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CONTENTS

Exploration of red rice land races from north western Himalayas for availability and interactions of anthocyanin and antioxidant nutrients

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ABSTRACT: In Himalayan region colored rice races are being cultivated since old age and these practices still continued. This study is focused on the nutritional value of pigmented rice land races grown in hillyregions of Uttarakhand as compare to white rice variety of tarai region. Eight local land races of pigmented rice from Thalisain (PauriGarhwal: Elevation: 1695 meter), Ranikhet (Kumaun: Elevation: 1869 meter) and one commonly grown white rice variety Pant Dhan 14 from tarai region were taken for the present study. All these pigmented rice land races as well as white rice variety were assessed for anthocyanin, protein, dietary fiber and secondary metabolites *viz,* phenol, flavonoids along with antinutritional factor phytic acid. Significant amount of anthocyanin was found in pigmented rice grain (0.3 to 1.5 mg/100g) and was not found in Pant Dhan14. High albumin and globulin protein concentrations was found in all of the pigmented rice land races but two (G6 and K1) as compared to Pant Dhan14. High dietary fiber (1.5%-2%) and high phenols (10-20mg/g) and flavonoids (9-15mg/g) and low phytic acid (0.5 - 1.5mg/g) content was found in all pigmented rice land races of Uttarakhand as compare to Pant dhan-14(dietary fiber(0.5%), phenols(8mg/g), flavonoids (7mg/g), phytic acids(3.2mg/g)). All above studies suggest the nutritional superiority of pigmented rice local land races of Uttarakhand hilly regions as compare to white rice variety. Highlighting this superiority aims to encourage the consumption of pigmented rice for better dietary health, support its cultivation to preserve traditional practices and biodiversity and enhance food security through diet diversification.

Key words: Anthocyanin, dietary fibers, flavonoids, phenols, phytic acid, protein

Rice is a fundamental food source for billions of people in Asia, providing approximately 27% of total calorie intake in low- and middle-income countries (Bhattacharya, 2013). Classified under the order *Glumiflorae* and the grass family *Poaceae*, domesticated rice comprises two primary species: *Oryza sativa* and *Oryza glaberrima*, which are native to tropical and subtropical regions of South Asia and Southeast Africa, respectively (Crawford and Shen, 1998).Rice also serves as a crucial crop that embodies significant cultural and social value in many societies. It plays a central role in traditional diets and is often involved in various cultural rituals and festivities. However, rice production faces numerous challenges, including the pressing need for increased yields to meet global food demands, the reduction of post- harvest losses and the adaptation to the impacts of climate change, which can alter growing conditions and affect crop viability.Moreover, enhancing the nutritional profile of rice is imperative for addressing malnutrition in vulnerable population (Bhullar and Gruissem, 2013). Among the numerous rice land races, various quality traits are critical for ensuring food security, including high caloric content, protein levels, disease resistance and overall nutritional value. Recently, there has been growing interest in exploring and evaluating pigmented rice land races, which offer additional health advantages due to their high content of bioactive compounds (Bhat *et al.*, 2020).In various states across India, colored rice has been cultivated for centuries. These strains owe their unique hues to the accumulation of anthocyanin pigment in the bran layer of the grain. These pigments can manifest in colors such as red, blue or purple, depending on pH conditions; for instance, anthocyanins appear red in acidic environments and blue in alkaline conditions (Brouillard, 1983; Giusti and Wrolstad, 2001). The presence of anthocyanins is particularly noteworthy because they are well-known for their antioxidant properties, which help mitigate oxidative stress and reduce the risk of chronic diseases such as cardiovascular ailments, diabetes and certain types of cancer (Tsuda, 2012; Wang *et al.,* 2023).

Additionally, research has suggested that anthocyanins may improve cognitive function and promote overall health by enhancing memory and reducing neuro-inflammation (Dos Santos *et al.,* 2019).Besidesanthocyanins, pigmented rice is also rich in other bioactive compounds, such as phenolic acids, flavonoids, dietary fiber and vitamins. Phenolic compounds, including ferulic acid and caffeic acid, exhibit strong antioxidant properties and have been linked to anti- inflammatory effects and improved heart health (ZduDska *et al.,* 2018). Flavonoids, which include compounds like quercetin and kaempferol, contribute to the anti-inflammatory and anti-cancer properties of rice (Wang *et al.,* 2023). Dietary fiber is another critical component found in pigmented rice, which aids in digestion, promotes satiety and helps regulate blood sugar levels (Slavin, 2013). High fiber intake is associated with reduced risks of various chronic diseases, including heart disease and type 2 diabetes (Anderson *et al.,* 2009). Furthermore, pigmented rice tends to have lower levels of phytic acid, an anti-nutritional factor that can inhibit the absorption of essential minerals such as iron and zinc (Hurrell and Egli, 2010).Thus, by and large consumption of pigmented rice, individuals may benefit from enhanced mineral bioavailability, contributing to better overall nutritional status.

Exploring the potential of pigmented rice varieties presents a promising solution to these challenges. By promoting the consumption of these nutritionally superior varieties, it is possible to improve public health outcomes and support sustainable agricultural practices. The higher antioxidant content and unique phytochemicals found in pigmented rice not only offer significant health benefits but also contribute to biodiversity in rice cultivation, which is crucial for resilience against pests and diseases. Ultimately, the integration of pigmented rice into agricultural systems and diets could play a pivotal role in enhancing food security and promoting health across communities.

MATERIALS AND METHODS

Plant material

In this study, eight pigmented localrice land races (G1, G2, G3, G4, G5, G6, G7, K1) originating from Thalisain and nearby areas (Pauri Garhwal, Elevation: 1695 meters) and Ranikhet (Kumaun, Elevation: 1869 meters), along with one commonly cultivated white rice variety, Pant Dhan 14 (C), from the Tarai region, were selected for analysis. The samples were stored in moisture-free chambers prior to further testing. In this nomenclature, "G" represents samples from the Garhwal region, "K" represents samples from the Kumaun region and "C" serves as the control. Table 1 provides details on the local names and collection locations of the rice samples.

Determination of total anthocyanin content

Total Anthocyanin content in the samples was determined using the method described by(Pal *et al.*, 2019) with some modifications. Briefly, 1 g of sample was homogenized with 5 ml acidified organic solvent (95% methanol: 1.5 N HCl, 85: 15, v/ v; pH = 1). The sample was extracted twice by keeping it overnight with solvent followed by centrifugation at 10000 rpm for 15-20 min in a chilling centrifuge. The final volume of supernatant was made to 10 ml and the absorbance was read at 535 nm. Total anthocyanin content of the samples was calculated as mg total anthocyanin per 100 g of sample using formula described below.

Total Anthocyanin(mg/100g)= Absorption × Molecular weight ×1000 /Molar absorptivity ×Path length = Absorption × 450 × 1000/26900 ×1

Extraction of phenolic compounds

Respective powdered samples (500 mg each) were

Table 1: Local name and locations of rice samples collected from

Rice samples	Name of village collected from	Local name
G1	Fafriana (Thalisain , PauriGarh.wal)	Jeeri
G2	Fafriana (Thalisain, PauriGarhwal	Chaurya
G3	Ainthi (Thalisain, PauriGarhwal)	MotaChawal
G4	Rauli (Thalisain, PauriGarhwal)	Jeeri
G5	Gadamason (Thalisain, PauriGarhwal)	Chaurya
G6	Maason (Thalisain , PauriGarhwal)	Chaurya
G7	Rangaon (Thalisain, PauriGarhwal)	Motachawal
K1	Ranikhet (Kumaun)	Lal chawal
C	NEB Crop research center, Pantnagar	Pant Dhan 14

mixed individually with 5 ml of 80 % methanol and ground thoroughly in pestle and mortar. The ground material was centrifuged at 600 rpm for 15 min. The volume was made to 50 ml by washing with 80 % methanol. The extract thus prepared was used for the estimation of total phenol and flavonoids(Dai and Mumper, 2010).

Determination of phenolic compounds

The total phenolic contents in respective flours were determined by Folin Ciocalteu reagent Ainsworth and Gillespie, (2007) and calculated from a standard calibration curve based on gallic acid (0–0.1 mg/ml). The results were expressed as gallic acid equivalents in mg GAE/g DW for total phenols.

Determination of phytic acid

Phytic acid (PA) contents in defatted flour samples were determined by the method of Wu *et al.*, 2009. The phytic acid content was calculated from a standard curve using phytate phosphorus salt in the range of 10–50 µg.

Determination of dietary fiber

Total dietary fiber was determined calorimetrically by following the alkali treatment method McCleary (2003).

Extraction of protein content

Rice flour (50 g) was defatted with 200 mL hexane.The defatted rice flour was dried under a hood at ambient temperature for 24 h. The flour was then extracted for albumin fraction of protein by shaking with 200 mL distilled water at 20ºC for 4 h and then centrifuged at 3000 rpm for 30 min. After water extraction, the flour was further used to globulin fraction extraction with 200 ml of 5% NaCl at 20ºC for 4 h and centrifuged at 3000 rpm for 30 min. The flour was then extracted for glutelin with 100 mL of 0.02 M NaOH (to pH 11.0), at 20 ºC for 30 min and followed by prolamin extraction with 100 mL of 70% ethanol at 20ºC for 4 h (Ju *et al.,* 2001).Each extraction was repeated three times in order to remove all the protein of each fraction.

Determination of protein content

Total protein content was determined using Bradford protein assay using bovine serum albumin as a standard. Different buffers were used for different protein. For albumin used water, for globulin used 5%NaCl, for glutelin used 0.03 M NaOH for prolamin used 70% ethanol as a buffer. Same buffers were used for preparation of standard for each protein. Used buffer as a blank and absorption was taken at 595 nm.

Statistical analysis

The data reported in all the figures were subjected to one-way-analysis of variance(ANOVA) using SPSS16.0.

RESULTS AND DISCUSSION

Nutritional properties

The total dietary fiber content of various test rice land races and control variety were analyzed and is presented in Figure 1. Among the tested samples, the G4 pigmented rice exhibited the highest dietary fiber content at 1.97%, followed closely by K1 with 1.83%. The remaining pigmented rice displayed slightly lower levels, with G6 at 1.69%, G7 at 1.67% and G2 at 1.57%. G1, G3 and G5 all had comparable fiber contents of 1.47% and 1.46%, respectively. The control variety (Pant Dhan 14) had the lowest dietary

Table 2: Correlation among Anthocyanin, Phenol, Flavonoid, Phytic acid , total Protein and Dietary fiber in the screened rice samples

Variables	Anthocyanin	Phenol	Flavonoid	Phytic Acid	Total Protein	Dietary Fiber
Anthocyanin	1.00	0.97*	0.81*	-0.43	-0.08	0.66
Phenol	0.97*	1.00	0.90*	-0.52	-0.27	0.71*
Flavonoid	0.81*	0.90	1.00	-0.58	-0.37	0.75*
Phytic Acid	-0.43	-0.52	-0.58	1.00	0.53	-0.89*
Total Protein	-0.08	-0.27	-0.37	0.53	1.00	-0.43
Dietary Fiber	0.66	0.71	0.75	-0.89	-0.43	1.00

(r=1, Positively correlated, r=-1, Negatively correlated), *=Indicates significant levels at 0.05

fiber content at 0.47% only. Liese *et al.*, 2003 stated that increased intake on total dietary fiber appears to be useful for the treatment of both type 2 diabetes and heart related problem. (Rebeira *et al.*, 2022) had reported the total dietary fiber content (1.16% to 6.79%) in pigmented rice varieties.These results are also in agreement with the values reported by (Amrinola *et al.,* 2021).

The total protein content ranged from 4.9 to 5.8 mg/g among the test and control rice samples studied(Figure2). The highest total protein was observed inland race G2 (5.8 ± 0.03 mg/g), followed closely by G1 (5.7 ± 0.10 mg/g) and G5 (5.5 ± 0.06 mg/g) land races. In contrast, G3 pigmented rice land raceexhibited the lowest total protein content (4.9 ± 0.03 mg/g). Albumin levels did not show a

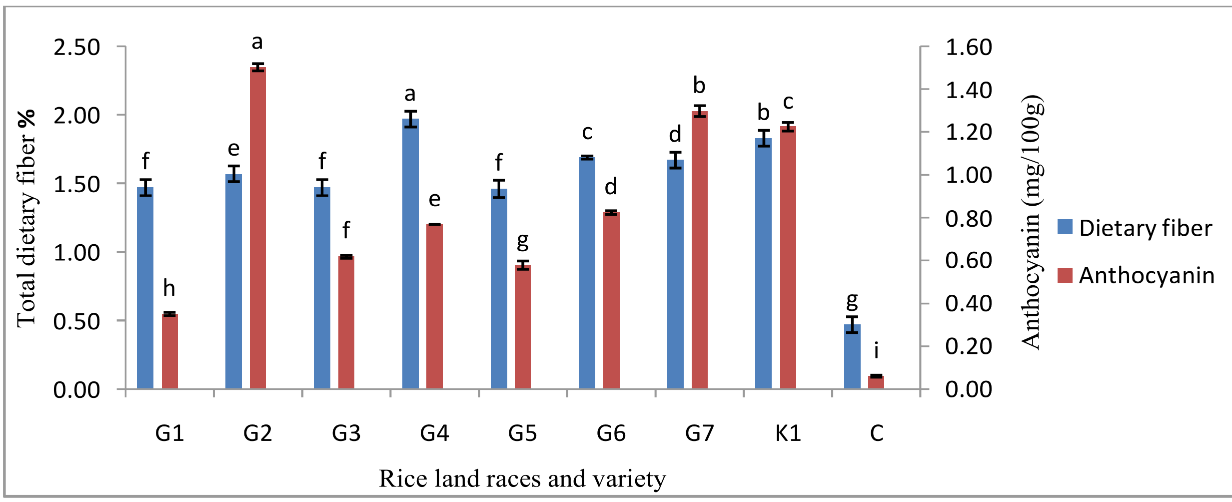

Fig.1: Total dietary fiber and total anthocyanin content in different pigmented riceland races(G1, G2, G3, G4, G5, G6, G7, K1) and white rice variety(C). Values are expressed as mean ± standard deviation (n = 3). Means with different letters (a, b, c, d, e, f, g, h and i) were significantly different at the level of P≤0.05

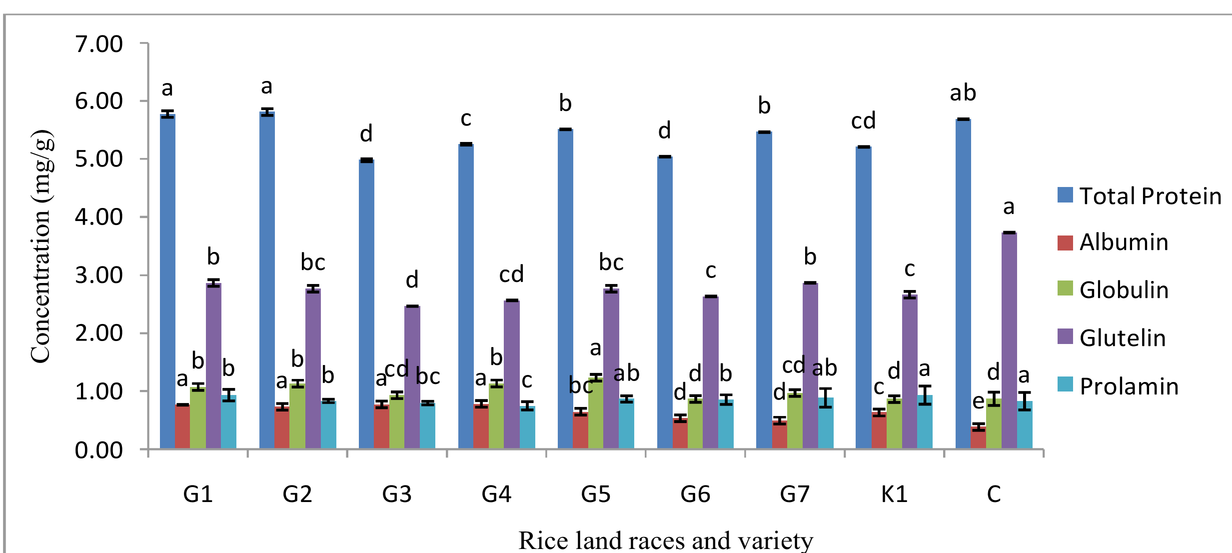

Fig.2: Total protein, Albumin, Globulin, Glutelin and Prolamin content of pigmented rice land races and white rice variety. Values are expressed as mean ± standard deviation (n = 3). Means with different letters (a, b, c, d, ab, cd and bc) were significantly different at the level of P≤0.05.

significant variation among the land races, with G1 land racepresenting the highest albumin content (0.76 ± 0.05 mg/g) and G6 the lowest (0.53 ± 0.005 mg/g). Notably, G4 and G2 pigmented rice land racesdemonstrated comparable albumin levels (0.78 ± 0.01 and 0.73 ± 0.05 mg/g, respectively), suggesting a relatively consistent presence of this protein fraction across different samples.Globulin content varied significantly, with G5 land raceexhibiting the highest value (1.20 ± 0.05 mg/g), indicating its potential for contributing to the overall protein intake. Conversely, G6 and G3land raceshad the lowest globulin levels (0.86 ± 0.05 mg/g and 0.93 ± 0.05 mg/g, respectively). The variations in globulin content among the rice varieties suggest differing capacities for nitrogen storage and may reflect genetic differences in protein synthesis pathways (Xinkang *et al.*, 2023). Glutelin content ranged from 2.46±0.05 mg/g in G3land race to 3.73±0.05mg/g in control rice variety, Pant Dhan 14.Prolamin content varied slightly across the tests and control samples of rice, with the highest value in pigmented riceland raceG1 (0.93 ± 0.05 mg/g) and the lowest in G6 (0.85 ± 0.005 mg/g) land race. These results are in agreement with the values reported by Ju *et al.*(2001). The presence of these protein fractions is significant, as they contribute to the nutritional profile and functional properties of rice.The results indicate that pigmented riceland races (particularly pigmented rice land racesG1 and G2) not only provide higher total protein content but also exhibit a favorable balance of protein fractions compared to the Pant Dhan 14 i.e., control white rice variety.

Anti-nutritional properties

The phytic acid content of various rice land races and control varietywere analyzed and is presented inFigure 3.The phytic acid concentrations ranged from 0.5 mg/g to 3.2 mg/g, with the highest concentration observed in control varietyi.e., Pant Dhan 14 (3.2 ± 0.12 mg/g) and the lowest in land races G3, G4 and K1, each showing a concentration of 0.5 ± 0.10 mg/g or 0.5 ± 0.11 mg/g.Similar range of phytic acid in pigmented rice was also reported by Goufo and Trindade, (2014). Phytic acid is considered as an antinutritional factor, for its ability to bind, precipitate and decrease the availability of di-and trivalent cationic minerals (Oatway *et al.*, 2001). Phytic acid was known to inhibit number of alimentary canal enzymes such as pepsin, ±-amylase and trypsin (Ravindran, 1992).The high levels of phytic acid in white rice variety i.e. Control, could suggest a reduced bioavailability of minerals, which

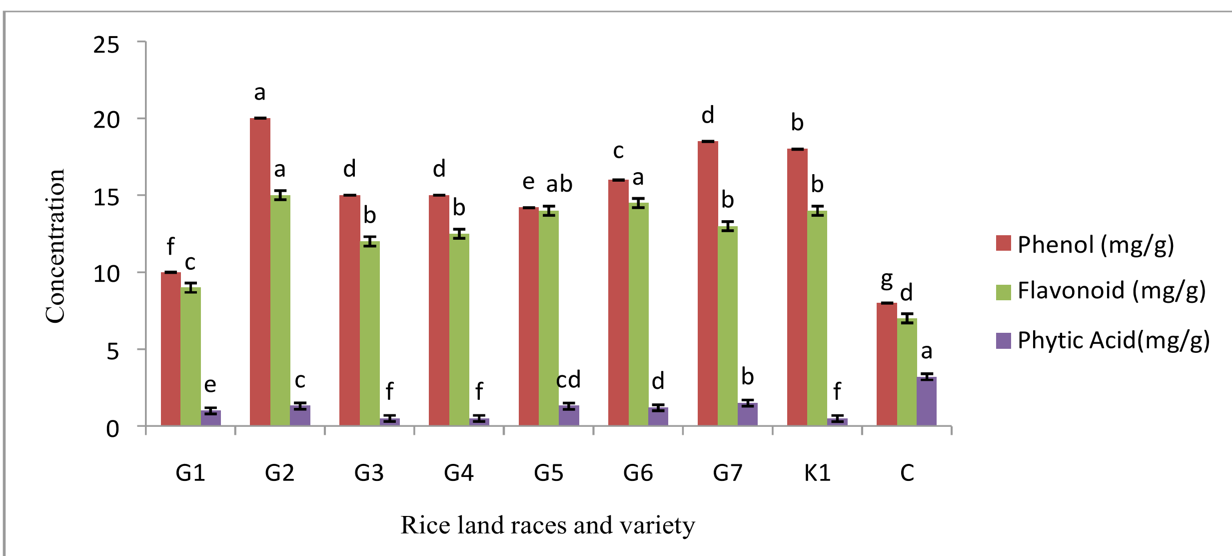

Fig.3: Total phenol, flavonoid and phytic acid content of pigmented riceland races and white rice variety Pant Dhan 14. Values are expressed as mean ± standard deviation (n = 3). Means with different letters (a, b, c, d, e, f, g, ab and cd) were significantly different at the level of P≤0.05

may impact dietary choices and nutritional strategies. On the other hand, the lower phytic acid levels in pigmented rice land races G3, G4 and K1 may enhance mineral absorption, making these varieties more favorable for human consumption in contexts where mineral deficiencies are prevalent(Kumar *et al.,* 2017).

Bioactive Compounds

The anthocyanin content across the evaluated varietiesand land races demonstrated notable variation, as shown in Figure 1. The anthocyanin concentrations ranged from 0.06 mg/100g in control Pant Dhan 14 to 1.50 mg/100g in G2 land race, the highest anthocyanin concentration indicating a substantial disparity among the different rice land races. High anthocyanin availability in pigmented rice suggests enhanced antioxidant properties, which are often attributed to higher anthocyanin levels(Tsai *et al.,* 2002). In contrast, in Control variety(Pant Dhan14) had lowest concentration at 0.06 ± 0.005 mg/100g, indicating a minimal contribution to antioxidant activity and consequent health benefits.Other localland raceshad also showed varying levels of anthocyanins. For instance, pigmented rice land races G7 and K1 contained 1.30 ± 0.025 mg/100g and 1.23 ± 0.02 mg/100g, respectively, positioning them as varieties with relatively high anthocyanin content. Pigmented rice land racesG4(0.70 ± 0.01 mg/100g), G3(0.60 ± 0.007 mg/100g) and G5 (0.57 ± 0.02 mg/100g) exhibited moderate anthocyanin levels while G1 had the lowest concentration among the tested land races (0.30 ± 0.007 mg/100g). These results are in agreement with the values reported by (Agustin *et al.*, 2021). Anthocyanins have antioxidant and antimicrobial properties, leading to improvement of visual and brain health and protecting against various non-communicable illness (Khoo *et al.,* 2017).

The total phenolic content across the evaluated samples is summarized in Figure 3**.** The phenolic concentrations varied significantly, ranging from 8.0 mg/g in white rice variety i.e., Control and then 20.0 mg/g in pigmented rice land race G2, indicating a marked diversity in phenolic compound levels among the tested samples. Pigmented rice land races were observed with highphenolic concentrations which also contribute to their potential health benefits, as phenolic compounds are known for their antioxidant properties and role in disease prevention (Fernez – Panchon *et al.,* 2008). Conversely, control white rice variety (Pant Dhan 14), displayed the lowest phenolic content at 8.0 ± 0.58 mg/g, suggesting a reduced capacity for antioxidant activity compared to the other land races. Other pigmented rice land races demonstrated varied phenolic levels as G6 (16.0 ± 0.17 mg/g), G7 (18.5 ± 0.32 mg/g) and K1 (18.0 ± 0.06 mg/g) showed relatively high concentrations, land races *viz;* G3 (15.0 ± 0.46 mg/g) and G4 (15.0 ± 0.29 mg/g) also exhibited slightly lower quantities of phenolic and least available phenolic content in G5 (14.2 ± 0.12 mg/g) indicated their potential utility in health- promoting applications (Bhuyan and Basu*,* 2017). These results are in agreement with the values reported by(Shen *et al.*, 2009).

The total flavonoid contents across the evaluated rice variety and local land racesare summarized in Figure 3. The concentrations of flavonoids varied significantly among the rice samples, with values ranging from 7.0 mg/g in control white rice variety, to 15.0 mg/g in pigmented riceland race G2, highlighting considerable diversity in flavonoid levels. Pigmented riceland race G2 exhibited the highest flavonoid concentration at 15.0 ± 0.21 mg/g, indicating its potential as a rich source of these bioactive compounds, which are associated with various health benefits, including antioxidant, anti-inflammatory and cardioprotective effects(Yi *et al.*, 2017). In contrast, Controlrice variety Pant Dhan 14 had the lowest flavonoid content at 7.0 ± 0.12 mg/g, suggesting limited health-promoting properties compared to other land races.Intermediate flavonoid levels were observed in some pigmented rice land races, including G6 (14.5 ± 0.33 mg/g), G5 (14.0 ± 0.05 mg/g) and K1 (14.0 ± 0.41 mg/g), all of which demonstrated significant concentrations that may enhance their functional food potential. Pigmented riceland racesG3 (12.0 ± 0.12 mg/g) and G4 (12.5 ± 0.12 mg/g) also showed moderate flavonoid levels.These results are in agreement with the values reported by Goufo and Trindade, (2014).The significant variation in flavonoid content among the tested rice land races suggests that genetic and environmental factors may influence flavonoid biosynthesis. The high flavonoid content in land racesG2 and G6, in particular, highlights their potential for development as functional foods aimed at improving health outcomes.

The correlation matrix presented in Table 2 reveals the interrelationships among six bioactive compounds: anthocyanin, phenol, flavonoid, phytic acid, total protein and dietary fiber**.** Strong positive correlations are observed between anthocyanin and phenol (r = 0.97), phenol and flavonoid (r = 0.90) and flavonoid and dietary fiber (r = 0.75), indicating a tendency for these compounds to co- occur in the same samples. In contrast, phytic acid exhibits significant negative correlations with both dietary fiber (r = -0.89) and anthocyanin (r = - 0.43), suggesting an inverse relationship between these variables. Total protein shows weak or negligible associations with most of the other variables, with a moderate positive correlation observed only with phytic acid (r = 0.53). These results indicate that anthocyanin, phenol and flavonoid may occurstogether, phytic acid and total protein may exhibit distinct and independent patterns of association with other components.

CONCLUSION

Incorporating pigmented red riceland races into the diet can offer enhanced nutritional benefits compared to white ricei. e., Pant Dhan 14, including higher fiber, antioxidant content and better overall nutrient density. These advantages make red rice a more beneficial choice for health-conscious individuals looking to improve their dietary intake and reduce the risk of chronic diseases. Present study showed that red pigmented riceland races contained high dietary fiber, albumin, globulin, anthocyanin, phenols, flavonoids as compare to white rice variety. Among antinutritional compound red pigmented riceland racescontains lower phytic acid content compare to white rice variety. All above studies suggest the nutritional superiority of red pigmented rice verities as compare to white rice variety. Overall, incorporating red pigmented rice into the diet can improve nutrient density and support better health outcomes compared to white rice.

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