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Evaluation of the Antioxidative Activities of Gamma-Oryzanol and Tocotrienol Rich Fractions Extracted from Rice Bran and Bran Oil

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ABSTRACT

The current study determined the antioxidant activities of γ-oryzanol rich fraction (OzF) and tocotrienol rich faction (TRF) derived from rice bran. Total phenolic content (TPC), total flavonoid content (TFC) and oryzanol content of OzF and TRF were examined. We explored hydrogen peroxide, hydroxyl radical, and 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging and reducing power assays. OzF and TRF's total antioxidant activity (TAA) were investigated using conventional procedure. Total antioxidant activity of OzF was 361.29±6.17 AAE mg/g and TRF was 273.88±5.31 AAE mg/g. Additionally, TPC and TFC of TRF (189.83±4.37 mg GAE/g and 97.64±0.22 CE mg/g respectively) were lower than those of OzF (256.34±7.68 mg GAE/g and 118.31±0.42 CE mg/g, respectively). DPPH, H_2O_2, hydroxyl radicals scavenging activities and reducing power were present in all concentrations of the extracts, the effects being dose-dependent. The current study provided crucial information for the development of food products with high antioxidant efficacy by utilizing TRF and OzF.

1. Introduction

Oxidative degradation is a major cause of the short shelf life of foodstuffs containing substantial lipids and a serious financial threat for the culinary business. When fatty acid is oxidized, it produces hydroperoxides, which then break down into subsequent byproducts [1]. Foods rich in lipids lose their sensory appeal over time due to this oxidation, which generates off-flavor and rancidity when the item is stored. Antioxidants might potentially extend the storage time of products by scavenging and stabilizing free radicals, which in turn slows down the rate of lipid hydrolysis as well as oxidation. Food items, particularly those containing lipids, can benefit from the addition of antioxidants as they can prolong their shelf life by delaying the stages of lipid peroxidation reaction, which is among the main factors that degrades foodstuffs during its preservation and processing [2].

Antioxidants' potential in treating and preventing diseases like coronary artery disease, heart failure, neurological disorders, wrinkles, cancer, diabetes, hypertension, and many more is at present the subject of much research. Some of the extensively employed antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), tert-butylhydroquinone (TBHQ), and other similar compounds. Nevertheless, in clinical trials, these

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synthetic antioxidants are thought to be the reason of cancers and liver damages [3, 4]. The health impact of different natural antioxidants extracted from herbs, spices, and teas that reduce oxygen radicals usually referred to as reactive oxygen species (ROS), have been the subject of several research nowadays. Under these circumstances, the generation and application of other potent antioxidants derived from natural sources is required [5].

A byproduct of the rice processing industry, the bran obtained from rice comprises 8%–11% of the rice grain and 8%–23% oil. The two primary methods used to obtain rice bran oil (RBO) are mechanical compressing and extraction with solvents. RBO has between 90% and 96% lipid components (free fatty acids, waxes, di-, and triglycerides) and 3% to 5% unsaponifiable (sterols, tocotrienols, and triterpene alcohols) [6]. Furthermore, RBO is a reliable origin of phytonutrients as well as polyphenols such as γ-oryzanol, tocopherols and tocotrienols, which naturally act as antioxidants. [7].

One of the significant micronutrients of RBO, γ-oryzanol has applications in the dietary supplement, cosmeceutical, and medicinal product sectors [8]. Oryzanol's molecular structure includes an aromatic phenolic nucleus of esterified ferulic acid [9]. The solubility of γ-oryzanol ($C_{40}H_{58}O_4$) in both polar and nonpolar solvents is due to the presence of an alcoholic group (-OH) in its ferulic acid component [10]. Because oryzanols have the antioxidant activity significantly larger than that of tocopherols and an enormous ability to decrease blood cholesterol, it finds widespread usage [11, 12]. Moreover, γ-oryzanol may combat free radicals which trigger carcinogenesis [13].

Tocotrienols are composed of three double bonds according to the R-configuration and have all trans-geometries, with a single stereocenter [14]. There is only one stereoisomeric carbon and one isomer for each structure due to the unsaturation in tocotrienol tails. At room temperature, tocotrienols are dense liquids that dissolve in lipids and lipophilic solvents but does not dissolve in the water. Because of their antioxidative qualities, tocotrienols have applications to cosmetics and sun blockers for protection against harmful ultraviolet (UV) rays in addition to aid in the moisturizing and repairing of the skin [15].

Antioxidant activities can be measured in the laboratory using a variety of ways. For dietary and pharmaceutical products, total antioxidative efficiency is a commonly used test metric. The capacity of a substance to prevent oxidative deterioration, such as lipid oxidation containing unsaturated structure, is one of the examples of this test [16]. Nevertheless, the DPPH radical detection test is the most straightforward and extensively documented technique for evaluating antioxidant activity in foodstuffs and numerous plant-based medications. The experiment involves the reduction of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical, a purple-colored chromogen, to pale yellow hydrazine through the action of antioxidants or reducing agents. Scavenging capacity tests against particular reactive oxygen species (ROS), that involve all extremely reactive, oxygen-containing molecules like hydroxyl radicals, hydrogen peroxide, etc., are other significant approaches [17].

It was found that, the evolution of reducing power is correlated with antioxidant capability, and that the reducing power of biologically active substances and food ingredients represents their ability to release electrons [18]. So, reducing agents and inactivators of oxidizing agents are two examples of biologically active chemicals having antioxidant properties.

The purpose of this project is to use scavenging and reducing assays to ascertain the antioxidative capability of γ- oryzanol and tocotrienol rich fractions extracted from rice bran and its oil.

2. Materials and Methods

2.1 Collection of materials

From a local plant of automated rice mill situated in Natore, Rajshahi, the freshly processed bran from rice was obtained immediately in December.

2.2 Chemicals and reagents

Chemical reagents like DPPH (2, 2-Diphenyl-1-picrylhydrazyl) and standard compounds for phenolic substances were purchased from Sigma Aldrich (Sigma Aldrich, St. Louis, MO, USA). Solvents of analytical quality like n-hexane, methanol and other required chemicals used in the present work were from Merck (Darmstadt, Germany or Mumbai, India), Supelco Chemical Co. (Bellefonte, PA, USA), Loba (India), etc.

2.3 Preparation of sample

2.3.1 Stabilization of rice bran

To optimize the moisture content to 21% [19] and to deactivate endogenous lipase enzyme, first of all the rice bran sample was sealed in a plastic bag that was suitable to the microwave and then the bag was heated in a microwave oven (MS 3042G, LG, China) at 2450 MHz, output power 850 W for three minutes. After being taken away from the oven chamber and let to cool down to room temperature, the bran was refrigerated at a temperature of -15 ^{o}C in polyethylene bags.

2.3.2 Extraction of oil sample

One of the important steps of the project was extracting the rice bran oil (RBO) from previously stabilized rice bran. This was done by utilizing n-hexane as solvent for extraction and a solid to solvent ratio of 1:10 (w/v) was used for 1 h in an orbital shaker device (Phoenix Instrument, RS-OS 20) at ambient temperature. Whatman No. 4 filter paper was used to filter the extract, and the residue again went through the extraction step using the identical solvent and the same setup. After combining the resulting extracts, the solvent was evaporated at 45 °C under a low pressure. Before being subjected to additional analysis, the oil sample was weighed to determine its yield and preserved in glass containers at -15 °C. The following formula was applied to calculate the oil yield (%):

$$(\text{weight of the oil} / \text{weight of the bran}) \times 100 \quad (1)$$

2.3.3 Preparation of γ- oryzanol (OzF) rich fraction from rice bran

The fraction rich in γ-oryzanol from rice bran was prepared using the methodology of Xu et al., [10]. Firstly, in a glass conical flask, the rice bran was suspended in distilled water. The mixture was mixed with ascorbic acid. After vortexing the ingredients, it was allowed to incubate for 30 minutes in a water bath at 60°C. Subsequent to the addition of the solvent combination, which included 50:50 hexane and isopropanol, the suspension was vortexed for 30 seconds, then the mixture was centrifuged at 200 RCF for 15 minutes. By using a separatory funnel, the organic portion was accumulated. After blending the residues with the same solvent system, another centrifugation was performed. The earlier collection and this organic part were then added and stored together. After that, the organic layer was rinsed with distilled water. The distilled water in the funnel was removed after allowing it to rest for ten minutes. There were two iterations of this rinsing process. The organic portion was subsequently collected into a conical flask. The used solvent was finally evaporated under reduced pressure operating a Lab Tech, EV311AD rotary evaporator instrument at 40 °C to obtain the extracted substance.

2.3.4 Preparation of tocotrienol rich fraction (TRF) from rice bran oil

TRF was isolated from extracted crude RBO by exploiting methanol solvent [20]. To put it briefly, RBO was mixed with methanol, stirred with the help of a magnetic stirrer at room temperature for one hour. Following one hour, the TRF-enriched solvent phase was collected and the methanol solvent was recovered by vacuum-evaporation at 65 °C using a rotary evaporating instrument.

2.4 Estimation of bioactive phytochemicals

Several techniques were implemented to determine the biologically active phytochemicals of lab-extracted RBO, OzF, and TRF. Total phenolic and total flavonoid contents of RBO, OzF and TRF were estimated by the strategies of Skerget et al., and, Wu and Ng, respectively [21, 22]. The content of oryzanol present in all of the samples was determined by the approach of Seetharamaiah and Prabakar [23].

2.4.1 Total phenolic content

With the Folin-Ciocalteau reagent (FCR), the total phenolic content (TPC) of the RBO, OzF, and TRF samples was ascertained by spectroscopy. To be specific, 2.5 mL of the reagent Folin-Ciocalteu (ten times diluted with distilled water) and 2 mL of 75 g/L Na_2CO_3 were mixed together with 0.5 mL of test sample. After incubating at 50 °C for five minutes, the sample was subsequently cooled. 0.5 mL of distilled water was tested in place of the sample as the control sample, measuring the absorbance at 760 nm. TPC measured as gallic acid equivalent (GAE) was calculated while the findings were represented as mg GAE/g sample.

2.4.2 Total flavonoid content

In short, 2.2 mL of deionized water, 0.15 mL of 5% sodium nitrite, and 0.15 mL of 10% aluminum chloride were added together with 0.5 mL of sample, and the mixture was allowed to react for six minutes. Following that, 2.0 mL of 4% sodium hydroxide solution was added to the combination. The combination was again allowed to react for fifteen minutes at room temperature before the absorbance at 510 nm was taken. The total flavonoid content, which was measured in terms of catechin equivalent (CE), was reported as mg CE/g in the results.

2.4.3 Estimation of oryzanol

After dissolving 10 mg of each sample in 10 mL of hexane, a UV spectrophotometer was used to measure the absorbance of the resulting solution in a 1-cm cell at 315 nm. A specific extinction coefficient of 358.9 was used to estimate the oryzanol concentration in the samples [23]. The content of oryzanol (g %) in the samples was calculated using the formula:

$$\{(A_1 \times V)/ W\} \times 100/358.9 \quad (2)$$

Here,
A_1= Absorbance of sample
V= Volume of hexane used
W= Weight of sample

2.5 Antioxidant activities

Antioxidative evaluation of the prepared samples was carried out by different antioxidant assays such as

phosphomolybdenum assay [24], DPPH radical scavenging assay [25], H_2O_2 scavenging activity [26], hydroxyl radical-scavenging activity [27], and reducing power [28].

2.5.1 Phosphomolybdenum assay (total antioxidant activity)

In order to measure the total antioxidant activity (TAA) a combination of 0.3 mL of sample and 3 mL of prepared reagent (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was put into a test tube and allowed to incubate at 95 ºC for 90 minutes. Upon cooling at 25 ºC the absorbance was measured at 695 nm using a spectrophotometer against a blank. Ascorbic acid equivalent (AAE) was the unit of measurement for total antioxidant activity. The outcomes were given as mg AAE/g of sample.

2.5.2 DPPH radical scavenging activity

In brief, 2.0 mL of the samples dispersed into the extraction solvents at various levels of concentration were mixed with 2.0 mL of freshly made 0.2 mM DPPH in methanolic solution. The resulting mixture underwent shaking and was allowed to stand at an ambient temperature for 30 minutes in dark conditions and after that, the absorbance at 517 nm was measured with respect to the blank control. In contrast to the test solution, a control sample comprised the same amount of 70% methanol. A formula as follows was used to estimate the percentage of the test samples that could inhibit the DPPH radical:

$$[(A_{control} - A_{sample}) / A_{control}] \times 100 \quad (3)$$

Here,
$A_{control}$ = Absorbance of control solution
A_{sample} = Absorbance of sample solution

2.5.3 Hydrogen peroxide scavenging activity

At first, a 40 mM H_2O_2 solution was made in a phosphate buffer solution (pH = 7.40, 0.2 mol/L). The solution of hydrogen peroxide (0.6 mL) was added to the extracted samples at varying concentrations. Following a 15-minute incubation period, the intensity of absorption at 230 nm was measured in contrast to a control solution that contained phosphate buffer saline but no hydrogen peroxide. The formula below was utilized to determine the scavenging activity or percent of inhibition:

$$[(A_0 - A_1) / A_0] \times 100 \quad (4)$$

Where,
A_0 = Absorbance of control solution
A_1 = Absorbance in presence of extract

2.5.4 Hydroxyl radical scavenging activity

With some modifications, the Smirnoff and Cumbes method was applied to examine the hydroxyl radical scavenging power of the antioxidant rich samples [29]. In a reaction test tube, the following reagents were added in the following order: 0.3 mL of 20 mM sodium salicylate, 1.0 mL of 1.5 mM $FeSO_4$, 1.0 mL of sample solution at different concentrations, and 0.7 mL of 6 mM H_2O_2. After a quick mixing of the ingredients, the reaction tubes were kept in a water bath set for one hour, at 37 ºC. The absorbance of the combination was measured in comparison to a blank at 510 nm. Here is the calculation to determine the hydroxyl radical scavenging ability (%) of the samples:

$$[(A_0 - A_1) / A_0] \times 100 \quad (5)$$

Where,
A_0 = Absorbance of control solution
A_1 = Absorbance in presence of extract

2.5.5 Reducing power

To 2.5 mL of 1.0% potassium ferrycyanide, 1.0 mL of the sample at various concentrations was added to 2.0 mL of phosphate buffer (0.2 M, pH 6.6). The resulting mixture was mixed with 2.5 mL of 10% trichloroacetic acid after being incubated for 20 minutes at 50 ºC. A part of the mixture (2.5 mL) was combined with 0.5 mL of 0.1% ferric chloride and 2.5 mL of distilled water. After 30 minutes of reaction time, the resultant solution's absorbance was recorded at 700 nm. The sample's reduction ability was determined by an increase in the absorbance with the concentration of the test mixture.

2.6 Statistical analysis

The results were represented as the mean and standard deviation (SD) of the three experiments. A one-way analysis of variance (ANOVA) was then executed on the data. The Duncan's multiple range test was applied to compare mean values at the $p<0.05$ significant level with the help of SPSS 11.5 software.

3. Results and Discussions

3.1 Extraction yield and bioactive phytochemicals

The first step of the project work, i.e., the extraction of crude oils, was very crucial, as oils obtained in the market may contain a significant amount of preservatives, which can affect the original value of the assay results and the process necessitates both effort and time. After extraction and complete removal of solvents, the yields were calculated and the results were reported (Table 1 and Table 2).

3.1.1 Extraction yield

The current study found that the yield of crude oil content in rice bran was 15.32%, which is somewhat lower than the estimated figure of 17.08% by Ali et al.,

[30]. The percentage yields of the fraction of rice bran and bran oil rich in oryzanol and tocotrienol were 19.58 and 8.75 g/100 g, respectively.

3.1.2 Total phenolic content (TPC) and total flavonoid content (TFC)

Phenolic substances can act as potent anti-oxidants and have the ability of adsorbing, neutralizing harmful free radicals, decomposing peroxides and quenching singlet and triplet oxygen to avoid oxidative deterioration. In addition, flavonoids are those phytochemicals containing phenolic hydroxyl groups in their molecular structures. These substances are capable of chelating transient metallic ions (such as Fe^{2+}, Cu^{2+}, and Zn^{2+} and so on), catalyzing transportation of electrons, and scavenging free radicals. The project effort involved determining the total phenolic content (TPC) and total flavonoid content (TFC) in order to evaluate how successful the samples will act as antioxidant.

Table 1. Extraction yield, total phenolic content and total flavonoid content of rice bran oil and antioxidant rich fractions.

Sample	Yield (g/100g)	TPC (mg GAE/g)	TFC (CE mg/g)
RBO	15.32±0.52^{b}	24.19±0.10^{a}	11.76±0.14^{a}
OzF	19.58±0.43^{c}	256.34±7.68^{c}	118.31±0.42^{c}
TRF	08.75±0.16^{a}	189.83±4.37^{b}	97.64±0.22^{b}

Each value is the mean ± standard deviation of triplicate determinations. Values within a column with the same letters are not significantly different at $p < 0.05$.

According to Table 1, total phenolic content (mg GAE/g) and total flavonoid content (CE mg/g) of crude RBO were 24.19±0.10 mg GAE/g and 11.76±0.14 CE mg/g respectively which are greater than the value reported by Ali et al., [30]. On the other hand, oryzanol rich fraction showed greater TPC (256.34±7.68 mg GAE/g) and TFC (118.31±0.42 CE mg/g) than that of the tocotrienol rich fraction 189.83±4.37 mg GAE/g and 97.64±0.22 CE mg/g respectively.

Table 2. Total antioxidant activity and oryzanol content of rice bran oil and antioxidant rich fractions.

Sample	Total antioxidant activity (AAE mg/g)	Oryzanol content (g/100g)
RBO	36.74±0.54^{a}	01.69±0.01^{a}
OzF	361.29±6.17^{c}	08.04±0.05^{c}
TRF	273.88±5.31^{b}	01.97±0.03^{b}

Each value is the mean ± standard deviation of triplicate determinations. Values within a column with the same letters are not significantly different at $p < 0.05$.

3.1.3 Oryzanol content

Oryzanol concentration of the samples was evaluated spectrophotometrically. According to Table 2, rice bran oil contained γ- oryzanol (1.69±0.01 g/100g) which was in excellent accordance with the value investigated by Ali et al., [30]. TRF extracted from rice bran oil showed 1.97±0.03 g/100g of oryzanol content. γ-Oryzanol fraction (OzF) of rice bran exhibited highest concentration of oryzanol (8.04±0.05 g/100g) compared to all other samples examined in this study.

The outcomes shows that, the amount of bioactive phytochemicals present in rice bran oil may not be significant. However, the solvent extraction of rice bran and bran oil typically concentrates bioactive substances such as phenolic acids, flavonoids, tocopherols, tocotrienol and γ-oryzanol. These phytochemicals have potent antioxidant properties. In contrast, while rice bran oil also contains some of these compounds, they are present in lower concentrations compared to the extracts. Moreover, the dissolvent used here like methanol, isopropanol, water, hexane etc. are effective solvent for extracting a wide range of antioxidant compounds from rice bran and bran oil. It can extract polar and non-polar compounds alike, including phenolics, tocopherols and so on, which contribute significantly to antioxidant activity. On the other hand, rice bran oil predominantly contains lipophilic compounds, which may not exhibit as strong antioxidant properties as those extracted in alcohols [31]. Additionally, extraction efficiency also has an impact on the properties of antioxidant rich fractions. Solvent extraction is designed to maximize the recovery of bioactive phytochemicals from rice bran and bran oil. It allows efficient extraction of both hydrophilic and lipophilic antioxidants, resulting in a more potent antioxidant extract compared to the oil, where the oil extraction process may not yield as high a concentration of these bioactives [32].

3.2 Antioxidant Activity Assay

3.2.1 Total antioxidant activity

Total antioxidant activity (TAA) is the primary measure for assessing the capability of a substance to act against oxidation. TAA of the samples was determined by phosphomolybdenum method. As shown in Table 2, TAA of crude RBO was greater (36.74±0.54 AAE mg/g) than the value reported by Ali et al., [30]. In addition, antioxidant OzF displayed better TAA (361.29±6.17 AAE mg/g) than that of the TRF (273.88±5.31 AAE mg/g).

Other antioxidant activities such as DPPH radical scavenging activity, hydrogen peroxide scavenging activity, hydroxyl radical scavenging activity and reducing power of antioxidant rich fractions i.e, OzF and TRF were performed and the results are plotted and shown in Figure 1, 2, 3 and 4 respectively.

3.2.2 DPPH radical scavenging activity

The DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging test relies on the transfer of electrons for generating a violet solution in ethanol.

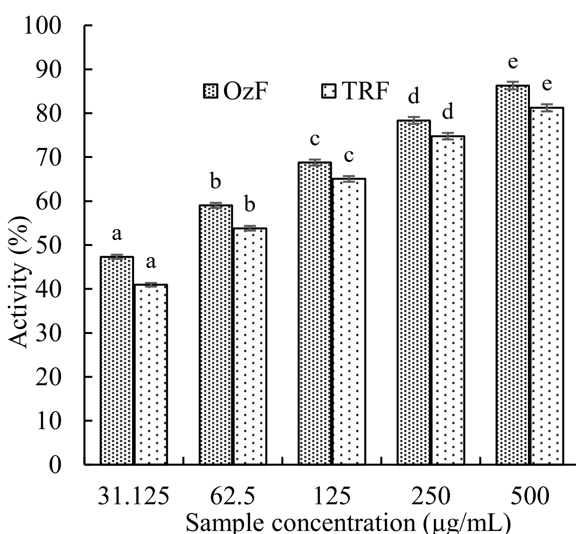

Figure 1. DPPH radical scavenging activity of antioxidant rich fractions. Each value is the mean ± standard deviation of triplicate determinations. Values within each sample with different letters on bar are significantly different ($p < 0.05$).

The presence of an antioxidant component reduces this free radical, which is stable at ambient temperature, resulting in a yellow colored suspension of ethanol. Antioxidant evaluation by spectroscopic analysis can be performed quickly and smoothly with the help of the DPPH test method.

For both OzF and TRF a clear increment in antioxidant activity were seen with concentration of the antioxidants. For all concentrations OzF showed better activity than TRF. Less than 40 µg/mL OzF was required to inhibit 50% of DPPH radical. On the contrary, 50% scavenging activity was recorded for TRF at concentration around 50 µg/mL (Figure 1).

3.2.3 Hydrogen peroxide radical scavenging activity

The hydroxyl radicals that hydrogen peroxide produces within cell membranes might be harmful to the cells. Rapid cellular membrane penetration of H_2O_2 can result in the formation of hydroxyl radicals within the cell when it reacts with Fe^{2+} or Cu^{+}, which can have a variety of detrimental consequences on neural cells [33].

In order to evaluate extracted antioxidant-rich fractions, H_2O_2 scavenging activity is an effective metric. From Figure 2 it is understood that, OzF and TRF could scavenge 50% of total hydrogen peroxide present in the test solution at concentrations less than 45 and 62 µg/ml respectively. Hence, OzF is more effective in showing hydrogen peroxide radical scavenging activity than TRF. Both of them showed a dose dependent action.

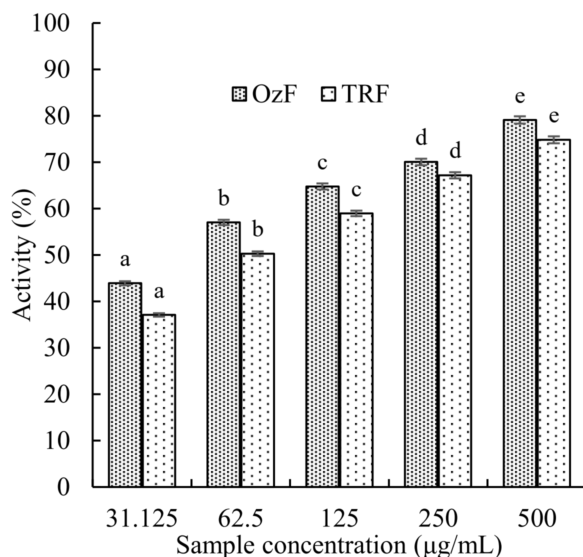

Figure 2. Hydrogen peroxide radical scavenging activity of antioxidant rich fractions. Each value is the mean ± standard deviation of triplicate determinations. Values within each sample with different letters on bar are significantly different ($p < 0.05$).

3.2.4 Hydroxyl radical scavenging activity

Hydroxyl radicals are one of the most damaging and short-lived radicals in the body. They are created by both the superoxide radicals and H_2O_2 to damage biological macromolecules such as proteins, lipids, nucleic acids and so on [34]. Figure 3 demonstrates the hydroxyl radical scavenging ability of the samples in various doses.

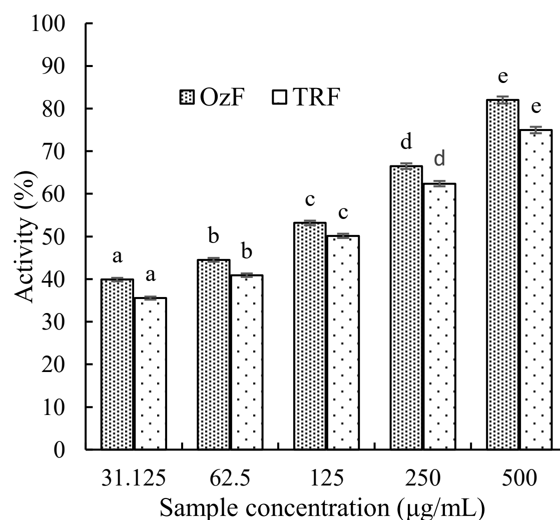

Figure 3. Hydroxyl radical scavenging activity of antioxidant rich fractions. Each value is the mean ± standard deviation of triplicate determinations. Values within each sample with different letters on bar are significantly different ($p < 0.05$).

It is seen that, the scavenging capacity magnifies with the intensification of the extracts concentrations. However, 50% hydroxyl radical scavenging activity was recorded by around 98 μg/mL OzF and slightly less than 125 μg/mL TRF sample concentrations.

3.2.5 Reducing power

A substance's antioxidant capacity is also indicated by its reducing power [35].

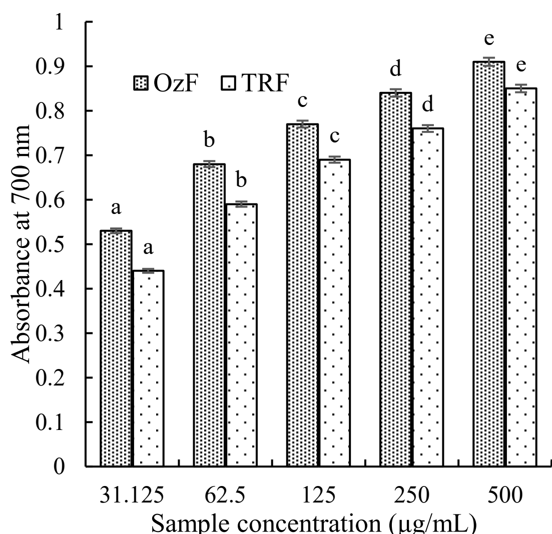

Figure 4. Reducing power of antioxidant rich fractions. Each value is the mean ± standard deviation of triplicate determinations. Values within each sample with different letters on bar are significantly different ($p < 0.05$).

As oxidized intermediates of lipid peroxidation steps can be reduced by electron donor chemicals, which are often thought of as reducing agents, they can function as primary or secondary antioxidants [16].
The reducing power is expressed as absorbance value in the Figure 4. The reported results justified that, reducing power was concentration related and increasing the dosage of extracts triggered a significantly improvement in reducing capability in the test samples. The reducing power of OzF and TRF at a concentration of 500 μg/mL were 0.91 and 0.85 respectively. Among the extracts, OzF showed higher reducing power at each concentration.

In the present work, tocotrienol rich fraction showed lower antioxidative activities than oryzanol rich fraction which is assumed to be because of the presence of more phenolic and flavonoid content in OzF. For the same reason total antioxidant activity (TAA) of TRF was observed to be higher than that of the rice bran oil. It is believed that phenolic compounds have the capacity to scavenge reactive radical species by donating their hydrogen atom and/or electron to the radical through various chemical pathways. Proton-coupled electron transfer processes are involved in this reaction. The presence, quantity, and configurations of hydroxyl groups in ring structures, the electron-withdrawing characteristics of the carboxylate group in benzoic acid, and the existence of a methoxyl group are the factors that determine the antioxidant activity of phenolic acids, which is contingent upon their chemical structures [36]. Furthermore, flavonoids—a class of widely substituted hydroxylated phenolic compounds—are widely accepted for their anti-oxidant capabilities. As with phenolic acids, flavonoids have the ability to function as chelating agents as well as transfer hydrogen or electrons to free radicals via several chemical routes [37]. On the contrary, in case of both antioxidant rich fractions of the present study, activities against DPPH, hydroxyl, hydrogen peroxide radicals increased with increasing extract's concentration. This trend was also observed by S. Yousuf et al., [38]. J. Bardhan et al., observed that, tocotrienol rich fraction extracted form rice bran oil can effectively scavenge DPPH free radical and can also retard lipid oxidation [2]. Even, methanolic, ethanolic and acetone extracts of rice bran or oil could effectively display metal ion reducing and free radical scavenging powers owing to the existence of phenolic and flavonoid components [39].

4. Conclusion

The study's antioxidative findings unequivocally establish that, fractions rich in γ-oryzanol and tocotrienol have the power of scavenging or inhibiting free radicals, similar to a primary antioxidant reacting with free radicals. This could mitigate the harm that free radicals do to the body. Strong hydrogen-donating capabilities and efficaciousness as scavengers of H_2O_2 and other free radicals can be credited for the extracts' diverse antioxidant methods. In addition, the extracts get engaged in competition with DPPH radicals and inhibits the production of chromogen. As a result, OzF and TRF both exhibit strong antioxidant activity against a variety of in vitro oxidative structures, making them valuable as prospective dietary supplements as well as easily accessible sources of naturally occurring antioxidants.
A compound's reducing capacity may be a useful predictor of its ability to exert antioxidant action and it is easily assumed that, these antioxidants will be able to prevent the development of lipid peroxides. According to the study, antioxidant-rich fractions' reducing capacity gets enhanced in proportion to concentration. As compared to tocotrienol rich fraction, all concentrations of γ-oryzanol rich fraction displayed a strong potential to serve as an antioxidant; this is possibly because of the high phenolic content of the former, which was discovered through the analysis of the samples' bioactive phytochemicals.
The samples are thought to be able to work competitively and synergistically against free radicals. Therefore, free

radicals can be stabilized or neutralized by antioxidant-rich components before they damage cells.

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