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Antioxidant properties of ten high yielding rice varieties of Bangladesh

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ABSTRACT

Objective: To study total phenolic content and antioxidant properties of 80% methanol extracts of ten high yielding rice varieties, five each from two different seasons namely *aman* and *boro* of Bangladesh. **Methods:** Total phenolic content was measured by Folin–Ciocalteau method while 1,1–diphenyl–2–picrylhydrazyl (DPPH) radical scavenging, hydroxyl ion scavenging, ferric reducing antioxidant power (Ferric reducing antioxidant power), and total antioxidant capacity (TAC) by ammonium molybdate, were used to analyze their Antioxidant properties. **Results:** Rice variaty BR5 of *aman* and BRRI dhan28 of *boro* season comparatively showed higher TPC and Antioxidant properties than the other rice varieties. BR22 of *aman* season showed the highest hydroxyl ion scavenging activity although it displayed the lowest TPC. Except for hydroxyl ion scavenging activity, *aman* rice varieties displayed comparatively higher total phenolic content and antioxidant property than the *boro* rice varieties. **Conclusions:** The results of the present study implies that the selected rice varieties possess moderate antioxidant capacity and therefore, can be considered as health supplements and nutraceuticals foods as rice is the staple food of Bangladesh.

1. Introduction

Rice is the staple food for nearly 50% of the world population[1] and Asia represents about 90% of global rice production and consumption. Bangladesh is the world's 6th largest rice–producer where people get more than 70% of their total calorie from staple food–rice, providing carbohydrate and some other proteins, vitamins and minerals. Rice has the potential to promote human health, due to its content of phenolic compounds that are able to inhibit the formation or reduction of the concentrations of reactive cell–damaging free radicals, thereby reducing the risk of coronary heart disease and cancer[2,3] and preventing oxidative damage of lipid and low–density lipoproteins[4]. It has been well accepted that natural antioxidants may inhibit lipid peroxidation in food products and improve food quality and safety[5] as well as improve the redox status in biological systems and reduce the risk of aging associated health problems[6–9].

In order to cope with the increasing population, food security, nutrition, urbanization, climate change and changing in food preferences, Bangladesh Rice Research Institute (BRRI) has introduced many high yielding rice varieties and till today they have released 57 new varieties which are growing in three different seasons namely *aush*, *aman* and *boro*. *Aman* is the main monsoon season in Bangladesh (July to November) and *aush* is a short season (April–May) that follows the dry season or *boro* (November– December to April–May). The selected high yielding rice varieties BR5, BR22, BRRI dhan34, BRRI dhan37, and BRRI dhan38 grows in *aman* season and BR7, BR16, BRRI dhan28, BRRI dhan29, and BRRI dhan50 grows in *boro* season.

In the present study, we examined antioxidant properties of ten high yielding rice varieties of Bangladesh, growing in two different seasons and determined the potential correlation between total phenolic content and the antioxidant properties among those rice varieties. This information is needed for production of value–added rice grain high in natural antioxidants.

2. Materials and methods

2.1. Chemicals and reagents

Sodium carbonate, disodium hydrogen phosphate, potassium dihydrogen phosphate, Folin–Ciocalteau's phenol reagent, sodium salicylate, trichloroacetic acid, methanol and iron (II) sulfate–7–hydrate were purchased from Merck Chemicals. 1, 1–diphenyl–2–picrylhydrazyl (DPPH) was purchased from Sigma Aldrich. All the

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reagents are analytical and HPLC grade.

2.2. Extraction of rice antioxidants

Ten rice samples were cleaned and milled on a Satake mill for separating into bran and brown rice fraction. Brown rice was then polished and five grams of polished rice were ground to 80 mesh and extracted for 20 h with 50 mL of 80% methanol (v/v) at ambient temperature. The extractions were filtered through Whatman-40 filter paper and were kept -20 ℃ until further analysis.

2.3. Total phenolic content

The total phenolic content of methanol extract was evaluated by Folin-Ciocalteau method[10]. Gallic acid was used for calibration. Results were expressed as mg of gallic acid equivalents (GAE) per 100 g of dry weight of the rice flour.

2.4. Ferric reducing antioxidant power

The reducing power of rice extracts was determined according to the method of Turkmen *et al*[11]. All the analysis were run in triplicate and averaged. Ferric reducing antioxidant power values were expressed as μM ascorbic acid equivalent (AAE) per 100 g of dry weight of the rice flour.

2.5. DPPH radical scavenging activity

The free radical scavenging activity of methanol extracts of rice was measured by 1,1-dipheyl-2-picryl-hydrazyl (DPPH) using the method described by Oktay *et al*[12]. All analysis was run in triplicate and averaged. Radical scavenging activity was expressed as inhibition percentage and was calculated using the formula:

% Radical scavenging activity = (Control OD-Sample OD/ Control OD) × 100.

2.6. Total antioxidant capacity

The assay is based on the reduction of Mo(VI)-Mo(V) by the extract and subsequent formation of a green phosphate/ Mo(V) complex at acidic pH[13]. TAC values were expressed as μM ascorbic acid equivalent (AAE) per 100 g of dry weight of the rice flour.

2.7. Hydroxyl ion scavenging activity

The scavenging capacity of rice extract on hydroxyl ion (OH.) was evaluated according to the reaction of sodium salicylate and residual hydroxyl radical. OH. scavenging activity was performed according to a literature procedure[14]. The scavenging activity was calculated using the following Eq. (1):

$$\text{Scavenging or inhibition rate } (\%) = [1-(A_1-A_2)/A_0] \times 100$$

where A_0 is the absorbance of the control (without extract), A_1 is the absorbance of the extract addition and A_2 is the absorbance without sodium salicylate.

2.8. Statistical analysis

All the statistical analysis was conducted by SPSS (ver. 16). Regression analysis was performed in Microsoft Excel-2007. Results were expressed as means±SD where all the analysis were done in triplicate.

3. Results

3.1.Total Phenolic Content (TPC)

Total phenolic content was determined by the Folin-Ciocalteau (FC) method and the results are presented in Table 1. TPC was expressed as mg GAE/100g of dry weight of the rice flour. For aman rice, TPC ranged between 13.58 ± 0.45 mg/100g (BR22) to 25.30 ± 0.52 mg/100g (BR5). BRRI dhan37 had the TPC value of 21.14 ± 0.09 mg/100g and BRRI dhan34 displayed 18.66 ± 0.98 mg/100g which was higher than BRRI dhan38 (17.42 ± 0.26 mg/100g). On the other hand, for boro rice, TPC ranged between 10.78 ± 0.71 mg/100g (BR16) to 18.42 ± 0.45 mg/100g (BRRI dhan28). TPC values of BR7 and BRRI dhan50 was registered as 16.62 ± 00 mg/100g and 15.87 ± 2.85 mg/100g, respectively. In this group, BR16 had the lowest TPC value of 10.78 ± 0.70 mg/100g. (Table 1)

3.2. Ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power measures the ability of the antioxidant to reduce ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) which is characterized by the formation of Perl's Prusian Blue. FRAP values were expressed as μM ascorbic acid equivalent (AAE) per 100 g of dry weight of the rice flour. Similar to the results of TPC, in aman rice group, BR5 had the highest amount of FRAP (195.78 ± 1.96 μM/100g) while

Table 1

Total phenolic content, ferric reducing antioxidant power, and total antioxidant capacity of methanol extracts of *aman* and *boro* rice.

Season	Variety	Total phenolic content (mg GAE/100g)	Ferric reducing antioxidant power (μM AAE/100g)	Total antioxidant capacity (μM AAE/100g)
aman	BR5	25.30±0.52^a	195.78±1.96^a	701.16±1.44^a
	BR22	13.58±0.45^b	112.87±1.90^b	373.07±1.50^b
	BRRI dhan34	18.66±0.98^c	155.71±1.48^c	516.37±2.68^c
	BRRI dhan37	21.14±0.09^d	147.70±1.97^d	585.45± 2.47^d
	BRRI dhan38	17.42±0.26^c	139.34±1.88^e	481.83± 7.32^c
boro	BR7	16.62±0.00^w	106.60±0.00^w	459.38±0.00^{wy}
	BR16	10.78±0.70^x	90.22±0.48^x	297.04±19.53^x
	BRRI dhan28	18.42±0.45^y	113.56±3.94^w	518.10±14.65^z
	BRRI dhan29	17.67±0.08^y	114.26±6.89^w	488.74±2.44^{yz}
	BRRI dhan50	15.87±2.85^w	142.83 ±4.92^y	440.38±81.15^w

Values of total phenolic content , ferric reducing antioxidant power and total antioxidant capacity are means±SD (n = 3). For each column, values followed by the same letter (a-e) and (w-z) are not statistically different at a $P < 0.05$, as measured by the Duncan test.

BR22 had the lowest (112.87 ± 1.90 μM /100g). FRAP values of BRRI dhan34, BRRI dhan37, and BRRI dhan38 were 155.71 ± 1.48 μM /100g, 147.70 ± 1.97 μM /100g, and 139.34 ± 1.88 μM /100g respectively. On the other hand, unlike TPC, in boro rice group, BRRI dhan50 displayed the highest FRAP (142.83 ± 4.92 μM /100g) followed by BRRI dhan29 (114.26 ± 6.89 μM /100g), BRRI dhan28 (113.56 ± 3.94 μM /100g), BR7 (106.60 ± 00 μM /100g), and BR16 (90.22 ± 0.48 μM /100g). On the basis of significant ($^{a}P < 0.05$) differences in their antioxidant activity, BRRI dhan50 was in the highest group, medium group comprised BRRI dhan29, BRRI dhan28, BR7 and BR16 was solely in the lowest group.

Table 2

IC_{50} values of DPPH radical scavenging activity of *aman* and boro rice.

season	variety	IC_{50} (mg/mL)
aman	BR5	6.01 ± 0.11^{a}
	BR22	14.47 ± 0.31^{b}
	BRRI dhan34	6.72 ± 0.04^{a}
	BRRI dhan37	7.30 ± 1.43^{a}
	BRRI dhan38	7.45 ± 0.92^{a}
boro	BR7	9.85 ± 0.74^{wx}
	BR16	12.28 ± 0.63^{y}
	BRRI dhan28	10.73 ± 0.66^{x}
	BRRI dhan29	7.65 ± 0.38^{z}
	BRRI dhan50	8.56 ± 0.15^{z}

In the column, values followed by the same letter (a–b) and (w–z) are not statistically different at a $P < 0.05$, as measured by the Duncan test.

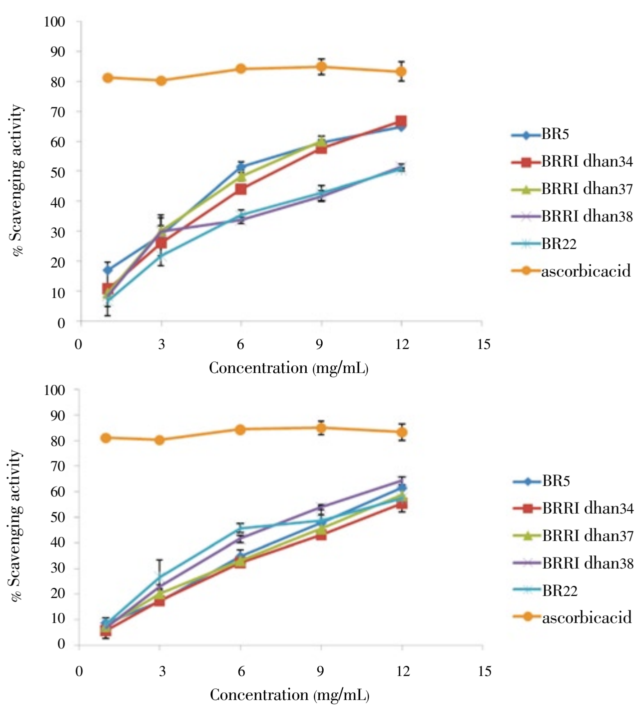

Figure 1. DPPH radical scavenging activity of *aman* (A) and boro (B) rice

3.3. DPPH radical scavenging activity

The results in the Figure 1 showed that all the extracts displayed potential scavenging activity in a dose dependent manner. From the half maximal inhibitory concentration (IC50; the effective concentration at which the DPPH radicals were scavenged by 50%) it was seen that for aman rice, BR5 showed the greatest scavenging activity with the value of 6.01 ± 0.11 mg/mL, followed by BRRI dhan34 (6.72 ± 0.04 mg/mL), BRRI dhan37 (7.30 ± 1.43 mg/mL), BRRI dhan38 (7.45 ± 0.92 mg/mL) and BR22 (14.47 ± 0.31 mg/mL). In the boro group, BRRI dhan29 showed the greatest scavenging activity with the value of 7.65 ± 0.38 mg/mL, followed by BRRI dhan50 (8.56 ± 0.15 mg/mL), BR7 (9.85 ± 0.74 mg/mL), BRRI dhan28 (10.73 ± 0.66 mg/mL) and BR16 (12.28 ± 0.63 mg/mL). (Table 2).

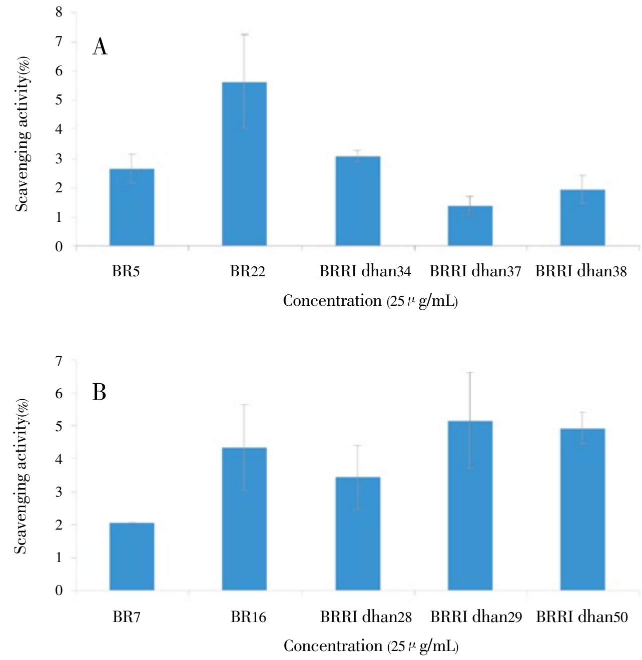

Figure 2. Hydroxyl ion scavenging activity of *aman* (A) and boro (B) rice.

3.4. Total antioxidant capacity (TAC)

Data presented in the Table 1 showed that all the rice varieties had potential antioxidant capacity. For aman rice, BR5 displayed the highest TAC (701.16 ± 1.44 μM /100g) which is nearly double than that of BR22 (373.07 ± 1.50 μM /100g) which displayed the lowest. BRRI dhan37 also showed notable antioxidant capacity with the value of 585.45 ± 2.47 μM /100g followed by BRRI dhan34 (516.37 ± 2.68 μM /100g) and BRRI dhan38 (481.83 ± 7.32 μM /100g). On the other hand, for boro rice, BRRI dhan28 showed the highest TAC (518.10 ± 14.65 μM /100g) followed by BRRI dhan29 (488.74 ± 2.44 μM /100g), BR7 (459.38 ± 00 μM /100g), BRRI dhan50 (440.38 ± 81.15 μM /100g), and BR16 (297.04 ± 19.53 μM /100g).

3.5. Hydroxyl ion scavenging activity

Hydroxyl ion scavenging activity was tested at 25 μg/mL concentration for all the varieties and the results are expressed as percent scavenging activity (Figure 2). BR22 displayed the highest antioxidant activity with the value of (5.62±1.46). Other than BR22, BRRI dhan34 displayed hydroxyl ion scavenging activity (%) with the value of (3.07±0.20) followed by BR5 (2.63±0.49), BRRI dhan38 (1.95±0.50), BRRI dhan37 (1.37±0.33). On the other hand, in the *boro* rice group, BRRI dhan29 showed the highest antioxidant activity (%) with the value of (5.16±1.46), followed by BRRI dhan50 (4.93±0.48), BR16 (4.36±1.30), BRRI dhan28 (3.44 ±0.97). Compared to the *aman* rice, *boro* rice showed comparatively higher antioxidant activity. Correlation

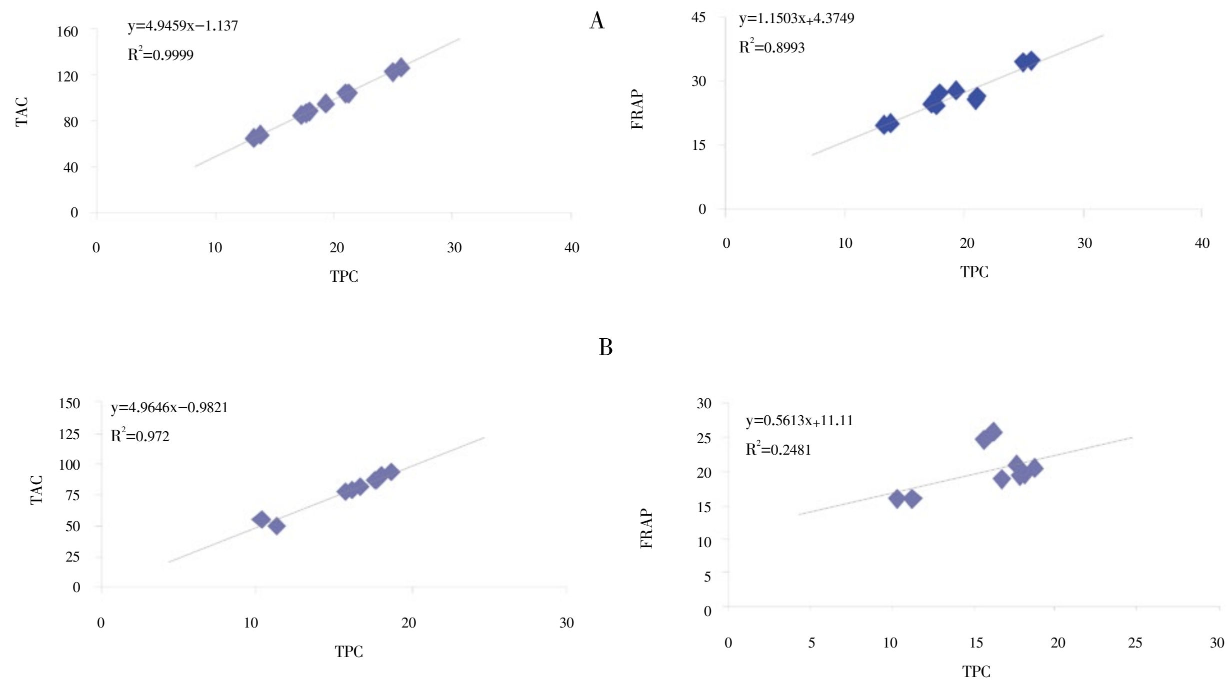

Figure 3. Correlation between total phenolic content and antioxidant activities of *aman* (A) and *boro* (B) rice. TAC: Total antioxidant capacity; FRAP: Ferric reducing antioxidant power.

between total phenolic content and antioxidant activities of *aman* (A) and *boro* (B) rice.

4. Discussion

Rice contains a variety of phenolic compound especially ferulic acid, p-coumaric acid and diferulate that are not present in significant quantities in fruits and vegetables[15]. Compared to *boro* rice, *aman* rice contained more phenolic compounds. The possible reason for this variation could be due to the several factors including difference in the rice variety, growing season, soil condition, degree of maturity etc.[16]. Recently Gunaratne *et al*[17], found similar level of total phenolic content in the polished rice of Sri lanka. On the other hand, Qiu *et al*[10] found higher Total phenolic content in commercial wild rice of China. In addition, Butsat *et al*[18] also found comparatively higher levels of phenolic compound in the milled rice of Thiland. The difference could be attributed to the polishing of rice as different countries follow different degree of polishing depending on their consumer choice which is supported by the earlier reports that brown rice, bran and husk contained very high amount of phenolic compound compare to the polished rice[18].

Significant difference ($P < 0.05$) was observed in Ferric reducing antioxidant power values between the *aman* and *boro* rice varieties. These data indicated that growing season as well as the difference in the variety might contribute to the difference in their antioxidant activity.

DPPH is commercially available nitrogen centered stable free radical which is destroyed by a free radical scavenger. The method is based on the measurement of the loss of deep purple color of DPPH after reaction with the test compound functioning as a proton radical scavenger or hydrogen donor[19]. Consistent with the results of Total phenolic content and Ferric reducing antioxidant power, *boro* rice varieties were found less potent in terms of IC_{50} values compare to the *aman* rice. It might be due to the effect of temperature variation as well as other environmental factors during cultivation and harvesting period of the rice varieties. During *aman* season (July to November) heavy rain fall occurs with occasional extreme heat and when harvest starts in early winter (November) temperature drops to 20 ℃ to 25 ℃. On the other hand, *boro* season starts in early winter and during the harvesting period (April-May) temperature is very high. Our findings are also supported by the previous reports of Wang and Zheng[7], showing that environmental temperature strongly alters antioxidant properties in strawberry. Furthermore, the observation of Yu *et al.*[20] who studied the effect of location on antioxidant activity of wheat also supports the findings of the present work. Although genetic variations among the rice varieties could play a vital role for these discrepancies, it should also be pointed out that cultivation of the *aman* varieties in *boro* season and vice verse was not possible as they are season specific.

Total antioxidant capacity was determined by phosphomolybdenum method which is based on the reduction of Mo(VI) to Mo(V) by the antioxidant and the formation of a green phosphate/ Mo(V) complex with a maximal absorption at 695 nm. As this method is simple, rapid and independent of other antioxidant measurements commonly employed, it was decided to extend its application to the rice extracts. No significant difference ($P > 0.05$) was observed between the *aman* and *boro* rice as analyzed by One-way ANOVA. Data on total antioxidant capacity of polished rice based on this method is not available.

Hydroxyl radical is the most reactive free radical in the biological system and it has been regarded as the highly damaging to almost every molecule found in the biological system. It can conjugate with nucleotides in DNA and cause strand breakage which leads to ultimately mutagenesis, carcinogenesis, and cytotoxicity[21]. For *aman* rice, interestingly BR22 displayed the highest hydroxyl ion scavenging activity which is in contrast to the results of the Total phenolic content, Ferric reducing antioxidant power and total antioxidant capacity. One of the possible reasons for this variation could be the difference in the mechanism of action of these methods[22]. Compared to the *aman* rice, *boro* rice showed comparatively higher antioxidant activity.

The data was subjected to statistical analysis using One-way ANOVA. The relationship between total phenolic content and the antioxidant activities among the rice varieties of the different seasons was evaluated by regression analysis (Figure 3). For *aman* rice, the statistical analysis showed a positive and highly significant (R^2 = 0.887, P < 0.001) correlation between Total phenolic content/Ferric reducing antioxidant power and (R^2 = 0.999, P < 0.001) for Total phenolic content/TAC. Our results are in agreement with the previous studies concerning the relationship between the total phenolic content and antioxidant activities[11, 23]. On the other hand for *boro* rice varieties, no relation was found between Total phenolic content and Ferric reducing antioxidant power (R^2 = 0.154, ^{d}P > 0.001). Our findings are supported by the previous studies by Kahkonen *et al*[24] where they did not find any correlation between total phenolic content and the antioxidant activities. Responses to Folin-Ciocalteau reagent by the different phenolic compound are different, so it is very difficult to correlate phenolic content with the antioxidant activities. However a strong significant correlation (R^2 = 0.972, ^{c}P < 0.001) has been found between total phenolic content and total antioxidant capacity. Gupta *et al*[25] also corroborated our findings.

This study shows that all the rice extracts are moderate in polyphenol content as well as antioxidant capacity. Overall highly significant correlations were found between the total phenolic content and the antioxidant activities. The demand for rice is constantly rising in Bangladesh with nearly 2.3 million people being added each year to its population of about 160 million. Considering the importance of rice in Bangladesh as it is the main staple food, if rice varieties with high level of phenolic compounds and antioxidant capacities can be invented, it will serve as dietary source of natural antioxidant for disease prevention and ultimately will make contribution in health promotion.

Conflict of interest

We declare that we have no conflict of interest.

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