRB) are a valuable source of many nutrients, including proteins, starch, dietary fiber, and oligosaccharides, and are used in various foods in Japan. In this study, we analyzed their polyphenol and procyanidin contents, DPPH radical scavenging activity, and reducing power. SRB (purple) had a higher polyphenol content and DPPH radical scavenging activity than those of SRB (black). The reducing power of SRB (black) (5.1 mg/g) was greater than that of SRB (purple) (4.1 mg/g). Both SRB (purple) and SRB (black) had greater levels of polymeric polyphenols (total Fra.II and Fra.III) than monomeric polyphenols (Fra.I), and inhibited the activity of α -glucosidase in a dose-dependent manner. SRB (black) showed higher α -glucosidase inhibitory activity (IC_{50} , 26.4 μ g/mL) than that of SRB (purple) (IC_{50} , 39.7 μ g/mL), and a mixed pattern of inhibition (non-competitive and uncompetitive). The α -glucosidase inhibitory activity was greater for polyphenols from the seed coat (>91%) than from the cotyledon (<0.1%) for SRB (purple) and SRB (black). Both 250 mg/kg and 750 mg/kg polyphenols from the coat of SRB (purple) effectively suppressed the elevation of blood glucose levels after the oral administration of starch in mice. These results suggest that the seed coat of SRB has useful properties and, in particular, has potential applications for the treatment of diabetes.

Keywords: scarlet runner bean, seed coat, polyphenol, α -glucosidase, blood glucose

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1. Introduction

Diabetes has received considerable attention owing to the increasing number of people suffering from this chronic disease [1]. Postprandial hyperglycemia is an important risk factor for the development of Type II diabetes [2]. Controlling the blood glucose level is the most effective method for preventing diabetes deterioration and hyperglycemia [3]. All dietary carbohydrates are hydrolyzed by enzymes to yield simple sugars, which can improve blood glucose levels [4]. α -Glucosidase is an intestinal cell membrane enzyme that can hydrolyze polysaccharides; hence, inhibiting the activity of α -glucosidase may be an effective way to treat pre-diabetes and slow the progression of diabetes [5].

Several synthetic α -glucosidase inhibitors, such as acarbose and voglibose, have been developed, but these have various side effects, such as digestion disorders, flatulence, and liver function disorders [6]. Recently, many studies have focused on natural α -glucosidase inhibitors from plants. Although it has been reported that polyphenolic compounds from legumes [7,8] could inhibit α -glucosidase activity, few studies have examined the

inhibitory activity of these polyphenolic compounds on both α -glucosidase and blood glucose levels.

In the present study, we examined the inhibitory effects of polyphenols from scarlet runner beans (SRB) (*Phaseolus coccineus* L.) on α -glucosidase in vitro. Moreover, we evaluated the effect of polyphenols from the coat of SRB (purple) on blood glucose levels after the oral administration of starch in mice in vivo.

2. Materials and Methods

2.1. Materials

SRB (purple) have black spots on a purple surface, and SRB (black) are purely black. Samples of SRB (purple) and SRB (black) were purchased from the Kawanishi Agricultural Cooperative Association (Obihiro, Japan). Diaion HP-20 columns and Sephadex LH-20 columns for chromatography were obtained from the Mitsubishi Chemical Corporation (Tokyo, Japan) and GE Healthcare Bio-Sciences AB (Uppsala, Sweden), respectively. All other reagents and chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), unless stated otherwise.

2.2. Extract Preparation and Fractionation

SRBs were ground into a powder, followed by extraction with 20 mL of 80% ethanol. After ultrasound treatment for 30 min, the mixture was centrifuged at $1,006 \times g$ for 10 min to obtain a supernatant. The same extraction process was repeated two more times. The residues were subjected to another three rounds of extraction with 70% acetone and the supernatant. Then, the supernatant was mixed, concentrated by rotary evaporation in a vacuum, and purified by chromatography through Diaion HP-20 columns. The columns were washed with distilled water and then eluted with methanol. The methanol solution was concentrated by rotary evaporation in a vacuum, and dissolved in 2 mL of methanol for the experiment. Part of the concentrate was dissolved in ethanol and fractionated by Sephadex LH-20 column chromatography. The column was successively eluted with ethanol, methanol, and 60% acetone to collect fraction I (Fra.I), fraction II (Fra.II), and fraction III (Fra.III), respectively.

2.3. Quantification of Polyphenols

Polyphenols were quantified using the Folin–Ciocalteu method [9]. The methanol fraction (HP-20 column) (100 μL) was treated with 300 μL of distilled water, 400 μL of Folin–Ciocalteu reagent, and 400 μL of a 10% Na_2CO_3 solution. The mixture was prepared in triplicate, incubated at 30 °C for 30 min, and centrifuged at $1,006 \times g$ for 10 min. The absorbance of the mixed supernatant was measured at 760 nm. The polyphenol content is expressed in mg of catechin equivalents per gram of beans (mg/g).

2.4. Quantification of Procyanidins

The procyanidin content of the methanol fraction (HP-20 column) was determined by the HCl-butanol method [10], using cyanidin as the standard equivalent.

2.5. Estimation of DPPH Radical Scavenging Activity

DPPH radical scavenging activity was evaluated by the method described by Brand-Williams et al. [11], with some modifications. A 50- μL aliquot of the sample was mixed with 100 μL of ethanol, and the mixture was supplemented with 150 μL of 0.5 mM DPPH in ethanol. The absorbance of the mixture was measured using a microplate reader at 517 nm. The DPPH radical scavenging activity is expressed in μmol trolox equivalents per gram of beans ($\mu\text{mol/g}$).

2.6. Estimation of Reducing Power

Reducing power was evaluated according to a previously reported method, with minor modifications [12]. Briefly, 250 μL of the methanol fraction (HP-20 column) was mixed with 250 μL of sodium phosphate buffer (pH 7.5) in a test tube, and 250 μL of 1% (w/v) potassium ferricyanide was added. The mixture was incubated at 50°C for 20 min. After the incubation period, 250 μL of 10% trichloroacetic acid was added and centrifuged at

$1,006 \times g$ for 10 min. The upper layer of the supernatant (500 μL) was mixed with 500 μL of distilled water and 100 μL of 0.1% (w/v) ferric chloride, and reacted under shading for 15 min. The absorbance of the reaction mixture was measured at 700 nm. Reducing power activity is expressed in mg of vitamin C equivalent per gram of beans (mg/g).

2.7. α -Glucosidase Inhibitory Activity

α -Glucosidase inhibition was analyzed following the methods of Matsumoto et al. [13], with modifications. Sucrose was broken down by α -glucosidase, and the amount of reducing sugar was calculated based on the α -glucose content. In total, 0.8 mL of the enzyme reaction solution (50 μL of 0.4% sucrose, 625 μL of 0.1 mol/L sodium phosphate buffer (pH 6.8), and 125 μL of 1% NaCl) was pre-incubated at 37 °C for 30 min. The methanol fraction (HP-20 column) (polyphenol concentration, 0–100 $\mu\text{g/mL}$) was added to 0.1 U/mL α -glucosidase solution (EC3.2.1.20; Oriental Yeast Co., Ltd., Tokyo, Japan) at 37 °C for 10 min. After pre-incubation, 200 μL of the mixture (polyphenol extract and α -glucosidase) was added to the enzyme reaction solution and incubated at 37 °C for 30 min. The reaction was terminated by adding 125 mL of 2 M NaOH, and 1% dinitrosalicylic acid was added in boiling water for 10 min. After incubation, the mixture was analyzed at 540 nm at room temperature. Enzyme inhibitory reactions, for all polyphenol extract concentrations, were replicated three times. The α -glucosidase inhibitory activity is expressed as the percent inhibition. The concentration of inhibitors required for the inhibition of 50% of the enzyme activity under the assay conditions was defined as the IC_{50} value.

The inhibitory kinetics of α -glucosidase by the polyphenol extract was determined by the Lineweaver-Burk equation. Sucrose was used as the substrate in the concentration range of 3.3–20.0 mM. The enzyme activity was measured at 0 and 10 $\mu\text{g/mL}$ polyphenols.

2.8. Oral Starch Tolerance Test in Mice

Male DDY mice (Japan SLC, Inc., Shizuoka, Japan) were housed in plastic cages at 23°C, a 12 h/12 h light/dark cycle, and a relative humidity of 60%. Mice had free access to feed (CE-2; CLEA Japan, Inc., Tokyo, Japan) and water for 1 week before the experiment. The feed had 344.9 kcal/100 g and contained (w/w %) 24.9% crude protein, 4.6% crude fat, 4.1% crude fiber, 6.6% crude ash, 51.0% NFE (nitrogen-free extract), and 8.9% moisture. After 1 week, the animals were randomly divided into experimental groups (8 mice per group). The mice were fasted for 24 h and blood was withdrawn from the tail vein and subjected to assays of blood glucose levels. The polyphenols from SRB (purple) were suspended in physiological solution and used doses of 250 and 750 mg/kg. After the oral administration of the suspension of polyphenolic compounds for 30 min, a single oral injection of 2 g/kg starch in physiological solution was administered. Blood was withdrawn from the tail vein at 0.5, 1, and 2 h, and blood glucose levels were analyzed using the OMRON Precision Exceed HEA-216 (Omron Healthcare Co., Ltd., Kyoto, Japan) according to the

manufacturer's instructions. The study was approved by the regulatory authority of the National University Corporation Obihiro University of Agriculture and Veterinary Medicine and it adhered to the standard principles described in the Guide for the Care and Use of Laboratory Animals.

2.9. Statistical Analysis

Values are presented as the means \pm standard error of 5 out of 8 mice in each group. 5 mice in each group. Statistical significance was evaluated by ANOVA and least significant difference (LSD) tests (SAS Enterprise Guide 5.1 system). Differences were considered significant when $p < 0.05$.

3. Results and Discussion

3.1. Polyphenol and Procyanidin Content, DPPH Radical Scavenging Activity, and Reducing Power

Table 1 summarizes the polyphenol and procyanidin contents, DPPH radical scavenging activity, and reducing power for SRB (purple) and SRB (black). The polyphenol contents were 9.9 and 7.6 mg/g and the DPPH radical scavenging activities were 41.7 and 39.3 $\mu\text{mol/g}$ for SRB (purple) and SRB (black), respectively. SRB (purple) showed a higher polyphenol content and DPPH radical scavenging activity than those of SRB (black) ($p < 0.05$). A positive correlation between the polyphenol content and DPPH radical scavenging activity for common beans has been reported by Ci et al. [14]. Polyphenols from the coat and cotyledon of SRB (purple) and SRB (black) were analyzed. Polyphenol ratios were higher for the coat (>96%) than for the cotyledon (<4%). The cotyledon contains the main reserve of substances, particularly proteins and carbohydrates. The seed coat, which acts as a protective barrier for the cotyledon, has a high concentration of

phenolic compounds [15,16]. The procyanidin contents were 3.9 and 3.8 mg/g for SRB (purple) and SRB (black). The reducing power was greater for SRB (black) (5.1 mg/g) than for SRB (purple) (4.1 mg/g).

3.2. Polyphenol Fractions

We performed Sephadex LH-20 column chromatography obtain three polyphenol fractions, i.e., Fra.I, Fra.II, and Fra.III, for each SRB (Figure 1). Fra.I represented 24% and 13%, and the total values of Fra.II and Fra.III were 76% and 87% for SRB (purple) and SRB (black), respectively.

According to Saito et al. [7], Fra.I contains monomeric polyphenols, and Fra.II and Fra.III contain polymeric polyphenols. Both SRB (purple) and SRB (black) had higher ratios of polymeric polyphenols than monomeric polyphenols.

3.3. α -Glucosidase Inhibitory Activity

We analyzed the inhibitory effects of polyphenols on α -glucosidase. Polyphenols from both SRB (purple) and SRB (black) inhibited the activity of α -glucosidase in a dose-dependent manner. Phenolic compounds from seven kinds of legume [7], soybeans [8], and the millet seed coat [17] inhibit α -glucosidase activity. SRB (black) showed greater α -glucosidase inhibitory activity (IC_{50} , 26.4 $\mu\text{g/mL}$) than that of SRB (purple) (IC_{50} , 39.7 $\mu\text{g/mL}$) (Table 1). SRB (black) showed greater percentages of Fra.II and Fra.III (Figure 1), and a high degree of polymeric polyphenols exhibits stronger α -glucosidase inhibitory activity than that of monomeric phenolic compounds. We also analyzed the inhibitory effects of polyphenols (20 $\mu\text{g/mL}$) on α -glucosidase for the seed coat and cotyledon of SRB (purple) and SRB (black). α -Glucosidase inhibitory activity levels of 91% and 94% were observed for the seed coat of SRB (purple) and SRB (black), and both were less than 0.1% for the cotyledon. We speculate a much higher degree of polymeric polyphenols in the seed coat than the cotyledon results in stronger α -glucosidase inhibitory activity.

Table 1. Summary of polyphenols, procyanidins, DPPH radical scavenging activity, reducing power, and inhibition of α -glucosidase for scarlet runner beans

	Polyphenols (mg/g)	DPPH radical scavenging activity ($\mu\text{mol/g}$)	Reducing power (mg/g)	α -Glucosidase inhibitory activity IC_{50} ($\mu\text{g/mL}$)
SRB (purple)	9.9 ^a \pm 0.1	3.9 ^a \pm 0.1	41.7 ^a \pm 0.7	39.7
SRB (black)	7.6 ^b \pm 0.2	3.8 ^a \pm 0.0	39.3 ^b \pm 0.3	26.4

Values followed by different letters in a column are significantly different ($p < 0.05$).

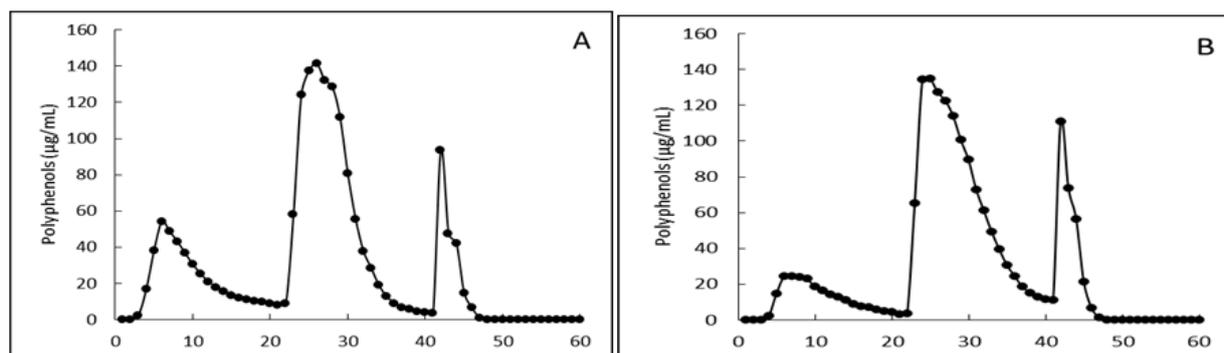


Figure 1. Scarlet runner bean polyphenols were applied to the LH-20 column for the successive elution of Fra.I (ethanol fraction, 1–20), Fra.II (methanol fraction, 21–40), and Fra.III (60% Acetone fraction, 41–60). A. SRB (purple), B. SRB (black)

We also performed an enzyme kinetic study of α -glucosidase inhibition. Figure 2 shows a Lineweaver–Burk plot of the α -glucosidase inhibitory activity of polyphenols from SRB (black) at 0 and 10 $\mu\text{g/mL}$, with different concentrations of sucrose (3.3–20.0 mM). The maximum velocity (V_{max}) was 0.59 mmol/min and the Michaelis–Menten constant (K_m) was 20.6 mM for sucrose. When the concentration of polyphenols was 10 $\mu\text{g/mL}$, V_{max} increased to 0.7 mmol/min, and K_m increased to 31.8 mM. In the presence of polyphenols from SRB (black), both V_{max} and K_m increased, implying that polyphenols exhibited a mixed type of inhibition towards α -glucosidase, i.e., non-competitive and uncompetitive inhibition. It has been reported that the structural factors of phenolic groups are crucial determinants of the inhibitory pattern of polyphenols on α -glucosidase [4]. In our study, both SRB (purple) and SRB (black) had higher levels of polymeric polyphenols than monomeric polyphenols. We speculate that polyphenols from SRB (purple) also have a mixed pattern of α -glucosidase inhibition. Different natural compounds have different inhibition patterns against α -glucosidase; for example, finger millet (*Eleusine coracana L.*) seed coat phenolics exhibit noncompetitive inhibition [17], and three flavonoids (quercetin, isoquercetin, and rutin) exhibit mixed noncompetitive and anticompetitive inhibition [18].

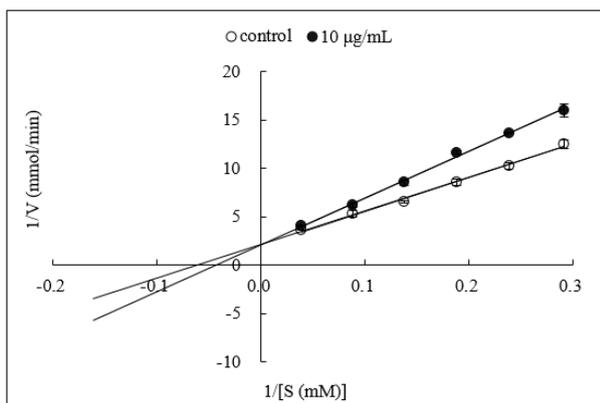


Figure 2. Lineweaver–Burk plots of polyphenols against α -glucosidase for SRB (black)

3.4. Inhibitory Effects of Polyphenols on Blood Glucose Levels after Oral Administration of Starch

Compared with those in the cotyledon, polyphenols of the seed coat of SRB (purple) showed more effective inhibition against α -glucosidase in vitro. The polyphenols from the seed coat of SRB (purple) were tested for their inhibitory effects on the elevation of blood glucose levels by the oral starch tolerance test in mice. After the administration of starch, the maximum increase in the blood glucose level was observed at 30 min in all mice, but mice treated with 250 mg/kg and 750 mg/kg polyphenols exhibited significantly lower blood glucose concentrations than those of the control group ($p < 0.01$) (Figure 3 A). At 60 min, the 250 mg/kg and 750 mg/kg polyphenol groups still showed significantly lower glucose levels than those of the control group ($p < 0.05$). At 120 min, the blood glucose concentrations recovered to

the levels observed at 0 min. SRB (black) was similar to SRB (purple) with respect to the polyphenol distribution and α -glucosidase inhibitory activity; accordingly, we speculated that SRB (black) also can effectively reduce blood glucose in vivo. Polyphenols from kidney beans [19] and black beans [20] also reduce blood glucose in rats.

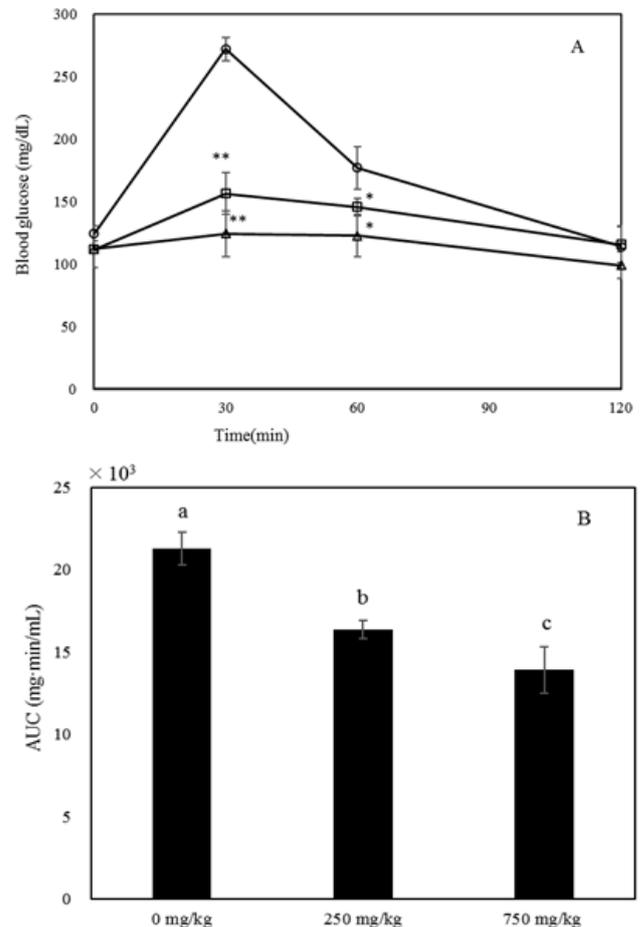


Figure 3. Oral starch tolerance test to monitor the inhibitory effect of polyphenols from SRB (purple) on blood glucose levels in mice (A) and AUC values (B). Values are presented as the means \pm standard error of 5 mice per group. * indicates a significant difference with respect to the control (** $p < 0.01$, * $p < 0.05$). Symbols: \circ , 0 mg/kg; \square , 250 mg/kg; \triangle , 750 mg/kg mouse. Values followed by different letters in a column are significantly different ($p < 0.05$)

The area under the curve (AUC) of the blood glucose level over a 120-min period is shown in Figure 3 B. The AUC value was significantly lower for the 750 mg/kg polyphenol group than for the other groups, and was significantly lower for the 250 mg/kg group than for the control group.

4. Conclusions

SRB (purple) had a higher polyphenol content and DPPH radical scavenging activity than those of SRB (black). Both SRB (purple) and SRB (black) showed high ratios of polymeric polyphenols and inhibited the activity of α -glucosidase in a dose-dependent manner. Moreover, polyphenols from the seed coat of SRB (purple) effectively suppressed the elevation of blood glucose levels in vivo, as shown by experiments of the oral

administration of starch in mice. These observations indicate that the seed coat of SRB may serve as a source for the development of nutraceuticals with anti-diabetic activity.

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Statement of Competing Interests

The authors have no competing interests.

List of Abbreviations

AUC: area under the curve
 DPPH: 2,2-diphenyl-1-picrylhydrazyl
 LSD: least significant difference
 SRB: scarlet runner beans

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