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Biochemical and antioxidant activities of pigmented landraces of *Oryza sativa* - Koraput District, Odisha, India

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Abstract

Ten pigmented landraces of rice were analyzed for chemical composition and antioxidant properties. The results of proximate composition and calcium, iron, zinc content showed intra variability among landraces. The amylose, total anthocyanin (TAC) and phytate contents were found to be in the range of 22.01 - 44.38%, 28.73 - 193.36 mg cyanidin-3-glucoside/100 g and 1.16 - 24.49 mg Fe/100 g respectively. Total phenol and polyphenol contents were in the range of 233.92 - 251.38 mg ferulic acid equivalent/100 g and 252.43 - 284.36 mg gallic acid equivalent/100 g, respectively. Antioxidant assays of landraces showed free radical scavenging activity.

Introduction

Asian cultivated rice, *Oryza sativa* is one among the three major food crops consumed worldwide and the staple food crop for almost the whole of Asia. Rice was domesticated between 8,000 and 10,000 years ago from its wild ancestor, *Oryza rufipogon*, a broadly distributed native species of Asia (Oka, 1988). The genetic diversity of the landraces of rice in the Jeypore tract of which Koraput district forms a part, of Odisha has been recorded by several researchers (Ramiah and Ghose, 1951; Govindaswami and Krishnamurty, 1959; Oka, 1974; Patra and Dhua, 2003; Tripathy *et al.*, 2005). These landraces are claimed to contain sources of useful genes for tolerance to physiological and ecological stress, disease and pest resistance and good quality traits (Sharma *et al*., 1996; Tripathy *et al.*, 2005). Japanese scientist, Oka (1988) had studied the population genetics of these landraces, wild rices of the area and their natural hybrids and regarded these landraces as forms intermediate between cultivated and wild types "still staying in the midst of differentiation" (Oka and Chang, 1962).

Landraces are rice cultivars that are intermediate from between wild rice and modern varieties that adapt to natural environment. Landraces of rice cultivated in remote agricultural areas by farmers mostly on a small scale for consumption, and are genetically diverse. There is a gradually decline in area under their cultivation due to various social and economic factors (Harlan, 1975). Over the past four decades, landraces of rice have been largely replaced by genetically uniform modern varieties in many parts of Asia including vast regions of China and Vietnam (Pingali and Rajaram, 1998). Owing to their diversified genetic variations, they are flexible, adapted to local and changing environment, farming practices and specific uses such as animal and human consumption (McCouch, 2004) making it the most immediately useful and valuable component of biodiversity (Wood and Lenne, 1997).

Considering the health-embracing aspects of these landraces, cultivating them in large scale and commercializing their consumption may result in reduction of causes of malnutrition. Since most of the cultivable lands have been converted for various purposes, these landraces which are flexible and adaptable to changing environment may serve as an eye-opener in the field of agriculture and nutrition. The present studies thus involve determination of chemical composition and antioxidant activities of ten pigmented landraces of rice and bring to light their health benefits and open possibilities of cultivation and commercialization on a large scale.

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Materials and Methods

Materials

Ten pigmented landraces of rice were collected from the Biju Patnaik Medicinal Plants Conservation plots, Jeypore from Koraput District, Odisha. The rice grain obtained were shade dried to remove the moisture content and samples dehusked manually to separate husk. 100 dehusked seeds and husk of each sample were weighed separately. 250 g of dehusked seeds of each sample was ground to fine powder using a table top mixture and stored at 4°C until further analysis.

Chemicals

Hydrogen peroxide from Sigma-Aldrich (Bengaluru, India); Sodium hydroxide, starch, ethylene diammine tetra acetic acid, ferulic acid, potassium chloride, sodium acetate, 2-deoxy-2-ribose, ascorbic acid, thiobarbituric acid, 2, 4, 6- tripyridyl-s-triazine (TPTZ), 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), 2,2'-diphenyl-1-picrylhydrazyl, sodium acetate trihydrate, potassium persulfate, ascorbic acid, ferric chloride and methanol from Himedia (Mumbai, India); Ethanol, acetic acid, trichloro acetic acid, nitric acid, dichloromethane, chloroform, potassium thiocyanate, ferric nitrate, glacial acetic acid, iodine, sodium carbonate from Merck (Bengaluru, India); Hydrochloric acid and HPLC water from Fisher Scientific (Mumbai, India); Folin–Ciocalteu reagent from Sisco Research Laboratories (Mumbai, India) were used for the experiment. All chemicals and solvents used for the experiments were of analytical and HPLC grade.

Analysis of proximate composition of landraces

Moisture content Ref. 934.01 at 95°C-100°C under pressure ≤ 100 mm Hg (ca 5 h), crude protein was analysed Ref. 984.13, fat (ether extract) Ref. 920.39, total ash was determined at 600°C for 2 h Ref. 942.05 and crude fiber Ref. 962.09 were all determined using AOAC (2006) methods. Total carbohydrate was calculated by difference [100- (crude protein + crude fat + ash + crude fiber)] (AOAC, 2006). Energy value was calculated by Pearson's method (Kirk and Sawyer, 1991).

Estimation of mineral content in landraces

The Ca, Fe and Zn analysis of ten landraces of rice were performed by AOAC method, Ref. 985.01 (AOAC, 2006). 1 g of dried and pulverized rice grain were weighed in a porcelain crucible and burnt to ash at 500°C for 2 h. The contents of the crucible were cooled to room temperature and moistened with 10 drops of water. Further 3 or 4 ml of (1+1) nitric acid was added to the crucible and nitric acid evaporated at 100°C on a hot plate. The crucible was returned to the furnace for an additional 1 h at 500°C. The contents of the crucible were allowed to cool to room temperature, dissolved in 10 ml of (1+1) hydrochloric acid, quantitatively transferred to a 50 ml volumetric flask, and brought to volume with distilled water. Quantification of metals was done by Inductively Coupled Plasma (ICP) spectroscopic method as indicated at the wavelength of 317.9 nm (calcium), 372.0 nm (iron) and 213.8 nm (zinc).

Determination of amylose content

1 ml of ethanol (95%) and 9 ml 1 N NaOH were added to 100 mg of flour. After mixing, the samples were heated for 10 min in a boiling water bath to gelatinize the starch, cooled and transferred to a 100 ml volumetric flask and 5 ml of starch solution and 1 ml 1 N acetic acid added to it. After addition of 2 ml iodine solution, the volume was adjusted to 100 ml with distilled water, mixed, and allowed to stand for 20 min. The absorbance was measured at 620 nm against a blank of distilled water using spectrophotometer (UVmini-1240, Shimadzu Corp., Japan). The amylose content was determined from the standard curve of potato amylose (Juliano, 1985) and classification of amylose content (IRRI, 2009). Amylose content was calculated using the formula:

$$\text{Amylose content} = (R/A)\ (a / r)\ (1/5)\ (100)$$

Where R-Sample reading, A- Standard reading, a- Amount of Standard Amylose (g), r- Amount of sample taken (g)

Determination of total phenolic content

1.5 g of each sample was transferred to centrifuge tubes and 85% methanol (25 ml) added and the mixture put in a shaker for 30 min to ensure uniform mixing. Then, the methanol layer in each tube was separated by centrifugation at 10000 g for 10 min. The solvent supernatant was transferred to a 50 ml falcon tube. The residue was again mixed with 15 ml methanol and centrifuged as described above. The supernatant was separated as previously described and mixed with the former supernatant. The tube containing supernatant was adjusted to 50 ml using methanol. The extracted solution was kept in 0°C until analysis (Sompong *et al*., 2011).

The TPC of extracts were determined using the Folin-Ciocalteu reagent (Singleton *et al*., 1999). Extract (120 µl) was added to 600 µl of freshly diluted 10-fold Folin–Ciocalteu reagent. 960 µl of sodium carbonate solution (75 g/l) was added to the

mixture after 2 min reaction time. The absorbance of the resulting blue colour was measured at 760 nm using spectrophotometer (UVmini-1240, Shimadzu Corp., Japan) against a blank of methanol after 5 min of reaction at 50°C. Ferulic acid was used as standard and TPC was expressed as mg ferulic acid (FA) equivalent per 100 g flour.

Determination of total anthocyanin content

1 g of each sample was transferred into a test tube (25 × 150 mm) to which 85% methanol (3 ml) was added, and the mixture vortexed for 30 sec. The tubes were capped and placed in a 60ºC water bath for 20 min. These test tubes were vortex mixed twice during incubation. Then, the methanol layer in each tube was separated by centrifugation at 10000 g for 10 min. The solvent supernatant was transferred to a 10 ml volumetric flask. The residue was again mixed with 3 ml of methanol. The supernatant was separated as previously described and combined with the previous supernatant. The tube containing supernatant was adjusted to 10 ml. The extracted solution was kept in 0°C until analysis (Jang and Xu, 2009).

The total anthocyanin content (TAC) was determined by the pH-differential method (Giusti and Wrolstad, 2001). 1 ml of the extract was made up to 10 ml using pH 1.0 potassium chloride and pH 4.5 Sodium acetate buffer respectively in sets of two and equilibrated for 15 min at room temperature. The absorbance of each dilution was measured at 510 nm and 700 nm against the blank cell filled with distilled water. All measurements were made between 15 min to 1 h after preparation of sample, since longer standing time tends to increase observed readings.

The absorbance of the diluted sample (A) is calculated as follows:

$$A = (A_{510nm}\text{-}A_{700nm})\ pH\ 1.0 - (A_{510nm}\text{-}A_{700nm})\ pH\ 4.5$$

The monomeric anthocyanin pigment of the samples were calculated using the following formula

$$\text{Monomeric anthocyanin pigment (mg/l)} = (A \times MW \times DF \times 1000)/ (\varepsilon \times l)$$

and was converted into mg of total anthocyanin content/100 g of sample.

Where MW is the molecular weight of cyanidin-3-glucoside, DF is the dilution factor, ε is the extinction co-efficient of cyanidin-3-glucoside, where MW = 449.2 (g/mol), DF = 10, ε = 26,900(L/mol cm) and L = 1(the path length in cm).

Estimation of phytate content

Different concentration of the ground sample was taken and 25 ml of 3% TCA was added and kept in a shaker for 30 min. It was then centrifuged at 10000 g for 10 min and 10 ml of the aliquot transferred to fresh tube and 4 ml of $FeCl_3$ added and kept in boiling water bath for 45 min. This was followed by centrifugation for 10 min and the supernatant decanted and the precipitate washed twice using 25 ml of 3% TCA. This was heated again and centrifuged, and the precipitate dispersed with few ml of water. 3 ml of 1.5 N NaOH was added. This volume was made up to 30 ml using water and kept in boiling water bath for 30 min. This mixture was filtered using Whatmann filter paper 2 and the precipitate dissolved using 40 ml of hot HNO_3. The flask was cooled and brought to room temperature. 2.5 ml of this aliquot was taken and made up to 35 ml using water and 10 ml of 1.5 M KSCN was added and the absorbance read at 480 nm within 1 min (Wheeler and Ferrel, 1971). The phytate content, P was calculated using the formula,

$$\text{Phytate (P) mg /100 g sample} = \text{mg Fe} \times 15 / \text{Weight of sample (g)}$$

Determination of polyphenol content

10 g of each sample were measured accurately and 10 mL of Methanol/water/ formic acid (250/ 242.5/ 7.5 ml) added and allowed to incubate for 24 h. After incubation, the samples were centrifuged at 10000 g for 15 min. The supernatant was transferred to fresh tubes and stored at 0ºC until further analysis (Gomez-Plaza *et al.*, 2000). Folin-Ciocalteau reagent was used for determination of polyphenol (Alvarez-Parrilla *et al*., 2007). Briefly, Folin Ciocalteau (FC) reagent was diluted with water in the ratio 1:9 (v/v). To the sample extract of different concentrations (10, 20, 30, 40 µL), 2.5 ml of the FC reagent was added. After 2 min of incubation at room temperature, 2 ml sodium carbonate (75 g/L) was added. The mixture was incubated at 50ºC for 15 min and allowed to cool in ice-bath. Within 15 min, absorbance was read at 760 nm by spectrophotometer (UVmini-1240, Shimadzu Corp., Japan) against a blank of methanol. The standard curve was obtained using gallic acid.

Antioxidant activity

Preparation of extract

1.5 g of each sample was transferred to centrifuge tubes and 85% methanol (25 ml) added and the mixture put in a shaker for 30 min to ensure uniform mixing. Then, the methanol layer in each tube was separated by centrifugation at 10000 g for 10 min. The solvent supernatant was transferred to a 50 ml falcon tube. The residue was again mixed with 15 ml methanol and centrifuged as before. The supernatant

was separated as previously described and mixed with the former supernatant. The tube containing supernatant was adjusted to 50 ml using methanol. The extracted solution was kept in 0°C until analysis (Sompong *et al*., 2011).

Determination of scavenging ability using DPPH assay

DPPH is used to estimate the ability of the extracts to scavenge free radicals. Four different concentrations of rice extract (1000, 2000, 3000 and 4000 µg/ml) were added to 2850 µl of 200 µM DPPH solution in methanol. After incubation in dark for 1 h, the absorbance at 517 nm was measured using spectrophotometer (UVmini-1240, Shimadzu Corp., Japan) against a blank of methanol. The standard solution for DPPH scavenging activity was ascorbic acid which was used in the range of 0-0.02 mg/ml (Hatano *et al*., 1988).

The percentage of radical-scavenging ability was calculated:

Scavenging ability (%) =
[Absorbance 515 nm of control - Absorbance 515 nm of sample/Absorbance 515 nm of control] × 100

Determination of ferric reducing antioxidant power (FRAP) assay

The FRAP assay is based on the reduction of Fe (III)-TPTZ complex to the ferrous form at low pH. This reduction is monitored by measuring the absorption change at 595 nm (Benzie and Strain, 1999). Briefly, four different concentrations (125, 250, 500 and 1000 µg/ml) of rice extract was mixed with 2960 µL of the FRAP reagent. Absorption was measured at 593 nm using a spectrophotometer (UVmini-1240, Shimadzu Corp., Japan) against a blank of methanol. FRAP reagent was prepared freshly and consisted of 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM FeCl3 in a ratio of 10:1:1 (v/v/v). FRAP values were obtained by comparing the absorption change in the test mixture with doses obtained from increasing concentrations of Fe(III) and expressed as mmol of Fe(II) equivalents per 100 g flour.

Determination of scavenging activity using ABTS assay

For ABTS assay, the method followed by Thaipong *et al.* (2006) was adopted with suitable modifications. The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in dark. The solution was then diluted by mixing 1 ml ABTS solution with 60 ml methanol. Rice extracts (150 µl) of different concentrations (1000, 2000, 3000 and 4000 µg/ml) was allowed to react with 1425 µl of the ABTS solution for 2 h in dark. Absorbance was measured at 734 nm using the spectrophotometer (UVmini-1240, Shimadzu Corp., Japan) against a blank of methanol. The standard curve of ascorbic acid was plotted. Additional dilution was needed if the ABTS value measured was over the linear range of the standard curve.

$$\text{ABTS activity (\%)} = [(A_c - A_t)/A_c] \times 100$$

A_c: Absorbance of the control
A_t: Absorbance of the extracts/standard

Determination of hydrogen peroxide (H_2O_2) assay

The ability of the extract to scavenge hydrogen peroxide was determined according to the method given by Ruch *et al*. (1989). A solution of 2 mM H_2O_2 was prepared in PB (pH 7.4). Rice extracts of different concentration (125, 250, 500 and 1000 µg/ml) were added to 600 µL hydrogen peroxide and incubated for 10 min. Then the absorbance at 230 nm was measured against a blank solution of PB using spectrophotometer (UVmini-1240, Shimadzu Corp., Japan) and compared with ascorbic acid.

$$\text{Hydrogen peroxide activity (\%)} = [(A_c - A_t)/A_c] \times 100$$

A_c: Absorbance of the control
A_t: Absorbance of the extracts/standard.

Determination of hydroxyl scavenging activity

The scavenging activity of extract was measured by the hydroxyl radical assay according to the method of Halliwell *et al.* (1987). The reaction mixture contained 1750 µl phosphate buffer solution (pH 7.4), 50 µl each of 2-deoxy-2-ribose (80 mM), EDTA (4 mM), $FeCl_3$ (4 mM), H_2O_2 (20 mM), ascorbic acid (4 mM) and 500 µL of rice extracts of various concentration (1000, 2000, 3000 and 4000 µg/ml). The above mixture was vortexed and allowed to incubate for 1 h at 37°C. Then 1mL of 2% trichloroacetic acid (TCA) and 1 ml of 1% thiobarbituric acid (TBA) were added and kept in a boiling bath for 15 min. After cooling, the pink chromogen revealing the formation of thiobarbituric reactive substances (TBARS) was read at 532 nm using spectrophotometer (UVmini-1240, Shimadzu Corp., Japan) against a blank of PBS.

The hydroxyl radical scavenging activity was

calculated according to the equation,

$$\text{Hydroxyl scavenging activity (\%)} = [(A_c - A_t)/A_c] \times 100$$

Where,
A_c = Absorbance of the control (without extract)
A_t = Absorbance of the extract/standard.

Statistical analysis

Results were recorded as mean ± standard deviation (n = 3) and subjected to one-way analysis of variance (ANOVA). The significance of the difference between means was determined by Duncan's multiple range test ($P < 0.05$) using SPSS 17.0 statistical software (SPSS South-Asia Pvt Ltd, Bangalore).

Results and Discussion

Proximate composition of pigmented landraces

The proximate composition of ten pigmented rice landraces is presented in Table 1. The total carbohydrate content exhibited by the landraces was generally found to be above 70% with the highest in Paradhan and the lowest in Kandulakathi, signifying that all landraces studied are good sources of carbohydrates. Fat values were significantly different in the range of 1.89-2.77% and the highest found in Bhatachudi and the lowest in Haladiganthi and Tikichudi. The fat values reported by Edeogu *et al.* (2007) in rice varieties from Ebonyi were found to be in the similar range of 0.5- 3.5%. The crude fibre content was found to be in the range of 0.19- 0.29% with no significant variation amongst the landraces. Protein content is responsible for the nutritional contribution and the protein range was found to be above 6%. Ash values were higher in Malli goindi and Kalachudi with 1.76% and 1.37%, respectively. Landraces are reservoirs of energy as all the energy values are above 360 Kcal/100 g.

Mineral content of pigmented landraces

The landraces analyzed exhibit high content of calcium, iron and zinc (Table 1). The highest calcium and zinc content was found in Paradhan, iron in Malli goindi. Calcium, iron and zinc content of two rice cultivars (Padovani *et al*., 2007) from Brazilian and USDA countries reported as 4 and 28 mg/100 g, 0.82 and 0.80 mg/100 g and 1.19 and 1.09 mg/100 g, respectively, which is much lower than the values reported in the landraces studied.

Amylose content of pigmented landraces

The amylose content of ten landraces is presented in Table 2. In general, it is reported that pigmented rice varieties exhibits high amylose content (Patindol *et al*., 2006). Landraces Bhatamalli, Beda gurumukhi and Bhatachudi were seen to express high amylose content of about 44.38%, 35.27% and 33.55% respectively compared to the commercial standard. High amylose content as reported by the present study is also reported in IR36 as 46.4% (Villareal *et al*., 1994) and 64.8% in a transgenic rice variety (Zhu *et al*., 2012). Such a variation of amylose content in indica rice varieties is about 8.0 - 40.71% and its formation is mainly attributed to genetic character and environmental conditions (Lin *et al*., 1993). High amylose content signifies lower glycemic index providing health benefits like weight reduction, reduced blood glucose level and favors diabetic individuals (Zhu *et al.*, 2012).

Total phenol content of tested landraces

Total phenol content (TPC) of the ten rice landraces studied is presented in Table 3. The highest TPC was found in Malli goindi. Generally rice bran is regarded as one of the natural resources containing high amounts of phytonutrients including phenolic compounds (Chen and Bergman, 2005). Although fruits and vegetables are major dietary sources of phenolic compounds, pigmented rice is reported to be an excellent source of phenolic compounds and categorized as natural antioxidants (Tian *et al*., 2004; Zheng and Wang, 2001). The values of landraces analyzed ranged from 233.92- 251.38 mg/100 g. A similar and lower TPC range was observed in 15 rice cultivars of Thailand from various categories were found to be 89.31 - 98.84 mg GAE/100 g, 101.03-104.94 mg GAE/100 g and 108.10 - 122.39 mg GAE/100 g for white, red and black rice bran extracts respectively (Muntana and Prasong, 2010).

Total anthocyanin content of pigmented rice

The landraces of the present study involved pigmented varieties and TAC of the landraces studied is presented in Table 3. From the result, Kandulakathi and Tikichudi showed maximum anthocyanin content which is natural as they were red rices. The anthocyanin content of pigmented rice as reported by Sompong *et al.* (2011) is lower compared landraces studied. Similar and higher anthocyanin content was reported from 10 black rice cultivars whose value ranged between 10 to 493 mg/100 g (Escribano-Bailon *et al*., 2004) and lower anthocyanin content range was observed in the grains of twelve Korean colored rice varieties whose values ranged between 10.21 and 122.65 mg/100 g (Seo *et al*., 2011). The high anthocyanin content in rice cultivars are considered as good sources of fiber, minerals and

Table 1. Proximate and mineral composition of ten pigmented landraces of rice

S.No.	Name of landraces	Carbohydrate (g/100g) [a]	Fat crude (g/100g) [a]	Fibre crude (g/100g) [a]	Moisture (g/100g) [a]	Protein crude (g/100g) [a]	Total ash (g/100g) [a]	Energy (Kcal/100g) [a]	Calcium (mg/100g) [a]	Iron (mg/100g) [a]	Zinc (mg/100g) [a]
1	Bhatamalli	78.34±0.93	2.42±0.21	0.22±0.03	10.45±0.18	6.35±0.30	0.89±0.17	360.9±1.73	35.48±1.81	13.73±0.14	2.46±0.69
2	Bhatachudi	78.67±0.49	2.77±0.33	0.19±0.02	10.17±0.31	7.74±0.81	0.97±0.12	364.7±1.58	236.39±1.37	6.84±0.83	3.92±0.14
3	Paradhan	79.85±0.28	2.38±0.32	0.21±0.03	10.83±0.69	6.42±0.37	0.48±0.09	366.2±2.34	635.52±2.62	22.73±0.76	8.67±0.08
4	Kandulakathi	77.56±0.59	2.39±0.19	0.25±0.04	10.29±0.11	9.56±0.56	1.05±0.16	364.8±2.64	235.67±1.85	7.25±0.62	4.74±0.11
5	Tikichudi	79.32±0.62	1.89±0.25	0.23±0.04	10.45±0.62	6.87±0.37	1.07±0.06	361.6±1.75	286.85±1.63	5.17±0.45	2.53±0.63
6	Beda Gurumukhi	78.53±0.73	2.42±0.35	0.21±0.06	10.26±0.57	7.19±0.62	0.88±0.18	361.4±2.32	36.57±1.75	40.44±0.26	3.44±0.17
7	Haladiganthi	79.54±0.52	1.89±0.25	0.27±0.05	10.19±0.85	7.36±0.53	1.03±0.10	364.1±2.65	227.29±2.36	46.64±0.86	2.07±0.26
8	Malli goindi	77.34±0.84	2.29±0.32	0.29±0.03	10.81±0.26	7.85±0.16	1.76±0.63	360.4±1.88	158.43±1.83	82.48±0.53	1.97±0.44
9	Kalachudi	78.87±0.18	2.65±0.31	0.19±0.04	10.92±0.64	7.54±0.26	1.37±0.24	363.3±2.25	167.79±1.65	43.36±0.66	2.51±0.17
10	Mallimakada	78.63±0.45	2.76±0.24	0.21±0.04	10.37±0.76	7.73±0.71	1.07±0.16	365.2±2.47	204.44±2.73	38.55±0.34	2.05±0.03

[a]Values are mean ± standard deviation of three replicates. Values are significant P < 0.05.

Table 2. Amylose and phytate content of ten pigmented landraces of rice

S.No.	Name of landraces	Amylose (%)[a]	Phytate (mg/100g)[a]
1	Bhatamalli	44.38±1.76	5.14±0.96
2	Bhatachudi	33.55±1.47	1.73±0.78
3	Paradhan	31.24±1.24	3.51±0.63
4	Kandulakathi	22.01±2.02	1.16±0.34
5	Tikichudi	27.93±0.35	8.57±0.84
6	Beda Gurumukhi	35.27±0.74	3.69±0.35
7	Haladiganthi	29.89±0.89	2.34±0.49
8	Malli goindi	31.45±0.65	4.63±0.78
9	Kalachudi	27.00±1.08	24.49±0.50
10	Mallimakada	26.81±0.94	2.51±0.67

[a]Values are mean ± standard deviation of three replicates. Values are significant P < 0.05.

several important amino acids (Zhang *et al*., 2005).

Phytate content of landraces

Table 2 signifies that the phytate content of the landraces studied ranged from 1.16 - 24.49 mg/100 g. The highest was observed in Kalachudi which is considered unfavorable for health owing to its anti-nutrient property. Ma *et al.* (2005) have reported phytate content for four rice varieties in the range of 55 mg/100 g to 183 mg/100 g which is higher than the values reported for the rice landraces studied. Similar range of phytic acid for rice bran was reported as 57.71 mg/100 g of phytate (Garcia-Estepa *et al*., 1999) which applies with the present case as maximum value of 25.06 mg/100 g for observed Kalachudi which is within the mentioned value.

Polyphenol in pigmented landraces of rice

The polyphenol content of the landraces studied is summarized in Table 3. The highest value was found in Malli goindi followed by Bhatachudi. The high values of polyphenols signify rich sources of flavonoid antioxidants as these are directly correlated with free radical scavenging activity and major antioxidant compounds in plant products (Escribano-Bailon *et al*., 2004).

Antioxidant activity of the tested landraces

The antioxidant activity of the dehusked rice grain extracts were studied using DPPH, FRAP, H_2O_2, ABTS and hydroxyl scavenging assays. The results of antioxidant activities of the ten landraces are presented in Table 3. In DPPH assay, the antioxidant activity of the samples percentage ranged from 19.37 to 38.10. Increase in extract concentration resulted in increase in the scavenging activity. As reported by Gorinstein *et al*. (2007), Jasmin rice and rice bran showed DPPH scavenging activity of 20% and 79%, respectively. Except Tikichudi, all other landraces showed activity greater than 20%. Haladiganthi and Tikichudi exhibited the highest and lowest DPPH scavenging activity with 38.10% and 19.37%, respectively. High yielding rice varieties from Bangladesh showed DPPH activity in the range 6.01 to 14.47% (Dutta *et al*., 2012). Sompong *et al.* (2011) have reported DPPH scavenging activity of red rice cultivars in the range of 13.0% to 76.4% which confirms the results of the present study.

The results of FRAP assay showed a significant increase in the scavenging activity with increasing concentration. Antioxidant activity of the samples ranged from 18.41 to 35.57%. Tikichudi exhibited the lowest activity while Haladiganthi exhibited highest activity. The Bangladesh varieties of BR16 and BRRI dhan34 showed an activity of 5.43% and 9.34%, respectively (Dutta *et al*., 2012) and red rice cultivars in the range of 1.40 to 6.80% (Sompong *et al.*, 2011).

The results of H_2O_2 assay indicated that the scavenging activity of the samples varied between 5.44 to 57.50%. Among the ten samples, Malli goindi showed the highest activity and Kandulakathi the lowest. The activity of all samples showed an increasing trend with increasing concentration. According to the report of Park *et al*. (2008), hydrogen peroxide scavenging activities of black rice were 18.39% at 10 µg/ml, 26.68% at 50 µg/ml, and 72.67% at 100 µg/ml, respectively.

Antioxidant activity of the samples as determined by ABTS assay show that the scavenging activity ranges from 28.54 to 44.44%. Kandulakathi has the highest antioxidant activity. The results are in accordance with those reported by Yodmanee *et al.* (2011) from the pigmented rice cultivars of southern Thailand. Also, Abru *et a*l. (2010) reported that water extracts of pigmented rice varieties exhibited ABTS scavenging activity.

Hydroxyl scavenging assay of the rice grain extracts shows an antioxidant activity that ranges between 12.52 to 46.42%. Dutta *et al*. (2012) reported

Table 3. Antioxidant activities, TPC, TAC and polyphenol content of ten pigmented landraces of rice

S. No.	Name of landraces	DPPH (%)[a]	H_2O_2 (%)[a]	FRAP (%)[a]	Hydroxyl scavenging (%)[a]	ABTS (%)[a]	TPC (mg FAE/100g)[a]	TAC (mg/100g)[a]	Polyphenol (mg GAE/100g)[a]
1	Bhatamalli	21.15±0.35	6.48±0.39	18.59±0.42	14.42±0.32	43.80±0.52	247.70±1.40	60.38±0.64	269.37±2.24
2	Bhatachudi	21.54±0.31	10.48±0.44	19.60±0.41	12.52±0.38	38.08±0.71	245.32±1.54	96.83±0.96	283.76±2.16
3	Paradhan	32.74±0.28	14.51±0.22	24.54±0.36	46.42±0.30	39.70±0.22	244.37±1.21	151.83±1.38	264.24±1.50
4	Kandulakathi	27.29±0.19	5.44±0.14	24.55±0.36	40.59±0.34	44.44±0.38	247.45±1.07	193.21±1.61	278.74±1.63
5	Tikichudi	19.37±0.26	34.62±0.33	18.41±0.20	22.56±0.27	28.54±0.32	237.04±0.80	193.36±2.31	277.38±1.46
6	Beda gurumukhi	26.19±0.22	55.47±0.37	25.95±0.10	35.50 ±0.34	37.45±0.44	238.73±1.43	106.53±1.56	270.19±2.86
7	Haladiganthi	38.10±0.25	50.63±0.26	35.57±0.38	34.59±0.28	43.03±0.91	233.92±1.29	66.89±0.63	260.01±2.32
8	Malli goindi	24.51±0.28	57.50±0.26	23.93±0.13	29.49±0.34	38.43±0.49	251.38±1.58	165.27±1.82	284.36±1.73
9	Kalachudi	29.05±0.74	47.53±0.28	27.52±0.32	30.58±0.18	37.17±0.80	246.62±2.07	28.73±0.49	276.79±2.28
10	Mallimakada	26.36±0.96	54.67±0.33	27.76±0.28	45.41±0.31	33.01±0.93	238.78±1.85	81.28±0.74	252.43±1.15

[a]Values are mean ± standard deviation of three replicates. Values are significant $P < 0.05$. (Abbreviations: DPPH, 1,1-diphenyl-2-picryl hydrazl; H_2O_2, Hydrogen peroxide; FRAP, Ferric ion reducing antioxidant power; ABTS, 2,2'-azino-bis-3-ethyl benzothiazoline 6- sulphonic acid; TPC, total phenol content; TFC, total flavonoid content; FAE, Ferulic acid equivalent; GAE, Gallic acid equivalent)

that high yielding rice varieties showed the higher antioxidant activity. Both the assays showed an increasing activity with increasing concentration. It is significant from the results of the present study that all these landraces are good sources of antioxidants.

Conclusion

Pigmented landraces of rice contain higher content of total anthocyanin, total phenol and polyphenol which signifies high antioxidant potential. Present investigation reveals that the landraces can be used as resources for future rice improvement programme and also serve to enhance the nutritive and health promoting potential of rice based diets. Mass cultivation of landraces will help humans to consume nutritious rice and remain healthy.

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