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Antioxidant Profiling of Rice Varieties for Use as Therapeutic Diet

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ABSTRACT

Nowadays, despite the production of a large number of new rice cultivars with improved yield and enhanced climate tolerance, the average person's nutritional needs are not being fulfilled. Although rice has higher antioxidant activity, which significantly improves human health. **Objectives:** To assess secondary metabolite composition and antioxidant potential of various local and mutant rice varieties. **Methods:** The Nuclear Institute of Agriculture (NIA) in Tandojam, Sindh, Pakistan, provided thirteen different rice cultivars. All experiments were carried out three times to find out the total phenolic content (TPC), total flavonoid content (TFC) and to analyze the antioxidant potential of rice extracts with various solvents by DPPH, FRAP, $Fe2+$ chelating activity and OH- radical scavenging activity. Graph-Pad Prism 7.0 was applied for analysis of the data. **Results:** It was found through the study that mutant rice cultivars differ significantly ($p<0.05$) from local rice varieties. Rice varieties such as Sonehri Sugdasi, Shandar, and Shua-92 had the highest TPC, while Jajai-77, IRI-6, and Shandar had the highest TFC. Shua-92, Mehak and Shadab were found to be best at their antioxidant potential. **Conclusions:** It was concluded that mutant rice varieties showed a significant difference from their parent varieties. The study highlights the antioxidant potential as phenolics known for their antioxidant properties, are of interest, with higher consumption linked to reduced cardiovascular and cancer risks. Notable varieties included Sonehri, Sugdasi, Shandar, Shua-92, Jajai-77, RI-6, and Shandar for the highest TPC and TFC, respectively. It was found through the study that mutant rice cultivars differ significantly ($p<0.05$) from local rice varieties.

INTRODUCTION

Rice is the most popular and nutritious staple food of not only Pakistan but also approximately 50% of the world's population. In Pakistan, rice is one of the main exports and the second most important cereal crop with a total production of 7.4 million metric tons [1, 2]. Rice also has a diverse range of antioxidant, anti-allergic, anti-inflammatory and anti-cancerous properties. Rice contains vitamins, fiber, minerals, and bioactive polyphenols and flavonoids (like ferulic acid, tocopherols, phytic acid) [2, 3]. These phytochemicals play an important role in inhibiting cellular oxidation via free radical scavenging and thus maintaining the proper ratio between antioxidants and oxidants [4]. In several ways, tissues and cells are being damaged by Oxidative stress, which is made possible by a disequilibrium in the balance due to the change in concentration of antioxidants and oxidants. Different mechanisms may be used by phenolic compounds to exhibit their antioxidant action [5]. As chain-breaking antioxidants, some reactive species, such as superoxide and hydroxyl radicals, are directly scavenged by them. Lipid peroxidation can be controlled or reduced by recycling more antioxidants, such as tocopherol. Some polyphenols are pro-oxidants which can bind with metals such as copper and iron, hindering the production of free radicals from these pro-oxidants while still conserving their ability to scavenge free radicals [6, 7]. Certain polyphenols are also associated with a rise in the activity of antioxidant enzymes and the activation of some antioxidant proteins. Phenols and flavonoids have been explored to have therapeutic potential for cancer and

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inflammation due to their extraordinary antioxidant capacity [8, 9]. The ions such as hydroxyl radical, superoxide ion and hydrogen peroxide, among them, can all be promptly scavenged by flavonoids, which serve as the most predominant plant secondary metabolites in rice [10, 11]. Therefore, some mutant rice varieties (such as Shadab, Shandar, etc.) with genetic variations have been developed to get higher yield, and these may have comparatively better nutrition in terms of polyphenols and flavonoid content.

This study aims to explore the therapeutic potential of rice varieties (in terms of their secondary metabolite content and antioxidant potential) for combating life-threatening diseases like cancer.

METHODS

The experimental study design was carried out for a comparative nutritional assessment and antioxidant potential of various mutant as well as local rice cultivars in Sindh province, Pakistan. The study duration was 2022-2023. Thirteen varieties of rice cultivars were procured from the Nuclear Institute of Tandojam, which include Sadagulab, Sugdasi Ratria, and Sonehri Sugdasi, IRI-6, Shadab, Shandar, Sarshar, IRI-8, Khushboo-95, Jajai-77, Shua-92, uper Basmati, and Mehak. Among them, IRI-6, Jajai-77, IRI-8 and Super Basmati were parent cultivars, whereas Shandar and Shadab were included as mutant varieties of IR6, Shua-92 and Sarshar as mutants of IRI-8, Khushboo-95 as a mutant of Jaijai-77 and Mehak as a mutant of Super Basmati. Three rice cultivars were selected as references, i.e., Sada gulab, Sugdasi Ratria and Sonehri Sugdasi. To justify a chosen sample size for rice research, a power analysis was conducted before the study, considering the effect size, desired power level (typically 80%), and significance level (usually 5%) to ensure sufficient statistical power to detect meaningful differences. The total phenolic and flavonoid content in rice varieties was analyzed using a control randomized sampling. Each sample was extracted with 20 times its weight of methanol 80% (v/v) that was acidified with 1% (v/v) hydrochloric acid. This was conducted by sonication at 25°C for 2 hours. After centrifugation at 2000 rpm (15 minutes), the supernatant was used to determine the total content of phenolics and flavonoids. A previous procedure [3] was followed to assess the TPC; for this, 200 µL supernatant, 1.5 mL of Folin-Ciocalteu's reagent, an incubation for five minutes and the addition of 1.5 mL of Sodium Carbonate (6% w/v) were used. The contents were incubated for one hour at 25˚C, and the data were gathered at 725 nm. Gallic acid equivalent (GAE) was used to measure the total polyphenol content (mg per gram protein). To identify total flavonoid content, the colourimetric technique was used but with minor changes [4]. 5% sodium nitrate of 75 µL was mixed with the sample of 250 µL and distilled water of 1.25 mL. Six minutes after the water was introduced, 150µL of AlCl3.H2O (10%) was added, and the solution remained untouched for 5 minutes. 2.5 mL of the solution was made up using 500µL of NaOH. The absorbance was determined on a UV-Visible spectrophotometer at 510nm. The catechin equivalents (CE, mg (+)-catechin/g sample) indicated the total flavonoid content. To test for antioxidant activity, de-husked rice was ground into flour. 1 gram of each flour was measured and soaked in 10 milliliters of distilled water for 5 minutes, after which a centrifuge (LXJ-IIC made by Shanghai Anting Scientific Instruments) was used to spin the solution at 14000 rpm for 15 minutes to get the supernatant. The DPPH, Fe2+ reducing power, OH radical scavenging and FRAP assays were performed using this supernatant [5]. For the DPPH assay, Rice samples (3 mL) were mixed with 150 µl DPPH solution (0.1 mM in 95% ethanol), then incubated in the dark for 30 minutes at 270°C. The absorbance at 517 nm was studied for every sample [3]. As blanks, ethanol and ascorbic acid were used in the experiment, and the results were compared with those. The percent of scavenging was calculated by the following formula. DPPH scavenging is measured as 100 times (1- the absorbance of the sample/the absorbance of the blank). To measure the ferrous ion-chelating activity [6], a 500 µl sample was mixed with 1 ml of Ferrozine (0.5 mM), vigorously shaken for 30 minutes at room temperature and the absorbance was measured at 562 nm when the shaking was completed. The experiment included EDTA to act as a positive control. % Binding Activity (Absorbance of blank – Absorbance of the sample) x 100. The sample (100 µl) was placed into 3 ml of FRAP reagent, vortexed and at this point, absorbance was measured immediately. The water bath was heated to 370C °C for 4 minutes, and the second reading was taken at 593 nm. FRAP value of the Sample (µM) = (The change in sample absorbance divided by the change in reference absorbance) ×2/absorbance of blank. When the FRAP value of the standard (ascorbic acid) is found to be 2. 0.2 ml of 10 mM FeSO4. 7H2O was incorporated with 10 mM EDTA, and 0.2 ml of deoxyribose solution (10 mM) was added to the mixture. After that, 0.1 M phosphate buffer (1 mL; PH 7.4) was mixed with the above solution, and 0.2 mL each of the sample and H2O2 10 mM were added to the mixture in a screw-capped tube. The mixture was then incubated at 37°C for one hour. When the incubation was over, 1 ml of trichloroacetic acid (2.8%) and 1 ml of Thiobarbituric acid (1.0%) were added to the mixture. What was left in the cuvette was boiled for 10 minutes, and the absorbance was collected at a wavelength of 520 nm after cooling. A positive control in the investigation was BHA. OH-Scavenging Activity (Absorbance of blank – Absorbance of the sample) x 100. Measure the absorbance of a blank. Data

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were shown using mean and standard deviation. Both ANOVA and T-test were applied to the data in Graph-Pad Prism 7.0 and Excel 2016. Any p-value less than 0.05 was considered to be significant.

RESULTS

Estimation of total phenolic content depicted that mutant cultivars, including Shua-92 and shandar, have high phenolic content as compared to their local varieties. Whereas among standard rice varieties, Sonehri Sugdasi showed highly significant phenolics content as compared to other reference varieties. The findings showed that overall polyphenol content varied considerably ($p<0.05$) amongst the rice cultivars (Figure 1).

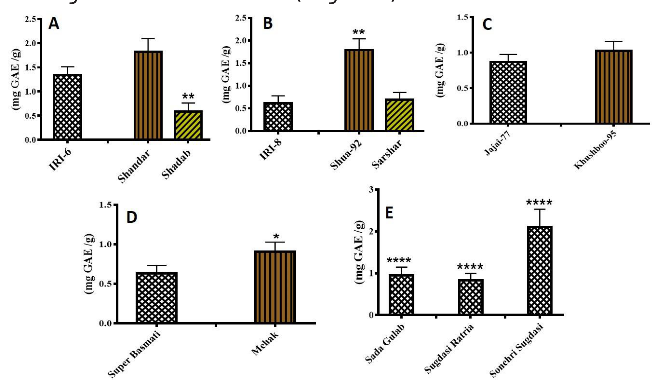

Figure 1: Comparison of Total Phenol Content in Local and Mutant Rice Varieties
****=p<0.001, ***=p<0.005, **=p<0.01. *=p<0.05

Determination of total flavonoid content revealed that Shua-92 and Mehak had higher total flavonoid content as compared to their local varieties, whereas Khushboo-95 and Shadab differed significantly from their parental varieties. All rice varieties taken as reference showed highly significant flavonoid content (Figure 2).

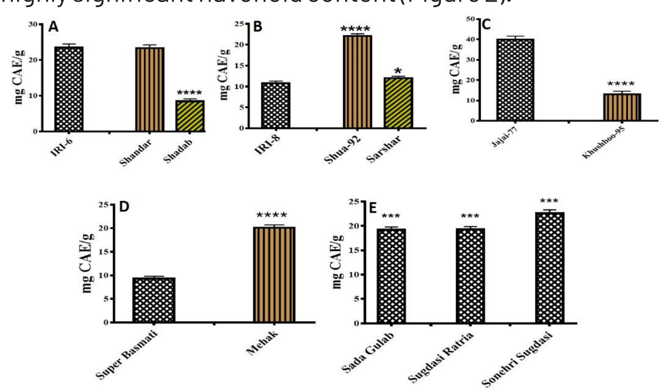

Figure 2: Comparison of Total Flavonoid Content in Local and Mutant Rice Varieties
****=p<0.001, ***=p<0.005, **=p<0.01. *=p<0.05

Results showed high DPPH in Sugdasi Ratria and Sonehri Sugdasi in comparison to Sada Gulab but three varieties were found significant. Mehak variety showed significantly high DPPH radical scavenging activity as compared to the parent variety. Moreover, OH scavenging results revealed that local line varieties Sugdasi Ratria were significantly higher than the Sada Gulab and Sonehri Sugdasi. Among mutant cultivars, shadab and khusboo-95 showed significantly high OH scavenging as compared to their local varieties (Table 1).

Table 1: DPPH Radical Scavenging Activity in Local and Mutant Rice Varieties

Sr. No	Rice Varieties	DPPH Radical Scavenging	p-value	OH Scavenging	p-value
1	IR-6	79.06 ± 2.83		38.47 ± 1.71	
2	Shandar	79.78 ± 2.71	<0.005	38.04 ± 1.61	<0.005
3	Shadab	50.57 ± 2.63		51.62 ± 1.92	
4	IR-8	54.57 ± 2.68		38.91 ± 1.76	
5	Shua 92	79.02 ± 2.96	<0.005	35.84 ± 1.56	<0.005
6	Sarshar	52.88 ± 2.79		38.41 ± 1.64	
7	Jajai-77	77.99 ± 2.67	>0.05	33.04 ± 2.54	<0.01
8	Khushboo-95	78.73 ± 2.57		55.98 ± 3.94	
9	Super Basmati	62.09 ± 2.46	<0.001	49.12 ± 2.38	>0.05
10	Mehak	82.33 ± 2.82		55.97 ± 3.59	
11	Sada Gulab	68.17 ± 2.43		49.25 ± 1.76	
12	Sugdasi Ratria	77.78 ± 3.65	<0.01	52.46 ± 1.85	<0.01
13	Sonehri Sugdasi	79.89 ± 3.52		44.39 ± 1.64	

Comparison of FRAP among rice varieties revealed that Mehak and Shua-92 had significantly higher FRAP activity, respectively, as compared to their local counterparts. Sugdasi Ratria showed increased FRAP values as compared to the other two local rice varieties, Sonehri Sugdasi and Sada Gulab. While comparing the Fe2+ chelating activity, the mutant cultivars. Local varieties like Sada Gulab, Khushboo-95 and Sarshar had Fe2+ chelating activity values (Table 2).

Table 2: FRAP and Fe (II) Chelating Activity in Local and Mutant Rice Varieties

Sr. No	Rice Varieties	FRAP	p-value	Fe (II) Chelating Activity	p-value
1	IR-6	15.77 ± 0.51		78.11 ± 0.95	
2	Shandar	17.79 ± 0.62	>0.05	79.92 ± 1.77	>0.05
3	Shadab	17.51 ± 0.53		81.14 ± 2.95	
4	IR-8	11.96 ± 0.97		71.89 ± 1.71	
5	Shua 92	17.78 ± 1.22	<0.01	74.09 ± 1.76	>0.05
6	Sarshar	12.59 ± 0.99		83.46 ± 2.89	
7	Jajai-77	16.41 ± 1.21	>0.05	70.63 ± 1.99	<0.01
8	Khushboo-95	17.78 ± 1.81		81.34 ± 2.35	
9	Super Basmati	14.49 ± 1.23	<0.005	79.18 ± 1.99	<0.01
10	Mehak	26.32 ± 1.34		69.38 ± 1.71	
11	Sada Gulab	16.41 ± 0.91		80.78 ± 1.98	
12	Sugdasi Ratria	28.41 ± 1.51	<0.001	72.58 ± 1.78	<0.01
13	Sonehri Sugdasi	18.62 ± 1.12		71.93 ± 1.97	

DISCUSSION

Rice polyphenols have received prominent attention in combating cellular oxidative damage due to their ability to scavenge free radicals and quench singlet oxygen [8]. In our study, mutant varieties showed a higher TPC (total phenolic content) than the local varieties. High Phenolic

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content is vital in oxidative stress regulation for the prevention of cancer, nervous, cardiovascular and other acute and chronic disorders, as evident from Various epidemiological research [10]. Shua-92 and Mehak are mutants that contain a lot of flavonoids. This varied flavonoid content in rice cultivars may be due to the genetic diversity between the different kinds of rice [12, 13]. Local varieties such as IRI-6SonehriSugdasi and Jajai-77 exhibited an increased flavonoid content; therefore, these varieties can be used for the treatment of coronary heart disease, gastrointestinal ulcers, cancer and rheumatic illnesses as flavonoids act together with the molecules involved in the cell growth signalling pathways and apoptosis [14, 15]. DPPH is a stable free radical, and its degree of radical scavenging is frequently associated with a sample's better antioxidant activity [16, 17]. Following the exclusion of hydrogen from antioxidants in the DPPH assay, DPPH's purple colour is lessened to a pale yellow colour. In our study, rice varieties were found to be different in terms of secondary metabolite composition and DPPH free radical scavenging capability. Differences in the amount of important chemicals in crops may be the result of genetic variation and changes in the environment [18]. The ferric reducing antioxidant power assay has been proposed to evaluate the capability of various dietary antioxidants to scavenge active free radicals. In our study, Mehak and Shua-92 had higher FRAP activity, possibly due to their higher flavonoid content, which helped them boost ferric ion reducing power [19]. A Fe2+ chelating agent caused a reduction in the red hue that appeared in the ferrous ion-chelating test. Hence, the ability of the chelator to bind metal ions could be seen by the reduction of colour. Chelating activity varied significantly among the species due to the difference in their plant secondary metabolite composition. Mutant cultivars such as Khushboo-95, Mehak and Shadab showed significant OH-scavenging activity. The free radicals, such as hydroxyl radicals, hydrogen peroxide and oxygen species, can cause DNA damage. In the OH scavenging assay, the Fenton reaction was observed releasing the hydroxyl radicals by cleavage of H2O2(due to electron transfer from ferrous ions), and these free OH radicals are reported to cause oxidative DNA damage [20].

CONCLUSIONS

It was concluded that mutant varieties such as Shua-92, Mehak and Shandar may be chosen as a high phenolic and flavonoid diet. Among local varieties, SonehriSugdasi, Jajai-77 and IRI-6 were found best in terms of phenolics and flavonoids content. Additionally, Khusboo-95 and Shadab displayed the highest chelating and OH scavenging power, respectively. Whereas, Shua 92 and Mehak were found best in terms of DPPH scavenging and FRAP. The current research may prove a useful tool for producing new rice varieties with substantial secondary metabolite composition for boosting their nutritive qualities.

Authors Contribution

Conceptualisation: ITA
Methodology: K, MAS
Formal analysis: BK, ZAA
Writing review and editing: ITA, BK, FNM

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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