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Nutritional Compositions and Antioxidant Activities of Non-Polar and Polar Extracts of Germinated Brown Rice

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ABSTRACT

The objective of this study was to investigate the nutritional compositions and antioxidant activities (AA) of non-polar and polar extracts of germinated brown rice (GBR). Nutritional compositions such as moisture, ash, carbohydrate, fat, protein and fibre were determined. Energy and minerals content were determined by using bomb calorimetry and Atomic Absorption Spectroscopy (AAS), respectively. Total phenolic content (TPC) and total flavonoid content (TFC) of the extracts were determined by Folin-Ciocalteu method and aluminium chloride colorimetric method. The AA was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and [2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] diammonium salt (ABTS) radical scavenging capacity assay, ferric reducing antioxidant potential (FRAP) assay, and β-carotene bleaching assay. Total energy content of GBR sample was 390.95±11.31 kcal/100g and carbohydrate (54.30±1.04 g/100 g) was the most abundant nutrient. The predominant minerals in the GBR sample were sodium, potassium and magnesium. The polar extract showed significantly higher ($p<0.05$) level in TPC, TFC and AA than non-polar extract except in β-carotene bleaching assay. Positive and strong correlations ($r>0.90$, $p<0.001$) existed between antioxidants (TPC and TFC) and AA. Therefore, polar extract was better than non-polar extract. The nutritional composition of GBR also provided an update for food composition database.

Keywords: Nutritional composition, antioxidant activities, germinated brown rice

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INTRODUCTION

Rice plays a prominent role in the food security of the world's population. It is a major staple food for more than half of the world population in general and some populous countries such as Asia and Africa in particular (Hu et al., 2012). Rice provided about third-quarter of the calorific intake for the population in Southeast Asia (Fitzgerald et al., 2009). It is also the main carbohydrate source for people in South-East Asia including Malaysia. In 2012, the worldwide rice production was about 926 million of metric tonnes, in which China was the highest rice production with 206 million of metric tonnes and Malaysia produced 2.75 million of metric tonnes of rice (FAO, 2014).

Recently, whole grain cereal has been associated with reduced risks of developing chronic diseases including coronary heart disease, diabetes mellitus and certain types of cancer (Hübner & Arendt, 2013). Germination of grain for a short period of time activates the hydrolytic enzymes and improves the contents of dietary fibre, minerals, vitamins, and phytochemicals including phenolic compounds and sterols (Chavan et al., 1989; Hübner & Arendt, 2013). Thus, it is believed that germinated grains rich in nutrients content and phytochemicals are better for the health-promoting benefits compared to non-germinated grains.

GBR has become a popular health food especially in Asian. GBR is produced by soaking brown rice grains in water to promote germination (Moongngarm & Saetung, 2010). It contains significantly higher amount of nutrients and is easier to be digested and absorbed, yet the texture is softer than brown rice (Chavan et al., 1989). Nutrients such as γ-amirobutyric acid (GABA), dietary fibre, inositols, ferulic acid, phytic acid, tocotrienols, some minerals, γ-oryzanol, and prolylendopeptidase inhibitor, are significantly increased in GBR (Latifah et al., 2010). In view of the nutritional value and the potential to become a novel functional food for human diet, there is a need to investigate the nutritional value of GBR for future industrial and domestic applications.

Thus, the objectives of this study were to determine the nutritional compositions of GBR and to evaluate the TPC, TFC and AA of non-polar and polar extracts of GBR in local brown rice varieties.

MATERIALS AND METHODS

Chemicals and Reagents

Methanol, n-hexane and dimethyl sulfoxide (DMSO), sodium acetate trihydrate, ferric trichloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ferrous sulphate ($FeSO_4 \cdot 7H_2O$) and hydrochloride acid (HCl) were purchased from Merck (Darmstadt, Germany). Sodium carbonate (Na_2CO_3), sodium nitrite, sodium hydroxide (NaOH), potassium persulphate, aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$), glacial acetic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium peroxodisulfate ($K_2S_2O_8$), ABTS [2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] diammonium salt, 2,4,6-tripyridyl-1,3,5-s-triazine (TPTZ), gallic acid, quercetin, 3,5-Di-tert-4-butylhydroxytoluene (BHT),

ascorbic acid and Folin-Ciocalteau reagent were purchased from Sigma Chemical Co (St. Louis, MO, USA). All chemicals and reagents used in the study were of analytical grade.

Sample Preparation

Brown rice (*Oryza sativa* L. mixed varieties MR219 and MR220), local rice varieties that are massive produced and sold in the market, was obtained from Padiberas Nasional Berhad (Bernas), Malaysia. The rice was germinated according to the pre-optimised conditions established in the Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia (UPM), Selangor, Malaysia as described previously (Sani et al., 2012). Briefly, brown rice was washed twice with clean tap water before incubation at 37°C for 24 hours until germinated. Then, the GBR was dried in an oven at 50°C. It was ground to powder using a stainless steel blender (Waring Commercial, Torrington, CT, USA) before further analysis.

Nutritional Composition Analysis

Moisture content was determined through direct drying method as described by Tee et al. (1996) using an air oven (Memmert Universal, Schwabach) set at 105ºC until constant weight of the sample was obtained. Meanwhile, lipid content was determined by using hexane extraction, facilitated by the Soxtec Avanti 2050 apparatus (Foss Tecator AB, Höganäs, Sweden), as described by AOAC (2006) (Official Method, 2003.05). Protein content was determined according to Kjeldahl method (AOAC, 2000) facilitated by the Kjeltec 2200 Auto Distillation Unit (Foss Tecator AB, Höganäs, Sweden), and the conversion factor used was 5.95. The total available carbohydrate content was determined using Clegg-anthrone method (Peris-Tortajada, 2004). Dietary fibre was determined using enzymatic-gravimetric method, as described by AOAC (1995) (Official Method 991.42 and 993.19), facilitated by the Fibertec system (Fibertec System E 1023 filtration module, Tecator, Höganäs, Sweden). Dry ashing method was used to determine the ash content by incinerating the sample in a furnace (Carbolite, Parsons Lane, Hope, UK) set at 550°C until whitish/greyish ash was obtained (Tee et al., 1996). The remaining inorganic material was cooled and weighed. The results were expressed as g/100 g of sample. Energy content was determined by bomb calorimetry (IKA C5003, IKA Werke, Germany) according to the manufacturer's protocol and expressed as kcal/100 g of sample. The resulting ash was further prepared for determination of mineral contents as described by Tee et al. (1996). The content of sodium, potassium, magnesium, calcium, iron and zinc were then measured using the Model nov AA 400 atomic absorption spectrometer (Analytik Jena AG, Jena, Germany). Calibration curves for each of the minerals were determined using the standard solutions before the readings of sample were obtained. The results for mineral contents were expressed as mg/100 g of sample.

Extraction of Non-Polar and Polar Components

Non-polar and polar components of GBR were extracted as described by Jang and Xu (2009) with slight modifications. Briefly, 10 g weighed sample was mixed with 30 ml of hexane in the ratio of 1:3 (w/v) and was vortex for 30 sec before placed in a 60ºC water bath for 20 min. Then, the mixture was centrifuged at 3500 rpm for 15 min before the supernatant was collected and filtered through filter paper (Whatman No.1). The extraction was repeated for another two times under the same condition. The three supernatants were combined into non-polar fraction and placed in a rotary evaporator (Büchi Rotavapor R-200, Büchi Labortechnik AG, Switzerland) below 40°C. Subsequently, the defatted GBR was spread on an aluminium foil and placed under a laboratory hood to dry before mixed with 30 ml methanol (1:3, w/v) to perform polar components extraction. The procedure for polar extraction was the same as the non-polar components extraction, except methanol was used as the extraction solvent. The extracts were reconstituted with DMSO before stored at -20°C until further analysis.

Measurements of Antioxidant Groups

TPC of GBR was determined based on Folin-Ciocalteu reagent (diluted 10-fold) method (Velioglu et al., 1998). After incubation at room temperature for 90 min with added of Na_2CO_3 solution (60 g/l), the reaction was measured at 725 nm versus blank using a spectrophotometer (UV-1800, Shimadzu Co., Japan). A standard calibration curve was plotted using gallic acid (0.005–0.25 mg/ml) for quantification purpose and the results were expressed as mg gallic acid equivalent (GAE)/100 g of sample.

TFC of GBR was determined by aluminium chloride colorimetric assay reported by Liu et al. (2008) with modification. Briefly, 0.6 ml of appropriately diluted extract was mixed with 0.06 ml of 5% sodium nitrite and incubated for 5 min. Then, 0.06 ml of 10% aluminium chloride was added to the mixture. After 6 min of incubation, 0.6 ml of 1 M sodium hydroxide was added to the mixture. The end volume of the reaction mixture was made up to 1.5 ml with DMSO. Absorbance of the reaction was read at 510 nm against a blank. Quercetin (6.25-100 µg/ml) was used to construct a calibration curve for quantification and the results were expressed as mg quercetin equivalent (QE)/100 g of sample.

Measurements of Antioxidant Activities

DPPH free radical scavenging assay was performed based on Brand-Williams et al. (1995). Briefly, 3.9 ml of 60 µM DPPH radical solution prepared in methanol and 0.1 ml of various concentrations of extract were mixed well. The absorbance of the mixture was measured at 515 nm by using the spectrophotometer after it was incubated at the room temperature for 30 min under dark condition. Reagent solution without test extract was used as the control. Ascorbic acid at different concentrations (7.8125-125 µg/ml) was used as the standard

of comparison. The DPPH free radical scavenging activity was calculated as follows:

$$\text{Scavenging activity (\%)} = \frac{A_{515}Control - A_{515}Extract}{A_{515}Control} \; x \; 100 \quad [1]$$

where, A_{515}Extract and A_{515}Control are the absorbance of reagent with extract and reagent without extract, respectively.

FRAP assay was adapted from the method described by Benzie and Strain (1996). The FRAP reagent contained 2.5 ml of 10 mM TPTZ solution in 40 mM HCl plus 2.5 ml of 20 mM $FeCl_3 \cdot 6H_2O$ and 25 ml of 300 mM acetate buffer at pH 3.6 in a ratio of 1:1:10 (v/v/v). The FRAP reagent was prepared fresh daily and warmed to 37°C in a water bath prior to use. FRAP reagent (3 ml) was mixed thoroughly with 0.1 ml of DMSO. Immediately, the absorbance was measured at 593 nm (0 min) using the spectrophotometer (A_0). Then, 3 ml FRAP reagent was mixed thoroughly with 0.1 ml of extract and incubated for 30 min at 37º C. Absorbance of the mixture was measured at 593 nm reference to blank (A_{30}). The absorbance of extract was the differences in absorbance (A_{30}) and absorbance (A_0). Ferrous sulphate (0.1-1.0 mM) was used to prepare the calibration curve for quantification. The FRAP value was expressed as mM of Fe (II) equivalent/100 g of sample.

ABTS radical scavenging assay was determined as described by Re et al. (1999). ABTS radical cation ($ABTS^{\bullet+}$) was obtained by reacting 7 mM ABTS in distilled water with 2.45 mM potassium peroxodisulfate and allowing the mixture to stand in the dark at room temperature for 12–16 hr before use. The $ABTS^{\bullet+}$ solution was diluted with ethanol to obtain an absorbance of 0.70±0.02 at 734 nm. Aliquots of various concentrations of extracts (20 µl) were allowed to react with 1 ml of the diluted ABTS solution and the absorbance was measured after 10 min incubation using the spectrophotometer. The ABTS scavenging capacity of the extract was compared with BHT (100 µg/ml). The percentage inhibition was calculated as follows:

$$\text{ABTS radical scavenging activity (\%)} = \frac{A_{734}Control - A_{734}Extract}{A_{734}Control} \; x \; 100 \quad [2]$$

where, A_{734}Extract and A_{734}Control are the absorbance of reagent with extract and reagent without extract, respectively.

β-Carotene bleaching method was performed as Jayaprakasha et al. (2007). β-Carotene solution was prepared by dissolving β-carotene (0.4 mg) in 0.4 ml of chloroform, 40 mg of linoleic acid and 400 mg of Tween 20 in round bottom flask. Chloroform was completely evaporated at 40°C using a vacuum evaporator. Then, 100 ml of distilled water was added with vigorous shaking. Aliquots (2 ml) of this emulsion were transferred into different test tubes containing 0.1 ml of extracts. The tubes were incubated at 50°C in a water bath and the absorbance was measured at 470 nm at zero time (t=0). After that, the

absorbance readings were recorded at 15 min intervals for 120 min at 470 nm by using the spectrophotometer. The same procedures were done on the control and blank. BHT (100 µg/ml) and ascorbic acid (100 µg/ml) were used for comparison purposes. The antioxidant activity (AA) was calculated as the percent of inhibition relative to the control using the following equation from Al-Saikhan et al. (1995), as follows:

$$AA\ (\%) = \frac{DR_{Control} - DR_{Extract}}{DR_{Control}}\ x\ 100 \quad [3]$$

where, $DR_{control}$ and $DR_{Extract}$ represent the degradation bleaching rates of β-carotene without and with the extract at 0 and 120 min, respectively.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD) of three replicates. Significant differences at $p< 0.05$ between the means were determined using independent T-test in IBM SPSS software version 21.0. Pearson correlation test was performed to determine the correlations between the antioxidant groups and AA of the GBR extracts.

RESULTS AND DISCUSSION

Nutritional compositions analysis

Germination of grains has not only changed the texture, flavour and taste of the grains, but also their content of nutrients and bioactive compounds (Kaukovirta-Norja et al., 2008). The nutritional compositions of GBR sample are listed in Table 1. The weights of GBR sample before and after moisture test were measured. The GBR sample consisted of 14.04% water content and 85.96% dry matter. The macronutrient compositions of GBR sample were total available carbohydrate, 54.30±1.04 g; lipid, 2.11±0.09 g; and protein, 11.03±0.10 g. The carbohydrate content of GBR sample was relatively lower than polished rice (79.00 g/100 g) and unpolished husked rice (75.98 g/100 g), as described in Nutrient composition of Malaysian foods (Tee et al., 1997). The decrease of total carbohydrate content in the germinated grains could be attributed by the utilisation of simple sugar as an energy source to start germination (El-Adawy, 2002). Meantime, previous studies reported that germinated red aromatic brown rice from Thailand (Wichamanee & Teerarat, 2012), GBR cultivar Koshihikari from Japan (Ohtsubo et al., 2005) and GBR cultivar RD-6 form Northeast of Thailand (Moongngarm & Saetung, 2010) contained higher carbohydrate contents than GBR sample in this study, with 70.69 g/100 g, 71.30 g/100 g and 77.70 g/100 g, respectively. A study done by Palmiano and Juliano (1972) revealed that the starch content of rice grain decreased progressively during germination. Thus, the germination time of the rice grain might be one of the possible factors that contributed to the differences in carbohydrate contents. In contrast, the content of protein in the GBR sample was higher than non-germinated rice with only 7.09 g/100 g in polished rice and 8.02 g/100 g in unpolished husked rice (Tee et al., 1997). At the same time, the

GBR sample also showed higher protein content than germinated rice from some previous studies [e.g., 5.49 g/100 g (Megat Rusydi et al., 2011), 8.2 g/100 g (Ohtsubo et al., 2005), 8.34 g/100 g (Wichamanee & Teerarat, 2012), 8.47 g/100 g (Mohd. Esa et al., 2011) and 8.98 g/100 g (Moongngarm & Saetung, 2010)]. Bau et al. (1997) suggested that the synthesis of enzyme proteins or a compositional change following the degradation of other constituents during germination could possibly explain the increase of protein content after germination. Meanwhile, the lipid content in the GBR sample was close to the study reported by Wichamanee and Teerarat (2012), who found value of fat of germinated red aromatic brown rice was 2.08 g/100g. Wichamanee and Teerarat (2012) and Megat Rusydi et al. (2011) suggested that the lipid content decreased after germination. This could be explained by the fact that lipid is used to produce energy during sprouting process (Wichamanee & Teerarat, 2012). However, the lipid contents of polished rice (0.49 g/100 g) and unpolished husked rice (1.78 g/100 g) were lower compared with the result in this study (Tee et al., 1997). Nevertheless, further validation studies are still needed. Hence, carbohydrate is the most abundant macronutrient in GBR, which majorly contributes to the GBR sample calories content with 390.95±11.31 kcal/100 g.

The total dietary fibre of GBR in this study was 9.18±0.91 g/100 g, where the content of insoluble dietary fibre (8.63±0.84 g/100 g) was significantly higher ($p< 0.05$) than soluble dietary fibre (0.55±0.12 g/100 g).

Table 1
Nutritional composition of GBR based on 100 g of raw sample

Components	Contents
Moisture (g/100 g)	14.04±0.10
Lipid (g/100 g)	2.11±0.09
Protein (g/100 g)	11.03±0.10
Carbohydrate (g/100 g)	54.30±1.04
Total dietary fibre (g/100 g)	9.18±0.91
Insoluble dietary fibre	8.63±0.84
Soluble dietary fibre	0.55±0.12
Ash (g/100 g)	1.19±0.01
Energy calories (kcal/100 g)	390.95±11.31
Minerals (mg/100 g)	
Sodium	34.41±0.85
Potassium	166.2± 5.77
Magnesium	86.43±7.15
Calcium	15.67±0.92
Iron	1.923±0.07
Zinc	1.848±0.01

The total dietary fibre of GBR was higher than non-germinated rice with only 0.38 g/100 g in polished rice and 0.48 g/100 g in unpolished husked rice (Tee et al., 1997). It can be explained by the formation of primary cell walls (pectin substances) in the middle lamella (Lee et al., 2007). These results were similar to other studies done on GBR (Megat Rusydi et al., 2011; Mohd. Esa et al., 2011), but higher than germinated rice in Thailand and Japan (Ohtsubo et al., 2005; Moongngarm & Saetung, 2010; Wichamanee & Teerarat, 2012). This also indicated that GBR in this study is a good fibre source among the rice

as intake of dietary fibre reduces the risk of developing several non-communicable diseases (Anderson et al., 2009). The ash content in this study was 1.19±0.01 g/100 g, which was higher than polished rice (0.49 g/100 g) (Tee et al., 1997) and unpolished husked rice (1.02 g/100 g) (Tee et al., 1997) but lower than those reported by Moongngarm and Saetung (2010) and Wichamanee and Teerarat (2012) on Thailand cultivars. Potassium was the highest mineral (166.2±5.77 mg/100 g) in GBR, followed by magnesium (86.43±7.15 mg/100 g), sodium (34.41±0.85 mg/100 g), calcium (15.67±0.92 mg/100 g), iron (1.923±0.07 mg/100 g) and zinc (1.848±0.01 mg/100 g). These results were similar those reported by Mohd. Esa et al. (2011).

Antioxidant Groups of Non-Polar and Polar Extracts

The phenolic compounds including flavonoids have been typically characterised as the compounds that exerted antioxidant activities which are important in the maintenance of health and protection from several diseases like coronary heart disease and cancer (Kähkönen et al., 1999). The results of antioxidant groups of non-polar and polar extracts of GBR sample are presented in Table 2. TPC was expressed in terms of gallic acid equivalent from a standard curve ($y = 6.4748x+0.0255$, $r^2 = 0.9996$). The polar extract of GBR sample contained significantly higher ($p< 0.05$) TPC (224.65±7.39 mg GAE/100 g of sample) than non-polar extract (41.60±2.15 mg GAE/100 g of sample). These results were implied that primarily the phenolics content in GBR were present in polar extract more than in non-polar extract. Phenolic acids such as ferulic, vanillic, caffeic, p-hydroxybenzoic, protocatechuic, p-coumaric and syringic acids are the most abundant polar compounds comprise in cereals (Kähkönen et al., 1999; Ma et al., 2013). The amount of TPC reported by Moongngarm and Saetung (2010) of methanolic extract on GBR cultivar RD-6 was only 84.3±6.35 mg GAE/100 g of the sample, which was lower than the TPC in this study. Alternatively, TFC was expressed in term of quercetin equivalent from a standard curve ($y = 1.2073x-0.0009$, $r^2 = 0.9995$). The TFC of GBR was similar to TPC, where the polar extract contained significantly higher ($p< 0.05$) TFC (125.31±11.91 mg QE/100 g of sample) than non-polar extract (11.06±1.67 mg QE/100 g of sample). TFC was represented about half of the TPC compounds in the polar extract and about one-quarter in the non-polar extract of GBR. Flavones such as apigenin, luteolin and tricetin are the predominant flavonoids in rice (Kim et al., 2008).

Antioxidant Activities of Non-Polar and Polar Extracts

Antioxidant compounds exert their activities through different ways. These include reducing agents, free radical scavengers, complexers of pro-oxidant metals and quenchers of singlet oxygen (Gordon, 1990). The existence of different antioxidant components in plant sources has become a relatively challenging task to measure their

AA. In this study, several methods have been employed to determine the AA of the non-polar and polar extracts of GBR sample.

In DPPH free radical scavenging assay, the absorbance of the DPPH radical decreased due to the presence of hydrogen donor from the antioxidant, resulting in the decolouration from purple to yellow. It is a rapid and highly sensitivity way in measuring the antioxidant efficiency of the sample (Blois, 1958). The DPPH free radical scavenging activity of the non-polar and polar extracts of GBR sample (Figure 1) was in dose-dependent manner, where the highest scavenging activity was at 5 mg/ml in non-polar extract (5.16±0.77%) and polar extract (29.94±1.17%), respectively. However, the ascorbic acid as a positive reference showed a better scavenging activity with 65.15±2.76% at concentration 125 µg/ml. In contrast, both non-polar and polar GBR extracts showed better AA in ABTS radical scavenging activity (Figure 2) compared to those obtained in DPPH reaction. The polar extract of GBR sample at 4 mg/ml (42.84±3.33%) and 5 mg/ml (47.01±1.55%) was shown to have better ability to reduce $ABTS^{\bullet+}$ than positive reference, BHT at 100 µg/ml (35.82±1.43%). In addition, there was a significant difference ($p< 0.05$) of scavenging activities between polar and non-polar extracts in both DPPH assay and ABTS assay. The scavenging activity of the non-polar extract was only shown at the concentration of 3 mg/ml and above in DPPH assay, indicating that the polar extract of GBR showed better AA than lipophilic extract.

The trend for the FRAP assay of GBR extracts (Table 2) did not vary markedly from their DPPH and ABTS results. The FRAP value was expressed as mM Fe (II) E/100 g of the sample with reference to ferrous sulphate ($y = 0.7075x+0.0118$, $r^2 = 0.9992$). The FRAP value of the non-polar extract (650.42±5.28 mM Fe (II) E/100 g of sample) was significantly lower ($p< 0.05$) than the polar extract (2063.13±37.40 mM Fe (II) E/100 g of sample). On the contrary, the β-carotene bleaching activity of the non-polar extract (37.84±4.32%) was not significantly different from polar extract (35.28±6.44%) in Table 2. The decrease in the absorbance of β-carotene in the presence of extracts with the oxidation of β-carotene and linoleic acid is shown in Figure 3.

Table 2

Comparison of the antioxidant groups and antioxidant activities of the non-polar and polar extracts of GBR

Antioxidant	Non-polar extract	Polar extract
Antioxidant groups		
TPC (mg GAE/100 g of sample)	41.60±2.15[a]	224.65±7.39[b]
TFC (mg QE/100 g of sample)	11.06±1.67[a]	125.31±11.91[b]
Antioxidant activities		
FRAP value (mM Fe(II)/ 100 g of sample)	650.42±5.28[a]	2063.13±37.40[b]
β-Carotene bleaching (%)	37.84±4.32[a]	35.28±6.44[a]

[a-b]Values within a row followed by different letters are significantly different at $p<0.05$. FRAP, ferric reducing antioxidant potential; TFC, total flavonoid content; TPC, total phenolic content.

Interestingly, the β-carotene bleaching activity of non-polar extract initially showed inferior activity than the polar extract but later it showed slightly better activity at 105 min and 120 min. This result suggested that although non-polar extract showed less effectiveness in antioxidant efficiency, it might have a more prolonged inhibition effect on the radical cation decolourisation than polar extract.

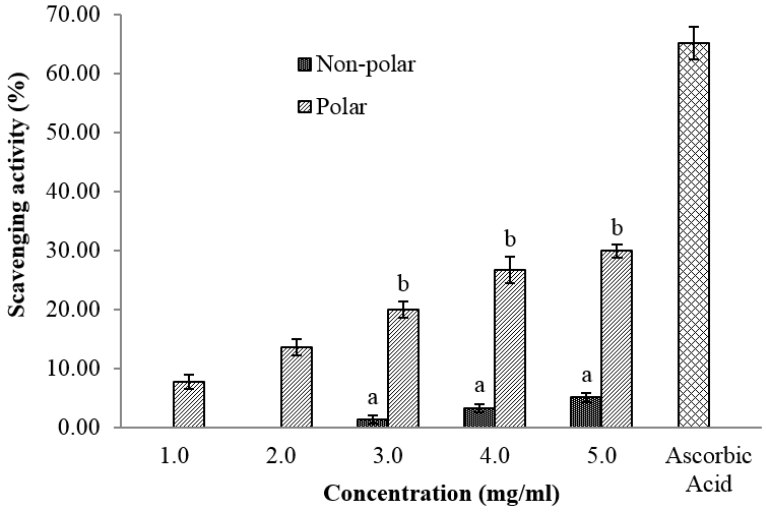

Figure 1. DPPH free radical scavenging activity of the non-polar and polar extracts of GBR at various concentrations and ascorbic acid (125 µg/ml). [a-b]Values with different letters are significantly different at $p< 0.05$ among the same concentration.

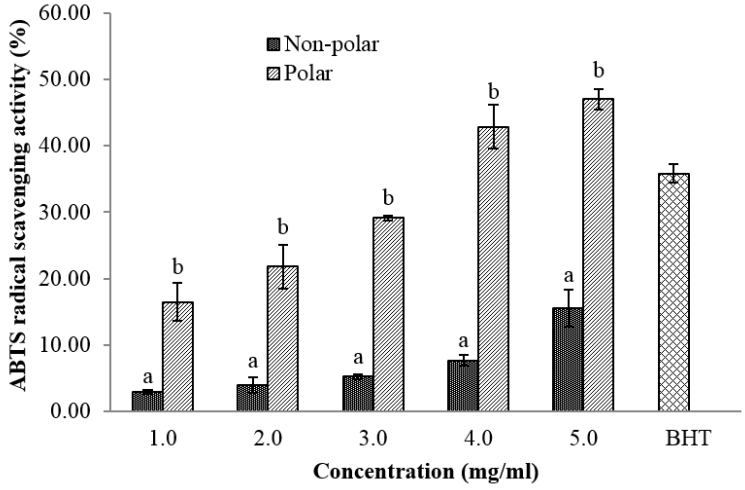

Figure 2. ABTS radical scavenging activity of the non-polar and polar extracts of GBR at various concentrations and BHT (100 µg/ml). [a-b]Values with different letters are significantly different at $p< 0.05$ among the same concentration.

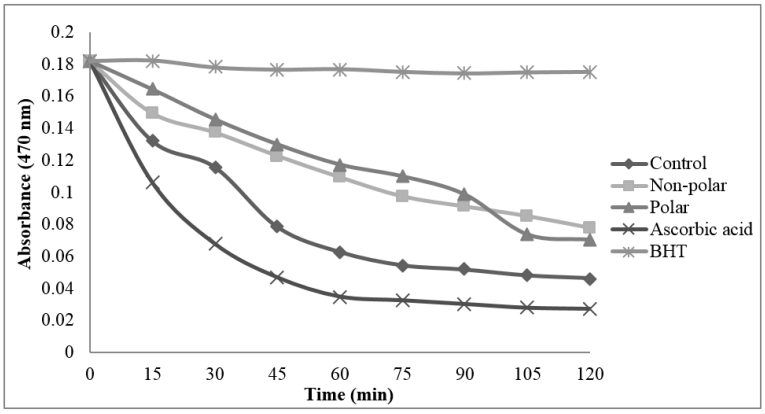

Figure 3. Absorbance value of the non-polar and polar extracts of GBR at 4mg/ml using β-carotene bleaching method. Higher absorbance at 470 nm indicates higher antioxidant activity.

In general, the results of this study concur with a previous report in 100 different kinds of food including fruits, vegetables, nuts, dried fruits, spices, cereals, infant and other foods, in which the AA of the non-polar extract of samples was lower than the polar extract (Wu et al., 2004). Jang and Xu (2009) also reported that the polar extracts of purple rice bran showed significantly higher phenolic content and free radical scavenging activity than the non-polar extract. Both the non-polar and polar extracts of GBR sample showed AA in this study; however, the polar extract was generally better. The antioxidant properties of rice sample is the result of combined activity of a wide range of compounds including vitamin E (α-tocopherol, α-tocotrienol, γ-tocopherol and γ-tocotrienol) (Xu et al., 2001), γ-oryzanol components (Xu et al., 2001), polyphenols (Hudson et al., 2000) and possibly other components.

Correlation between Antioxidant Groups and Antioxidant Activities

The relationships between antioxidant groups (TPC and TFC) and AA (DPPH free radical scavenging activity, FRAP value, ABTS radical scavenging activity and β-carotene bleaching activity) of the GBR sample are shown in Table 3. Very strong and positive correlations were observed between the antioxidant groups and AA ($r>0.90$ and $p<0.001$) according to Guildford's (1973) Rule of Thumb. This might relate to the phenolic and flavonoid compounds present in the non-polar and polar extracts. The presence of the hydroxyl group of the phenolic compounds and the second hydroxyl group in the *ortho* and *para* position forming resonance-stabilised phenoxyl radicals (Chen & Ho, 1997) clarified the antioxidative activity of the extracts. Moreover, flavonoids are one of the most diverse and widespread group of natural compounds in phenolics (Agrawal,

Table 3
Correlation coefficient (r) between antioxidant groups and antioxidant activities of GBR extracts and between assays

Antioxidant assay	TFC	DPPH	FRAP	ABTS	β-Carotene bleaching
TPC	0.997*	0.987*	0.997*	0.990*	-0.304
TFC	1	0.975*	0.989*	0.981*	-0.357
DPPH	0.975*	1	0.996	0.996*	-0.203
FRAP	0.989*	0.996*	1	0.996*	-0.251
ABTS	0.981*	0.996*	0.996*	1	-0.186
β-Carotene bleaching	-0.357	-0.203	-0.251	-0.186	1

*Correlation was significant at the 0.01 level (2-tailed). ABTS, ABTS radical scavenging capacity; DPPH, DPPH radical scavenging capacity; FRAP, ferric reducing antioxidant potential; TFC, total flavonoid content; TPC, total phenolic content.

1989). This also explained the reason of a strong and positive relationship existed between TPC and TFC ($r=0.997$, $p<0.001$). However, no significant relationship ($p>0.05$) was found between antioxidant groups with β-carotene bleaching.

Furthermore, very strong and significant correlations were also found between the various methods used to determine the AA of the GBR extracts ($r>0.90$ and $p<0.001$). However, just like the correlation between antioxidant groups with β-carotene bleaching, no significant correlation was found between β-carotene bleaching with other methods ($p>0.05$). Unlike the others, the β-carotene bleaching assay is based on hydrogen atom transfer, which might explain the divergence between the results obtained with the β-carotene bleaching assay and those obtained with other assays that are based on single-electron transfer mechanism (Huang et al., 2005).

CONCLUSION

The nutritional composition of the GBR sample obtained in this study is useful to provide update for food composition database. GBR could be used as a new functional food as it is low in carbohydrate but high in protein and fibre contents. The level of TPC and TFC in the GBR polar extract and their AA was significantly higher than that in the non-polar extract. Further studies could focus on their AA using *in vivo* model.

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