Evaluation of bran extracts of rice (*Oryza sativa*) and selected bean (*Phaseolus vulgaris* L.) varieties for their antioxidative and anti-hyperglycemic potentials

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Abstract

Search of therapeutic potential of natural and locally available food has become a trend due to increasing health concerns among consumers around the world. In this study, extracts of rice bran (*Oryza sativa* L.) and selected beans (red bean, red kidney bean, and white bean) (*Phaseolus vulgaris* L.) were obtained using 80% ethanol-water mixture to compare their anti-hyperglycaemic and anti-oxidative potentials. The total phenolic content (TPC), ferric reducing antioxidant power (FRAP), 2,2'-azino-bis (3-ethylbenzothiazoline-6- sulfonic acid (ABTS) radical scavenging activity, 2,2-diphenyl-1-picryl-hydrazylhydrate (DPPH) radical scavenging activity, and the carbohydrate hydrolyzing enzyme inhibitory potentials of the extracts were studied in-*vitro* using relevant assays. The highest phenolic content (0.122 mg of Gallic Acid Equivalent /g of extract) was

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found with red bean extract. FRAP values of the extracts were found to range from 48.98 to 75.94 µmol $FeSO_4$ /g bran extract. The highest ferric reducing power (75.94 µmol $FeSO_4$/g) was displayed by the bran extract of red kidney bean. The highest inhibitory effect against α-amylase (96.18%) was displayed by rice bran extract and the highest inhibitory effect against α-glucosidase (39.57%) was exhibited by red bean extract. This study concluded that the bran extracts of rice and the selected beans were potent sources of natural antioxidants and good postprandial hyperglycaemia regulators.

INTRODUCTION

Diabetes mellitus is a chronic endocrine disorder that affects the metabolisms of carbohydrates, proteins, and lipids. As a chronic ailment, diabetes might affect an individual in numerous ways. Some of the serious health complications caused by diabetes are diabetic neuropathy, cardiovascular diseases, and retinopathy (Kumar *et al.*, 2012). The overall prevalence of diabetes mellitus in Malaysia was reported as 22.9% of the total population (Wan Nazaimoon *et al.*, 2013). Failure to arrest this rising trend in diabetes in the country can lead to serious social and economic problems. Diabetes can be generally categorized into several individual sub-types, but type I and type II are considered to be more prevalent worldwide (Agarwal and Gupta, 2016; Bhutkar and Bhise, 2012).

There is so much effort taken by countries around the world to address this severe health challenge. Strategies of treating diabetes include stimulation of insulin secretion, enhancement of the action of insulin at the target tissue and inhibition of the carbohydrate hydrolysing enzymes to control spiking of blood glucose (Funke and Melzing, 2006). Decrease of the postprandial hyperglycaemia has been identified as an effective therapeutic approach against non-insulin dependent diabetes. This is usually achieved by retarding the glucose absorption in the small intestine. The inhibition of carbohydrate hydrolyzing enzymes present in human digestive tract would play a significant role in this scenario (Bhutkar and Bhise, 2012). Pancreatic α-amylase catalyzes the initial hydrolysis of starch into oligosaccharides (Kandra, 2003; Tangphatsomruang *et al.*, 2005) while α-glucosidase which is a membrane-bound enzyme present in the small intestine will convert oligosaccharides to glucose (Abubakar

et al., 2017; Agarwal and Gupta, 2016). Moreover, the maintenance of the balance between free radicals and radical scavenging capacity of the body is also an important factor when coming to diabetes. Oxidative stress of a diabetic patient might also have the ability to get involved in the diabetic associated complication up to a certain extent (Yao *et al.*, 2010).

At present, evaluation of antidiabetic effect of natural plant sources has received much attention from researchers engaged in chronic ailments. It has been noted that the allopathic drugs used for treating diabetes might cause various gastrointestinal side effects (Bhutkar and Bhise, 2012). This claim has been debated by the research community repeatedly. According to the recent literature, seed coats of different beans and grains are reported to exert considerable anti-oxidative and anti-hyperglycaemic effects, which is beneficial for managing diabetes (Abubakar *et al.*, 2017; Adekola *et al.*, 2017). In this study, we aimed to evaluate the anti-oxidative properties and anti-hyperglycaemic potentials of the extracts of rice bran and seed coats of selected beans grown in Malaysia. Up-to-date, a study on the anti-hyperglycaemic potential of rice bran of Cl220 hybrid type with different bean seed coats has rarely been investigated. The outcome of this kind of study might help to gain a broad idea with regard to the use of rice bran and different seed coats as precursors for developing functional foods to manage diabetes.

MATERIALS AND METHODS

Materials

Three samples of rice bran (Cl220 hybrid type) were obtained from BERNAS Company Sungai Baru Complex, Alor Setar, Malaysia. Three samples of white bean, red kidney bean and red bean were obtained from a local supermarket in Semenyih, Malaysia. Sigma-Aldrich company, Malaysia supplied Porcine pancreatic α-amylase and α-glucosidase.

Preparation of extracts

In order to collect the seed coat extracts, all the bean types were soaked in water for 48 h and subjected to air drying. Rice bran was dried in the same form of purchased from the market. The dried bran of beans and rice were finely ground into powder form and extracted with 80% ethanol-water mixture. The extracts were concentrated using rotary evaporator and freeze-dried subsequently (Abubakar *et al.*, 2017).

Antioxidant properties evaluation

Determination of TPC

The TPC content of crude extracts were determined as reported by Singleton *et al.* (1999). Accurately, 20 µL of sample, 110 µL of 10% Folin-Ciocalteu reagent and 70 µL of 7.5% Na_2CO_3 was mixed in a 96-well micro-plate. After incubating the samples for 30 min at room temperature (25 ± 2 °C), value of the absorbance at 765 nm was recorded. Gallic acid was used as the standard. TPC of the individual sample was expressed as mg gallic acid equivalents per 1 g dry weight of the crude extract.

Determination of FRAP

The method reported by Benzie and Szeto (1999) was used with slight modification to carry out the assay. In order to prepare FRAP reagent, acetate buffer (300 mM, pH 3.6), $FeCl_3.H_2O$ (20 µM), and TPTZ (10 mM) solution were mixed in the ratio of 10:1:1 and was heated at 37 °C. A 20 µL sample extract was mixed with 30 µL acetate buffer, 150 µL FRAP reagent and incubated for 8 min at room temperature in a 96-well microplate. Then, the value of absorbance at 600 nm was recorded. The FRAP values were expressed as µmol of $FeSO_4$ / g of crude extract.

ABTS radical scavenging activity

The protocol described by Re *et al.* (1999) was used to carry out the assay in a 96-well microplate. A solution of 7.8 mM of ABTS in potassium persulphate was kept at 37 °C in dark condition for 16 h to prepare a stable ABTS radical cation stock solution. A 40 µL of seven times diluted ABTS stock solution and 160 µL samples extracts were incubated at 25 ± 2 °C for 10 min in 96 microplate. The value of absorbance was recorded at 734 nm for each sample. The results were expressed as Trolox equivalents antioxidant capacity in µmol of Trolox / g of crude extract.

Determination of DPPH activity

Determination of DPPH radical scavenging activity was carried out in a 96-well microplate in accordance with the protocol described by Blois (1958). A 125 µM of DPPH radical and 75 µM samples extracts were incubated for 15 min at 25 ± 2 °C in a 96-well microplate. The absorbance was recorded at 517 nm. Results were expressed as µmol of Trolox / g of crude extract.

Enzyme inhibitory assays

Alpha-amylase inhibitory activity

This assay was carried out according to the protocol given by Bernfeld (1955) with a slight modification. Briefly, a 50 μL portion of samples extract (200 μg/mL), 50 μl of α-amylase enzyme (13 mg/mL), 40 μL of starch in 100 mM sodium acetate buffer (pH 6.0) were incubated at 40 °C for 15 min. Thereafter, 0.5 mL of DNS (3,5-dinitrosalicyclic acid) reagent was added, placed in a boiling water bath for 5 min, and allowed to cool in ice. The value of absorbance of each sample was recorded at 540 nm. The control experiments were carried out following the same procedure by replacing the samples extracted with 50 μl of acetate buffer. Percentage inhibition of amylase activity of each sample was calculated according to the following equation:

Percentage of Inhibition = $[(\delta A_C - \delta A_S)/ \delta A_C]$ x 100

Where, δA_C = Absorbance of control –Absorbance of control blank, δA_S = Absorbance of sample – Absorbance of sample blank

Alpha-glucosidase inhibitory activity

The assay was performed by adopting the protocol described by Matsui *et al.* (2001) with a slight modification. Briefly, 50 mU/mL of α-glucosidase enzyme, 4 mM p-nitrophenyl-β-D-glucopyranoside, 40 μL of samples solution (200 μg/mL)   in the total reaction volume of 100 μL in sodium acetate buffer (50 mM, pH 5.8) were mixed in 96 micro-well plate followed by incubation for 30 min at 37 °C. The reaction was then terminated by adding 50 mL of 0.1 M Na_2CO_3. The value of absorbance at 405 nm was recorded for each sample. The percentage of glucosidase inhibitory activity of each sample was calculated using the following equation:

Percentage of Inhibition = $[(\delta A_C - \delta A_S)/ \delta A_C]$ x 100

Where, δA_C = Absorbance of control –Absorbance of control blank, δA_S = Absorbance of sample – Absorbance of sample blank

Statistical analysis

For all measurements, triplicate data (n=3) were taken. IBM SPSS software package (version 21.0) was used to analyze the data adopting one-way analysis of variance (ANOVA). When *F* values were significant, mean differences were compared using Duncan's multiple range test at 5% level of probability.

RESULTS AND DISCUSSION

The yields of the bran extracts obtained from rice and selected beans are shown in Table 1. According to the data, the highest yield was observed for rice bran (5.01%) while the lowest was recorded for white been (1.05%). The yield data were found to follow the ascending order of white bean< red bean< red kidney bean< rice.

Table 1: Yield (%) of bran extracts of rice and selected bean seed coats.

Sample	Initial weight (dry basis) (g)	Weight of extract (g)	Yield (%) (w/w)
Red bean	100.00	3.00	3.00
Red kidney bean	100.00	3.54	3.54
White bean	100.00	1.05	1.05
Rice	100.00	5.01	5.01

Total phenolic compounds

The TPC of the extracts of rice bran and selected beans are shown in Table 2. According to data in Table 2, the TPC of the bran extracts were varied from 0.026 ± 0.001- 0.122 ± 0.004 mg of gallic acid equivalent (GAE)/g of bran extract. Although the TPC values were found in the ascending order of white bean< rice< red kidney bean< red bean, there was no significant difference ($p>0.05$) between the TPC of red bean and red kidney bean. The low phenolic content of white bean extracts can be due to its less coloured seed coats (Madhujith and Shahidi, 2005). According to some previous studies, the colours of the bran of grains are imparted by phytochemicals present in them which are known to possess antioxidant properties (Abubakar *et al.*, 2017; Yao *et al.*, 2010). Adekola *et al.* (2017) reported that red kidney bean seed coat was found to contain the highest TPC compared to seed coats of red bean, black eyed pea, and black bean. However, the results of the current study showed that the TPC of red bean was slightly higher than that of red kidney bean. This variation in results could be due to the differences in sample preparation methods or extracting solvents, or even the geographical origin of the sample. Arab *et*

al. (2011) previously commented that the rice bran extract might have the presence of tocotrienols and tocopherols.

Table 2: TPC and FRAP values of bran extracts of rice and selected beans

Bran extract	Antioxidant property	
	TPC (mg gallic acid equivalent (GAE)/g bran extract)	FRAP (µmol $FeSO_4$/g bran extract)
Red Bean	0.122 ± 0.004[a]	74.260 ± 0.551[b]
Red Kidney Bean	0.120 ± 0.001[a]	75.943 ± 0.575[a]
White Bean	0.026 ± 0.001[c]	48.980 ± 0.235[d]
Rice	0.049 ± 0.001[b]	59.020 ± 0.551[c]

Data represented as mean ± standard deviation. The mean values within each column bearing different superscripts are significantly different at $p < 0.05$.

Ferric Reducing Antioxidant Power (FRAP)

The FRAP values of seed coats of beans and rice bran are compared as shown in Table 2. According to the data, the highest FRAP value was displayed by red kidney bean (75.943 ± 0.575 µmol $FeSO_4$/g) while the lowest value was shown by white bean (48.980 ± 0.235 µmol $FeSO_4$/g). The values were found to follow the order of white bean< rice < red bean < red kidney bean while being significantly ($p<0.05$) different from each other. Zuo and Chang (2014) reported that the ferric reducing power is affected by the presence of condensed tannins in a sample. Moreover, it is reported that the tannin contents of dry beans are found to vary according to the bean species and the colour of seed coats.

ABTS radical scavenging activity

Dose-dependent ABTS radical scavenging activity values of the extracts of rice bran and beans are compared as depicted in Table 3. The results obtained at 50 µg/ml and 100 µg/mL concentration levels of all bran extracts were significantly different ($p<0.05$). However, no significant

difference (p>0.05) was noticed between the results obtained for rice and white bean at 200 µg/mL concentration level. At 50 µg/mL, the highest ABTS activity was exhibited by the extract of rice bran while the lowest activity was displayed by red bean. At 50 µg/mL level, the activity could be aligned in the order of rice > white bean > red kidney bean > red bean. At 100 µg/mL level, the highest activity was observed for white bean, while the lowest was observed for red bean. The ABTS values of the extracts tend to follow the descending order of white bean > rice > red kidney bean > red bean. At 200 µg/mL level, both white bean and rice bran exhibited the highest ABTS activity while red bean showed the lowest activity. The ABTS values at 200 µg/mL were found to follow the order of white bean ≈ rice > red kidney bean > red bean. Overall, all bran extracts showed a dose-dependent radical scavenging activity for the ABTS assay.

Table 3: ABTS values of bran extracts of rice and selected beans

Bran extract	Dose-dependent ABTS radical scavenging activity		
	50 (µg/mL)	100 (µg/mL)	200 (µg/mL)
Red Bean	6.820 ± 0.252[d]	9.533 ± 0.407[d]	11.987 ± 0.455[c]
Red Kidney Bean	9.287 ± 0.131[c]	11.463 ± 0.287[c]	12.810 ± 0.537[b]
White Bean	36.450 ± 0.476[b]	47.05 ± 0.440[a]	49.423 ± 0.296[a]
Rice	39.027 ± 0.839[a]	42.647± 0.488[b]	49.317 ± 0.415[a]

Data represented as mean ± standard deviation. The mean values within each column bearing different superscripts are significantly different at p < 0.05.

DPPH radical scavenging activity

A dose-dependent DPPH value of extracts of rice bran and beans is shown in Table 4. According to the data, both red kidney bean and red bean extracts did not display ant significant (p>0.05) difference at the 50 µg/mL and 100 µg/mL concertation levels. Nevertheless, all the extracts displayed significant (p<0.05) differences of each other at the 200 µg/mL concentration level. The DPPH values of the bran extracts at 200 µg/mL followed the order of white bean> rice> red bean> red kidney bean. However, white bean extract displayed the highest DPPH value at all levels.

Overall, the bran extracts showed a dose-dependent response to DPPH activity. Further, the exhibited values for DPPH assay were comparatively higher than the respective values obtained for the ABTS assay. Previously, Adekola *et al.* (2017) also reported higher DPPH values than ABTS values in the case of red bean and red kidney bean seed coats. However, according to another study, red kidney bean is reported to show higher ABTS scavenging activity than DPPH scavenging activity (Fahad *et al.*, 2014).

Table 4: DPPH values of bran extracts of rice and selected beans

Bran extract	Dose-dependent DPPH radical scavenging activity		
	50 (µg/mL)	100 (µg/mL)	200 (µg/mL)
Red Bean	12.333 ± 0.722[c]	15.903 ± 0.856[c]	22.200 ± 0.716[c]
Red Kidney Bean	12.467 ± 0.492[c]	15.373 ± 0.290[c]	20.700 ± 0.542[d]
White Bean	83.193 ± 0.920[a]	112.203 ± 0.276[a]	117.243 ± 0.410[a]
Rice	82.163 ± 0.818[b]	94.163 ± 0.519[b]	110.187 ± 0.477[b]

Data represented as mean ± standard deviation. The mean values within each column bearing different superscripts are significantly different at $p < 0.05$.

According to some other studies, the phenolic compounds present in grains were greatly contributing to their antioxidant activity (Yao *et al.*, 2010). In the present study, however, the non-phenolic constituents present in the extracts might have largely contributed to the radical scavenging activities (Ojha *et al.*, 2019). This fact is further confirmed by two other studies where non-phenolics were claimed to display high antioxidant activities in foods such as pomegranate (Mekni *et al.*, 2013) and *Buddleja asiatica* (Mortada *et al.*, 2008). The selected items were reported to contain proteinaceous inhibitors of the alpha-amylase (Le Berre *et. al.*, 1997; Yamada *et. al.*, 2001). Moreover, they are reported to have anti-obesity and extensive anti-diabetes potentials (Wang *et. al.,* 2011).

Inhibitory activity against alpha-amylase

Comparative inhibitory activity of the extracts of rice bran and selected beans against α-amylase is shown in Table 5. Percentage inhibitory activity

of the different extracts against α-amylase was found to range from 48.96% to 96.18% where rice bran showed the highest inhibitory effect while the white bean showed the lowest. In fact, significant ($p < 0.05$) differences were observed within the extracts and the order of the potency was found to be as rice bran > red bean > red kidney bean > white bean.

Table 5: Inhibitory activity (%) of bran extracts of rice and selected beans against Alpha-amylase and alpha-glucosidase

Bran extract	Anti-amylase and anti-glucosidase activity	
	α-Amylase inhibition (%)	α-Glucosidase inhibition (%)
Red bean	94.487 ± 0.545[b]	39.567 ± 0.722[a]
Red Kidney Bean	66.383 ± 0.592[c]	31.103 ± 0.380[c]
White Bean	48.957 ± 0.792[d]	34.573 ± 0.983[b]
Rice	96.180 ± 0.551[a]	11.373 ± 0.617[d]

Data represented as mean ± standard deviation. The mean values within each column bearing different superscripts are significantly different at $p < 0.05$.

Inhibitory activity against alpha-glucosidase

Comparative inhibitory activity of the extracts of rice bran and selected beans against α-glucosidase is shown in Table 5. In fact, the percentage inhibitory activities were found to range from 11.37 to 39.57% where the highest percentage inhibitory activity was claimed for red bean while the lowest inhibitory activity was shown by rice bran. The α-glucosidase inhibitory activities of bran extracts were significantly ($p < 0.05$) different from each other, and the values tend to follow the order of red bean > white bean > red kidney bean > rice. Accumulated evidence from various studies showed that the coloured grains are rich in anthocyanins and reckoned as competitive α-glucosidase inhibitor due to their structural similarities to acarbose, which is a known α-glucosidase inhibitor (Yao *et al.*, 2010). The flavonoid contents in them could partly contribute to their inhibitory activities against the two carbohydrate hydrolyzing enzymes (Marikkar *et al.*, 2016). Further, Barret and Udani (2011) stated that anthocyanins present in red kidney bean have the potential to inhibit α-glucosidase

enzyme activity. The present study demonstrated that the extracts of rice bran and selected beans were found to exert a stronger inhibitory effect against α-amylase than α-glucosidase. Nonetheless, Adekola *et al.* (2017) previously reported that the α-glucosidase enzyme inhibitory potency of the seed coats of red bean and red kidney bean were higher than their respective α-amylase inhibitory activity. Moreover, they stated that the seed coat of red kidney bean had the higher α-amylase and α-glucosidase inhibitory activity than red bean seed coat. This variation in results could be due to multitude of factors such as differences in sampling, cultivars and geographical influences.

CONCLUSIONS

This study results showed that the bran extracts of rice and the selected beans can be identified as rich sources of phenolic compounds. They displayed differences in their antioxidant properties and inhibitory potency against α-amylase and α-glucosidase. Hence, the brans used in this study have the potential to be used as natural antioxidants which has the capability to control postprandial hyperglycaemia. Therefore, this study will help to broaden the use of these brans of seeds as novel functional foods targeting the management of postprandial hyper-glycaemia in diabetic patients.

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DECLARATION OF CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

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