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Antioxidant and Antidiabetic Activities of Black Mung Bean (*Vigna radiata* L.)

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Supporting Information

ABSTRACT: Interest in mung bean as a functional food is growing. The objective of this study was to characterize the phenolic compounds, antioxidant activities, and antidiabetic activities of black mung beans. Five black mung beans were selected, and one green mung bean was included for comparison. The free phenolic acid and bound phenolic acid contents ranged from 16.68 to 255.51 μg/g and from 2284.53 to 5363.75 μg/g, respectively, whereas the total anthocyanin contents ranged from 0 to 810.55 μg/g with cyanidin-3-glucoside as the most dominant form, respectively. Among the mung beans tested, black mug bean Xiaoqu 7110 had the highest content of bound phenolic acids and exhibited the strongest antioxidant capacities (1,1-diphenyl-2-picrylhydrazyl, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt and oxygen radical absorbance capacity) as compared to the other tested mung beans. Jiheilv 27-3 exhibited higher antidiabetic activities (inhibition of α-glucosidase and advanced glycation end products).

KEYWORDS: *black mung bean, antioxidant, antidiabeitc, phenolic acid*

INTRODUCTION

Mung bean (*Vigna radiata* L.) is native to the northeastern India−Burma (Myanmar) region of Asia. There is now much interested in it for its physiological functionalities, such as anti-angiotensin I-converting enzyme (ACE),[1] antitumor,[2] antioxidant,[3] antidiabetic,[4] and antimelanocytes.[5]

Pigmented grain contains many secondary metabolites, such as phenolic acids and flavonoids. Phenolic acids represent the most common form of phenolic compounds and make up one of the major and most complex groups of phytochemicals in the grain.[6] These acids are present mainly in bound forms, linked to cell wall structural components such as cellulose, lignin, and proteins through ester bonds.[7] The bound phenolics are considered to have greater antioxidant capacity, because they can escape from upper gastrointestinal digestion along with cell wall materials and are absorbed into blood plasma during digestion by intestinal microflora.[8] Anthocyanins as a class of flavonoids are water-soluble glycosides of polyhydroxy and polymethoxy derivates of 2-phenylbenzopyrylium or flavylium salts and are responsible for the red, purple, and blue colors of many fruits, vegetables, and grain kernels.[9]

Antioxidants refer to compounds possessing free radical scavenging activity, transition metal-chelating activity, and/or singlet oxygen-quenching capacity.[10,11] Accumulated evidence has suggested that diabetic patients are under oxidative stress with an imbalance between the free radical-generating and radical-scavenging capacities. The increased free radical production and reduced antioxidant defense may partially mediate the initiation and progression of diabetes-associated complications.[12] The antioxidant activities and phenolic compounds of mung beans have been extensively reported in numerous studies,[13,14] as well as our earlier papers.[4,15] However, there are various black mung beans available in the market for human consumption. The diversity of phenotypes of food legumes indicates that complex phytochemical profiles and health benefits may differ in various legume varieties. It is important to quantify and compare functional components and investigate health benefits of black mung beans; therefore, the present study was conducted to (i) compare phenolic acids, anthocyanins, and total phenolics contents in black mung beans and green mung bean and (ii) systemically evaluate antioxidant and antidiabetic activities using a combination of several tests.

MATERIALS AND METHODS

Materials. Standards of Trolox, fluorescein sodium salt, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 2,2-azobis(2-amidinopropane) dihydrochloride (AAPH), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), rat intestinal acetone powder, bovine serum albumin (BSA), D-glucose, methylglyoxal (MGO), and ferulic, syringic, chlorogenic, caffeic, *p*-coumaric, and gentisic acids were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cyanidin-3-glucoside was obtained from Polyphenols (Sandnes, Norway). All of the chemicals were of analytical grade and were obtained from Beijing Chemical Reagent (Beijing, China). All of the analytical grade solvents for high-performance liquid chromatography (HPLC) were purchased from Fisher Chemicals (Shanghai, China).

Mung Bean Samples and Sample Preparation. Five black mung bean varieties (Jiheilv 27-3, Hei 15-2, Hun1029, Xiaoqu 7110, and Hei 45-1) were provided by Professor Jing Tian from Hebei Academy of Agriculture and Forestry Sciences (Hebei, China), and

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one green mung bean (Zhonglv 5) was provided by Professor Cheng from the Chinese National Genebank (Beijing, China) as a referemce sample. Each sample was milled into fine powder with a 60 mesh screen, thoroughly mixed, cooled immediately, and stored at −20 °C until analyzed.

Extraction of Free Phenolic Acids. Free phenolic acids in mung bean flour were extracted according to a previously reported method.[16] A 1.0 g sample of bean flour was extracted with 20 mL of 80% chilled ethanol. Tubes containing samples were shaken on a shaker at room temperature for 10 min. After centrifugation at 2500*g* for 10 min, the supernatant was transferred into a new tube, and extraction was repeated once more for the residue. Supernatants were pooled together, evaporated at 45 °C to <5 mL, and reconstituted into 10 mL with distilled water. The extracts were stored at −20 °C.

Extraction of Bound Phenolic Acids. Bound phenolic acids were extracted according to the method of Zhang et al.[17] Fifteen milliliters of distilled water and 5 mL of 6 M NaOH were added to test tubes with the residue after the extraction of free phenolic compounds and stirred for about 16 h at room temperature using a magnetic stirrer. The solution was then adjusted to pH 2, and liberated phenolic acids were extracted three times with 15 mL of a mixture of cold diethyl ether (DE) and ethyl acetate (EA, 1:1 v/v). DE/EA layers were combined and evaporated to dryness, and the residue was dissolved in 1.5 mL of methanol. Acid hydrolysis was then performed by adding 2.5 mL of concentrated 12 M HCl into the test tube and incubating in a water bath at 85 °C for 30 min after completion of the above alkaline hydrolysis. The sample was then cooled and adjusted to pH 2, with DE/EA extraction performed in the same manner as for alkaline hydrolysis.

Determination of Individual Phenolic Acids. Individual phenolic acids in the bean extracts were analyzed by a Shimadzu LC-20A high-performance liquid chromatograph equipped with a UV detector and Alltima C18 column (4.6 × 250 mm, Metachem Technologies, Inc., Torrance, CA, USA). The mobile phase of water with 0.05% trifluoroacetic acid (solvent A) and 30% acetonitrile, 10% methanol, 59.95% water, and 0.05% trifluoroacetic acid (solvent B) was used at a flow rate of 1 mL/min. Total run time was 50 min, and the gradient program was as follows: 10−12% B for 16 min, 12−38% B for 9 min, 38−70% B for 7 min, 70−85% B for 8 min, and 85−10% B for 10 min. The time of postrun for reconditioning was 5 min. The injection volume was 1 μL. Detection was done at 280 nm via the UV detector. Identification and quantification of phenolic acids in samples were performed by comparison with chromatographic retention times and areas of external standards. The stock solutions were stored in darkness at −18 °C. All determinations were performed in triplicates.

LC-MS Analysis of Individual Anthocyanidins. The LC-MS analysis of anthocyanins was performed by an Agilent 1200 series liquid chromatograph containing an autosampler coupled with a 6300 series ion-trap mass spectrometer (Agilent, Santa Clara, CA, USA). An aliquot of 3 μL of the sample was injected onto an analytical scale Alltima C18 column (4.6 × 250 mm, Metachem Technologies, Inc. maintained at 25 °C. The mobile phase was a mixture of solvent A (HPLC water containing 0.1% formic acid) and solvent B (acetonitrile containing 0.1% formic acid). The elution started with 5% B with a linear gradient to 25% B in 38 min and then to 90% B from 38 to 55 min. The flow rate was set at 0.3 mL/min, and the injection volume was 3 μL. Diode array detector (DAD) detection with 280 and 520 nm as detection wavelengths was performed. Anthocyanins and phenolic acids were detected using an ion trap in the positive ion mode. Used MS parameters were as follows: nebulizing pressure, 30 psi; source temperature, 110 °C; desolvation temperature, 350 °C; desolvation gas flow, 11 L/min nitrogen; scan ranger, m/z 100−1500; smart parameter setting; compound stability, 20%; trap drive level, 100%. The concentrations of individual anthocyanins were evaluated using the calibration curve of cyanidin-3-glucoside.

Evaluation of Antioxidant Activity Using the DPPH Method. The DPPH radical-scavenging activity was determined using the method reported by Yao and Ren.[18] DPPH (100 μM) was dissolved in 96% ethanol. The DPPH solution (1 mL) was mixed with 1 mL of the bean extracts. The mixture was shaken and allowed to stand at room temperature in the dark for 10 min. The decrease in absorbance of the resulting solution was monitored at 517 nm after 10 min. The results were expressed in micromolar Trolox equivalents (TE) per gram. All determinations were performed in triplicates.

Evaluation of Antioxidant Activity Using the ABTS$^{\bullet+}$ Method. The determination of ABTS$^{\bullet+}$ free radical-scavenging activity was determined as described.[19] Briefly, ABTS was dissolved in redistilled water at a concentration of 7 μM/L. ABTS$^{\bullet+}$ radical cation was produced by reacting ABTS stock solution with 2.45 mM/L potassium persulfate and kept at room temperature in the dark for 16 h. For the study of infusion, the resulting solution containing the ABTS$^{\bullet+}$ solution was diluted with redistilled water to an absorbance of 0.70 (±0.02) at 734 nm and equilibrated at 30 °C. Then a reagent blank reading was taken. After the addition of 3.0 mL of diluted ABTS$^{\bullet+}$ solution ($A_{734\ nm}$ = 0.70 ± 0.02) to 30 μL of the extracts or Trolox (prepared in DMSO for use as standard), the absorbance was taken exactly 6 min after initial mixing. The results were expressed in micromolar TE per gram. All determinations were performed in triplicate.

Evaluation of Antioxidant Activity Using the Oxygen Radical Absorbance Capacity (ORAC) Method. ORAC activity was determined as described previously with some slight modification.[20] Fifty microliters of the bean extract was mixed with 50 μL of fluorescein solution in a 96-well microplate, and then 150 μL of AAPH was added to each well rapidly. To build the blank decay curve and Trolox standard decay curve, 50 μL of blank (methanol) or Trolox standard solution was added instead of the sample solution. The microplate was immediately placed into the Synergy microplate fluorescence reader (Bio-Tek Instruments, Inc., Winooski, VT, USA) and recorded every minute for 80 min. The fluorescence filters of the plate reader were set at 485 nm with a tolerance of ±20 nm for the excitation wavelength and at 530 nm with a tolerance of ±20 nm for the emission wavelength. The temperature of the plate reader was set at 37 °C. Each ORAC value of the samples was calculated by using a regression equation between the Trolox concentration and the net area under the fluorescence decay curve (AUC). The net AUC corresponding to a sample was calculated by subtracting the AUC corresponding to the blank. ORAC values were expressed as micromolar TE per gram. All determinations were performed in triplicates.

Measurement of α-Glucosidase Inhibition Activity. The α-glucosidase inhibition activity was determined as previously described.[21] The α-glucosidase solution was prepared by mixing 0.1 g of rat intestinal acetone powder with 3 mL of 0.1 M phosphate buffer (pH 7.0) in a plastic test tube extracted with ultrasound at 4 °C for 0.5 min, repeated 12 times, and then centrifuged at 3500*g* for 5 min. The supernatant was the α-glucosidase solution. The reaction mix contained 50 μL of total phenolic compounds extracts and 100 μL of 0.1 M phosphate buffer (pH 7.0) containing α-glucosidase solution and was incubated in 96-well plates at 37 °C for 10 min. After preincubation, 50 μL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside in a 0.1 M phosphate buffer (pH 7.0) was added to each well. The reaction mixtures were incubated at 37 °C for 5 min. The absorbance readings were recorded at 490 nm on a microplate reader before and after incubation (Bio-Rad, IMAX, Hercules, CA, USA). The results were expressed as a percent of α-glucosidase inhibition, and the inhibition activity was calculated according to the following equation: $(A_{control} - A_{sample})/A_{control} \times 100\%$.

Evaluation of Glycation End Product (AGE) Inhibition Activity. BSA−glucose and BSA−MGO models were used for the evaluation of the inhibition effect of the extracts on the formation of AGE. The BSA−glucose assay was carried out according the method reported by Peng et al.[14] Briefly, 5 g of BSA and 14.4 g of D-glucose were dissolved in 1.5 M phosphate buffer (pH 7.4) to obtain the control solution with 50 mg/mL BSA and 0.8 M D-glucose. Two milliliters of the control solution was incubated at 37 °C for 7 days in the presence or absence of 1 mL of bean extracts in a 1.5 M phosphate buffer (pH 7.4). After 7 days of incubation, fluorescent intensity (excitation at 330 nm and emission at 410 nm) was measured. Percent inhibition of AGE formation by each extract was calculated using the

Table 1. Phenolic Acid Profile (μg/g) of Mung Bean Varieties[a]

		variety					
		Jiheilv 27-3	Hei 15-2	Hun1029	Xiaoqu 7110	Hei 45-1	Zhonglv 5
free	caffeic	109.11 ± 8.46 a	nd	54.98 ± 3.27 c	16.68 ± 2.12 d	85.71 ± 4.52 b	11.23 ± 0.93 d
	syringic	88.43 ± 3.07 c	78.43 ± 4.13 c	nd	nd	169.86 ± 10.58 b	220.29 ± 19.41 a
	subtotal	197.54 ± 11.58 c	78.43 ± 4.16 d	54.98 ± 3.29 e	16.68 ± 2.13 f	255.57 ± 15.21 a	231.52 ± 20.37 b
bound	ferulic	nd	5.86 ± 0.42 b	0.24 ± 0.07 c	17.70 ± 0.83 a	nd	nd
	caffeic	21.36 ± 1.50 d	20.28 ± 1.63 d	nd	394.44 ± 17.20 a	201.56 ± 10.45 b	72.44 ± 2.97 c
	chlorogenic	23.24 ± 0.55 c	26.29 ± 1.02 c	39.99 ± 2.11 b	111.25 ± 12.34 a	16.41 ± 2.01 c	nd
	syringic	3572.71 ± 325.40 a	1504.36 ± 100.71 d	1890.16 ± 59.46 c	3027.34 ± 217.66 b	1748.73 ± 113.25 d	1915.45 ± 89.41 c
	p-coumaric	1601.88 ± 20.87 b	1488.71 ± 13.48 c	1538.07 ± 19.46 c	1686.78 ± 23.47 a	1271.93 ± 77.54 d	158.19 ± 8.92 e
	gentisic	25.39 ± 2.33 d	34.74 ± 1.79 cd	108.22 ± 8.54 b	126.24 ± 9.15 a	48.88 ± 5.07 c	138.45 ± 14.20 a
	subtotal	5244.58 ± 350.65 a	3080.24 ± 119.05 c	3576.68 ± 89.64 b	5363.75 ± 280.65 a	3287.51 ± 208.34 c	2284.53 ± 115.50 d

[a]Data are expressed as mean ± standard deviation of triplicate samples. Values in the same row sharing different letters are significantly different ($p < 0.05$). nd, not detected.

Table 2. Anthocyanin Profiles (μg/g) of Mung Bean Varieties[a]

variety	cyanidin-3-glucoside	unknown peak	peonidin-3-glucoside	pelargonidin-3,6-malonylglucoside	pelargonidin-3-glucoside	total anthocyanins
Jiheilv 27-3	476.53 ± 3.40 a	18.82 ± 0.11 a	8.42 ± 0.16 a	12.49 ± 0.39 a	294.29 ± 3.51 b	810.55 ± 7.52 a
Hei 15-2	303.32 ± 1.64 b	12.89 ± 1.30 c	7.62 ± 0.09 c	12.35 ± 0.50 a	350.71 ± 2.51 a	686.89 ± 6.10 a
Hun1029	362.94 ± 4.13 b	12.40 ± 1.64 c	5.43 ± 0.04 d	9.17 ± 0.14 c	147.62 ± 2.67 c	537.56 ± 8.64 b
Xiaoqu 7110	256.32 ± 2.03 c	14.98 ± 0.26 b	7.57 ± 0.27 c	8.53 ± 0.01 d	150.25 ± 2.01 c	437.65 ± 4.59 c
Hei 45-1	381.19 ± 4.13 b	20.38 ± 0.26 a	8.04 ± 0.02 b	11.60 ± 0.06 b	129.16 ± 1.39 c	550.37 ± 5.47 b
Zhonglv 5	nd	nd	nd	nd	nd	nd

[a]Data are expressed as mean ± standard deviation of triplicate samples. Values in the same column sharing different letters are expressed as significantly different ($p < 0.05$). nd, not detected.

following equation: (1 − (fluorescence of the solution with inhibitors/fluorescence of the solution without inhibitors)) × 100%.

The BSA−MGO assay was carried out according to the method reported by Yao et al.[15] Briefly, 40 mg of BSA was mixed with 31 μL of MGO in a 0.1 M phosphate buffer (pH 7.4) to obtain the control solution with 1 mg/mL BSA and 5 mM MGO. Two milliliters of the control solution was incubated at 37 °C for 6 days with or without 1 mL of the bean extracts in phosphate buffer. The percent inhibition was calculated on the basis of the equation applied in the BSA−glucose assay as described above (excitation at 340 nm and emission at 420 nm).

Statistical Analysis. All values were expressed as mean ± SD. Statistical analysis was performed using SPSS (version 16.0), and Dunnett's multiple-range tests were used to determine the significant differences between group means at $p < 0.05$. A Pearson correlation test was conducted to determine the correlation between variables.

RESULTS AND DISCUSSION

Phenolic Acids. Many studies have shown that dietary phenolics have high antioxidant activities. In our present work the average concentration of free phenolic acids was 120.64 μg/g, making up only 2.86% of the phenolic acids determined in black mung bean samples (Table 1). Syringic accounted for 55.82% of the free phenolic acids, whereas caffeic accounted for 44.18% of the total of this class. There were no detectable amounts of the free phenolic acid components ferulic, chlorogenic, coumaric, and gentisic. Lopez et al. found ferulic acid (46.14 μg/g) was the most abundant phenolic acid, followed by sinapic (17.34 μg/g) and *p*-coumaric acids (6.01 μg/g) in common beans.[16]

The average concentration of bound phenolic acids in black mung bean samples was 4110.55 μg/g, making up 97.14% of the phenolic acids determined, among which syringic accounted for 57.21% of it, whereas ferulic, caffeic chlorogenic, *p*-coumaric, and gentisic accounted for 0.11, 3.10, 1.06, 36.92, and 1.67% of it, respectively. Bound phenolics are more likely to survive upper gastrointestinal digestion and can be released from the colon through microflora digestion activity. Thus, the mung bean phenolics are more likely to exert health benefits in the colon where they are released.

Hei 45-1 had the highest free phenolic acid concentration (255.57 μg/g), followed by Zhonglv 5 (231.52 μg/g) and Jiheilv 27-3 (197.54 μg/g), whereas Xiaoqu 7110 had the lowest concentration (16.68 μg/g) (Table 1). Jiheilv 27-3 had high caffeic, but low syringic, concentrations, whereas Hei 15-2 had low syringic concentration. Hun 1029 had medium caffeic concentrations. Hei 45-1 had medium caffeic and syringic concentrations. Zhonglv 5 had low caffeic concentration, but high syringic concentration. Xiaoqu 7110 had the highest bound phenolic acid concentration (5363.75 μg/g), followed by Jiheilv 27-3 (5244.58 μg/g), whereas Zhonglv 5 had the lowest (2284.53 μg/g) concentration (Table 4). Jiheilv 27-3 had low concentrations of caffeic, gentisic, and chlorogenic and a medium concentration of *p*-coumaric but a high concentration of syringic, whereas Hei 15-2 had medium ferulic and *p*-coumaric concentrations and low concentrations of caffeic, syringic, and gentisic. Hun 1029 had low ferulic concentration, but medium concentrations of chlorogenic, syringic, and *p*-coumaric. Xiaoqu 7110 had high ferulic, caffeic, chlorogenic, *p*-coumaric, and gentisic concentrations, but medium syringic concentration. Hei 45-1 had medium caffeic and gentisic concentrations, low ferulic, chlorogenic, and *p*-coumaric concentrations. Zhonglv 5 had medium caffeic and syringic concentrations and low *p*-coumaric concentration, but high gentisic concentration. Significant differences ($p < 0.05$) were observed between green mung bean and black mung beans in

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the contents of both free phenolic acids and bound phenolic acids.

Individual Anthocyanidins. We used HPLC-MS to quantify the individual anthocyanin of these mung beans (see the Supporting Information). Table 2 showed the contents of individual anthocyanidins. According to our results, black mung beans were found to contain a wide concentration range of total anthocyanins from 437.65 to 810.55 μg/g. Jiheilv 27-3 had the highest concentration of total anthocyanins, 810.55 μg/g. Cyanidin 3-glucoside was found as the most dominant form of anthocyanin followed by pelargonidin-3-glucoside in these black mung beans. Pelargonidin-3,6-malonylglucoside, pelargonidin-3-glucoside, and an unknown anthocyanin were detected only in trace amounts. However, the anthocyanins were not detected in green mung bean. Our results are similar to the data reported by Lopez-Martinez et al.,[22] who reported that anthocyanins have not been detected in white, yellow, and orange maize kernels. Previously, we have reported that black soybean contains five species of anthocyanins, including cyanidin-3-glucoside, delphindin-3-glucoside, malvidin-3-glucoside, petunidin-3-glucoside, and peonidin-3-glucoside. However, the content of total anthocyanins of black soybean is quite higher than that of the black mung bean.[23]

Antioxidant Activity. DPPH radical, $ABTS^{\bullet+}$ radical, and ORAC radical cation assays, expressed as TEAC value, were used for the evaluation of free radical-scavenging properties of the six varieties of mung bean. The use of more than one method is recommended to give a comprehensive prediction of antioxidant efficacy.[24] The results are shown in Table 3. Total antioxidant activities, measured by the DPPH method, ranged from 18.34 μM TE/g in Zhonglv 5 to 61.20 μM TE/g in Xiaoqu 7110. The DPPH in green mung bean fell by Xu and Chang's[13] observation (16.8 μM TE/g), but was lower than in our previous paper (45.36 μM TE/g).[15] The differences between current and previous results may be attributed to the differences in the raw materials. Significant differences ($p < 0.05$) were found between green mung bean and black mung beans. All black mung beans had a value >25 μM TE/g, as measured by DPPH method. Except Xiaoqu 7110, no significant differences were found between black mung beans. The relatively stable organic radical, DPPH, has been widely used in the determination of the antioxidant activity of different plant extracts. Although the DPPH method is simple and rapid, it has generally a relatively small linear reaction range of only 2−3-fold. Furthermore, the DPPH radical is decolorized by reducing agents as well as H transfer, which may contribute to inaccurate interpretations of antioxidant capacity.[25]

Table 3. Antioxidant Activity of Mung Bean Varieties[a]

variety	DPPH	ABTS	ORAC
Jiheilv 27-3	33.62 ± 0.69 b	17.60 ± 1.46 b	70.01 ± 5.47 c
Hei 15-2	29.18 ± 0.38 b	12.09 ± 2.10 c	55.76 ± 2.84 cd
Hun1029	27.55 ± 0.83 b	11.31 ± 1.55 c	47.92 ± 2.81 e
Xiaoqu 7110	61.20 ± 1.18 a	21.19 ± 2.15 a	166.40 ± 17.28 a
Hei 45-1	39.47 ± 2.66 b	13.87 ± 1.07 c	99.64 ± 8.04 b
Zhonglv 5	18.34 ± 0.19 c	8.76 ± 1.82 d	28.61 ± 4.85 f

[a]Data are expressed as mean ± standard deviation of triplicate samples. The antioxidant activity is expressed as μM TE/g. Values in the same column sharing different letters are significantly different ($p < 0.05$).

Total antioxidant activities, measured by the $ABTS^{\bullet+}$ method, ranged from 8.76 μM TE/g in Zhonglv 5 to 21.19 μM TE/g in Xiaoqu 7110. These results confirm previous research[26] that $ABTS^{\bullet+}$ is more sensitive to phenolic-containing compounds than DPPH. Significant differences ($p < 0.05$) were found between green mung bean and black mung beans. The synthetic nitrogen-centered $ABTS^{\bullet+}$ radical is not biologically relevant but is often used as an "indicator compound" in testing hydrogen donation capacity and thus antioxidant activity.[27]

ORAC is the only assay that combines both inhibition time and degree of inhibition into a single quantity. The ORAC values of mung beans have not been extensively studied excluding one paper.[13] Xiaoqu 7110 exhibited the strongest ORAC value (166.40 μM TE/g). The current ORAC value of green mung bean (Zhonglv 5) is much smaller than the literature value. This difference may be due to the different extraction reagents; the green mung bean was extracted by acetone.

In this research, the antioxidant activity of black mung beans was higher than that in normal green mung bean. Xiaoqu 7110 exhibited the highest antioxidant activities as assayed by DPPH, $ABTS^{\bullet+}$, and ORAC methods. The possible reason is that the anthocyanin content in black mung beans contributes mainly to antioxidant activity.[28] The antioxidant capacities of black mung beans have been reported here for the first time.

α-Glucosidase Inhibition Activities. Jiheilv 27-3 was the most active (60.61%), followed by Hei 45-1 (60.30%) (Table 4). Significant differences ($p < 0.05$) were found between green mung bean and black mung beans. Previously we have determined the α-glucosidase inhibition activities in black soybean (content of anthocyanins = 7.4 μg/g); the IC_{50} is >1000 mg/mL, which is higher than that of black mung beans. Lower IC_{50} values indicate more potent postprandial blood glucose inhibitory activity of a given anthocyanin. Anthocyanins have been implicated in the inhibition of α-glucosidase by berry extracts and sweet potato extracts. Gordon and Derek[29] investigated the antidiabetic effects of berry on digestive enzymes, and they suggested that the mechanism of α-glucosidase inhibition action of anthocyanins is similar to that of acarbose; that is, anthocyanins act as a competitive α-glucosidase inhibitor because of the structural similarity between the normal substrate maltose and the glucosyl group, which is β-linked to the anthocyanin. A combination of acarbose and anthocyanin-rich berry extracts could inhibit α-glucosidase activity, suggesting that anthocyanins could substitute for or be used in conjunction with pharmaceutical agents in maintaining glycemic control, which may be useful in the treatment of type 2 diabetes.[30] In addition, identifying

Table 4. Antidiabetic Activity of Mung Bean Varieties[a]

variety	α-glucosidase inhibition (%)	BSA−glucose (%)	BSA−MGO (%)
Jiheilv 27-3	60.61 ± 1.18 a	65.05 ± 1.55 bc	66.07 ± 0.47 a
Hei 15-2	45.35 ± 0.38 b	64.79 ± 2.15 bc	60.87 ± 1.28 bc
Hun1029	58.19 ± 2.66 a	62.58 ± 1.07 c	57.48 ± 0.81 bcd
Xiaoqu 7110	42.98 ± 2.69 b	64.85 ± 1.46 bc	62.34 ± 0.84 ab
Hei 45-1	60.30 ± 0.83 a	67.24 ± 2.10 ab	55.29 ± 2.04 d
Zhonglv 5	18.62 ± 0.19 c	51.76 ± 3.82 d	46.45 ± 4.85 e

[a]Data are expressed as mean ± standard deviation of triplicate samples. Values in the same column sharing different letters are significantly different ($p < 0.05$).

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Table 5. Correlation between Phytochemicals and Bioactivities of Mung Bean Varieties[a]

	bound phenolic acids	total anthocyanins	DPPH	ABTS	ORAC	α-glucosidase inhibition	BSA−glucose	BSA−MGO
free phenolic acids	−0.390	−0.206	−0.448	−0.360	−0.378	−0.077	−0.257	−0.455
bound phenolics acids		0.544	0.747	0.941**	0.698	0.482	0.567	0.837*
total anthocyanins			0.251	0.446	0.182	0.861*	0.861*	0.881*
DPPH				0.906*	0.995**	0.282	0.599	0.539
ABTS					0.881*	0.380	0.610	0.755
ORAC						0.232	0.558	0.464
α-glucosidase inhibition							0.865*	0.676
BSA−glucose								0.781

[a]Correlation is significant at (*) $p < 0.05$ level (two-tailed) or (**) $p < 0.01$ level (two-tailed).

natural components with high α-glucosidase but lower α-amylase inhibitory potential could prevent certain side effects of acarbose, which are largely due to undigested, but readily fermented, starch reaching the colon.[31]

Advanced Glycation End Product Formation Inhibition Activities. AGEs are part of a major pathogenic process in diabetic complications including neuropathy, nephropathy, retinopathy, atherosclerosis, and cataracts.[14] BSA−glucose and BSA−MGO models were used for the evaluation of the inhibition effect on the formation of AGEs. The inhibition measured by BSA−glucose and BSA−MGO varied significantly among mung beans (Table 4). Hei 45-1 and Jiheilv 27-3 had the highest inhibition measured by the BSA−glucose and BSA−MGO methods, respectively. Significant differences were found between green mung bean and black mung beans. Our previous study confirmed that vitexin and isovitexin extracted from mung bean seeds are effective in inhibiting the formation of AGEs in vivo; thus, mung beans have positive effects in diabetic nephropathy.[4] Beans have been recommended as suitable foods for diabetic patients in the past mainly on the basis of their high fiber and protein contents. Recently, it has been reported that beans contained considerable bioactive phytochemicals, including phenolic compounds, which offer extra benefits for amelioration of diabetes and alleviating diabetic complications.[32] Cyanidin-3-α-*O*-rhamnoside and pelargonidin-3-α-*O*-rhamnoside, isolated from acerola fruit, have the potential of inhibiting AGE formation. Purple sweet potato can improve spatial learning and memory ability in D-gal-treated old mice by decreasing advanced AGE formation and AGE receptor expression.[33]

Correlation of TAC with DPPH and α-Glucosidase Inhibition Activities. The linear correlation coefficients between phytochemicals of mung beans and their bioactivities are shown in Table 5. Bound phenolic acid contents of mung beans exhibited significantly linear correlations with ABTS and significantly correlated with BSA−MGO; meanwhile, the total anthocyanins also exhibited significantly linear correlations with overall antidiabetic activities (α-glucosidase, BSA−glucose, and BSA−MGO). Among the three chemical antioxidant assays, DPPH exhibited significantly linear correlations with ABTS and ORAC; ABTS also significantly correlated with ORAC. α-Glucosidase exhibited significantly linear correlations with BSA−glucose. These results indicated that phenolic components, especially anthocyanins, played an important role in the overall antioxidant activities (DPPH, ABTS, and ORAC) and antidiabetic activities (α-glucosidase, BSA−glucose, and BSA−MGO) of mung bean.

According to the obtained data, it could be said that a higher content of phenolic compounds in mung beans contributes to their higher antioxidant activity. These results are in good accordance with literature data.[34] Lopez-Martinez et al.[22] also found that pigmented maize showed relatively higher radical scavenging activity than nonpigmented samples. The differences in the antioxidant capacity are possibly related to the specific composition of anthocyanin derivatives such as simple or acylated glycosides of cyanidin, pelargonidin, or peonidin.[35] The radical scavenging capacity of different anthocyanins depends mainly on the number of hydroxyl groups and their position on the molecule. Additionally, glycosylation of the anthocyanidins may modulate the antioxidant capacity depending on the aglycons.[36] In Peng's previous investigations, they found the correlation of the total phenolic content and inhibition effect on the formation of AGEs in legumes. This result is attributed to phenolic compounds inhibiting the formation of AGEs by inhibition of free radical generation in the glycation process and subsequent inhibition of protein modifications.[14]

In summary, various varieties of mung beans showed considerable variations in their phytochemical profiles in terms of free phenolic acids, bound phenolic acids, total phenolic and anthocyanin contents, overall antioxidant activities, and antidiabetic activities. Correlation analyses between phytochemicals and bioactivities indicated that overall antioxidant capacities of mung beans may be predominantly contributed by phenolic compositions, whereas anthocyanins play major roles in the antidiabetic activities. The free phenolic acid and bound phenolic acid contents ranged from 16.68 to 255.51 μg/g and from 2284.53 to 5363.75 μg/g, respectively, whereas the total anthocyanin contents ranged from 0 to 810.55 μg/g. Among the mung beans tested, black mung bean Xiaoqu 7110 had the highest content of bound phenolic acids and exhibited the strongest antioxidant capacities (DPPH, ABTS, and ORAC) as compared to the other tested mung beans. The α-glucosidase and glycation end product inhibitory effects of black mung beans were first discovered here. Black mung beans were found to be excellent dietary sources of natural antioxidants for health promotion; meanwhile, these mung bean sources exhibited great potential to be developed into functional foods or nutraceutical ingredients for maintaining glycemic control and alleviating diabetic complications.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

HPLC chromatogram of phenolic compounds in mung bean. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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