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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, Kaw quder*, and *Shel kaw*) were the highest in 100% methanol, and those of black pigmented bran (*Samarkand*), lightly blackish (*Kaw kareed*) and brown (*Gull zag, Teli zag*), were the highest in 80% and 90% methanol. The higher flavonoid contents in non-pigmented *Gull 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 kew* with higher value in 90% methanol, while, among the light colored brown rice brans, *Kaw kareed* showed higher activities in 80% methanol and *Teli zag* in 90% methanol.

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 phenolics 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-

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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 Sluis 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 Folin-Ciocalteu 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 *Zag, Shel kew*, and *Kaw quder. Tilla zag* and *Gull zag* are sparsely red-colored cultivars with red rice kernels dispersed in many white-colored cultivars, while *Samarkand* and *Kaw kareed* 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) Na_2CO_3 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: $y = 0.675x + 0.087$ ($r^2 = 0.999$), 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% $NaNO_2$ solution. After 6 min, 0.3 ml of 10% (w/v) $AlCl_3.6H_2O$ solution was added and kept for 5 min; afterwards, 1.0 ml of 1 M 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:

$$\text{Total anthocyanin content} = \left(\frac{A \times MW \times DF \times 1000}{\in \times 1}\right),$$

where A (absorbance) = [(A515-A700) pH 1 - (A515-A700) pH 4.5]; MW is molecular weight of cyaniding 3-glucoside (449.2 g/ml); DF is the dilution factor of the sample (8); $\in$ is extinction coefficient of cyaniding 3-glucoside, equal to 26900.

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-μm pore-size syringe-driven filter before injection. Then, 20-μl 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×4.6 mm i.d., 5-μm, 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 9% solvent acetonitrile; from 5 to 15 min, 9% 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 hydroxycinnamic 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:

$$\text{DPPH radical scavenging\%} = \left(1 - \frac{\text{A515 nm, sample}}{\text{A515 nm, control}}\right) \times 100.$$

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 2.5ml 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 phosphomolybdinum 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 $FeSO_4$ (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 1.1% 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, 0.1ml 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.

$$\%\text{Inhibition} = \left(1 - \frac{A532, \text{Sample})}{A532, \text{Control}}\right) \times 100.$$

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 *Kaw quder*. The bran of red-colored *Zag* rice was found to possess two phenols that included Dicaffeoylquinic acid (m/z 101) and thymol, while *Teli zag* and *Gull zag* were found to possess Ellagic acid deoxyhexoside (m/z 301) and phloretic acid with m/z 165. Ellagic acid (m/z 229) and (E)-Coniferaldehyde (m/z 177) were identified in *Kaw kareed*.

Hydrocinnamic 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 *Kaw 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 *Kaw quder*, and procyanidin (m/z 427) in *Shel kew* and *Kaw 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)90*(46)80*(45)	Flavonoid glycoside	*Samarkand*
3.78	450	Ferulic acid hexose derivative	100(13)90(14)80(16)	Hydroxycinammic acid derivative	*Samarkand*
4.62	695	Proanthocyanidin trimer	100(15)90(13)80(12)	Flavonoid	*Samarkand*
7.57	457	Epigallocatechin- 3-*O*-gallate	100(9.5)90(9)80(8.5)	Flavonoid	*Samarkand*
3.02	293	2"-*O*-pentosyl-8-C-hexosyl-apigenin	100(17)90(16)80(14.5)	Flavonoid	*Samarkand*
4.88	145	*p*−Coumaroyl glucose	100(12)90(14)80(15.5)	Hydroxycinammic acid derivatives	*Samarkand*
9.84	137	*p*−hydroxybenzoic acid	100(10)90(11.5)80(13)	Hydroxybenzoic acid derivatives	*Samarkand*
8.91	507	Quercetin-3-*O*-(6-acetyl) glucoside.	100(11)90(10)80(9.5)	Flavonoid	*Samarkand*
8.92	203	(epi)-catechin	100(5)90(3)80(3.5)	Flavonoid	*Samarkand*
17.78	229	Ellagic acid	100(3)90(5)80(6)	Phenol	*Samarkand*
5.22	256	Luteolin-7-*O*-glucoside	100(6)90(5.5)80(4)	Flavonoid glycoside	*Samarkand*
6.55	161	Caffeic acid	100(4)90(5.5)80(6.5)	Hydroxycinammic acid	*Samarkand*
4.61	287	Cyanidin-3-*O*-galactoside	100(42)90(45.5)80(47)	Anthocyanin	*Samarkand*
3.55	279	Myrecitin	100(14)90(15)80(13)	Flavonoid	*Samarkand*
1.74	671	dicaffeoyl-protocatechuic acid diglucoside	100(10)90(8)80(9.5)	Hydroxybenzoic acid derivatives	*Kaw quder*
7.90	287	Cyanidin-3-*O*-rutinoside	100(37)90(40)80(43)	Anthocyanin	*Kaw quder*
7.92	427	Procyanidin	100(67)90(63)80(60)	Flavonoid	*Kaw quder*
7.95	165	Phloretic acid	100(12)90(9)80(10.5)	Phenol	*Kaw quder*
10.08	271	Pelargonidin-3-*O*-diglucoside	100(40)90(43)80(46)	Anthocyanin	*Kaw quder*
11.33	284	Luteolin	100(45)90(43)80(41)	Flavonoid	*Kaw quder*
11.33	131	Thymol	100(22)90(19)80(20.5)	Phenol	*Kaw quder*
11.34	106	Quercetin-3-*O*-rhamnoside	100(12)90(10)80(11)	Flavonoid	*Kaw quder*
18.28	148	Apigenin	100(22)90(20)80(18.5)	Flavonoid	*Kaw quder*
5.52	160	5-*O*-feruloylquinic acid	100(24)90(22)80(20)	Flavonoid	*Kaw quder*
7.92	695	Proanthocyanidin trimer	100(48)90(46)80(47)	Flavonoid	*Kaw quder*
4.61	403	apigenin-6,8-di-C-glucoside	100(18)90(15)80(17)	Phenol	*Kaw quder*
5.52	177	(E)- Coniferaldehyde	100(13)90(10)80(8)	Flavonoid	*Kaw quder*
2.53	191	Chlorogenic acid	100(78)90(76)80(77)	Hydroxybenzoic acid derivatives	*Kaw quder*
11.73	465	Quercetin-3-*O*-rutinoside	100(5)90(7)80(8.5)	Anthocyanin	*Kaw quder*
5.42	933	Castalagin derivative	100(6.5)90(4)80(5.5)	Hydroxycinammic acid	*Zag*
5.30	163	*p*−Coumaric acid	100(95)90(92)80(90)	Flavonoid	*Zag*
5.31	148	Apigenin	100(87)90(84)80(85.5)	Hydroxycinammic acid	*Zag*
5.33	101	Dicaffeoylquinic acid	100(63)90(61)80(62)	Phenol	*Zag*
5.67	131	Thymol	100(76)90(73)80(74.5)	Phenol	*Zag*
3.78	184	quinicquinic- caffeic acid ester	100(34)90(31)80(32)	Hydroxycinammic acid	*Zag*
4.15	187	*p*−coumaroylhexose	100(35)90(31)80(33)	Hydroxycinammic acid derivative	*Zag*
4.63	407	tricaffeoyl-hydroxyferulic acid	100(9.5)90(6.5)80(8)	Hydroxycinammic acid	*Zag*
2.92	308	Luteolin-7-*O*-rutinoside	100(8)90(6)80(4.5)	Flavonoid	*Teli zag*
11.33	589	Quercetin pentosyl-pentoside	100(14)90(12)80(10)	Flavonoid	*Teli zag*
11.98	161	Caffeic acid	100(47)90(49.5)80(44)	Hydroxycinammic acid	*Teli zag*
4.61	492	5- pyranopelargonidin- 3-*O*-glucoside	100(38.5)90(40)80(42)	Anthocyanin	*Teli zag*
7.54	170	Luteolin-7-*O*-glucoside	100(14)90(12)80(10.5)	Flavonoid	*Teli zag*
7.57	162	Quercetin hexoside	100(12)90(10)80(8.5)	Flavonoid	*Teli zag*
2.28	250	Apigenin-7-*O*-glucoside	100(3.5)90(2.5)80(1)	Flavonoid	*Teli zag*
3.47	228	Quercetin-3-*O*-galactoside	100(3)90(1)80(0.5)	Flavonoid	*Teli zag*
4.17	503	Pelargonidin-malonylrhamnoside	100(10)90(13)80(15)	Anthocyanin	*Teli zag*
4.63	407	Tricaffeoyl-hydroxyferulic acid	100(6.5)90(4)80(8)	Hydroxycinammic acid derivative	*Teli zag*
4.58	431	Dihydrogallic acid derivative	100(10)90(8)80(12.5)	Hydroxybenzoic acid	*Teli zag*
4.60	301	Ellagic acid deoxyhexoside	100(4)90(6.5)80(2.5)	Phenol	*Teli zag*
5.85	245	(epi)catechin	100(4.5)90(3)80(1.5)	Flavonoid	*Teli zag*
9.83	137	*p*−hydroxybenzoic acid	100(21)90(23)80(18.5)	Benzoic acid	*Shel kew*
7.92	287	Cyanidin-3-*O*-rutinoside	100(36)90(39)80(43)	Anthocyanin	*Shel kew*
7.92	427	Procyanidin	100(53)90(51)80(48.5)	Flavonoid	*Shel kew*
10.08	271	Pelargonidin-3-*O*-diglucoside	100(40)90(43)80(47)	Anthocyanin	*Shel kew*

*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.

Table 1. LC-MS analysis of different polyphenolic compounds of colored rice bran at varying concentrations of extraction solvent (methanol) (continued).

Rt (min)	MS (m/z)	Existing compounds	Compound percentage	Compound class	Cultivars
11.33	284	Luteolin	100*(42)90*(40)80*(38)	Flavonoid	*Shel kew*
18.28	148	Apigenin	100(20.5)90(18)80(16)	Flavonoid	*Shel kew*
5.52	160	5-*O*-feruloylquinic acid	100(20)90(24)80(18)	Hydroxycinammic acid	*Shel kew*
11.73	465	Quercetin-3-*O*-rutinoside	100(5)90(3)80(1.5)	Flavonoid	*Shel kew*
11.98	161	Caffeic acid	100(3)90(6)80(1.5)	Hydroxycinammic acid	*Gull zag*
4.61	492	5-pyranopelargonidin- 3-*O*-glucoside	100(37)90(40)80(42)	Anthocyanin	*Gull zag*
7.54	170	Luteolin-7-*O*-glucoside	100(12)90(10)80(8.5)	Flavonoid	*Gull zag*
7.57	162	Quercetin hexoside	100(13)90(11)80(9.5)	Flavonoid	*Gull zag*
2.28	250	Apigenin-7-*O*-glucoside	100(57)90(55)80(52.5)	Flavonoid	*Gull zag*
3.47	228	Quercetin-3-*O*-galactoside	100(3)90(2)80(1)	Flavonoid	*Gull zag*
7.92	165	Phloretic acid	100(8)90(11)80(6)	Phenol	*Gull zag*
4.62	407	tricaffeoyl-hydroxyferulic acid	100(6)90(8)80(4.5)	Hydroxycinammic acid derivative	*Gull zag*
18.26	148	Apigenin	100(16)90(14)80(12)	Flavonoid	*Gull zag*
4.60	301	Ellagic acid deoxyhexoside	100(4)90(6)80(2.5)	Phenol	*Gull zag*
17.78	229	Ellagic acid	100(3)90(1.5)80(5)	Phenol	*Kaw kareed*
11.31	284	Luteolin	100(35)90(32)80(30)	Flavonoid	*Kaw kareed*
6.55	161	Caffeic acid	100(2.5)90(1.5)80(4)	Hydroxycinammic acid	*Kaw kareed*
4.61	287	Cyanidin-3-*O*-galactoside	100(2)90(6)80(8)	Anthocyanin	*Kaw kareed*
3.55	279	Myrecitin	100(8)90(6)80(5)	Flavonoid	*Kaw kareed*
4.75	145	Ferulic acid	100(8)90(5.5)80(10.5)	Hydroxycinammic acid	*Kaw kareed*
5.54	177	(E)-Coniferaldehyde	100(9.5)90(6.5)80(11)	Phenol	*Kaw kareed*
1.74	671	Dicaffeoyl-protocatechuic acid diglucoside	100(20)90(18)80(22)	Hydroxybenzoic acid derivative	*Kaw kareed*
9.81	137	*p*-Hydroxybenzoic acid	100(5)90(8.5)80(10)	Hydroxybenzoic acid	*Kaw kareed*

*100, 90 and 80 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 kareed* and its derivative dihydrogallic acid in *Teli zag* (m/z 431) was found to increase with increasing dilution of methanol, despite dicaffeoyl-protocatechuic 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 rhamnoside, 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 quder* depicted higher value for TPC (4.31 mgGAE/g) in 100% methanol and *Shel kew* had lower values of TPC (3.69 mgGAE/g) in 100% methanol. However, the methanol at 80% concentration showed higher values of TPC in the case of *Kaw quder* (4.29 mgGAE/g) and *Zag* (4.28 mgGAE/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 quder*) 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

Table 2. Effect of solvent concentrations on the TPC and TFC of rice bran extract*.

Cultivars	Total phenolic content (mg GAE/g)			Total flavonoid content (mg RE/g)		
	100% M	90% M	80% M	100% M	90% M	80% M
Shel kew	3.69 ± 0.15^{b}	3.70 ± 0.06^{a}	2.82 ± 0.02^{c}	12.40 ± 0.04^{a}	8.49 ± 0.02^{a}	7.31 ± 0.06^{a}
Zag	4.30 ± 0.03^{a}	3.62 ± 0.01^{b}	4.28 ± 0.03^{a}	3.00 ± 0.06^{d}	1.73 ± 0.07^{e}	0.78 ± 0.02^{e}
Samarkand	3.22 ± 0.01^{c}	3.23 ± 0.01^{c}	3.24 ± 0.04^{b}	3.37 ± 0.04^{c}	2.28 ± 0.05^{c}	2.01 ± 0.05^{c}
Kaw quder	4.31 ± 0.02^{a}	3.63 ± 0.01^{b}	4.29 ± 0.02^{a}	12.43 ± 0.02^{a}	8.55 ± 0.01^{a}	7.32 ± 0.02^{a}
Gull zag	1.27 ± 0.02^{e}	1.77 ± 0.04^{d}	0.64 ± 0.02^{f}	6.18 ± 0.02^{b}	2.53 ± 0.02^{d}	1.64 ± 0.01^{d}
Kaw kareed	$1.76\pm .01^{d}$	1.15 ± 0.01^{e}	2.26 ± 0.01^{d}	2.36 ± 0.02^{e}	0.88 ± 0.01^{f}	0.38 ± 0.01^{f}
Teli zag	1.30 ± 0.02^{e}	0.77 ± 0.02^{f}	1.81 ± 0.01^{e}	6.22 ± 0.02^{b}	2.56 ± 0.01^{d}	1.68 ± 0.01^{d}

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

Means in a column with different superscripts are significantly different ($p \leq 0.05$).

their percentage in *Zag* including apigenin (87%), dicaffeoylquinic acid (63%), thymol (76%), quinicquinic-caffeic acid ester (34%), *p*-coumaroylhexose (35%), and tricaffeoyl-hydroxyferulic 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 diglucoside (10%), phloretic 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, Zag*) 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 zag* had higher TPC in 90% methanol (1.77 mgGAE/g) and the lowest in 80% methanol (0.64 mgGAE/g). Contrary to this, *Teli zag* 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 zag* (6.18 mgRUE/g) and *Teli zag* (6.22 mgRUE/g) higher than those in some pigmented rice, such as *Zag* (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 significantly different ($p \leq 0.05$) at different concentrations 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 anthocyanin 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 phosphomolybdenum 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	48.23±1.54^{c}	50.53±2.12^{c}	52.87±1.96^{c}
Zag	3.58±0.01a	3.52±0.01^{b}	3.58±0.00a	4.91±2.64^{d}	7.14±1.54^{d}	10.08±1.52^{e}
Samarkand	2.78±0.01^{b}	1.98±0.05^{d}	2.12±0.05^{c}	101.51±2.4^{b}	110.37±2.7^{b}	1 15.27±1.85^{b}
Kaw quder	2.518±0.02^{c}	2.37±0.01^{c}	2.38±0.0‘^{b}	116.18±1.4^{a}	116.62±2.2^{a}	120.63±2.33^{a}
Gull zag	0.65±0.01^{f}	0.53±0.01g	0.48±0.00^{f}	ND	ND	ND
Kaw kareed	1.88±0.01^{d}	1.78±0.01^{e}	1.87±0.01^{d}	7.58±1.17^{d}	10.07 ±1.4^{d}	13.991±1.30^{d}
Teli zag	0.98±0.07^{e}	1.12±0.01^{f}	1.11±0.01^{e}	ND	ND	ND

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

Means in a column with different superscripts are significantly different ($p \leq 0.05$), ND= Not Detected.

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*-diglucoside, 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 91.6 6% at 100% methanol, while, in the case of 90% methanol, it ranged from 60.52 to 85.14% with *Kaw kareed* 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* (77.78%). The superior DPPH radical scavenging activity of 80% methanol extracts in *Kaw kareed* (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±0.15^{cd}	82.05±0.10^{b}	78.14±0.17^{d}	70.26±0.11^{cd}	72.02 ±0.21^{b}	68.11±0.23^{d}
Zag	91.66±0.10a	85.14±0.08^{a}	89.14± 0.10^{a}	81.62±0.14^{a}	75.16±0.06^{a}	79.18±0.07^{a}
Samarkand	78.90±0.07^{d}	77.05±0.05^{f}	78.71±0.04^{b}	68.83±0.12^{d}	66.73±0.65^{d}	68.68±0.07^{b}
Kaw quder	80.81±0.04^{c}	77.23±0.04^{e}	78.50±0.07^{c}	70.75±0.03^{c}	67.17±0.05^{d}	68.43±0.05^{c}
Gull zag	89.41±0.04^{b}	80.01±0.04^{d}	78.14±0.06^{d}	79.41±0.04^{b}	70.01±0.04^{c}	68.17±0.04^{d}
Kaw kareed	67.30±2.35^{f}	60.52±0.05^{g}	77.83±0.05^{e}	57.27±0.03^{f}	50.48 ±0.06^{e}	67.80±0.05^{e}
Teli zag	76.81±0.03^{e}	80.20±0.06^{c}	77.787±0.04^{e}	66.74±0.08^{e}	70.17±0.07^{c}	67.68±0.04^{e}

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

Means in a column with different superscripts are significantly different ($p \leq 0.05$).

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 diversibility of these cultivars. According to a report by Oki et al. [30], procyanidin 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 zag* could be justified due to their greater amount of the antioxidant compositions, mainly polar phenolic compounds such as thymol, quinicquinic-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 dicaffeoylquinic acid possessing 7-hydroxyl groups in *Zag* 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 *Kaw 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

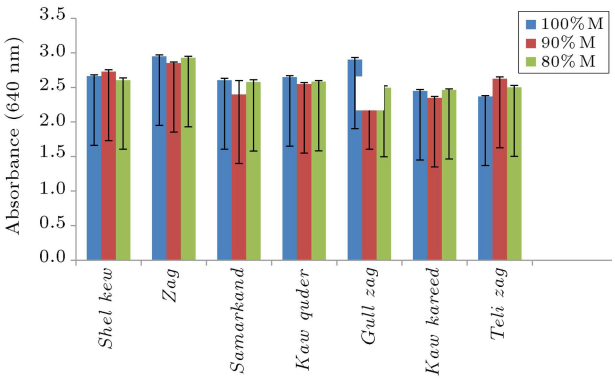

Figure 1. Effect of different solvent concentrations on the reducing power of rice bran extracts from seven different traditional rice cultivars.

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, Fe^{3+}/ferricyanide complex is reduced to the ferrous form by the antioxidant components present in the bran extracts resulting in the generation of Fe^{2+} 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 *Zag* (2.95) extracted by 100% methanol; the absorbance reduced in 90% methanol (2.85), yet increased in 80% methanol (2.93). The greater reducing power of *Zag* and *Kaw 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 *Kaw kareed*. The reducing power in *Samarkand* and *Kaw 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 hydracinammic acid, that have the ability to quench O^{2-} 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 Procyanidin containing multiple -OH groups that have been reported to donate hydrogen and quench O^{2-}, as reported by Fukumoto and Mazza [18]. The contrasting 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 *Zag*, *Samarkand*, and *Kaw 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 phosphomolybdenum 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% *Zag* (3.58 mgAAE/ml) and 80% *Zag* (3.58 mgAAE/ml) with no significant variations in their values. The pigmented rice cultivars had higher values of phosphomolybdenum activity in 100% methanol, except red-colored *Shel 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 phosphomolybdenum activity and DPPH radical scavenging activity. A variety of phenolic compounds present in rice, especially ferulic acid, *p*-coumaric acid, and diferulate, are present in higher quantities in bran. The variation in the total antioxidant activity as assessed by phosphomolybdenum 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. *Zag* showed the highest inhibition activity in 100% methanol (81.62%), 90% methanol (75.163%), and 80% methanol (79.18%), while *Kaw kareed* had the lowest inhibition activity in 100% methanol (57.27%) and 90% methanol (50.48%). However, at 80% methanol, the lipid peroxidation inhibition of non-pigmented *Teli zag* (67.68%) did not differ significantly from that of *Kaw kareed* (67.80%).

Saenkod 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 zag* (70.17%), *Gull zag* (79.41%), and *Kaw kareed* (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 *Shel kew* and *Teli zag* 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 hydrophobic 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 PC_1, PC_2, and PC_3 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, phosphomolybdenum 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 ($PC_2 = 28.82$) 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		
	1	2	3
TPC 100% methanol	0.88	-0.39	0.16
TPC 90% methanol	0.84	-0.50	0.08
TPC 80% methanol	0.73	-0.20	0.51
TFC100% 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
Phosphmolybdenum assay 100% methanol	0.90	0.00	-0.26
Phosphmolybdenum assay 90% methanol	0.86	0.08	-0.50
Phosphmolybdenum 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

Total variance explained

PC	Initial Eigen value	Variance (%)	Cumulative Eigen value	Cumulative (%)
1	11.87	56.56	11.87	56.56
2	6.05	28.82	17.93	85.38
3	2.27	10.83	20.20	96.22

and negatively with TPC and anthocyanin content at the selected concentrations of methanol. The third component (PC_3) 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	Phosphomolybdinum assay
PCA	Principal Component Analysis
AAE	Ascorbic Acid Equivalent

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