Extraction solvent concentration affecting the anthocyanins and other phytochemicals profile and antioxidant properties of bran extracts of pigmented rice cultivars

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Abstract. Different concentrations (100%, 90%, and 80%) of extracting solvent methanol were used to determine antioxidant capacities of seven pigmented rice cultivars bran on the basis of phytochemicals and other antioxidants. TPCs of red-colored bran (Zag, Kow quder, and Shel kow) were the highest in 100% methanol, and those of black pigmented bran (Samarkand), lightly blackish (Kow kareed) and brown (Gult zag, Teli zag), were the highest in 80% and 90% methanol. The higher flavonoid contents in non-pigmented Gult zag and Teli zag resulted from luteolin-7-O-glucoside, quercetinhexoside, apigenin-7-O-glucoside, quercetin-3-O-galactoside, apigenin and (epi)catechin rather than anthocyanins in pigmented rice. Higher anthocyanin contents of extracts in lower methanol concentration resulted from higher percentage of Cyanidin-3-O-rutinoside, Pelargonidin-3-O-diglucoside, and cyanidin-3-O-galactoside. The antioxidant activity showed a similar trend in which the pigmented cultivars showed higher antioxidant activity in 100% methanol, except red-colored Shel kow with higher value in 90% methanol, while, among the light colored brown rice bran, Kow kareed showed higher activities in 80% methanol and Teli zag in 90% methanol.

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1. Introduction

Pigmented rice varieties have been shown to have beneficial effect on improving human health due to their excessive amount of phenolic compounds, such as proanthocyanidin, anthocyanins, flavonols, and other phenolics, with significant antioxidative and free radical scavenging properties [1]. The polyphenols present in rice bran were found to be associated with various health benefits such as their ability to act as a reducing agent by quenching free oxygen radicals and donating free hydrogen ions. Accordingly, these polyphenols in rice protect the cell constituents from oxidative damage and protect the human body against cardiovascular, inflammatory and carcinogenic diseases [2]. Researchers have reported that colored rice bran varieties contain anthocyanin that inhibits enzyme reductase and protects the body from various diabetic complications, such as neuropathy and retinopathy [3].

The consumption of pigmented rice cultivars, including red-, purple- or black-colored rice varieties, has accelerated due to their higher amounts of hy-
drophilic phenolic compounds and flavonoids, such as anthocyanin and proanthocyanidin, which act as chelators of metal ion besides free radical scavengers and reducing agents. Thus, the pigmented rice has been found to be associated with various health-promoting benefits [4]. Rice bran of pigmented rice cultivars has been found to possess higher amounts of phenolic content and antioxidant properties than non-pigmented rice varieties [5]. The total phenolic and flavonoid contents with their antioxidant properties have been found to be proportional to the intensity of bran color. These colored rice cultivars were used in the past in medication as in ayurveda and unani practices [6].

Traditional rice cultivars have occupied an important place in the valley of Kashmir since prehistoric times and are still cultivated due to their unique health and nutritional benefits. Various researchers have done extensive studies on evaluating the antioxidant properties of enormous rice varieties by utilizing different organic solvents in different concentrations. The main objective of this research is to determine and identify the phenolic and flavonoid contents along with various antioxidant properties of bran isolated from different rice cultivars by using different concentrations of methanol. The evaluation of antioxidant capacity using different ratios of organic solvents has not been proposed before, and this is the first attempt to select the best solvent combination to extract the antioxidant or other functional components of interest from the rice cultivars. Methanol was used in three different ratios of pure methanol (without water), 90% methanol, and 80% methanol to obtain bran extracts of different rice cultivars. Methanol was chosen as an extracting solvent at room temperature due to its greater extraction capacity, compared to other solvents as evidenced by previous researches [7]. Research indicated that thermal processing conditions might accelerate their oxidation and other degenerative reactions, which could result in the loss of natural antioxidants. Thus, heating temperature is of much consideration during processing. Van der Shuis et al. [8] reported that an accelerated shelf-life test at 80°C for 4 days resulted in 20-40% decrease in the antioxidant activity of the apple juice. The antioxidant activity of wheat bran decreases up to 61% by heating at 100°C for 9 days [9]. Combination of methanol and water was reported to be the most efficient solvents for the extraction of antioxidants due to their better solvation of antioxidant compounds in rice bran. The solvation resulted from interactions between the polar sites of the antioxidant molecules and the solvent, leading to the hydrogen bond formation. The present study was, therefore, carried out to investigate the antioxidant status of these pigmented varieties and impact of different solvent concentrations on their extraction pattern, component profile, and antioxidant capacities.

2. Materials and methods

2.1. Chemicals and reagents

The chemicals and solvents used in this research work were of analytical and HPLC grade. Methanol solvents and chemicals, i.e., sodium nitrite, aluminum trichloride, and ascorbic acid, were obtained from Merck, India Ltd, Mumbai (India). Water used for methanol dilution was distilled. The chemicals and reagents needed for determining antioxidant activities, such as FolinsCiocalteu reagent, 2,2’-diphenyl-1-picrylhydrazyl (DPPH), Gallic acid, rutin, potassium ferricyanide, and potassium phosphate [ dibasic and monobasic] (used in making phosphate buffer), were purchased from Sigma-Aldrich Pvt, Ltd (India).

2.2. Preparation and stabilization of rice bran

Rice bran was prepared from seven variably colored rice cultivars procured from different breeding centers (managed by SKAUST) of Kashmir valley by milling the rice grains in a laboratory-scale polisher (Agrosa, Pvt. Ltd, India), which separated bran from the rice kernels due to abrasive action of the emery roller, fitting the polisher. The red-colored cultivars consist of Zayg, Skel kow, and Kaw qider. Tilla zayg and Gull zayg are sparsely red-colored cultivars with red rice kernels dispersed in many white-colored cultivars, while Samarland and Kaw karned are dark and light black-colored cultivars. The bran collected was then stabilized by subjecting it to microwave heating in polythene bags at a temperature of 120°C for duration of 3 min followed by cooling at room temperature overnight. This process was repeated three times to ensure complete stabilization of the rice bran. The stabilized rice bran was then stored at 4°C for further analysis.

2.3. Extraction of polyphenolic compounds

Extraction of phenolic compounds was carried out by the method of Finocchiaro [10]. One gram of stabilized rice bran was extracted with 5 ml of 100%, 80%, and 90% rates of methanol at room temperature by suspending the test tubes in a water bath with continuous shaking for 2 h. The mixture was subjected to filtration through Whatman No. 1 filter paper. The remaining residue was re-extracted twice in their respective solvents and, then, filtered, as mentioned above. The prepared bran extracts of respective solvents were combined and dried under vacuum by means of a rotary evaporator and, then, weighed to determine the extraction yield. The vacuum dried residues were dissolved in methanol at a concentration of 1 mg/mL and were used for analysis of antioxidant activity and LC-MS determination of bioactive components.

2.4. Determination of Total Phenolic Content (TPC)

The total phenolic content was determined using the
Folin-Ciocalteu reagent, as described by Amerine and Ough [11]. To 1 ml of the rice bran extract, 9 ml of distilled water and 1 ml of the Folin-Ciocalteu reagent were added. Shortly after, 10 ml of 7% (w/v) Na₂CO₃ solution was added followed by 25 ml of distilled water with continuous stirring. The mixture was given a rest period of 90 minutes and the absorbance was measured against the reagent blank at 750 nm by spectrophotometer (Shimadzu, Japan). TPC was calculated through the mathematical relationship between gallic acid at different concentrations (mg) and their corresponding absorbance given as:

\[
y = 0.675x + 0.057 \quad (r^2 = 0.999),
\]

where \( y \) is absorbance and \( x \) is concentration.

Results were expressed as mg Gallic acid equivalents in 1 g of dried sample (mg GAE/g).

2.5. Determination of Total Flavonoid Content (TFC)

TFC was determined using colorimetric method by following the procedure of Abu Bakar et al. [12]. The extracts (0.5 ml) prepared using different concentrations of methanol were mixed with 2.25 ml of distilled water, followed by addition of 0.15 ml of 5% \( \text{NaNO}_2 \) solution. After 6 min, 0.3 ml of 10% \( (\text{w/v}) \) \( \text{AlCl}_3 \) \( \cdot \text{H}_2\text{O} \) solution was added and kept for 5 min; afterwards, 1.0 ml of 1 M \( \text{NaOH} \) was added with thorough mixing. The absorbance was instantly measured at 510 nm by spectrophotometer, and the results were expressed in mg unit of Rutin equivalents (RUE)/g. A mathematical relationship was established between Rutin at different concentrations and their corresponding absorbance, given as follows:

\[
y = 0.217x + 0.039 \quad (r^2 = 0.995).
\]

2.6. Determination of Total Anthocyanin Content (TAC)

The total anthocyanin content was determined by the pH differential method used by Hosseinian et al. [13] with slight modification:

\[
\text{Total anthocyanin content} = \left( \frac{A \times M \times W \times DF \times 1000}{\xi \times 1} \right),
\]

where \( A \) (absorbance) = [(A515-A700) pH 1 - (A515-A700) pH 4.5]; \( M \) is molecular weight of cyaniding-3-glucoside (449.2 g/ml); \( DF \) is the dilution factor of the sample (8); \( \xi \) is extinction coefficient of cyaniding-3-glucoside, equal to 26000.

To 0.5 ml of the extract, 3.5 ml of 0.025 M potassium chloride buffer (pH 1.0) was added with continuous mixing and given an incubation period of 15 min followed by measurement of absorbance at 515 and 700 nm against distilled water (blank) in a spectrophotometer. The optical density was found to be maximum at 515 nm. The extract was mixed with sodium acetate buffer (0.025 M, pH 4.5) according to the similar procedure as mentioned for KCl buffer given above. The absorbance was measured at the same wavelength, and the results were expressed as mg cyaniding-3-O-glucoside equivalents of sample.

2.7. Liquid Chromatography-Mass Spectrometry (LC-MS)

Polyphenolic compounds in the rice bran of different rice cultivars extracted at different concentrations of methanol were estimated by means of LC-MS method. All of the methanol extracts at different dilution rates were filtered through a 0.45-\( \mu \)m pore-size syringe-driven filter before injection. Then, 20-\( \mu \)l of the extracted solution of the rice bran was separated using a Shimadzu HPLC system equipped with a diode array detector on a 150 mm \( \times \) 4.6 mm i.d., 5-\( \mu \)m Cosmosil 5C18-MS-II, C18-ODS analytical column (waters). The mobile phase included acetonitrile and double distilled water with 0.1% trifluoroacetic acid (TFA) maintained at a flow rate of 0.8 ml/min. The gradient elution was done in the following manner: from 0 to 5 min, linear gradient from 5 to 95% solvent acetonitrile; from 5 to 15 min, 95% solvent acetonitrile; from 15 to 22 min, linear gradient from 9 to 11% solvent acetonitrile; and from 22 to 35 min, linear gradient from 11 to 18% solvent acetonitrile. Column temperature was set to 40°C. Hydroxybenzoic acid compounds were detected at a wavelength of 280 nm and hydoxybenzene acid compounds at 325 nm. Phenolic compounds in the extracted rice bran samples were identified by comparing their \( m/z \) values and UV-vis spectra with authentic compounds and were detected using an external standard method.

2.8. In vitro antioxidant activity of phenolic extracts

2.8.1. DPPH free radical scavenging activity assay

The DPPH radical scavenging activity of bran extracts was determined, as described by Sanchez-Moreno et al. [14]. To 0.1 ml of the extract solution, 3.9 ml of DPPH solution prepared by dissolving 2.3 mg of DPPH radical in 100 ml methanol was added and mixed thoroughly. The solution was incubated for 30 min in dark followed by measurement of the absorbance at 515 nm against reagent blank (control). The DPPH radical scavenging activity was calculated by the following equation:

\[
\text{DPPH radical scavenging\%} = \left( 1 - \frac{A_{515 \text{ nm, sample}}}{A_{515 \text{ nm, control}}} \right) \times 100.
\]

2.8.2. Reducing power assay

The reducing power was determined through the method used by Yen and Duh [15] with slight modification. 2.5 ml of phosphate buffer (2.0 M, pH 6.6)
was added to 2.5 ml of the rice bran extract along with 2.5 ml of 1% potassium ferricyanide. The mixture was given a heat treatment at 50°C for 20 min followed by addition of 2.5 ml of 10% solution of trichloroacetic acid, and the mixture was centrifuged at 2000 × g for 10 min. Then, 2.5 ml of the resulting solution was mixed with 2.5 ml distilled water and 0.5 ml of ferric chloride (0.1%) followed by measurement of absorbance at 700 nm by UV VIS spectrophotometer. The absorbances were compared with each other to determine the strength of the reducing power.

2.8.3. Phosphomolybdenum reduction assay (PMA)

The total antioxidant activity of the rice bran extracts by phosphomolybdenum assay was determined by following the method of Khan et al. [16] with slight modification. To 0.3 ml of the prepared rice bran extract, 3 ml of reagent solution prepared by using sulphuric acid (0.6 M), sodium phosphate (28 mM), and ammonium molybdate (4 mM) was added. The mixture was incubated at 95°C in a water bath for 90 min. After cooling to room temperature, absorbance was recorded at 695 nm against reagent blank containing 0.3 ml methanol in place of extract. Antioxidant capacity in terms of phosphomolybdenum reduction assay was calculated through the mathematical relationship established between ascorbic acid concentration and their corresponding absorbance as follows:

\[ y = 0.842x \ (r^2 = 0.997) \]

where \( y \) is absorbance and \( x \) is concentration. Total antioxidant capacity was calculated as ‘ascorbic acid equivalents’.

2.8.4. Inhibition of lipid peroxidation in egg yolk homogenate

Inhibitions of lipid peroxidation in the egg yolk were determined using the thiobarbituric acid-reactive species (TBARS) assay, as described by Badmus et al. [17]. To 0.5 ml of egg yolk homogenate (10% in distilled water, \( v/v \)), 0.1 ml of bran extract was mixed thoroughly in a test tube and the volume was made up to 1 ml by adding distilled water. Briefly, 0.05 ml of FeSO₄ (0.07 M) was added to the above mixture and incubated for 30 min to induce lipid peroxidation. Thereafter, 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% TBA (\( w/v \)) in 11.1% Sodium Dodecyl Sulfate (SDS) and 0.05 ml 20% TCA were added, thoroughly mixed, and then heated in a boiling water bath for 60 min. Upon cooling, 5.0 ml of butanol was added to each tube and centrifuged at 3000 rpm for 10 min. For control, 0.1 ml of methanol was used in place of bran extract in the prepared sample. The absorbance of the organic upper layer was measured at 532 nm, and percent inhibition was calculated by the following equation.

\[ \% \text{Inhibition} = \left(1 - \frac{A_{532, \text{Sample}}}{A_{532, \text{Control}}} \right) \times 100. \]

2.9. Statistical analysis

All the experiments were done in triplicate and tabulated as mean ± standard deviation. Data were statistically analyzed by analysis of variance (ANOVA), and Duncan’s multiple range test (\( p < 0.05 \)) using SAS software version 9.1 was performed to determine significant differences among the mean values. The principle component analysis was performed by means of Statistical XLSTAT package version 2014 to assess the correlation between different parameters.

3. Results and discussion

3.1. Identification and quantification of different phenolic, flavonoid, and anthocyanins in rice bran

Table 1 shows the identification and quantification of the phenolic acids, flavonoids, hydroxycinnamic acid derivatives, hydroxybenzoic acid derivatives, and the pigmented (anthocyanins) compounds based on their mass spectra as determined by LCMS method. These compounds were found from the values of their mass-to-charge ratio (\( m/z \)) by comparing \( m/z \) values with those already published by several researchers [3,14,18]. A number of phenolic compounds identified in the bran of the seven rice cultivars included ellagic acid (\( m/z \) 229) in black colored Samarkand, phloretic acid (\( m/z \) 165), thymol (\( m/z \) 131), proanthocyanidin trimer (\( m/z \) 695), and apigenin-6,8-di-C-glucoside (\( m/z \) 403) in red-colored Kow quder. The bran of red-colored Zag rice was found to possess two phenols that included Dicafeoyluquinic acid (\( m/z \) 101) and thymol, while Teli zag and Gull zag were found to possess Ellagic acid deoxyhexose (\( m/z \) 301) and phloretic acid with \( m/z \) 165. Ellagic acid (\( m/z \) 229) and (E)-Coniferaldehyde (\( m/z \) 177) were identified in Kow kareed.

Hydroxycinnamic acid identified in the given rice brans plays an important role in determining their antioxidant capacity and aids in enhancing the shelf life and color of foods containing these compounds. Ferulic acid (\( m/z \) 145) identified in Kow kareed rice cultivars along with p-coumaric acid (\( m/z \) 163) in Zag was reported by Tian et al. [19] as one of the major soluble phenolic compounds in brown rice. The majority of the flavonoids identified in these rice cultivars were found to decrease in percentage with increasing concentration of methanol. The flavonoids, proanthocyanidin trimer (\( m/z \) 695) in black-colored Samarkand and red-colored Kow quder, and procyanidin (\( m/z \) 427) in Shek kew and Kow quder were reported by Gu et al. [20] as very strong antioxidants and act as anti-carcinogens by preventing oxidative damage, thus reducing the
Table 1. LC-MS analysis of different polyphenolic compounds of colored rice bran at varying concentrations of extraction solvent (methanol).

| Rt (min) | MS (m/z) | Existing compounds | Compound percentage | Compound class | Cultivars |
|----------|----------|--------------------|--------------------|----------------|-----------|
| 2.30     | 308      | Luteolin-7-O-rutinoside | 100* (48; 80* (45) | Flavonoid glycoside | Samarkand |
| 3.78     | 450      | Ferulic acid hexose derivative | 100 (31) | Hydroxybenzamic acid derivative | Samarkand |
| 4.02     | 605      | Proumnonidin trimer | 100 (17) | Hydroxymannosidic acid | Samarkand |
| 7.57     | 457      | Epigallocatechin-3-O-gallate | 100 (5) | Flavonoid | Samarkand |
| 3.02     | 263      | 2'-O-pentosyl-2'-O-glucosyl apigenin | 100 (17) | Hydroxymannosidic acid | Samarkand |
| 4.98     | 145      | p-Coumaroyl glucose | 100 (17) | Hydroxybenzamic acid derivatives | Samarkand |
| 9.84     | 137      | p-Hydroxybenzonic acid | 100 (17) | Hydroxybenzonic acid derivatives | Samarkand |
| 9.91     | 567      | Quercetin-3-O-(6-acetyl) glucoside | 100 (36) | Flavonoid | Samarkand |
| 8.02     | 203      | (epi) catechin | 100 (35) | Flavonoid | Samarkand |
| 10.78    | 220      | Ellagic acid | 100 (3) | Flavonoid | Samarkand |
| 5.22     | 256      | Luteolin-7-O-glucoside | 100 (5) | Flavonoid glycoside | Samarkand |
| 6.05     | 161      | Caffeic acid | 100 (20) | Hydroxymannosidic acid | Samarkand |
| 4.61     | 287      | Cyanidin-3-O-glucoside | 100 (20) | Anthocyanin | Samarkand |
| 3.05     | 270      | Myricetin | 100 (8) | Flavonoid | Samarkand |
| 1.74     | 671      | dicaffeoyl-proanthocyanidin acid diglucoside | 100 (10) | Hydroxybenzonic acid derivatives | Kwa quder |
| 7.00     | 287      | Cyanidin-3-O-rutinoside | 100 (8) | Anthocyanin | Kwa quder |
| 7.02     | 427      | Procyanidin | 100 (6) | Flavonoid | Kwa quder |
| 7.05     | 165      | Phloretic acid | 100 (6) | Phenol | Kwa quder |
| 10.08    | 271      | Pelargonidin-3-O-rutinoside | 100 (4) | Anthocyanin | Kwa quder |
| 11.33    | 284      | Luteolin | 100 (4) | Flavonoid | Kwa quder |
| 11.33    | 131      | Thymol | 100 (4) | Phenol | Kwa quder |
| 11.34    | 106      | Quercetin-3-O-rhamnoside | 100 (4) | Flavonoid | Kwa quder |
| 18.28    | 448      | Apigenin | 100 (4) | Flavonoid | Kwa quder |
| 5.52     | 160      | 5-O-Caffeoylquinic acid | 100 (4) | Flavonoid | Kwa quder |
| 7.92     | 695      | Proumnonidin trimer | 100 (4) | Flavonoid | Kwa quder |
| 4.61     | 403      | apigenin-6-O-glucoside | 100 (4) | Phenol | Kwa quder |
| 5.52     | 177      | (E)- Coniferaldehyde | 100 (4) | Flavonoid | Kwa quder |
| 2.53     | 191      | Chlorogenic acid | 100 (4) | Hydroxybenzonic acid derivatives | Kwa quder |
| 11.73    | 405      | Quercetin-3-O-rutinoside | 100 (4) | Anthocyanin | Kwa quder |
| 5.42     | 933      | Castachin derivative | 100 (4) | Flavonoid | Zag |
| 5.50     | 163      | p-Coumaric acid | 100 (4) | Flavonoid | Zag |
| 5.31     | 148      | Apigenin | 100 (4) | Flavonoid | Zag |
| 5.33     | 101      | Dicaffeoylquinic acid | 100 (4) | Phenol | Zag |
| 5.67     | 131      | Thymol | 100 (4) | Phenol | Zag |
| 3.78     | 184      | quercetin-3-O-cafeic acid ester | 100 (4) | Hydroxybenzonic acid derivatives | Zag |
| 4.15     | 187      | p-Coumaroylhexose | 100 (4) | Hydroxybenzonic acid derivatives | Zag |
| 4.63     | 407      | tricaffeoyl-hydroxyferulic acid | 100 (4) | Hydroxybenzonic acid derivatives | Zag |
| 2.62     | 308      | Luteolin-7-O-rutinoside | 100 (4) | Flavonoid | Teli zag |
| 11.33    | 580      | Quercetin pentosyl-pentoside | 100 (4) | Flavonoid | Teli zag |
| 11.98    | 161      | Caffeic acid | 100 (4) | Hydroxybenzonic acid derivatives | Teli zag |
| 4.61     | 492      | 5a-pyranopelargonidin-3-O-glucoside | 100 (4) | Anthocyanin | Teli zag |
| 7.54     | 170      | Luteolin-7-O-glucoside | 100 (4) | Phenol | Teli zag |
| 7.57     | 162      | Quercetin hexoside | 100 (4) | Phenol | Teli zag |
| 2.28     | 250      | Apigenin-7-O-glucoside | 100 (4) | Phenol | Teli zag |
| 3.47     | 228      | Quercetin-3-O-galactoside | 100 (4) | Phenol | Teli zag |
| 4.17     | 503      | Pelargonidin-malonylhexoside | 100 (4) | Anthocyanin | Teli zag |
| 4.63     | 407      | Tricaffeoyl-hydroxyferulic acid | 100 (4) | Hydroxybenzonic acid derivatives | Teli zag |
| 4.98     | 431      | Dihydroxygallic acid derivative | 100 (4) | Hydroxybenzonic acid derivatives | Teli zag |
| 4.00     | 301      | Ellagic acid deoxyhexoside | 100 (4) | Phenol | Teli zag |
| 5.85     | 245      | (epi) catechin | 100 (4) | Phenol | Teli zag |
| 9.63     | 137      | p-Hydroxybenzonic acid | 100 (4) | Phenol | Teli zag |
| 7.92     | 287      | Cyanidin-3-O-rutinoside | 100 (4) | Anthocyanin | Teli zag |
| 7.92     | 427      | Procyanidin | 100 (4) | Phenol | Teli zag |
| 10.08    | 241      | Pelargonidin-3-O-glucoside | 100 (4) | Anthocyanin | Teli zag |

*100, 90 and denotes 100% methanol, 90% methanol and 80% methanol.

The values in parenthesis are the percentage of polyphenolic compounds. Rt (min): Retention time; m/z: mass to charge ratio.
formation of free radicals inside the human body due to prolonged exposure to smoking and pollution. The presence of p-hydroxybenzoic acid (m/z 137) in bran of *Samarkand* and *Kaw kared* and its derivative dihydrogallic acid in *Teli zag* (m/z 431) was found to increase with increasing dilution of methanol, despite dicaffeoyl-prococatecholic acid diglucoside (m/z 671) and Chlorogenic acid (m/z 191) that depicted varying trends in their concentrations at different dilution rates. p-Hydroxybenzoic acid and its derivatives are associated with many pharmaceutical, antipyretic, and antifungal characteristics.

The pigmented compounds consisting of mainly anthocyanins identified in the analysed bran samples, such as cyanidin-3-O-galactoside, cyanidin-3-O-rutinoside, ferulic acid hexose derivative, quercetin-3-O-galactoside, quercetin-3-O-rutinoside, 5-pyranopelargonidin-3-O-glucoside, pelargonidin-3-O-glucoside, quercetin-3-O-galactoside, and pelargonidin-malonyl rhamnose, were found to increase in concentration with increasing dilution of the extracting solvent methanol.

### 3.2. Effect of solvent concentration on Total Phenolic Content (TPC)

The total phenolic content determined by modified Folin-Ciocalteu reagent method was expressed as gallic acid equivalents (Table 2). The results of antioxidant capacity among the rice bran extracts showed significant variations at different concentrations of the solvent used. The red-colored pigmented bran showed higher TPC in pure 100% methanol as compared to black-colored *Samarkand* and other light or non-pigmented cultivars. Among the red-colored bran samples, *Kaw qader* depicted higher value for TPC (4.31 mg GAE/g) in 100% methanol and *Shel kew* had lower values of TPC (3.69 mg GAE/g) in 100% methanol. However, the methanol at 80% concentration showed higher values of TPC in the case of *Kaw qader* (4.29 mg GAE/g) and *Zag* (4.28 mg GAE/g), while *Shel kew* had higher TPC in 90% methanol. This fluctuation in values of TPC may be due to changes in the activity coefficient of dissolved compounds that influenced the composition of the extracting solvent. The interaction between the extracted polyphenols and the extracting solvent modified the activity coefficient of the components, thus their solubility in the extracting solvent, as proposed by Tan et al. [21].

The higher TPC values in bran of pigmented red (*Zag, Kaw qader*) rice cultivars extracted from 100% methanol could be attributed to the higher percentage of phenolic compounds as determined by means of LC-MS in these rice brans at different methanolic concentrations. Different phenolic compounds along with

| Rt (min) | MS (m/z) | Existing compounds | Compound percentage | Compound class | Cultivars |
|----------|----------|--------------------|--------------------|----------------|----------|
| 11.33    | 284      | Luteolin           | 100 *(42) 90 *(40)* | Flavonoid      | Shel kew |
| 18.28    | 148      | Apigenin           | 100 *(20.5) 180 *(16) | Flavonoid      | Shel kew |
| 5.52     | 160      | 5-O-feruloylquinic acid | 100 *(20) 80 *(18) | Hydroxyimaric acid | Shel kew |
| 11.73    | 465      | Quercetin-3-Arutinoside | 100 *(50) 130 *(1.5) | Flavonoid      | Shel kew |
| 11.08    | 161      | Caffeic acid       | 100 *(3) 98 *(1.5) | Hydroxyimaric acid | Gall zag |
| 6.61     | 492      | 5-pyranopelargonidin-3-O-glucoside | 100 *(37) 90 *(8.5) | Anthocyanin | Gall zag |
| 7.54     | 170      | Luteolin-5-O-glucoside | 100 *(12) 90 *(8.5) | Flavonoid      | Gall zag |
| 7.57     | 162      | Quercetin hexoside | 100 *(13) 90 *(0.5) | Flavonoid      | Gall zag |
| 2.28     | 250      | Apigenin-5-O-glucoside | 100 *(57) 90 *(22.5) | Flavonoid      | Gall zag |
| 3.47     | 228      | Quercetin-3-O-galactoside | 100 *(3) 90 *(1) | Flavonoid      | Gall zag |
| 7.92     | 165      | Phloretic acid     | 100 *(8) 90 *(0.6) | Phenol         | Gall zag |
| 4.62     | 467      | tricaffeoyl-hydroxyferulic acid | 100 *(6) 90 *(4.5) | Hydroxyimaric acid derivative | Gall zag |
| 18.26    | 148      | Apigenin           | 100 *(6) 90 *(12) | Phenol         | Gall zag |
| 4.60     | 301      | Ellagic acid oxeyxoside | 100 *(4) 90 *(2.5) | Phenol         | Gall zag |
| 17.78    | 229      | Ellagic acid       | 100 *(1) 90 *(0.5) | Phenol         | Kaw kared |
| 11.31    | 284      | Luteolin           | 100 *(35) 90 *(10) | Flavonoid      | Kaw kared |
| 6.55     | 161      | Caffeic acid       | 100 *(2) 98 *(1.5) | Hydroxyimaric acid | Kaw kared |
| 4.61     | 287      | Cymidin-3-O-galactoside | 100 *(6) 90 *(8) | Phenol         | Kaw kared |
| 3.55     | 270      | Myricetin          | 100 *(8) 90 *(0.5) | Phenol         | Kaw kared |
| 4.75     | 145      | Ferulic acid       | 100 *(8) 90 *(10.5) | Hydroxyimaric acid | Kaw kared |
| 5.54     | 217      | (E)-Coniferaldehyde | 100 *(2) 90 *(1) | Phenol         | Kaw kared |
| 1.74     | 671      | Dicaffeoyl-prococatecholic acid diglucoside | 100 *(4) 90 *(10) | Hydroxyimaric acid derivative | Kaw kared |
| 9.81     | 187      | p-Hydroxybenzoic acid | 100 *(5) 90 *(10) | Hydroxyimaric acid | Kaw kared |

*100, 90 and 80 denotes 100% methanol, 90% methanol and 80% methanol.

The values in parentheses are the percentage of polyphenol compounds. Rt (min): Retention time; m/z: mass to charge ratio.

### Table 1. LC-MS analysis of different polyphenolic compounds of colored rice bran at varying concentrations of extraction solvent (methanol) (continued).
their percentage in Zaq including apigenin (87%), di-
caffeoylquinic acid (63%), thymol (76%), quinicic-
caffeic acid ester (34%), p-coumaroylhexose (35%),
and tricaffeoyl-hydroxymufelic acid (9.5%) were found
to be higher than 100% methanol; likewise, the Kaw
quder possessed higher concentration of phenolic
compounds such as dicaffeoyl-protocatechuic acid di-
glucoside (10%), plinertic acid (12%), thymol (22%),
proanthocyanidin trimer (48%), and apigenin-6,8-di-C-
glucoside (18%).

Higher value of TPC was observed in black-
colored Samarkand in 80% methanol (3.24 mgGAE/g)
followed by 90% methanol (3.23 mgGAE/g) and 100%
methanol (3.22 mgGAE/g), which might be due to the
presence of ferulic acid hexose derivative (16%), ellagic
acid (6%), and caffeic acid (6.5%) in 80% methanol.
Bran of red rice cultivars (Shel kew, Kaw quder, Zaq)
contained higher total phenolic content than the bran
of black rice cultivar (Samarkand) as determined by
Folin-Ciocalteu method and was evidenced also by
Butchan et al. [22]. It was observed that, in the case
of the light colored (Kaw kareed) rice bran, higher
TPC was found in 80% methanol (2.26 mgGAE/g)
and the lowest in 90% methanol (1.15 mgGAE/g).
While the non-pigmented Gull zaq had higher TPC
in 90% methanol (1.77 mgGAE/g) and the lowest in
80% methanol (0.64 mgGAE/g). Contrary to this,
Teki zaq depicted higher TPC in 80% methanol (1.81
mgGAE/g) and the lowest in 90% methanol (0.77
mgGAE/g). This fluctuation in results might be due
to higher extraction rate of phenolics in more polar
solvents [23].

3.3. Effect of solvent concentration on Total
Flavonoids Content (TFC)
The results presented in Table 2 showed that total
flavonoid content of rice bran of different varieties
with methanol (100%) ranged from 2.36 to 12.43
mgRUE/g with Kaw kareed, exhibiting the lowest TFC
and Kaw quder having the highest TFC. The results
of TFC showed a decreasing trend with a reducing
concentration of methanol. Herein, the values varied
from 0.88 to 8.55 mgRUE/g in 90% methanol and
0.38 to 7.31 mgRUE/g at 80% methanol, wherein Kaw
kareed and Kaw quder showed lower and higher values
of TFC at both 80% and 90% methanol, respectively.
According to Shen et al. [24], the flavonoid contents
of black pigmented rice were higher than those of
red and non-pigmented rice varieties. However, the
flavonoid contents in non-pigmented Gull zaq (6.18
mgRUE/g) and Teki zaq (6.22 mgRUE/g) higher than
those in some pigmented rice, such as Zaq (3.00
mgRUE/g) and Samarkand (3.37 mgRUE/g), might
be due to the presence of greater amount of some
flavonoid compounds such as luteolin-7-O-glucoside,
quercetin hexoside, apigenin-7-O-glucoside, quercetin-
3-O-galactoside, apigenin and (epi)catechin rather than
anthocyanins present in pigmented rice. In this
experimental work, bran of red rice cultivars with higher
TPC also exhibited higher total flavonoid contents
than black rice bran extracts. This is in agreement
with the report of Butchan et al. [25]. This could
also be attributed to the presence of naturally present
colored substances that are mostly the members of
flavonoid [26].

3.4. Effect of solvent concentration on total
anthocyanin content
The anthocyanin contents of the red, black, and
brown brans of different rice cultivars were signifi-
cantly different (p ≤ 0.05) at different concentra-
ations of methanol, as shown in Table 3. The
anthocyanin content showed an increasing trend with
decreasing concentration of methanol. The dilution
of extracting solvent methanol by water was reported
to cause lysis of the cell membrane, resulting in
simultaneous dissolving and stabilizing of the antho-
cyanin pigmented groups, as validated by Naczk and
Shahidi [27]. The highest amount of anthocyanin was
found in red-colored Kaw quder (120.63 mg cyaniding-
Table 3. Effect of different solvents on the phosphomolybdenuem reduction assay activity and anthocyanin content of rice bran.

| Cultivars    | Phosphomolybdenum reduction assay | Total anthocyanin content (mg/g) |
|--------------|----------------------------------|---------------------------------|
|              | 100% M  | 90% M  | 80% M  | 100% M  | 90% M  | 80% M  |
| Shel kew     | 3.60±0.00a | 3.61±0.00a | 3.58±0.02a | 40.23±1.54a | 50.33±2.12a | 52.87±1.96a |
| Zag          | 3.58±0.01a | 3.52±0.01b | 3.58±0.00a | 4.91±2.64d | 7.14±1.54d | 10.08±1.52a |
| Samarkand    | 2.78±0.01b | 1.98±0.05c | 2.12±0.05c | 101.51±2.4b | 110.37±2.7b | 115.27±1.85b |
| Kaw quder    | 2.51±0.02c | 2.37±0.01f | 2.38±0.00b | 116.18±1.4d | 116.62±2.2d | 120.63±2.3ad |
| Gull zag     | 0.65±0.01f | 0.53±0.01g | 0.45±0.00f | ND     | ND     | ND     |
| Kaw kared    | 1.88±0.01d | 1.78±0.03d | 1.87±0.01d | 7.38±1.17d | 10.07±1.4d | 13.90±1.30d |
| Teli zag     | 0.98±0.07m | 1.12±0.01f | 1.10±0.01m | ND     | ND     | ND     |

*n = 3, results are expressed as mean values ± standard deviations.

Means in a column with different superscripts are significantly different (p ≤ 0.05).

3-O-glucoside equivalents) followed by black-colored Samarkand (115.27 mg cyaniding-3-O-glucoside equivalents) at 80% methanol. The main pigmented compounds identified in the bran of red and black rice cultivars, including Cyanidin-3-O-rutinoside, Pelargonidin-3-O-dihexoside, and cyanidin-3-O-galactoside, were found to increase their percentage with increasing dilution of methanol.

The content of anthocyanin in rice bran extract was found to increase in the order of 80% methanol > 90% methanol > 100% methanol. The lower anthocyanin content was found in Zag (4.910 mg cyaniding-3-O-glucoside equivalents) at 100% methanol. Since anthocyanins were water soluble; hence, its extraction increased with increasing the dilution of methanol with water. Water-soluble extract of rice bran was found to have antioxidant effect on the body cells by reducing cellular damage from the UV radiations, as reported earlier by Santa-Maria et al. [28].

3.5. Polyphenolic compounds and in vitro antioxidant properties as affected by extraction solvent concentration

3.5.1. DPPH free radical scavenging activity assay

DPPH free radical scavenging activities of the rice bran methanolic extracts at different concentrations revealed a significant difference in their scavenging activities. Table 4 illustrates a reduction in scavenging activity of pigmented rice bran with increased dilution of solvent. The DPPH scavenging activity varied from 67.30 to 91.66% at 100% methanol, while, in the case of 90% methanol, it ranged from 60.52 to 85.14% with Kaw kared and Zag depicting the lowest and highest values, respectively. The results of DPPH in 80% methanol showed maximum activity in Zag (89.14%) and minimum activity in Teli zag (77.72%). The superior DPPH radical scavenging activity of 80% methanol extracts in Kaw kared (77.83%) and 90% methanol in Shel kew (82.05%) and Teli zag (80.20%)

Table 4. Effect of different solvent concentrations on the DPPH scavenging activity and lipid peroxidation inhibition of rice bran.

| Cultivars    | DPPH scavenging activity (%) | Lipid peroxidation inhibition (%) |
|--------------|------------------------------|----------------------------------|
|              | 100% M | 90% M | 80% M | 100% M | 90% M | 80% M |
| Shel kew     | 80.30±0.15d | 82.05±0.10b | 78.14±0.17d | 70.36±0.11f | 72.02±0.21b | 68.11±0.23d |
| Zag          | 91.66±0.10a | 85.14±0.08a | 89.14±0.10b | 81.62±0.14a | 75.16±0.06b | 79.18±0.07b |
| Samarkand    | 78.90±0.07d | 77.05±0.05f | 78.71±0.04b | 68.83±0.12d | 66.73±0.65d | 68.68±0.07b |
| Kaw quder    | 80.81±0.01c | 79.23±0.04d | 78.50±0.06c | 70.75±0.03d | 67.17±0.05d | 68.43±0.05c |
| Gull zag     | 89.41±0.04b | 80.01±0.04d | 78.14±0.06b | 79.41±0.04b | 70.01±0.04c | 68.17±0.04d |
| Kaw kared    | 67.30±2.35f | 60.52±0.05f | 72.83±0.05n | 57.27±0.03f | 50.48±0.06b | 67.90±0.05c |
| Teli zag     | 76.81±0.03m | 80.20±0.06a | 77.78±0.04m | 66.74±0.08a | 70.17±0.07m | 67.68±0.04a |

*n = 3, results are expressed as mean values ± standard deviations.

Means in a column with different superscripts are significantly different (p ≤ 0.05).
may be due to its higher efficacy for retaining extractable antioxidant compounds at this concentration, as proposed earlier by Shon et al. [29]. The variation in antioxidant activity of the colored rice cultivars resulted from genetic diversities of these cultivars. According to a report by Oki et al. [30], procyandin in red rice bran is the major components involved in scavenging DPPH radicals. The superior antioxidant activities in 100% methanol in the case of pigmented (except Shel kew) bran and non-pigmented Gull zog could be justified due to their greater amount of the antioxidant compositions, mainly polar phenolic compounds such as thymol, quinonemine-caffeic acid ester, tricaffeoyl-hydroxyferulic acid, and Chlorogenic acid at this concentration of methanol.

The presence of hydroxyl groups in a phenolic compound plays an important role in determining the scavenging activity as hydroxyl groups are found to donate hydrogen atoms to the free radicals, forming stable phenoxyl radicals. The presence of dicafeoylquinic acid possessing 7-hydroxyl groups in Zog with higher percentage in 100% methanol (63%) could account for its higher scavenging activity at this concentration. The higher scavenging activity in 80% methanolic extract of Kau kareed could be attributed to the presence phenolic compounds with a greater number of hydroxyl groups at higher percentage such as ellagic acid (4-OH group), caffeic acid (3-OH group), Ferulic acid (2-OH group), and p-hydroxybenzoic acid. The higher antioxidant activity found in the bran extracts at different concentrations of methanol could be related to ability of water at the given ratio to cleave covalent bonds of the biopolymers and release some bound antioxidants, such as polyphenols, flavonoids, flavoprotein, carotene, etc., as proposed earlier by Iqbal et al. [31].

3.5.2. Reducing power assay

The reducing powers of rice bran extracts at different concentrations of methanol are shown in Figure 1. The reducing power of rice bran extracts at 1 mg/ml showed a fluctuating order for the different cultivars at varying dilutions of the extracting solvent. In the analysis of reducing power, Fe³⁺/ferrocyanide complex is reduced to the ferrons form by the antioxidant components present in the bran extracts resulting in the generation of Fe²⁺ with navy blue color, the absorbance of which can be measured accurately at 700 nm, as reported by Gupta and Prakash [32]. The maximum absorbance values of bran were observed in Zog (2.95) extracted by 100% methanol; the absorbence reduced in 90% methanol (2.85) yet increased in 80% methanol (2.93). The greater reducing power of Zog and Kau kareed in 100% methanol could be linked with caffeic acid, ellagic acid, p-coumaric and ferulic acids that had been reported to donate electrons, thus having higher reducing power capacity (see Medina et al. [33]). The absorbance values in a similar manner at the three given concentrations of methanol were also found in Kau kareed. The reducing power in Samarkand and Kau quder was found to have higher values in 80% methanol followed by 100% methanol and exhibited lower absorbance values at 90% methanol. This could be attributed to the presence of multiple -OH groups, containing flavonoids and hydracinaminic acid, that have the ability to quench O²⁻ and chelate metals by donating hydrogen, as proposed by Sasidharan et al. [34]. The validation of this result was evidenced by Zubair et al. [35], reporting that the reducing power of bran extract in 80% methanol was higher than 100% methanol. Shel kew was found to have higher and lower reducing powers in 90% methanol (2.73) and 80% methanol (2.60), respectively, while the value of the reducing power in 100% methanol depicted an intermediate value of 2.66, which might be due to Procyandin containing multiple -OH groups that have been reported to donate hydrogen and quench O²⁻, as reported by Fukumoto and Mazza [18]. The contrastings results regarding the reducing power among the bran extracts at different concentrations could be linked to the differences in dissolution of some compounds of rice bran extracted at these concentrations such as tocopherols that can act as electron donors, resulting in the termination of radical chain reactions as reported earlier by Nam et al. [36]. The highest reducing power of Zog, Samarkand, and Kau quder in methanol could be attributed to the presence of caffeic acid, chlorogenic acid, p-coumaric and ferulic acids as reported to be able to donate electrons [36].

3.5.3. Phosphomolybdenum assay (PMA)

The antioxidant activity evaluated in terms of phosphomolybdenum assay at various concentrations of extracting solvent showed a concentration-dependent activity in a similar manner as revealed in the case of DPPH radical scavenging activity and lipid per-
oxidation. In phosphenolylbenzen method, Mo (VI) was reduced to Mo (V) by the bran extract with the formation of a green Mo (V) complex at a low pH, as investigated earlier by Pan et al. [37]. As shown in Table 3, bran extract of Zag cultivar showed higher antioxidant activity in 100% Zaz (3.58 mgAAE/ml) and 80% Zaz (3.58 mgAAE/ml) with no significant variations in their values. The pigmented rice cultivars had higher values of phosphenolylbenzen activity in 100% methanol, except red-colored Shed kew that had shown higher antioxidant activity in 90% methanol (3.65 mgAAE/ml) followed by 100% methanol (3.60 mgAAE/ml). The higher content of total phenols in the rice cultivars at varying concentrations accounts for the better results found in their phosphenolylbenzen activity and DPPH radical scavenging activity. A variety of phenoic compounds present in rice, especially ferulic acid, p-coumaric acid, and delinate, are present in higher quantities in bran. The variation in the total antioxidant activity as assessed by phosphenolyl-benzen assay in bran of the same color at different concentrations could be attributed to variation and solubility of phenolic compounds. Zhu et al. [38] proposed that the use of different extracting solvents was reported to affect the composition of the extracting compounds; likewise, the extracting solvent at different concentrations resulted in differences in antioxidant compositions, thus the antioxidant activities of the bran extracts. The determination of antioxidant activity by PMA gives a direct estimation of reducing the capacity of antioxidants in the rice bran extracted at different concentrations. The differences in the results could be due to the several factors including varietal differences, climate of growing season, and topographical differences, as reported by Natella et al. [39].

3.5.4. Lipid peroxidation inhibition assay
The inhibition capacity of rice bran extracts at different concentrations was determined using egg yolk homogenate, inhibiting peroxide radicals production in the peroxidation of egg yolk (Table 4). The inhibitory activity of rice bran extracts against lipid peroxidation was shown to decrease with decreasing concentration methanol. The results of lipid peroxidation inhibition followed a trend similar to that exhibited by DPPH scavenging activity. Zaz showed the highest inhibition activity in 100% methanol (81.62%), 90% methanol (75.163%), and 80% methanol (79.18%), while Kaw karoed had the lowest inhibition activity in 100% methanol (57.27%) and 90% methanol (55.48%). However, at 80% methanol, the lipid peroxidation inhibition of non-pigmented Teli zaz (67.68%) did not differ significantly from that of Kaw karoed (67.80%).

Saehood et al. [40] proposed that the variation in lipid peroxidation inhibition in different rice cultivars and at varying concentrations could be due to the diminution of the lipid oxidation products of egg yolk homogenate, especially the conjugated dienes. The greater inhibition of Teli zaz (70.17%), Gull zaz (79.41%), and Kaw karoed (67.80%) in 90% methanol, 100% methanol, and 80% methanol could be attributed to the presence of higher percentage of hydrophilic phenolic compounds at these concentrations, shown to correlate positively with inhibition of lipid peroxidation inhibition by donating hydrogen ions to lipid peroxides radicals that are the major propagators of lipid peroxidation process, as reported by Gulcin et al. [41].

The higher lipid peroxidation inhibition of Shed kew and Teli zaz in 90% methanol resulted from lesser polar phenolic compounds, such as caffeic acid, p-hydroxybenzoic acid, and ellagic acid deoxyhexoside, which were found to act more effectively with lipids in polar solvent mixture and, thus, have higher efficiency in inhibition of lipid peroxidation as proposed earlier by Porter [42]. These phenolic compounds were also reported by Lizcano et al. [43] to approach the hydrophilic portions easily as these possess higher partition coefficients and, thus, inhibit free radical attack on the lipids. The bran from pigmented rice exhibited greater inhibition of peroxyl and alkoxyl radicals produced by lipid peroxidation. These radicals damage the cell membranes and lead to glutathione depletion, thereby causing cytotoxicity as reported by Shen et al. [24].

3.6. Principle Component Analysis (PCA)
PCA was done on the results obtained in this study to determine the effect of different concentrations of methanol on the TPC and antioxidant properties of several rice bran cultivars. PCA as a multivariate data analysis tool reduces an original greater amount of data to limited multivariate data, showing maximum variability present in the data matrix. The factor loadings of the first three principal components, including Eigen values, variance% along with cumulative Eigen values, and cumulative%, are shown in Table 5. The first three principal components depicted 96.22% of variance for all the seven rice bran varieties, where PC1, PC2, and PC3 accounted for 56.56%, 28.82%, and 10.83% of variance, respectively. As per the PCA loading of components shown in Table 5, it was found that 56.56% of variability in PC1 was positively correlated with parameters, including TPC, DPPH activity, lipid peroxidation inhibition, phosphenolylbenzen assay, and the reducing power at three given concentrations of methanol; however, it was shown to correlate negatively with anthocyanin content at all concentrations of methanol. The second component (PC2 = 28.82) was observed to correlate positively with DPPH scavenging activity (0.60) and lipid peroxidation inhibition (0.61) in 80% methanol.
Table 5. Principal component loading of the first three components and PCA component analysis of factors.

| Factor loading                               | Principle components |
|----------------------------------------------|----------------------|
| TPC 100% methanol                            | 0.88 -0.39 0.36      |
| TPC 90% methanol                             | 0.84 -0.50 0.08      |
| TPC 80% methanol                             | 0.73 -0.20 0.51      |
| TFC 100% methanol                            | 0.25 -0.82 -0.45     |
| TFC 90% methanol                             | 0.27 -0.84 -0.42     |
| TFC 80% methanol                             | 0.23 -0.87 -0.40     |
| DPPH scavenging 100% methanol               | 0.97 0.03 0.21       |
| DPPH scavenging 90% methanol                | 0.95 -0.22 0.03      |
| DPPH scavenging 80% methanol                | 0.74 0.60 0.25       |
| Lipid peroxidation inhibition 100% methanol  | 0.97 0.03 0.21       |
| Lipid peroxidation inhibition 90% methanol   | 0.95 -0.22 0.02      |
| Lipid peroxidation inhibition 80% methanol   | 0.74 0.61 0.25       |
| Reducing power 100% methanol                | 0.96 0.24 0.15       |
| Reducing power 90% methanol                 | 0.89 0.13 -0.29      |
| Reducing power 80% methanol                 | 0.90 0.40 0.18       |
| Phosphomolybdenum assay 100% methanol       | 0.90 0.00 -0.26      |
| Phosphomolybdenum assay 90% methanol        | 0.86 0.08 -0.50      |
| Phosphomolybdenum assay 80% methanol        | 0.87 0.11 -0.46      |
| Anthocyanin content 100% methanol           | -0.05 -0.90 0.42     |
| Anthocyanin content 90% methanol            | -0.05 -0.90 0.41     |
| Anthocyanin content 80% methanol            | -0.08 -0.88 0.45     |

and negatively with TPC and anthocyanin content at the selected concentrations of methanol. The third component (PC3) with lower contribution of 10.83% correlates positively with anthocyanin content at all the given concentrations of methanol and negatively with TFC and phosphomolybdenum assay at the given concentrations of methanol, including reducing power (-0.29) at 90% methanol.

4. Conclusion

This study was conducted to investigate the concentration of the extracting solvent suitable for maximum extraction of phenolic and antioxidant compounds from a given rice cultivar. The antioxidant properties of the traditional rice cultivars, as revealed by utilizing three different concentrations of methanol, exhibited a significant difference. Further to that, both pigmented and non-pigmented rice cultivars were found to exhibit higher phenolic content and antioxidant properties at varying concentrations of methanol. The only general rule analyzed in this study was that the total anthocyanin content was found to increase with the decreasing concentration of methanol, and total flavonoid content showed a reducing trend with increasing dilution of methanol.

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Abbreviations

TFC  Total Flavonoid Content
TPC  Total Phenolic Content
GAE  Gallic Acid Equivalents
RUE  Rutin equivalent
TBARS Thiobarbituric acid-reactive species
DPPH 2, 2-diphenyl-1-picrylhydrazyl
PMA    Phosphomolybdenum assay
PCA    Principal Component Analysis
AAE    Ascorbic Acid Equivalent

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