RESEARCH PAPER

Comparative Analysis of Antioxidant Activity Attributed to Anthocyanin Content in Diverse Cereals

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ABSTRACT
Cereals and legumes are major dietary staples containing bioactive phytochemicals that contribute to antioxidant potential. Pigmented cereals, particularly those rich in anthocyanins, are increasingly recognized as functional foods. This study comparatively evaluated eleven cereals and legumes for total phenolic content (TPC), total flavonoid content (TFC), total protein content (TPrC), total amine content (TAmC), and antioxidant activity.TPC was determined using Folin–Ciocalteu and Gibbs methods, TFC by aluminum chloride colorimetric assay, TPrC by Biuret method, and TAmC using MBTH–NQS assays. Antioxidant activity was evaluated using the DPPH radical scavenging method.Black rice and ragi exhibited the highest TPC (9.60 and 7.02 mg GAE/2 g DW, respectively) and TFC (4.69 and 3.30 mg RE/2 g DW, respectively). Kidney beans showed the highest protein content (7.77 mg BSAE/2 g DW), while black chana exhibited elevated amine content (6.89 mg HE/2 g DW). DPPH radical scavenging activity was highest in black rice (86%), followed by ragi (80%) and black chana (73.3%).The results demonstrate that pigmented cereals possess superior antioxidant capacity and significant nutraceutical potential, supporting their use in functional food development.
Keywords: *Anthocyanins, Phenolic content, Flavonoids, Antioxidant activity, DPPH assay*
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INTRODUCTION

Cereals are staple foods consumed worldwide and represent a vital source of carbohydrates, proteins, vitamins, minerals, and bioactive compounds [1]. Beyond their nutritional role, cereals such as rice, maize, wheat, and millets are increasingly recognized for their functional properties due to the presence of phenolics, flavonoids, and other secondary metabolites [2]. Technological advancements have further enhanced the retention and bioavailability of these bioactive components in cereal-based foods[3].

Pigmented cereals, including black rice, red rice, colored wheat, and finger millet (ragi), contain higher concentrations of anthocyanins and polyphenolic compounds located primarily in the bran layer [4-6]. Comparative studies have demonstrated that pigmented rice varieties exhibit significantly higher phenolic and flavonoid contents than non-pigmented varieties, correlating strongly with antioxidant activity [7-9]. Finger millet has also been reported to contain considerable levels of phenolic compounds contributing to oxidative stress reduction [10].

Maize and other cereal crops similarly contain phenolic acids and antioxidants, although their concentrations vary depending on genotype and environmental conditions [11-13]. Reviews have emphasized the importance of colored wheat and lesser-known cereal crops in improving nutritional security and promoting sustainable agriculture[14-17]. Variability in phytochemical composition among wheat, barley, and rye has also been reported [18].

Phenolic compounds present in whole grains are associated with reduced risk of chronic diseases such as type 2 diabetes and cardiovascular disorders [19]. The antioxidant activity of cereals has been extensively evaluated using in vitro assays including DPPH radical scavenging methods[20-24]. Functional food components derived from cereals also demonstrate medicinal properties and potential health-promoting effects [25].

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Additionally, bioactive peptides and secondary metabolites contribute to the nutraceutical value of cereals [26].

Despite the growing body of literature, comparative biochemical profiling of multiple cereals within a single analytical framework remains limited [27], Recent advances highlight the need for detailed phytochemical characterization of cereals to support their application in functional foods and health-oriented dietary formulations [28-30]. Therefore, the present study was designed to evaluate total phenolic content (TPC), total flavonoid content (TFC), total protein content (TPrC), total amine content (TAmC), and antioxidant activity of eleven cereal and legume samples to provide a comprehensive understanding of their nutritional and functional potential.

METHODOLOGY

Materials and Methods

Analytical grade solvents and reagents were procured . For phenolic and flavonoid estimation, distilled water, methanol, HCL (hydrochloric acid) (for acidified methanol), gallic acid (standard), sodium carbonate, FC-GB Reagents, rutin (standard), 10% aluminium chloride, 5% sodium nitrate, and 1 M NaOH were used. Protein estimation employed bovine serum albumin (BSA, standard) and biuret reagent, while amine estimation involved histamine (standard), MBTH, NQS, and sodium hydroxide buffer (pH 13). Antioxidant activity was assessed using DPPH (0.1 *mM* solution) in methanol. The instruments and equipment used included volumetric flasks (Borosil), manual glass pipettes, double beam UV-visible spectrophotometers (ELICO-210 and Systronic 2203), digital weighing balance, centrifuge, glass funnels, and beakers.

Preperation for Reagents

(a). 2% Folin–Ciocalteu reagent : 2 ml of FC reagent was diluted to 10*ml* distilled water

(b). 7.5% Sodium chloride [Na_2CO_3]: 7.5*g* of sodium chloride in 100*ml* distilled water

(c). Gibbs reagent: taken 0.1*g* of gibbs reagent powder in 100*ml* volumetric flask and now, add ethanol up to the mark.

(d). 2% Aluminium chloride [$AlCl_3$] :2*g* of aluminium chloride in 100 *ml* methanol

(e). 5% Sodium acetate: T 5.00*g* sodium acetate in a 100 *ml* volumetric flask, add 80ml of distilled water stirr thoroughly until dissolved and make up volume till upto the mark with distilled water

(f). Biuret reagent : (alkaline copper sulfate solution) for protein estimation.

(g). MBTH Reagent : taken 0.5*g* MBTH reagent in 100 *ml* volumetric flask and diluted with distilled water to the mark and stored at 5c freeze temp for 10 min.

(h). NQS Reagent : taken 0.5g NQS in 100*ml* volumetric flask and add distilled water upto the mark

(i). Ferric chloride: taken 1.35*g* of ferric chloride in 100*ml* distilled water to prepare a 0.1M solution.

(j). DPPH 0.1mM solution: Taken 3.94 *mg* DPPH rapidly (light-sensitive). Dissolved in ~80 *mL* methanol with mild swirling (no heating).

METHODOLOGY

Extraction

Cereal samples were first soaked in distilled water for 48 hours to soften the matrix and release soluble components. The hydrated material was then triturated using a mortar and pestle, followed by acidification (hydrochloric acid) with methanol to facilitate the extraction of phenolic and flavonoid compounds. The mixture was sonicated to enhance cell disruption and centrifuged to separate the soluble fraction. The resulting supernatant was carefully filtered and subjected to mild heating at 100 °*C* to remove residual solvents, yielding a concentrated extract suitable for subsequent spectrophotometric analyses.

TOTAL PHENOLIC CONTENT

FC-GB method with minor changes is used for estimation of TPC . 1 *mL* of extract was combined with 2.5 *mL* of diluted 10-fold Folin–Ciocalteu reagent in distilled water. After 10 *min,* 2 *mL* of 7.5% odium carbonate solution was added, and the reaction mixture was kept at room temperature for 40 min. Absorbance was measured at 620 *nm* in a UV–Vis spectrophotometer. Gallic acid was utilized as calibration standard, and the results were calculated as mg gallic acid equivalents (GAE) per 2 gram dry weight (DW) as indicated in table 1. To further confirm phenolic groups, Gibbs reagent was also utilized, and the absorbance was recorded at 420 *nm.* The wavelengths for gallic acid using FC reagent are optimized at 620nm as indicated from figure 1 following a number of trials with varying timings and reagents.

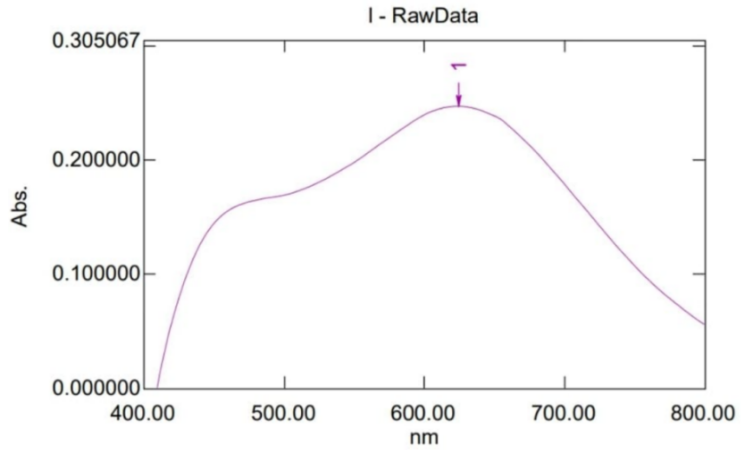

Fig. 1.- Spectrum of gallic acid obtained with folin ciocalteu reagent at 620nm

The wavelengths for gallic acid with gibbs reagent are optimsed at 420 *nm* with good peak as shown in figure 2 after several trial with different timings and reagents.

Table .1. Representing concentration and absorbance values of various chemical constituents.

SNO	CON.	ABSORBANCE				
		TPC (G vsFC)	TPC (G VS GI)	TFC	TPrC	TAmC
1.	10	0.112	0.1124	0.101	0.542	0.15
2.	20	0.157	0.145	0.189	0.615	0.29
3.	30	0.198	0.168	0.246	0.691	0.43
4	40	0.215	0.199	0.298	0.73	0.58
5	50	0.275	0.21	0.33	0.812	0.72

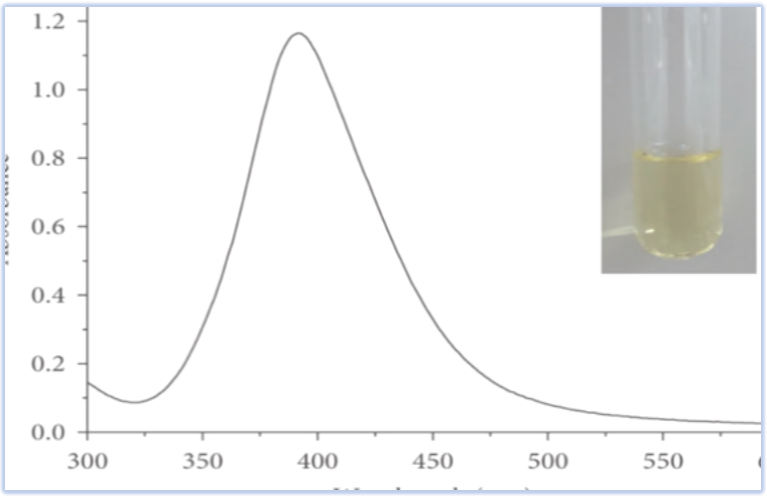

Fig. 2.-Spectrum of gallic acid obtained with gibb's reagent at 420nm

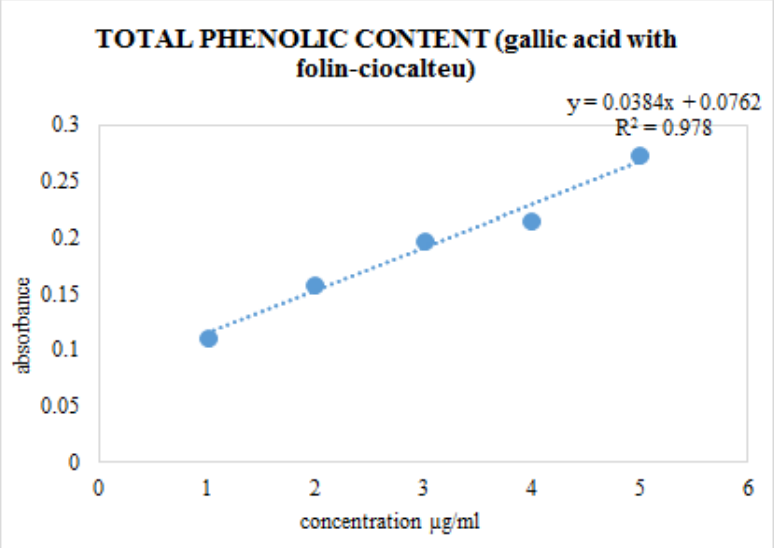

Fig. 3. Calibration Curve of gallic acid with fc reagent.

They illustrate how absorbance increases with concentration (*µg/ml)*. Figure 3 was developed using the FC method, while Figure 4 was obtained using Gibb's reagent.

Total Flavanoid Content

TFC estimated by the aluminum chloride method. In brief, 1 *mL* of extract was mixed with 1 *mL* of 2% aluminum chloride solution and 1ml of 120mM potassium acetate solution and 2ml of distilled water and incubated for 30 min at room temperature. Absorbance was measured at 410 *nm.* Rutin was used as the reference standard, and values were expressed as mg rutin equivalents (RE) per 2 gram DW.

After several trial with slidly modification such as temperature change and with different concentrate solvent it was not aquire but the peak of rutin is optimised at 410nm.

Total Protein Content

TPC was estimated according to the Biuret method. To 1 *mL* of extract sample, 4 *mL* of Biuret reagent (prepared by dissolving sodium hydroxide, potassium sodium tartrate, and copper sulfate solution) was added. The mixture was then incubated at room temperature for 45 *min.* The absorbance at 560 *nm* was taken using a UV–Vis spectrophotometer. Bovine Serum Albumin (BSA) was employed as a standard, and protein content was calculated as mg BSAE/2g DW..

After several trial with slidly modification such as temperature change and with different solvent it was not aquire but the peak of BSA is optimised at 560 *nm.*

Total Amine Content

TAC was determined using MBTH–NQS colorimetric method. Briefly,1 mL of 0.1% MBTH solution was mixed with 1 *mL* of cereal extract, and incubated for 20 min at freezer temperature. Subsequently, 1 *mL* of 0.1% NQS reagent and 1 mL of 1% NaOH (pH 13) were added to initiate the reaction. The mixture was allowed to stand for 20 min in the freeze, using a UV–Visible spectrophotometer absorbance was recorded at 520 nm . Standard calibration was performed using histamine, and results were expressed as mg histamine equivalent (HE)/2g DW.

After several trial with slidly modification such as temperature change to room temp and with different solvent it was not optimised but the peak of Histamine with MBTH reagent is optimised with cooling temperature 1C at 560 nm .

After several trial with slidly modification such as temperature change to room temp and with different solvent it was not optimised but the peak of Histamine with NQS reagent is optimised with cooling temperature to 1C at 520 nm as shown in figure 10 against all solvent mixture without histamine as a blank.

The calibration curve of concentrations with increasing absorbance of Histamin with MBTH reagent which used as standard for total amines content

Figure 12 is the calibration curve of concentrations with increasing absorbance of Histamin with NQS reagent which used as standard for total amines content.

Determination of Antioxidant Activity

Cereal samples were first soaked in distilled water for 48 hours to soften the matrix and release soluble components. The hydrated material was then triturated using a mortar and pestle, followed by acidification (perchloric acid) with methanol to facilitate the extraction of Antioxidant compounds. The mixture was sonicated to enhance cell disruption and centrifuged to separate the soluble fraction. The resulting supernatant was carefully filtered and subjected to mild heating at 100 °C to remove residual solvents, yielding a concentrated extract suitable for subsequent spectrophotometric analyses.

DPPH Method

Antioxidant activity of the cereal extracts was determined by the DPPH radical scavenging procedure. 1 mL aliquot of extract was added with 2 mL of 0.1 mM DPPH solution made in methanol. The mixture was incubated in dark for 45 min at room temperature. Absorbance was recorded at optimised wavelength 516 nm using methanol blank. Radical scavenging activity was determined by the formula.

$$\% \text{ Inhibition} = Ac - As * 100 / Ac$$

where :

Ac - Absorbance of DPPH solution without extract

As - Absorbance with extract.

Results were expressed as % inhibition.

Statistical Analysis

All experimental data were analyzed using coSTaT software (version 6.303). Statistical evaluation was performed using analysis of variance (ANOVA) based on a completely randomized design (CRD) with three replications. The significance of differences among sample means was determined using the Least Significant Difference (LSD) test at a 5% probability level ($p < 0.05$).

All results were expressed as mean ± standard deviation (SD). Correlation among total phenolic content (TPC), total flavonoid content (TFC), total protein content (TPRC), total amine content (TAmC), and antioxidant activity (DPPH %) was evaluated using Pearson's correlation coefficient to determine the relationship between biochemical composition and radical scavenging activity.

RESULTS

The TPC of the cereal extracts was measured using the Folin–Ciocalteu method. Black rice (7.03 mg GAE/g) and ragi (7.03 mg GAE/g) showed the highest TPC, whereas maize (1.2 mg GAE/g) and lentils (1.56 mg GAE/g) exhibited the lowest values. Using Gibbs reagent, ragi (5.60 mg GAE/2g) and black rice (4.01 mg GAE/2g) showed maximum TPC, while black chana (0.25 mg GAE/2g) and corn (0.29 mg GAE/2g) had the lowest content as shown in Table 8.

Table .2 absorbance and of TPC (mg of GAE/2g) of samples extract with folin ciocalteu and gibbs reagent

S.no	Samples	Absorbance		TFC (mg GAE/2g) = c*v/wt	
		FC reagent	Gibb's reagent	Fc reagent	Gibb's reagent
1	Kidney beans	0.387	0.295	4.04 mg (GAE/2g)	1.52 mg GAE/2g
2	Brown beans	0.267	0.180	2.48mg (GAE/2g)	1.76 mg GAE/2g
3	Black chana	0.212	0.158	1.76 mg (GAE/2g)	2.52 mg GAE/2g
4	Green moong	0.315	0.105	3.109 mg (GAE/2g)	0.259 mg GAE/2g
5	Red rice	0.434	0.184	4.6 mg (GAE/2g)	1.89 mg GAE/2g
6	Black rice	0.821	0.292	9.6mg (GAE/2g)	4.01 mg GAE/2g
7	Red wheat	0.389	0.641	4.07 mg (GAE/2g)	2.71 mg GAE/2g
8	Brown barley	0.452	0.374	4.89 mg (GAE/2g)	1.383 mg GAE/2g
9	Corn	0.168	0.107	1.2 mg (GAE/2g)	0.29mg GAE/2g
10	Lentils	0.197	0.110	1.56 mg (GAE/2g)	0.35 mg GAE/2g
11	Ragi	0.61	0.371	7.02 mg (GAE/2g)	5.60mg GAE/2g

Total flavonoid content (TFC) estimated using the aluminum chloride method showed that black rice (4.69 mg RE/2g) and ragi (3.32 mg RE/2g) had the highest flavonoid levels, whereas corn (0.87 mg RE/2g) and brown barley (0.108 mg RE/2g) had the lowest.

S.no	Samples	Absorbance	TFC = c*v/wt
1	Kidney beans	0.3121	2.19mg RE/2g
2	Brown beans	0.387	2.8mg RE/2g
3	Black chana	0.226	1.44mg RE/2g
4	Green moong	0.4166	3.12mg RE/2g
5	Red rice	0.328	2.3mg RE/2g
6	Black rice	0.595	4.69mg RE/2g
7	Ragi	0.437	3.3mg RE/2g
8	Brown barley	0.186	0.108mg RE/2g
9	Corn	0.1621	0.87mg RE/2g
10	Lentils	0.4166	3.12mg RE/2g
11	Red wheat	0.381	0.2806mg RE/2g

Protein content (TPRC) measured using the Biuret method indicated that kidney beans (7.77 mg BSAE/g) and black chana (4.64 mg BSAE/g) contained the highest protein concentrations as shown in Table 9.

Table 3. Absorbance and of TPrC BSAE/2g of samples extract

S.no	Samples	Absorbance	TPrC = c*v/wt
1	Kidney beans	1.55	7.77mg BSAE/2g
2	Brown beans	0.964	3.68mg BSAE/2g
3	Black chana	1.09	4.64mg BSAE/2g
4	Green moong	0.952	3.59mg BSAE/2g
5	Red rice	0.829	2.63mg BSAE/2g
6	Black rice	0.558	0.58mg BSAE/2g
7	Red wheat	0.772	2.21mg BSAE/2g
8	Brown barley	0.76	2.12mg BSAE/2g
9	Corn	0.564	0.62mg BSAE/2g
10	Lentils	0.65	1.36mg BSAE/2g
11	Ragi	0.837	2.71 mg BSAE/2g

The total amine content (TAmC) of the cereal extracts was measured using the MBTH method. Black chana (6.80 mg HE/2g) and Black rice (6.71 mg HE/2g) showed the highest TAmC, whereas corn (0.06 mg HE/2g) and brown beans(1.56 mg HE/2g) exhibited the lowest values as shown in table 10 at 490 nm

Using NQS reagent, kidney beans (2.54 mg HE/2g) and Ragi (1.90 mg HE/2g) showed maximum TAmC, shown in Table 10.

Table 4 Absorbance and Total Amine Content mg HE/2g of samples extract

S.no	Samples	Absorbance		TFC = c*v/wt	
		MBTH	NQS	MBTH	NQS
1	Green moong	0.387	0.685	3.02 mg HE/2g	1.90 mg HE/2g
2	Brown beans	0.267	0.281	1.16 mg HE/2g	0.76 mg HE/2g
3	Red rice	0.212	0.225	2.16 mg HE/2g	0.60 mg HE/2g
4	Kidney beans	0.553	0.912	3.84 mg HE/2g	2.54 mg HE/2g
5	Black chana	0.989	0.251	6.89 mg HE/2g	0.67 mg HE/2g
6	Lentils	0.727	0.357	5.06 mg HE/2g	0.97 mg HE/2g
7	Red wheat	0.562	0.325	3.90 mg HE/2g	0.88 mg HE/2g
8	Brown barley	0.521	0.345	3.61 mg HE/2g	0.94 mg HE/2g
9	Corn	0.015	0.214	0.06 mg HE/2g	0.57 mg HE/2g
10	Black rice	0.962	0.363	6.71 mg HE/2g	0.99 mg HE/2g
11	Ragi	0.813	0.821	5.66 mg HE/2g	

Antioxidant capacity assessed via DPPH radical scavenging assay revealed that Black rice (86%), Ragi (80%) and Black chana (73.3%), exhibited the highest activity, whereas corn showed the lowest antioxidant activity (13.3%) as shown in table 5.

Table 5. Absorbance and percentage % of antipxidant of samples extract

S.no	Samples	Absorbance	% antioxidant
1	Kidney beans	0.387	46.6%
2	Brown beans	0.267	42%
3	Brown barley	0.212	66%
4	Black rice	0.2610	86%
5	Red rice	0.492	68.9%
6	Green moong	0.553	66.6%
7	Red wheat	0.629	60%
8	Ragi	0.3527	80%
9	Corn	1.3347	13.3%
10	Lentils	0.4010	73%
11	Black chana	0.4109	73.3%

Correlation Between Biochemical Composition and Antioxidant Activity

Pearson correlation analysis indicated a strong positive relationship between TPC, TFC, and DPPH radical scavenging activity. Samples with higher phenolic and flavonoid contents (black rice and ragi) exhibited significantly greater antioxidant activity.

Protein and amine contents showed moderate correlation with antioxidant activity, suggesting that phenolic compounds are the primary contributors to radical scavenging capacity in these cereals and legumes.

Table 6. Summary of Key Findings

Parameter	Highest Sample	Lowest Sample	Parameter
TPC (FC)	Black rice, Ragi	Maize	TPC (FC)
TFC	Black rice, Ragi	Maize	TFC
TPRC	Kidney beans	Maize	TPRC
TAmC	Black chana	Corn	TAmC
DPPH %	Black rice (86%)	Maize (13.3%)	DPPH %

DISCUSSION

The high TPC observed in black rice and ragi may be attributed to the presence of pigmented outer layers rich in anthocyanins and polyphenols[22], which are known for strong antioxidant properties. Similarly, the elevated flavonoid content in pigmented cereals supports the association between pigmentation and bioactive compound accumulation[40-43]

Protein content was highest in legumes such as kidney beans and black chana, reflecting their nutritional importance as plant-based protein sources[32-34]. Variation in amine content among cereals may result from differences in amino acid composition and metabolic activity, which could influence functional and bioactive properties[35-39]

The determination of primary amine content in cereals and legumes using MBTH and NQS methods revealed marked variation among the samples[27-29]. In general, the MBTH method consistently showed higher values compared to NQS method, indicating that MBTH is more sensitive and effective in detecting amines in cereals[30,31].

The highest antioxidant activity observed in Black rice (86%), Ragi (80%) and Black chana (73.3%) may be attributed to their richness in phenolic compounds, flavonoids, and other bioactive molecules that effectively scavenge free radicals. Pigmented cereals such as red rice (68.9%), Lentils (73%), and Green moon (66.6%) also exhibited considerable activity, supporting the role of anthocyanins and polyphenols in enhancing antioxidant potential. In contrast, kidney beans (46.6%) and brown beans (42%) showed moderate activity, while corn recorded the lowest antioxidant activity (13.3%), reflecting its poor phenolic contribution. These findings highlight the nutritional significance of legumes and pigmented cereals as natural sources of antioxidants with potential health-promoting properties.

CONCLUSION

The present study highlights the nutritional and functional potential of diverse cereals, with a special emphasis on their antioxidant properties. Among the analyzed samples, pigmented cereals such as Ragi, Black rice and Black chana, Kidney beans exhibited significantly higher phenolic and flavonoid contents, which directly contributed to their strong antioxidant activities.

Overall, the findings suggest that the inclusion of anthocyanin-rich cereals in the daily diet can enhance antioxidant intake, thereby offering protective effects against oxidative stress–related disorders. This study underscores the value of promoting pigmented cereals as functional foods for improving nutritional security and preventing chronic diseases.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

This statement does not apply to this article

Human and Animal Research

This research did not invole human participant, and therefore, informed consent was not required.

INFORMED CONSENT STATEMENT

This research did not invole human participant, and therefore, informed consent was not required.

AUTHOR CONTRIBUTION

The three authors was responsible for methodology,analysis, writing and final approval of the manuscript.

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