Colour based nutraceutical potential of some traditional rice (*Oryza sativa* L. ssp. *indica*) varieties of India

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Consumption of unpolished grain, rather than polished grain has become a modern trend and consumers are mainly putting their thoughts and effort to consume products with more antioxidant capacity. Rice is the main staple food and apart from being considered as the main source of energy, it contains many nutraceutical properties because of its enriched secondary metabolites. This study is an effort to bring back the indigenous traditional rice landraces that almost disappeared from the farm fields after the advent of the Green Revolution in India. This article focuses on colour-based nutritional properties of six coloured and four non-coloured indigenous rice varieties based on antioxidant potential, total phenol and flavonoid content along with secondary metabolites profiling by high performance liquid chromatography. The biochemical uniqueness of these varieties has been explored that opens the gate for the conservation of more indigenous rice varieties for food security, as a cheap source of nutritional food and to construct a better niche for public health in developing country like India.

**Keywords:** Antioxidant, Coloured rice, High performance liquid chromatography, Secondary metabolites.

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**Introduction**

South Asia is a great repository of genetic diversity of rice with more than 100,000 folk landraces of the *indica* group\(^1\). Different parameters are taken into consideration for the selection of different rice varieties for their breeding purposes, for various gastronomic preferences and culinary practices in different food cultures from time immemorial\(^2\). As a staple food of more than half of the people in the world, it supplies the nutritional values in our daily diet. With the increasing commercialization of rice crop, we are gradually compromising the nutritional properties of rice by milling out the pericarp and aleurone layer, commonly known as bran. Most of the important nutritional compounds are bound to this bran layer\(^3,4\), which is the main site of micro-and macro-nutrients and a range of important phytochemicals, such as oryzanols, tocopherols, phenolic compounds, flavonoids, and anthocyanin that impart an overall nutritional property of rice\(^5,6\). Nowadays, consumers are increasingly showing their preferences for consumer products with more antioxidant activities and a trend has started toward the consumption of unpolished whole grain rice\(^7,8\).

India is home to thousands of extant rice landraces (*Oryza sativa* L. ssp. *indica*), with various natural hues of black, purple, red, brown, and white grains. The colour content varies with the composition of secondary metabolites. Rice contains many unique polyphenolic compounds present both as bound with the cell wall polysaccharide and in free form. Normally, grains with red and black pericarp colours have a higher concentration of phenolic compounds compared to those with a light brown pericarp colour\(^9\). About 38%\(^{ref\,10}\) to 60%\(^{ref\,11}\) of the total polyphenol content is present in grains of light brown rice. In contrast, the red and black pericarp of coloured grains contains around 81% of the polyphenols\(^11\). The natural hues of rice bran colour have a profound effect in determining the nutritional content of grains. The free phenolics, mostly concentrated in the pericarp, are usually eliminated with the bran during milling\(^3,4\). Thus, the functional dietary potential and the protective effect of the rice grain in the prevention of age-related degenerative diseases become reduced during processing\(^12\). The present article highlights the antioxidant potential of coloured and non-coloured indigenous rice varieties which are still growing in different remote marginal farms in India\(^2\).

**Materials and Methods**

**Samples procurement**

Ten rice landraces (six coloured and four non-coloured) were selected based on the agronomic and morphological characters (Fig. 1). Samples were collected from the germplasm bank of Basudha farm (www.cintdis.org/basudha), located in the Rayagada

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district of Odisha (19°33'06" N, 83°23'28.14" E), where each of these landraces is conserved in situ and characterised (Table 1). We cultivated these landraces in the Kharif season (June to December) in the University Agricultural Experimental Farm (22°22'03.5" N and 88°26'07.2" E). Rice varieties were grown in separate plots at a density of 16 hills/m² in the tropical climate of the lower Gangetic plain of West Bengal by regular watering but without application of chemical fertilizers, pesticides and herbicides.

Evaluation of antioxidant potential

Rice grains were dehulled with a traditional small hand-made rice pounder followed by winnowing the whole grains with bran and were crushed into a fine powder. Rice powder was soaked overnight with methanolic water (7:3 v/v) and kept in refrigeration. Total phenolic content (TPC) of the methanolic extract of the samples was estimated by the Folin-Ciocalteu method, measuring spectrophotometrically at 765 nm (Jasco V-630, USA) and expressed as mg of gallic acid (Sigma-Aldrich Chemistry, USA) equivalent (GAE) per 100 g dry weight. Total flavonoid content (TFC) was estimated by adding methanolic extract, 10% aluminium chloride, 5% sodium nitrite and water in a ratio of 1:1:1:7 \textsuperscript{(ref 15)}. Optical Density was measured spectrophotometrically at 510 nm (Jasco V-630, USA) after 25 minutes incubation and the result was expressed as mg of quercetin (ChromaDex, USA) equivalent (QE) per 100 g dry weight of rice grain. Antioxidant potential (AP) of the samples was measured by the radical scavenging property of 2-diphenyl-1-picrylhydrazyl (DPPH) \textsuperscript{16}. Aqueous methanolic (3:7 v/v) DPPH (0.16 mM) was added with the sample aliquot in 3:1 ratio and the degree of decolourisation was measured at 517 nm in a UV-spectrophotometer (Jasco V-630, USA) after 30 minutes of incubation in the dark. The IC50 value of the sample was calculated against the degree of decolourization in respect of ascorbic acid (Sigma, USA) and the AP was expressed as the inverse of the IC50 value. The experiment was repeated thrice in all cases.

Analysis of secondary metabolites

Rice powder (whole grain) was treated with 1:2:1 chloroform, methanol and water and kept for 72 hours under refrigeration for the removal of lipid, following which the chloroform phase was discarded. The methanolic phase was concentrated by speed vac (Thermo SPD 2010) and subjected to high pressure...
liquid chromatography (HPLC) (Agilent, USA) for the analysis of phenolic acids and flavonoids following the modified method of Dey et al., (2015)[ref 17]. HPLC was attached with Zorbax SB-C18 column (4.6x150 mm, 3.5µ, Agilent USA) and equipped with a photo-diode array detector. Gradient of two mobile phases i.e., methanol (A) and water with 0.02% aqueous H₃PO₄ (B) were set at 25% A + 75% B for 5 minutes> 30% A + 70% B for 10 minutes> 45% A + 55% B for 30 minutes> and 80% A + 20% B for 60 minutes. The injection volume was 20 µL. The flow rate was kept at 0.4 mL/min and analytes were scanned at 280 nm wavelength. The peaks were identified by comparing the relative retention time with proper peak integration, co-chromatography with standard and calibration against absorption spectra obtained from the authentic compounds (Sigma-Aldrich Chemistry), USA; Chroma Dex, USA). Analytes were estimated using an external method following standard validation guideline and finally, the amounts of the analytes were expressed as µg/g of the dry weight of the sample.

Statistical evaluation

The correlations between TPC, TFC, and IC50 value were plotted on 3D planes and elucidated by Pearson's product-moment correlation test following PAST3 software.

Results and Discussion

Antioxidant potential and secondary metabolites of rice

AP was expressed in terms of the quantitative assessment of secondary metabolites for scavenging reactive oxygen species. The 3D interpolation based on the TPC, TFC, and IC50 value for DPPH reduction projected the interrelationship among these three variables (Fig. 2). AP was found to be the highest with antioxidant capacity (AOC) of 1.46 mg/mL in B56 and lowest AP with AOC of 58.91 mg/mL in G04. A high correlation was observed between TPC and IC50 of DPPH with the coefficient value of –0.671. Similarly, a high correlation with Pearson product coefficient of -0.9009 for TFC and IC50 was also obtained (Fig. 2). A strong positive relationship (r =0.856) was also obtained between TPC and TFC. Therefore, it can be concluded that antioxidant potentiaility is highly influenced by the presence of TPC and TFC. All the correlation values are highly significant at P<0.05. It was revealed from the above interpretation that all the coloured rice landraces (B09, B72, B56, K69, P23, SH01) had higher AP than the non-coloured one.

A total of 14 different secondary metabolites, namely gallic acid (GA), protocatechuic acid (PRCA), 4-hydroxybenzoic acid (4HBA), vanillic acid (VA), caffeic acid (CA), syringic acid (SY), p-coumaric Acid (PCA), ferulic acid (FA), rutin (RU), o-cresol (OCRE), p-cresol (PCRE), myricetin (MY), 3,4-xylenol (3,4XYL), and quercetin (Q) were identified and quantified from different cultivars (Fig. 3). GA is present in almost all the studied samples, but more prominently in a higher amount in all non-coloured rice grains whereas coloured grain had a higher abundance of PRCA and RU. CA and FA are ubiquitously present in all samples with a more or less equal amount. It is found that 4HBA and PCA are prevalent in non-coloured samples than the coloured ones. One striking feature is that SY is only
present in non-coloured samples. The quantitative profile of secondary metabolites examined in these ten landraces indicated their “uniqueness” for nutritional benefits (Fig. 4). Wanyo et al.\textsuperscript{18} found that GA, PRCA, \textit{para}-hydroxybenzoic acid, VA, chlorogenic acid, CA, SY, PCA, FA and sinapic acid are present in 105 rice varieties of northeastern Thailand.

For overproduction of grains by developing high-yielding varieties and by modern grain processing technology, we are gradually compromising with the nutritional quality of rice. The mechanized husking and polishing process of rice grains remove nutritionally rich pericarp, testa and aleurone layer, commonly known as bran\textsuperscript{3,4}. Rice bran is reported to contain a diverse array of phenolic acids and varied classes of flavonoids\textsuperscript{19}. The pigmented rice contains a more diverse form and higher quantity of phenolic acids and FAIs is the principal component of bran conferring black colour of the grain\textsuperscript{20}. The AOC in rice is directly influenced by the quality and quantity of flavonoids, reported by several authors\textsuperscript{21,22}. The six coloured varieties contain higher proportions of AOC, TPC, and TFC (Fig. 2). Among the whole flavonoid class, anthocyanin is the major compound responsible for the pigmentation of rice grain\textsuperscript{23}. Shikimic acid pathway in combination with the acetate pathway is responsible for the biosynthesis of many flavonoids, flavono-lignans, and isoflavonoids\textsuperscript{23} which ensure the colour variation of the rice grains.

Phenolic compounds confer variability in taste, flavour, and health-promoting properties in cereals, vegetables, and fruits\textsuperscript{24}. It has been reported earlier that phenolic substances, besides their known antioxidant capacity, serve a protective role in the human body against many chronic metabolic disorders and chronic diseases such as cardiovascular disease and hyperlipidemia by upregulating the expression of nuclear factor-erythroid 2

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\textbf{Fig. 4} — Radar chart generated based on the concentration of secondary metabolites representing a unique profile of 10 different landraces. The numeric values in the radar chart represent the amount of each secondary metabolites expressed in μg/g of dry weight of the sample.
indicative of the additional nutritive value of the grains.

The authors tried to interpret the ‘uniqueness’ of these ten landraces in terms of the amounts of phenolic acid, polyphenols, and flavonoids (Fig. 4). Coloured rice showed a high amount of AP in all cases. Therefore, the direct co-relation between AP and coloured grain is indicative of the additional nutritive value of the grains.

**Conclusion**

The present findings may hopefully promote an extensive and comprehensive exploration of the nutritional potential of more indigenous landraces, which have remained largely neglected in mainstream rice research. These findings may encourage further conservation of these rare and evanescent landraces to provide nutritional security for people, especially in the rice-eating part of the Globe.

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**Conflict of interest**

Authors have no conflict of interest.

**Reference**