

# STUDY OF ANTIOXIDANT PROPERTIES AND ACTIVITY OF TWO RICE BRAN EXTRACTS FROM LOCAL CULTIVARS IN INDIA

KISHOR S. NAKTODE\*

Department of Chemistry, Nevjabai Hitkarini College Bramhapuri, Maharashtra - 441 206, INDIA

e-mail: naktode.kishor98@gmail.com

ORCID ID: <http://orcid.org/0000-0003-0799-2498>

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\*Corresponding  
author

## ABSTRACT

The rice bran varieties native to India, *Oryza sativa* L. CV. Mohara (RB-1) and *Oryza sativa* L. CV. Kedarnath (RB-2), which are extensively cultivated, exhibit a noteworthy abundance of antioxidant components. Employing spectrophotometric techniques, the antioxidant attributes, including total phenolic content, 1, 1-diphenyl-2-picryl-hydrazil (DPPH) scavenging, and 2, 2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical-scavenging, of rice bran extracts were assessed. The present study revealed that the isopropanolic extracts from rice bran yielded promising outcomes, with EC-50 values in the DPPH and ABTS assays for RB-1 measuring  $0.64 \pm 0.06$  and  $0.63 \pm 0.05$  mg/mL, respectively, and for RB-2,  $0.69 \pm 0.03$  and  $0.55 \pm 0.03$  mg/mL, respectively. The highest levels of total phenolic content within rice bran extracts were detected in RB-1, specifically  $4.34 \pm 0.062$  mg gallic acid eq/g. As a result, it is conceivable that isopropanolic extracts derived from these rice bran varieties hold potential utility as natural antioxidants

## INTRODUCTION

Rice, a staple food in India obtained from paddy seeds through milling, yields valuable by-products: rice husk and rice bran. Of these, rice bran carries global commercial significance due to its nutritional and biological merits. The extraction of rice bran's oil content through solvent extraction and refinement produces edible oil. Notably, rice bran contains essential proteins and antioxidants such as vitamin E,  $\gamma$ -oryzanol, tocopherols, ferulic acid, inositol, and phytic acid (Punia *et al.*, 2021). Research indicates that rice bran antioxidants, like vitamin E and  $\gamma$ -oryzanol, can effectively reduce serum cholesterol and low-density lipoprotein levels (Ha *et al.*, 2005). Specially antioxidants in rice bran extract shows the colon Anticancer activity (Faizah *et al.*, 2023). Components like -tocopherol, flavonoid isovitexin, and -oryzanol in rice bran exhibit antioxidant activity similar to the commonly used food preservative butylated hydroxyanisole (BHT) (Duvernay *et al.*, 2005; Ramarathnam *et al.*, 1989). Colored rice bran extracts also show efficacy against reactive oxygen species and free radicals (Saikia *et al.*, 2006; Hirawan *et al.*, 2011; June *et al.*, 2012). Also, very recently Ghasemzadeh *et al.* (2018) showed that the Black rice bran exhibited the most significant phytochemical constituents, showcasing robust antioxidant and antiproliferative activity against breast cancer cells. (Ghasemzadeh *et al.*, 2018).

Recent research by S. Khomdram *et al.* (2010) demonstrated the antioxidant potential of various fruits rich in natural antioxidants and essential vitamins like ascorbic acid (vitamin C). Notably, certain wild fruits like *Emblica officinalis* (amla) displayed exceptional vitamin C concentrations (379.7 mg/

100 g) and potent antioxidant activity with a low IC50 value (181  $\mu$ g/mL) assessed through DPPH assay. Very recent study of quantification of total phenolics and total flavonoids of colored rice bran extracts shows black coloured rice has more activity than the red and light coloured rice extracts (Min *et al.*, 2012; Walter *et al.*, 2013; Guo and Beta, 2013). The findings from Peanparkdee *et al.* (2019) on four local cultivars of Thai rice revealed that extracts from colored rice varieties exhibited a higher concentration of profoundly polar compounds compared to those from uncolored varieties. However, despite research in antioxidant activity of whole grain white rice (Gorinstein *et al.*, 2007; Chotimarkorn *et al.*, 2008) and rice bran extract (Iqbal *et al.*, 2005; Devi *et al.*, 2007), a gap persists in investigating antioxidant activities of rice bran extract from local cultivars in Maharashtra, India. Therefore, this paper aims to explore the antioxidant potential of two native rice bran varieties, *Oryza sativa* L. CV. Mohara (RB-1) and *Oryza sativa* L. CV. Kedarnath (RB-2), cultivated in India. The study employs spectrophotometric techniques to evaluate their antioxidant attributes, revealing promising results in terms of DPPH and ABTS scavenging, alongside specific phenolic content levels. This investigation bridges a research gap, enhancing our understanding of local rice bran varieties' antioxidant capabilities and their potential significance for human consumption.

## MATERIALS AND METHODS

Folin-Ciocalteu reagent, sodium carbonate, potassium persulfate, phosphate buffered saline (PBS, pH 7.4), Butylated hydroxytoluene (BHT) and methanol were purchased from

Merck, Gallic acid from Fluka 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ABTS from Sigma-Aldrich without further purification.

Rice bran powders of two varieties, namely *Oryza sativa* L. CV. Mohara (RB-1) and *Oryza sativa* L. CV. Kedarnath (RB-2) was obtained by milling rice grain in a local grinding mill in Bramhapuri, Maharashtra.

#### Preparation of Rice Bran Extract

It is reported that with increase in temperature extract of rice bran increases in isopropanol solvent (Zigoneanu *et al.*, 2008). We have also prepared rice bran extraction from isopropanol solvent. In two volumetric flask, 20 g of rice bran of each sample (RB-1 and RB-2) were weighed and the 60 mL isopropanol was added to each of them then the flasks were kept in water bath at 40 °C with constant shaking for about 30 minutes. After cooling for 20 minutes, the solution was filtered off using filter paper (Whatman No.1). The excess solvent was evaporated under vacuum. Further, the remaining crude extract kept at low temperature (-20 °C). This extraction process was repeated three times. For the measurement of antioxidant properties 1 g of rice bran extract was dissolved in 25 mL of methanol and sonicated for 15 minutes to get standard solution. These extract solutions used for the estimation of total phenolic contents, DPPH and ABTS radical scavenging ability.

#### Estimation of Total Phenolic Content (TPC)

Total phenolic content in both rice bran extract of was measured by using Folin-Ciocalteu method (Dewanto *et al.*, 2005). Firstly, 1% Standard-1 solution was prepared by dissolving 1 g of Gallic acid in 100 ml of methanol. Different concentrations (0.01, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45 and 0.5 mg/mL) of gallic acid are prepared by dilutions of standard-1 solution of gallic acid. To each of these dilutions 5 ml of 4 % Folin-Ciocalteu reagent and 5 mL of 7.5 % sodium carbonate was added. Then 2 mL of each of these solutions were taken and makeup the volume up to 5 mL by adding distilled water and kept in dark place for 1 hours. The absorbance was measured at 760 nm spectrophotometrically. A standard gallic acid curve was plotted. Similar process was repeated with the rice bran extract solution of all the two formulations. The total phenolic content of the two rice bran extracts was calculated and compared with gallic acid (mg GAE/g). Similar procedure was repeated three times to calculate Mean and standard deviation. Table.1 and fig. 1 is the concentration and absorbance of various standard gallic acid solutions used for plotting curve and regression equation which is used to calculate total phenolic content of the extracts.

#### DPPH radical assay

For the measurement of DPPH radical scavenging ability of rice bran, method reported by Williams *et al.* (2005) and K. S. Nagalapur *et al.* (2010) were used. According to which different concentration (20, 40, 60, 80 and 100 g/mL) of rice bran extract were prepared in methanol then the 3 ml of 4 % DPPH solution was added equally. These mixtures were kept for 30 min in dark place before measuring absorbance at 515 nm using spectrophotometer. Butylated hydroxytoluene (BHT)

Scavenging ability(%) =  $\left[ \frac{\text{absorbance}_{515\text{nm of control}} - \text{absorbance}_{515\text{nm of sample}}}{\text{absorbance}_{515\text{nm of control}}} \right] \times 100$

was used as standard for comparison. Following formula is used to calculate scavenging ability.

The graph was plotted against percentage of inhibition and concentration. By using regression equation EC50 was calculated. Similar procedure was repeated for other two rice bran extracts.

#### ABTS radical cation assay

ABTS radical assay of rice bran was determined by method described by Arts *et al.* (2004). ABTS radical solution was made ready by adding 2.45 mM potassium persulfate into the 7 mM ABTS stock solution in equal volume. This solution was then kept for stand at room temperature in dark place for about 12 hours. Further 2.45 mM ABTS radical cation solution was added to 5 mM phosphate buffered saline (PBS, pH 7.4). Prepared 3 ml ABTS solution and 20, 40, 60, 80 and 100  $\mu\text{l}$  sample of rice brans are mixed well. Further this solution was kept in dark place for 7 minutes and then the absorbance was recorded at 734 nm using colorimeter. To compare the ABTS radical scavenging activity of rice bran Butylated hydroxytoluene (BHT) was used. Following formula was used for calculation of ABTS radical scavenging ability.

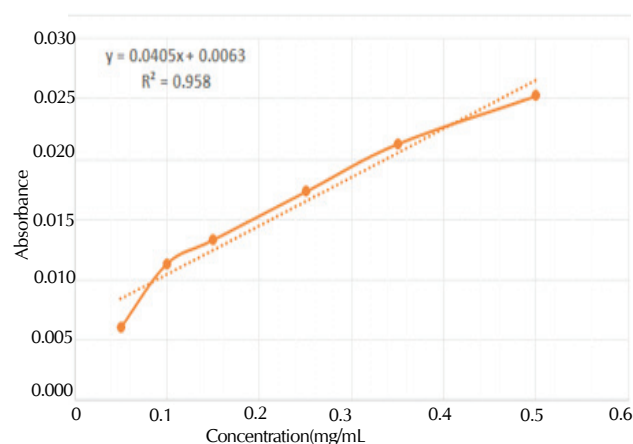
scavenging ability(%) =  $\left[ \frac{\text{absorbance}_{515\text{nm of control}} - \text{absorbance}_{515\text{nm of sample}}}{\text{absorbance}_{515\text{nm of control}}} \right] \times 100$

## RESULTS AND DISCUSSION

The antioxidant attributes of extracts derived from two distinct rice bran varieties, *Oryza sativa* L. CV. Mohara (RB-1) and *Oryza sativa* L. CV. Kedarnath (RB-2), indigenous to local cultivars, were investigated employing the specified methodologies. The ensuing outcomes are as follows. The quantification of total phenolic content for RB-1 and RB-2 yielded values of  $4.34 \pm 0.062$  and  $3.31 \pm 0.087$  mg gallic

**Table 1. concentration and absorbance of various standard gallic acid solutions.**

Concentration (mg/mL)	Absorbance (Mean) $\lambda_{\text{max}} = 510 \text{ nm}$
0.05	0.0060
0.1	0.0113
0.15	0.0133
0.25	0.0173
0.35	0.0213
0.5	0.0253



**Figure: 1. Standard gallic acid solutions curve.**

**Table 2: Yield, Total Phenolic content of isopropyl alcohol extract from 2 rice bran.**

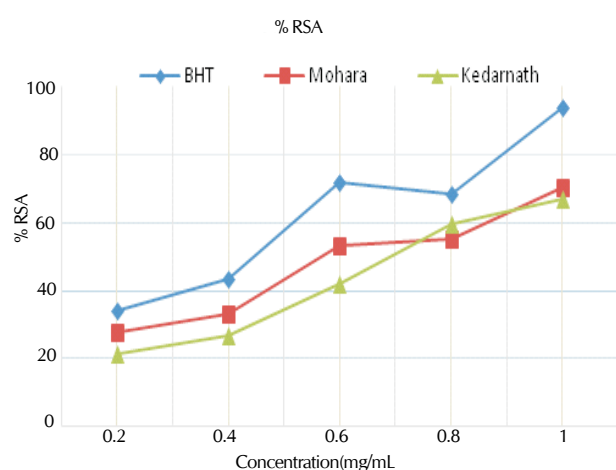
2 rice bran	RB-1	RB-2
Total phenolic content	4.34 ± 0.062	3.31 ± 0.087

Values are mean ± SD (n = 3). Different letters in the same row are significantly different by Duncan's multiple test (P < 0.05) Total phenolic content expressed as mg gallic acid equivalent per mg of extract. RB-1- *Oryza sativa* L. CV. Mohara, RB-2- *Oryza sativa* L. CV. Kedarnath.

**Table 3: EC50 values of Two rice bran extracts**

Antioxidant Activity	BHT (Standard)	RB-1	RB-2
Scavenging ability on DPPH radicals	0.23 ± 0.036	0.64 ± 0.06	0.69 ± 0.036
Scavenging ability on ABTS radicals	0.19 ± 0.016	0.55 ± 0.030	0.63 ± 0.057

Values are mean ± SD (n = 3). Different letters in the same row are significantly different by Duncan's multiple test (P < 0.05) DPPH and ABTS activity expressed as mg/ml. (RB1- *Oryza sativa* L. CV. Mohara, RB 2- *Oryza sativa* L. CV. Kedarnath, BHT as Standard



**Figure 2:** DPPH radical-scavenging activity (% RSA) of different amounts of isopropanolic rice bran extracts from *Oryza sativa* L. CV. Mohara (■, RB-1), *Oryza sativa* L. CV. Kedarnath, (▲, RB 2), *Oryza sativa* L. CV. (×, RB-3) and BHT (●) by 1,1,-diphenyl-2-picrylhydrazyl (DPPH) radicals

acid equivalent per gram of rice bran, respectively, as delineated in Table 2. Remarkably, the rice bran subtype RB-1 exhibited the most substantial concentration of phenolic compounds. This observation aligns harmoniously with the findings established by P. M. Pradeep *et al.* (2014). Similar kind of investigation conducted on rice bran varieties of Reziq rice and SunRice from Australia demonstrated comparable activity (238 to 277 GAE/100g) verifying the present findings (Saji *et al.*, 2019). Additionally, a study undertaken by Peanparkdee *et al.* (2018) revealed a higher total phenolic content in Thai rice cultivars and whereas by Jung *et al.* (2017) in South Korea shows comparable to current study's outcomes observation (31.32 to 156.08 mg GAE/g).

DPPH free radical assay is most commonly used method for analysing the antioxidant activity and also used to estimate reducing species, where methanolic solution of DPPH reduced by proton-donating substance to get diamagnetic molecules (Laskar *et al.*, 2010). The DPPH radical scavenging activity was compared with the commercially available antioxidant standard BHT. From above obtained results, the DPPH radical scavenging effects of all extracts increased with increasing of concentration as shown in Figure 2. DPPH radical-scavenging at 50 % capacity, EC50 values of rice bran extracts obtained from regression analysis as shown in Table 3.

Between the two extracts derived from rice bran, *Oryza sativa* L. CV. Mohara (RB-1) and *Oryza sativa* L. CV. Kedarnath (RB-

2), it was discerned that RB-1 exhibited superior activity concerning EC50 values for DPPH scavenging, registering at  $0.64 \pm 0.06$  mg/mL, whereas RB-2 displayed comparably lower activity ( $0.69 \pm 0.036$  mg/mL) ( $p < 0.05$ ). The attained proportions of percentage radical scavenging activity notably corroborated the findings of A.S.V.C. Reo *et al.* (2010) for

Njavara and Jyothi rice bran extracts from Kerala and Hyderabad, India, respectively, as well as with A. Halee *et al.*'s study (Halee *et al.*, 2018). It is worth noting that the DPPH assay outcomes for both rice bran extracts were on par with the established antioxidant standard, BHT ( $0.23 \pm 0.036$  mg/mL), at the same concentration. The DPPH radical assay's potency in the rice bran extract stems from its capacity to donate hydrogen atoms, as elucidated by Shimada *et al.* (1992). Furthermore, the outcomes of this study are in accordance with the recent investigation conducted by Surin *et al.* (2020) as elucidated that the IC50 value derived from the assessment of DPPH radical scavenging activity is  $1.05 \pm 0.01$  mg/mL, while the IC50 value obtained from the evaluation of ABTS scavenging activity is  $2.82 \pm 0.03$  mg/mL.

At a concentration of 100  $\mu$ g/ml, the scavenging activity of RB-1 and RB-2 stood at  $70.56 \pm 0.97$  % and  $67.05 \pm 0.84$  %, respectively. In comparison, the reference BHT exhibited a radical scavenging activity of  $93.97 \pm 0.26$  % at the same concentration. A parallel inquiry conducted by S. Seema and colleagues into the antioxidant activity of ethanolic extract of *Cyamopsis tetragonolobus* fruits revealed an 80% inhibition on DPPH free radicals at a concentration of 0.5 mg (Seema *et al.*, 2010).

ABTS assay was determined according to method described by Arts *et al.* (2004). The EC50 value for ABTS (2, 2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging effect of RB-1 was found to  $0.26 \pm 0.012$ ,  $0.64 \pm 0.003$  and  $0.91 \pm 0.013$  mg/mL and the standard, BHT was  $0.19 \pm 0.016$  mg/mL. It is observed that RB-1 has comparable higher ABTS radical scavenging activity ( $0.26 \pm 0.012$  mg/mL) to that of BHT ( $0.19 \pm 0.016$  mg/mL) whereas RB-2 and RB-3 ( $0.64 \pm 0.003$  and  $0.91 \pm 0.013$  mg/mL respectively) has less ABTS radical scavenging activity that RB-1 and BHT. The correlation of total phenolic content and DPPH radical scavenging activity as well as ABTS radical cation scavenging ability of all three rice bran extracts are in well agreement with previously reported phenolic compounds Jun *et al.*, (2012). However, previous research has shown that various factors like extraction methods, cultivars, and solvent used for extraction are responsible for the antioxidant ability of rice varieties (Pengkumsri *et al.*, 2015; Verma and Srivastav, 2020).

As observed from the database the two rice bran from local cultivars were extracted by using isopropanol. The extract from these rice bran shows total phenolic, DPPH radical scavenging ability and ABTS antioxidant activity. The RB-1 *Oriza sativa* CV-Mohara showed the highest total phenolics content and antioxidant activity than the rice bran extract of other two sample, especially with respect to DPPH radical scavenging ability. From our comparative study it was concluded that RB-1 contain high amount of phenolic compounds with high antioxidant activities than other two rice variety and this might be due to different phytochemical composition. Therefore, we recommend that Mohara variety could be used for cultivation and rice bran content of it can be a good natural source of antioxidant. However, further studies are needed to evaluate precisely the mechanism of these extracts as antioxidant in food systems.

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