Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2018; 10(12); 367-375

ISSN: 0975-4873

Research Article

Antioxidant Constituents and Properties of Bran from Selected Rice Genotypes Available in North- West India

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Available Online:25th December, 2018

ABSTRACT

Rice bran, a co-product of milling process is highly nutritious with promising antioxidant properties that can be used as a food supplement. Therefore, characterization of biochemical and antioxidant potential of bran from 16 rice genotypes was carried out to help the breeders' to identify rice varieties possessing improved nutritional value. Significant genotypic variations in the content of phytochemicals and their antioxidant potential in the bran of different rice genotypes grown in replicate trial during *Kharif* 2016 were observed. The bound phenolic content was higher than free phenolic content, while significant variation was seen in total phenolics, flavonoids, *o*- dihydroxyphenols, proanthocyanidins, and phytate content. Bran from rice genotypes under evaluation showed a high positive correlation between total phenolics and DPPH free radical scavenging activity. Wide variations in reducing power, metal chelating power, free radical $ABTS^{\cdot+}$ scavenging activity and ferric reducing ability power of rice bran extracts was observed. Significant differences in the content of flavonoids, phytate, phenolics and proanthocyanidins in the bran from different rice genotypes significantly effects antioxidant potential which could have applications in the food industries. It would also enable the breeders' to breed for new genotypes with high nutrient level.

Keywords: Rice bran; phenolics; flavonoids; phytate; proanthocyanidins; antioxidant capacity.

INTRODUCTION

In the wake of the second green revolution, researchers are not only concerned with the high yielding varieties for self-sufficiency but are also focused on nutritional security that can ensure protection against chronic oxidative stress-related diseases. Some 795 million people donot get enough food each day (State of Food Insecurities in world Report 2015), while 2 billion are under scourge of micronutrient deficiency fighting with a number of diseases. Objectives towards comprehensive nutritional security can be fulfilled by harnessing nutritional benefits of agriculture products and not wasting their co-products endowed with bounty of antioxidants and micronutrients. Rice (*Oryza sativa* L.) is an important staple food of about half the worlds' population (IRRI 2006) and influences the livelihood and economics of several billion people. The commercial and nutritional importance of rice is not limited to grain. On milling of paddy, major product is 70% of endosperm (rice). Also, the coproducts such as 20% husk, 2% germ and 8% bran are produced (Van Hoed *et al* 2006)[1]. Paddy husk has no food value but has several industrial uses.

Rice bran, which is predominantly used as an animal feed, can be successfully exploited as human food supplement and as a source of edible oil. Rice bran is a good source of fatty acids, proteins, dietary fiber and minerals (Mccaskill and Zhang 1999)[2]. Prevalence of oxidative stress as the etiological agent aging and chronic degenerative diseases has led to the search of phytochemicals due to their potential to prevent them. These phytochemicals are mostly redox active molecules called as antioxidants. Antioxidants present in food bring about beneficial health effects by acting as inducers of various mechanisms related to defence system by antioxidants, longevity, cell maintenance and DNA repair (Astley *et al* 2004)[3]. An animal cell has very limited *de novo* antioxidant production. Also, as a consequence of disease processes (eg, inflammation) and from pollutants, tobacco smoking, drugs and radiation, an increased quantity of reactive nitrogen species and reactive oxygen species (RNS/ ROS) are formed in animal cells. If not eliminated, they may damage intracellular or extracellular components. The dietary intake of antioxidants through the consumption of fruit and vegetables has been suggested to reduce oxidative stress as they are known to be important dietary source of phenolic compounds including free phenolic compounds and their glycosides and substantial amount of insoluble phenolic compounds, mostly bound to polysaccharides in the cell wall (Scalbert and Williamson 2000)[4]. A group of antioxidants possessing different chemical properties is required for proper protection against oxidative damage. Cereal grains are reported to exhibit various phytochemicals with antioxidant properties. Among all these phytochemicals, phenolic compounds have been claimed to provide health benefits when present in diet (Liu 2007)[5]. However, the intake of phenolic compounds from cereal grains like rice and wheat is limited, since these are concentrated mostly in the bran layer and are lost

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with the separation of seed coat during processing. Rice bran representing 8-10% of the brown rice contains minerals like magnesium, phosphorus, iron, 13% crude protein, 11. 5% fibres (approximately) and 20% oil (Oliveira *et al* 2011)[6]. All over world, 63-76 million tons of rice bran is produced per annum, out of which 90 percent or more is used as animal feed (Heikal *et al* 2015)[7]. Rice bran has great potential to be used for human nutrition but that potential is still not utilized to the fullest. The bran layers consists of the testa, nucellus, pericarp and aleurone layer and are a rich source of health promoting compounds in the lipophilic, hydrophilic and cell – bound fractions (Goffman and Bergman 2004)[8]. These bioactive compounds include steryl ferulate esters (commonly referred to as Oryzanols), phenolic acids such as *p*-coumaric, diferulate and ferulic acid (Adom and Liu 2002)[9], tocopherols, tocotrienols (Nicolosi *et al* 1994)[10], dietary fibre and phytic acid (Seo *et al* 2008)[11]. These bioactive compounds apart from having a potential antioxidant role, are also reported to possess chemo-preventive effect. Trials in which animals were feeded with diet rich in cell – wall bound phenolic fraction of rice bran caused reduction in hyper-tension, hyperlipidemia and hyperglycaemia (Wells 1993)[12].

The genotypic diversity of some phytochemicals like flavonoid, anthocyanins (Abdel-Aal *et al* 2006)[13], phytic acid and proanthocyanids in rice bran have been reported in literature. Studies on the genotypic variation in rice bran phytochemicals from the widely grown sustainable rice varieties would provide valuable basis to the breeders' to improve nutrient composition and thus their health potential. Rice bran with improved phytochemical components will also serve as a profitable source for the production of functional foods.

MATERIAL AND METHODS

The chemicals used for antioxidant assays, such as TPTZ (2,4,6-tris(2-pyridyl)-1,3,5-triazine), DPPH (2,2´-Diphenyl,1-picrylhydrazyl), 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) catechol, gallic acid, L-glycine, sodium phytate, ascorbic acid and rutin were obtained from Sigma- Aldrich , India. Sulphuric acid, ferric chloride, sodium tungstate, aluminium chloride, phenol, ferric ammonium sulphate, bipyridine used in the present investigation were of analytical grade and were procured from Sisco research laboratories, India.

Seeds of 16 rice genotypes including ten non-basmati and six basmati varieties were procured from Rice Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India. Rice genotypes were grown in the experimental fields of the department using recommended package of practices in a randomized block design/replicate trial during *Kharif* 2016. Plants were harvested at maturity. Paddy was dried to moisture content of 14% and cleaned to make it free from extraneous matter. Cleaned paddy was stored in cloth bags at 25 ◦C for three months. To obtain bran, paddy was dehulled in Satake Rice Machine (Kitashiba electronics Co.ltd.,Japan) and then milled with Satake Test Mill (Kitashiba electronics Co.ltd.,Japan) to obtain bran. The bran so obtained was dried, powdered and passed twice through a 425 µm sieve screen.

Extraction of free and bound phenolic compounds

To the powdered bran samples (0.5 g) added 80% methanol (10 mL) in a ratio of 1:20 (w/v). The mixture was put for 1 h on a shaker at 250 rpm. The mixture was centrifuged at 11,000g for 15 min at 4 ◦C (Remi Cenrtifuge VCBS-2252, Vasai, India). The supernatant collected was stored at -20 ◦C for the quantification of free phenolic and flavonoids (Yu *et al* 2016)[14]. The residue was dried at room temperature and subjected to hydrolysis by alkali for the extraction of bound phenolics (Qiu *et al* 2010)[15]. Briefly, 15 mL of 4 M NaOH was added to the dried residue and hydrolyzed on a shaker at 4 ◦C at 250 rpm for 4 h. The pH of the resultant mixture was adjusted between 1.5 and 2.0 with 6 N HCl and then extracted with 15 mL ethyl acetate three times. Every time following addition, ethyl acetate was separated from the aqueous layer by centrifugating it at 11,000 g at 4 ◦C for 5 min (Remi Cenrtifuge VCBS-2252, Vasai, India). The supernatants were pooled and evaporated to dryness at 35 ◦C under vacuum using a rotary evaporator. The dried residue was redissolved in 5mL of 50% methanol and stored at -20◦C till analysis of bound PC.

Estimation of phenolic content

The content of free and bound phenolics was determined by Folin–Ciocalteau reagent using the method of Swain and Hillis (Swain and Hillis 1959)[16]. Dissolved 0.1 ml of extract in 6.4 ml distilled water. To it, 0.5 ml of Folin Ciocalteau reagent was added and mixed thoroughly. After 5 min, 1 ml of saturated sodium carbonate solution was added, vortexed and kept for 1 hour at room temperature. The blue color developed was read at 725 nm against the reagent blank. The free and bound phenolic content was calculated using gallic acid as standard and expressed in mg g^{-1} DW.

Estimation of flavonoids

An aliquot of 0.5 mL from the methanolic extract obtained above was added to 2 mL distilled H_2O and mixed with 0.15 mL 5% $NaNO_2$. After 5 minutes, 0.15 mL 10% $AlCl_3.6\ H_2O$ was added and allowed the mixture to stand for another 5 min followed by the addition of 1 mL 1M NaOH. The contents were mixed and incubated at room temperature for 15 minutes. The absorbance was read at 415 nm. Flavonoid content was calculated from the standard curve prepared using rutin and expressed as mg g^{-1} DW (Bao *et al* 2005)[17].

Estimation of o-dihydroxyphenols

An aliquot from the methanol extract obtained above was evaporated to complete dryness and residue was re-dissolved in 1 ml distilled water. Sequentially added 0.3 ml of 10 % trichloroactetate, 1 ml 10 % sodium tungstate, 0.5 ml of of 0.5 N HCl and 1 ml of freshly prepared 0.5 % sodium nitrite. After 5 min, 2 ml of 0.5 N NaOH was added. The colour developed was read after 15 min at 540 nm against the reagent blank. The standard curve using catechol was used to calculate the content of *o*-dihydroxyphenols and expressed as mg g^{-1} DW (Nair and

Vaidyanathan 1964)[18].

Extraction and estimation of proanthocyanidins

To 0.5g of the bran sample added 5 ml of extraction solvent consisting of acetone, water and acetic acid in a ratio of 70: 29.5: 0.5 (v/v/v) and kept at 25ºC for 16 hours in the dark. The supernatant was obtained by centrifugation at 11,000 rpm for 20 minutes at 20ºC. The residue was washed with the same extraction solvent and the supernatants were pooled. Proanthocyanidin content was estimated according to the method reported by Sun *et al* 1998[19]. An appropriate amount of the supernatant was evaporated to dryness. The dried extract was redissolved in 1 ml of 80% methanol and added 2.5 ml of 1% vanillin and 2.5 ml of 9.0 M sulphuric acid. The contents were mixed well and the absorbance was recorded at 500 nm. A substrate blank for every sample was prepared to eliminate interference of anthocyanins, by replacing 2.5 ml of 1% vanillin with 2.5 ml methanol. The amount of proanthocyanidins was calculated from the standard curve using (+) catechin as standard and expressed as mg g^{-1} DW (Furukawa *et al* 2007)[20].

Extraction and estimation of anthocyanins

Bran sample (1.5g) was mixed with 15 ml of acidified ethanol and incubated overnight in the dark at room temperature. The mixture was centrifuged at 10,000g (Remi Cenrtifuge VCBS-2252, Vasai, India) for 15 minutes at 4 ◦C. The resultant supernatant was adjusted to pH 1 with 1 N HCl and made to 20 ml volume with acidified ethanol (Abdel – Aal and Hucl 1999)[21]. Absorbance was read at 535 nm against a reagent blank. Total anthocyanin concentration was expressed as mg g^{-1}DW and was calculated as cyanidin 3- glucoside. The molecular weight of cyanidin 3- glucoside is 449 and its molar absorptivity is 5,965 cm^{-1} M^{-1}.

Extraction and estimation of phytate

Bran sample (1.0 g) was shaken with 25ml of 0.2 N HCl for three hours at 250 rpm. It was filtered through Whatman No. 1 and final volume was made upto 25 ml with 0.2 N HCl. The extract **(**0.2 ml) was mixed with 1 ml of ferric ammonium sulphate solution and kept in a boiling water bath for 30 minutes. Tubes were transferred to ice cold water, followed by the addition of 2 ml of bipyridine solution. The colour developed was read at 519 nm against water blank. Concentration of phytate present in bran was calculated from the standard curve of sodium phytate and expressed as µg g^{-1} DW (Zemel and Shelef 1982)[22].

Antioxidant Assays

Extraction was done using 80% methanol (1:10 w/v). The mixture was put for 12 hours on a shaker at room temperature and then centrifuged at 1000g for 30 minutes at 4 ◦C (Remi Cenrtifuge VCBS-2252, Vasai, India). This process was repeated twice. The supernatants obtained each time were pooled to make the volume with 80% methanol. The extract so obtained was analyzed for DPPH radical scavenging activity, radical cation $ABTS^{\cdot+}$ scavenging activity FRAP, metal chelating assay and reducing power assay.

Scavenging ability by the DPPH radical assay

To 0.2 ml of extract, 2.8 ml of 0.1mM DPPH solution was added and incubated in dark at room temperature for 30 min. After incubation, absorbance was read at 515 nm, against 3 ml of 80% methanol as blank. The control used was prepared by adding 0.2 ml of 80% methanol to 2.8 ml of 0.1mM DPPH (Williams *et al* 1995)[23]. The scavenging ability was calculated using formula: ({absorbance $_{515nm\ of\ control}$ - absorbance $_{515nm\ of\ sample}$) / absorbance $_{515nm\ of\ control}$} x 100.

Scavenging ability of the $ABTS^{\cdot+}$ radical cation assay

To 30 µl of methanolic extract added 3 ml ABTS radical cation solution (absorbance of 0.70 ±0.02), mixed thoroughly, and left to stand in dark for 6 minutes at room temperature. The absorbance was immediately recorded at 734 nm. Trolox standard solution in 80% ethanol was prepared and assayed under the same conditions. Results were expressed as Trolox equivalents antioxidant capacity (TEAC) in mmol of Trolox per g of dry weight (Arts *et al* 2004)[24].

Reducing power

An aliquot of 0.1 ml methanolic extract was mixed with 2.5 ml 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml 1% potassium ferricyanide. The mixture was incubated at 50 ◦C for 20 minutes. To the mixture added 2.5 ml 10% TCA and centrifuged at 2000 g at 25 ˚C for 5 minutes. A volume of 2.5 ml from the upper layer was mixed with 5.0 ml distilled water and 0.5 ml 0.1 % ferric chloride. The absorbance was read at 700 nm. Reducing power of rice bran extract was calculated using ascorbic acid as standard and expressed as mg g^{-1} DW (Oyaizu 1986)[25].

Ferric reducing antioxidant power assay (FRAP)

To 20 µl of extract, 2 ml of freshly prepared FRAP reagent was added. Incubated at room temperature for 30 min and absorbance was read at 593 nm. The results were calculated from the standard curve prepared with known concentration of $FeSO_4$ (15-30 µM), and expressed as µmoles $FeSO_4$ g^{-1} DW and expressed as µmoles $FeSO_4$ g^{-1} DW (Benzie and Strain 1996)[26].

Metal chelating assay

To 0.5 ml of extract sequentially added 4.2 ml 80 % methanol, 0.1 ml 2 mM $FeCl_2$ and 0.2 ml 5 mM ferrozine. The mixture was mixed vigorously and incubated at room temperature for 10 minutes. The absorbance was read at 562 nm, against 80% methanol as blank. The control was prepared by adding 4.7 ml of 80% methanol, 0.1 ml 2mM $FeCl_2$ and 0.2 ml 5mM ferrozine (Decker and Welch 1990)[27]. The chelating ability was calculated using the formula: scavenging ability (%) = {(Absorbance $_{562nm\ of\ control}$ - absorbance $_{562nm\ of\ sample}$) / absorbance $_{562\ nm\ of\ control}$} x 100

Statistical analysis

Data was collected in triplicate and analysis of variance (ANOVA) technique was used to analyze the data by completely randomized design (CRD) using SAS (9.3) software. Post- hoc test Tukey was performed to compare the mean difference of sample.

RESULTS AND DISCUSSION

Phenolic Constituents

Plant phenols have attracted the attention of researchers for their ability to provide health benefits. Phenolic content (PC) in the free fraction, bound fractions and the sum of

Table 1: Content of phenolic constituents in the bran from rice genotypes

Varieties	Free phenols (mg g $^{-1}$ DW)	Bound Phenols (mg g $^{-1}$ DW)	Total phenols (mg g $^{-1}$ DW)	Flavonoids (mg g^{-1} DW)	*o*-dihydroxyphenols (µg g^{-1} DW)
PR 121	5.79 ± 0.02^{d}	12.83 ± 0.02^{g}	18.62 ± 0.04^{f}	0.19 ± 0.43^{m}	0.104 ± 0.21^{b}
PR 122	4.52 ± 0.24^{f}	14.31 ±0.06^{e}	18.83 ± 0.3^{f}	0.38 ± 0.93^{l}	0.061 ± 0.23^{h}
PR 123	15.85 ± 0.035^{a}	6.73 ± 0.06^{l}	22.58 ± 0.095^{d}	0.61± 1.91^{k}	0.067 ± 0.24^{g}
PR 124	6.66 ± 0.02^{c}	22.07 ± 0.07^{a}	28.73 ± 0.09^{a}	0.23 ± 0.55^{m}	0.037 ± 0.36^{k}
PR 125	7.11 ± 0.02^{b}	10.21 ± 0.02^{i}	17.32 ± 0.04^{h}	0.79 ± 1.36^{j}	0.072 ± 0.21^{f}
PR 126	6.77 ± 0.05^{c}	10.58 ± 0.03^{h}	17.35 ± 0.08^{h}	0.92 ± 0.99^{i}	0.1 ± 0.11^{c}
PAU 201	4.58 ± 0.02^{ef}	20.78 ± 0.05^{c}	25.36 ± 0.07^{c}	1.29 ± 1.07^{h}	0.032 ± 0.25^{l}
RYT 3388	4.71 ± 0.02^{ef}	17.61 ± 0.03^{d}	22.32 ± 0.13^{e}	1.39 ± 1.58^{g}	0.031 ± 0.21^{l}
RYT 3402	5.63 ± 0.04^{d}	21.53 ± 0.09^{b}	27.16 ± 0.11^{b}	0.17 ± 0.92^{m}	0.112 ± 0.21^{a}
RYT 3316	4.73 ± 0.01^{e}	7.62 ± 0.03^{k}	12.35 ± 0.04^{j}	0.62 ± 0.53^{k}	0.025 ± 0.31^{e}
PB 2	3.88 ± 0.04^{h}	7.72 ± 0.01^{k}	11.60 ± 0.05^{k}	2.24 ± 1.41^{b}	0.042 ± 0.32^{j}
PB 3	4.18 ± 0.03^{g}	14.12 ± 0.09^{f}	18.3 ± 0.12^{g}	2.32 ± 0.88^{a}	0.023 ± 0.42^{m}
PB4	3.97 ± 0.04^{h}	7.62 ± 0.04^{k}	11.59 ± 0.08^{k}	1.91 ± 1.41^{d}	0.072 ± 0.21^{f}
PB 5	3.11 ± 0.04^{i}	9.41 ± 0.02^{j}	12.52 ± 0.06^{j}	1.42 ± 0.64^{f}	0.089 ± 0.22^{d}
PB 1509	3.88 ± 0.02^{h}	10.56 ± 0.09^{h}	14.44 ± 0.11^{i}	1.54 ± 0.53^{e}	0.042 ± 0.32^{j}
PB 1121	3.28 ± 0.04^{i}	9.28 ± 0.015^{j}	12.56 ± 0.055^{j}	2.04 ± 1.01^{c}	0.051 ± 0.21^{i}
Mean	5.54	12.68	18.22	1.13	0.06
Range	3.11 – 15.85	6.73 – 22.07	11.59 – 28.73	0.17-2.32	0.023 – 0.112
HSD	0.2	0.17	0.24	0.92	0.93

Values are mean ± SD of three replicates. Means with the same letter in the same column are not significantly different.

Table 2: Antioxidant capacity of bran from different rice genotypes

Varieties	DPPH free radical-scavenging (% remaining)	Ferrous ion-chelating activity (%)	Reducing Power (mg g $^{-1}$ DW)	TEAC (mMg^{-1} DW)	FRAP (µM Fe(II)g $^{-1}$ DW)
PR 121	92.01 ± 0.79^{a}	43.07 ± 0.16^{l}	2.16 ± 0.02^{c}	1.31 ± 0.01^{a}	20.15 ± 0.04^{a}
PR 122	91.44 ± 0.21^{ab}	78.49 ± 0.11^{g}	1.79 ± 0.01^{e}	1.16 ± 0.01^{c}	12.56 ± 0.05^{h}
PR 123	92.59 ± 0.64^{a}	59.19 ± 0.42^{k}	1.24 ± 0.015^{h}	1.02 ± 0.02^{ef}	13.01 ± 0.02^{g}
PR 124	84.09 ± 0.07^{cd}	74.91 ± 0.17^{i}	2.44 ± 0.027^{a}	1.24 ± 0.01^{b}	15.22 ± 0.04^{c}
PR 125	87.03 ± 0.78^{c}	79.54 ± 0.16^{f}	2.30 ± 0.01^{b}	0.99 ± 0.01^{ef}	12.35 ± 0.06^{i}
PR 126	72.46 ± 0.84^{g}	84.52 ± 0.09^{d}	1.81 ± 0.015^{e}	0.97 ± 0.01^{f}	14.72 ± 0.08^{e}
PAU 201	87.94 ± 0.83^{bc}	87.35 ± 0.15^{c}	2.47 ± 0.01^{a}	1.05 ± 0.02^{ed}	11.99 ± 0.06^{j}
RYT 3388	82.63 ± 0.24^{d}	89.36 ± 0.23^{b}	2.01 ± 0.02^{d}	0.77 ± 0.01^{g}	16.98 ± 0.05^{b}
RYT 3402	86.19 ± 0.42^{cd}	85.11 ± 0.35^{d}	1.24 ± 0.02^{gh}	1.09 ± 0.02^{d}	12.64 ± 0.0^{h}
RYT 3316	84.4 ± 1.08^{cd}	87.08 ± 0.41^{c}	1.23 ± 0.01^{h}	1.10 ± 0.02^{d}	13.16 ± 0.04^{gf}
PB 2	75.69 ± 0.25^{efg}	89.25 ± 0.54^{b}	1.84 ± 0.01^{e}	0.97 ± 0.01^{f}	11.26 ± 0.05^{k}
PB 3	74.53 ± 0.24^{efg}	13.61 ± 0.38^{m}	1.69 ± 0.015^{f}	1.01 ± 0.02^{ef}	14.94 ± 0.08^{d}
PB 4	73.86 ± 0.89^{gf}	94.36 ± 0.13^{a}	1.81 ± 0.01^{e}	0.74 ± 0.01^{gh}	13.24 ± 0.17^{f}
PB 5	74.76 ± 0.87^{efg}	76.23 ± 0.31^{h}	1.3 ± 0.01^{gh}	0.61 ± 0.06^{i}	8.31 ± 0.02^{m}
PB 1509	76.65 ± 0.31^{ef}	68.43 ± 0.34^{j}	1.06 ± 0.01^{i}	0.54 ± 0.01^{j}	10.51 ± 0.04^{l}
PB 1121	77.88 ± 1.47^{e}	81.93 ± 0.31^{e}	1.31 ± 0.06^{g}	0.69 ± 0.035^{h}	10.67 ± 0.05^{l}
Mean	82.14	74.53	1.73	0.95	13.23
Range	72.46 – 92.59	13.61 – 94.36	1.06 – 2.47	0.54 – 1.31	8.31 – 20.15
HSD	0.93	0.90	0.07	0.06	0.20

Values are mean ± SD of three replicates.
Means with the same letter in the same column are not significantly different.

these two fractions (total) is shown in Table 1. Among rice genotypes, PR 123 had highest content of free PC (15.85 mg GAE g^{-1}DW) followed by PR 126 (6.77 mg GAE g^{-1}DW) and PR 124 (6.66 mg GAE g^{-1}DW) indicating wide variation in free PC. Apart from free phenolics, significant amounts of bound phenolics ranging from 6.73 – 22.07 mg GAE g^{-1}DW (Table 1) were found in rice bran. PR 124, RYT 3402 and PAU 201 contained significantly higher bound PC than other genotypes. It has been reported that most phenolic antioxidants exist in free forms in fruits and vegetables, while in cereals grains such as rice, significant amount of phenolics are found bound to the cell wall (Decker and Welch 1990)[24]. The only exception in our study was rice bran from PR 123 where free PC was found to be higher (15.85 mg GAE g^{-1}DW) compared to bound PC (6.73 mg GAE g^{-1}DW, Table 1).

Table 3: Antioxidant constituents in bran from different rice genotypes

Varieties	Phytate (mg g $^{-1}$ DW)	Anthocyanins (mg kg $^{-1}$ DW)	Proanthocyanidins (mg g $^{-1}$ DW)
PR 121	29.3 ± 0.03^{f}	0.012 ±0.30^{h}	7.75 ± 0.035^{a}
PR 122	31.20 ± 0.01^{d}	0.020 ± 0.31^{d}	4.33 ± 0.02^{de}
PR 123	34.80 ± 0.02^{b}	0.015 ± 0.18^{gf}	3.76 ± 0.04^{ef}
PR 124	36.70 ± 0.04^{a}	0.016 ± 0.02^{f}	4.27 ± 0.02^{ed}
PR 125	32.90 ± 0.01^{c}	0.015 ± 0.17^{g}	5.37 ± 0.03^{c}
PR 126	27.50 ± 0.04^{g}	0.017 ± 0.19^{e}	6.16 ± 0.03^{b}
PAU 201	21.60 ± 0.01^{i}	0.022 ± 0.31^{c}	6.11 ± 0.04^{b}
RYT 3388	23.70 ± 0.02^{h}	0.020 ± 0^{d}	4.86 ± 0.03^{cd}
RYT 3402	27.80 ± 0.03^{g}	0.015 ± 0.32^{g}	0.86 ± 0.05^{j}
RYT 3316	32.30 ± 0.02^{c}	0.021 ± 0.31^{d}	2.77 ± 0.03^{gf}
PB 2	27.20 ± 0.02^{g}	0.020 ± 0.03^{d}	3.39 ± 0.02^{gf}
PB 3	28.70 ± 0.03^{f}	0.025 ± 0.17^{b}	2.35 ± 0.02^{hi}
PB 4	27.30 ± 0.01^{g}	0.027 ± 0.18^{a}	2.59± 0.02^{hi}
PB 5	35.40 ± 0.03^{b}	0.018 ± 0.31^{e}	2.23 ± 0.01^{i}
PB 1509	30.30 ± 0.03^{e}	0.022 ± 0.31^{c}	2.93 ± 0.04^{hg}
PB 1121	30.70 ± 0.02^{ed}	0.025 ± 0.47^{b}	4.12 ± 0.02^{e}
Mean	29.83	0.019	3.99
Range	21.6 – 36.7	0.012 – 0.027	0.86 – 7.75
HSD	0.132	0.20	0.60

Values are mean ± SD of three replicates., Means with the same letter are not significantly different.

The total PC of the rice bran samples was found to be in the range of 11.59 – 28.73 mg GAE g^{-1}DW with the highest amount being present in PR 124 and the lowest in Punjab Basmati
2 and Punjab Basmati 4. The differences in the total PC among rice brans were mostly due to differences in bound PC because differences in free PC were quiet less (Table 1). The contribution of bound PC to total PC has been reported by Adom and Liu (2002)[9], to be 62% and 50% in rice grain and light brown bran respectively, while it was 63% in whole grain rice (Yu *et al* 2016)[14]. In our study, the bound PC in bran accounted for upto 82% of total PC. These differences may be because of use of different extraction solvents and differences in extraction procedures. The choice of the extraction solvent is of utmost importance because both the content of antioxidants and antioxidant activity is influenced by solubility in the extraction solvent (Devi *et al* 2007)[28]. Despite the differences in the contribution of bound TP among various studies, rice brans contain significant content of bound phenolics. Since the bound phenolics can be enzymatically released by microbiota in the colon during digestion and therefore can exert their antioxidant activity after absorption, inclusion of bound phenolics in the estimation of total PC in cereals and evaluation of their antioxidant effects is important (Adom and Liu 2002)[9].
Flavonoids act as strong antioxidants due to their ability to donate electrons as well to stop chain reactions. Therefore, they are implicated for their therapeutic role in protection against viral diseases, cardiovascular diseases, inflammation, ulcers and cancer (Min *et al* 2011)[29]. Total flavonoid concentration (FC) was found to be in the range of 0.17-2.32 mg GAE g^{-1}DW with a mean of 1.13 mg GAE g^{-1}DW. The highest total FC was present in Punjab Basmati 3 while the lowest was found in RYT 3402. The content of *o*- dihydroxyphenols varied from 0.023- 0.111 mg g^{-1}DW with a average of 0.063 mg g^{-1}DW. The location and number of the phenolic OH groups are important for the antiradical efficacy of flavonoids. Flavonoids consist of a 15- carbon skeleton, organized in two aromatic rings (A and B rings) interlinked by a three carbon chain structure (C_6- C_3-C_6). Flavonoids having an *ortho*- 3,4-dihydroxy structure at B- ring, hydroxyl groups in position *meta* eg 5,7 dihydroxy at ring A as well as a double bond between C_2 and C_3 hydroxyl group at ring C, exhibit strong radical scavenging activity.
Phytic acid (PA, myo-inositol hexakisphosphate, IP_6) is the primary phosphorous reserve present in plants and accounts for upto 85% of the total phosphorous in cereals and legumes. In our study, the phytate content varied from 21.60-36.70 mg g^{-1}DW with an average of 29.90 mg g^{-1}DW with significant variation at $P<0.05$. Lehrfeld (1994)[30] reported phytate content of 6.55% in rice bran while Kasim and Edwards (1998)[31] reported it to range from 5.94 – 6.10%. PA content may be influenced by genetics, environment, location, irrigation conditions, soil type and fertilizer application. The utilization of rice bran is limited due to its high phytic acid content. The antinutritional effect of PA is attributed to its ability to interact with minerals, proteins and starch causing reduced mineral bioavailability and altered protein functionality. However, it provides therapeutic effect by acting as an antioxidant by virtue of suppressing oxidative reactions catalyzed by iron and may lower the incidence of inflammatory bowel diseases, diabetes, and colonic cancer.
Anthocyanins are a group of purple to reddish water – soluble flavonoids extensively researched in fruits and

Table 4: Correlation coefficient between phytochemicals and antioxidant activities

	Free phenolics	Bound phenolics	Total phenolic content	Flavonoid	*o*-dihydroxyphenols	DPPH free radical scavenging activity	FRAP	Reducing power	Metal Chelating activity	$ABTS^{\cdot+}$ scavenging activity
Free phenolics										
Bound phenolics	-0.137									
Total phenolic content	0.404	0.850**								
Flavonoids	-0.441	-0.367	-0.574*							
o-dihydroxyphenols	0.188	-0.064	0.0415	-0.438						
DPPH free radical scavenging activity	0.510*	0.305	0.553*	-0.739**	0.103					
FRAP	0.192	0.299	0.378	-0.365	0.085	0.337				
Reducing power	-0.045	0.468	0.408	-0.149	-0.117	0.245	0.479			
Metal Chelating activity	-0.178	-0.005	-0.099	-0.07	0.066	-0.072	-0.366	0.043		
$ABTS^{\cdot+}$ scavenging activity	0.324	0.393	0.535*	-0.652**	0.105	0.647**	0.593*	0.488	-0.239	0.1

* represents significant at P<0.05, **represents significant at P<0.01 respectively

scavenging activity and total phenolic content (r = 0.535, Tables 2 and 4). Among the rice varieties with brown pericarp the mean $ABTS^{\cdot+}$ scavenging activity reported by Shen *et al* (2009)[35] was 0.196 mM TEAC, and the range was 0.012-0.413 mM TEAC. Both $ABTS^{\cdot+}$ scavenging activity and DPPH radical scavenging activity are electron transfer based reactions in which they involve one redox reaction with the oxidant as an indicator of the reaction end point (Huang *et al* 2005)[36]. In rice bran, a large number of bioactive compounds exist which may possess scavenging activity against $DPPH^{\cdot}$ or $ABTS^{\cdot+}$ depending upon concentration of individual bioactive compounds in rice bran extracts and their synergistic effects. FRAP values of bran from rice genotypes studied ranged from 8.31 - 20.15 µM Fe(II)g^{-1} DW with mean of 13.23 µM Fe(II)g^{-1} DW (Table 2). PR 121 exhibited highest FRAP value of 20.15 µM Fe(II)g^{-1} DW, while lowest FRAP value was observed in Punjab Basmati 5 (8.31 µM Fe(II)g^{-1} DW). FRAP is sensitive to single electron transfers while DPPH radical scavenging activity test is sensitive to both single electron transfer and hydrogen atom transfer.

In our study, the reducing power capacity was in the range of 1.06-2.47 mg g^{-1}DW with mean of 1.73 mg g^{-1}DW (Table 2). Pusa Basmati 1509 (1.06 mg g^{-1} DW) exhibited lowest reducing power while PAU 201 (2.47 mg g^{-1} DW) possessed highest reducing capacity. Reducing power assay measures the ability of an antioxidant to donate electrons and oxidize intermediates of lipid peroxidation process. Antioxidants do not act only by chain- breaking mechanisms, but also display preventive role through which they can retard the rate of oxidation of free radicals. One of most important mechanisms of action of preventive or secondary antioxidants is complexation/ chelation of pro-oxidant metal ions. The metal chelating ability of bran extracts from genotypes under investigation was found in the range of 13.61 – 94.36% with the maximum being present in Punjab Basmati 4 while the minimum was found in Punjab Basmati 3. Significant variations in the metal chelating ability were observed among genotypes (Table 2). An extract with high chelating power reduces the free ferrous ion concentration by forming a stable Fe (II) chelate, thereby decreasing the extent of Fenton reaction which causes a number of diseases.

CONCLUSION

The significant differences in antioxidative activities of rice bran obtained from different genotypes indicated that genetic diversity in phenolic constituents may allow for breeding new rice cultivars with enhanced health promoting ingredients. It also provides a basis for developing rice bran as valuable food additive by virtue of its phytochemical composition and anti-oxidant activity. Future studies may be focused on the effect of location, growth conditions and environmental factors on the content of phytochemicals and their antioxidant potential in rice bran.

ACKNOWLEDGEMENT

The authors thank Dr. Pritpal Singh for his valuable help and keen guidance during the statistical analysis of the

Table 5: Designations, shape and size of rice genotypes used in present study

S.no	Genotypes	Designation	Grain size and shape classification
1	PR 121	PR 121	Long slender
2	PR 122	PR 122	Long slender
3	PR 123	PR 123	Extra long slender
4	PR 124	PR 124	Long slender
5	PR 125	PR 125	Medium slender
6	PR 126	PR 126	Long slender
7	PAU 201	PAU 201	Long slender
8	RYT 3388	2k10 – 23 – 451 – 2 – 164 – 127 – 0 – 0	Long slender
9	RYT 3402	2k10 – 23 – 54 – 4 – 76 – 40 – 0 – 0	Long slender
10	RYT 3316	2k10 – 322 – 5 – 1 – 1 – 9 – 1 – 1 – 1 – 1 - 1	Long slender
11	Punjab Basmati 2	Punjab Basmati 2	Extra long slender
12	Punjab Basmati 3	Punjab Basmati 3	Extra long slender
13	Punjab Basmati 4	Punjab Basmati 4	Extra long slender
14	Punjab Basmati 5	Punjab Basmati 5	Extra long slender
15	Pusa Basmati 1509	Pusa Basmati 1509	Extra long slender
16	Pusa Basmati 1121	Pusa Basmati 1121	Extra long slender

data.

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