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Full Length Article

Effect of Extraction Solvents on Phenolics and Antioxidant Activity of Selected Varieties of Pakistani Rice (*Oryza sativa*)

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ABSTRACT

This paper reports the effect of different extraction solvents (aqueous & pure methanol, aqueous & pure ethanol & aqueous & pure isopropanol) on the effective recovery of phenolics and antioxidant activity of 10 varieties of rice) using orbital shaker technique. The extraction yields of antioxidant components from the tested varieties of brown (unpolished) rice ranged from 1.56 g/100 g in Basmati 2000 to 3.45 g/100 g in Basmati 370. The contents of total phenolics (TP) were determined to be highest (293.2 mg GAE/kg) in Basmati Pak while lowest (130.2 mg GAE/kg) in Irri 6 rice. The antioxidant activity, following the determinations of 2,2-diphenyl-1-picrylhdrazyl (DPPH) free radical-scavenging capacity, reducing power and ferrous ion-chelating activity, revealed that Basmati Pak, Basmati 2000 and Basmati 515 rice varieties have greater antioxidant potential. Overall, aqueous isopropanol (80:20 isopropanol: water v/v) and aqueous methanol (80:20 methanol: water v/v) were noted to be the most effective solvent systems offering higher extraction yields, TP and antioxidant activity for the tested rice varieties. This study advocates the use of appropriate extraction solvents to recover maximum amount of rice antioxidants. It was concluded that the tested varieties, especially the Basmati cultivars, of Pakistani rice are a potential source of valuable antioxidant components and thus could be explored as ingredients for functional foods and nutraceuticals.

Key Words: Rice cultivars; Aqueous solvents; Phenolics extraction; Colorimetric; DPPH radical scavengers; Reducing power

INTRODUCTION

Oxidative stress related deterioration of cell components such as lipids, protein and DNA may result into chronic disorders such as carcinogenesis, atherosclerosis, aging and inflammation (Miller, 2001). Some synthetic antioxidants are in use to preserve the foods and biological systems from oxidative deterioration, however, their applications are restricted due to safety concerns (Alia *et al.*, 2011). On the other hand, natural antioxidants such as phenolics derived from cereals, vegetables and fruits, have gained much attention due to their health potential. Plant phenolics are valuable secondary metabolites and possess multiple biological and physiological properties such as antioxidant, anti-aging, anti-carcinogenic, anti-inflammatory and anti-atherosclerosis (Han *et al.*, 2007). Phenolics can be categorized in to different classes such as phenolic acids (hydroxybenzoic derivatives & cinnamic acid derivatives), flavonoids (flavonols, flavanol, flavons, isoflavones, anthocynines) and lignins depending upon the number of aromatic rings as well as structural moieties (Butterfield *et al.*, 2002; Han *et al.*, 2007).

Cereals, being rich source of phenolics and other antioxidant components, not only provide valuable nutrients for human diet but also play a vital role in protecting against oxidative stress related diseases (Slavin, 1994). Phenolics and other minor bioactives are mainly distributed in the seed pericarp tissues of cereals and thus contribute to the physiochemical (appearance, taste, odor & oxidative stability) and antioxidant attributes and potential health benefits of such food crops (Naczk *et al.*, 2004). The amount of such components in food crops is also affected due to post harvest factors (storage conditions & processing) as well as due to changes in sample preparation and analysis regimes (Hamauzu & Chachin, 1995; Rupérez *et al.*, 2001; Eitenmiller & Lee, 2004).

Rice is a good source of natural antioxidants including phenolic compounds. Both, phenolic and flavonoids compounds have potential as antioxidants to act as free radical scavengers, reducing agents, and/or metal ion chelators, thus contributing to human health benefits (Dai & Mumper, 2010). The analysis of antioxidative phytochemicals of rice involved their extraction using various solvents systems such as methanol (80% & 100%),

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isopropanol and ethyl acetate (Hu *et al.*, 1996; Chen & Bergman, 2005; Iqbal *et al.*, 2005; Devi & Arumughan, 2007; Chotimarkorn *et al.*, 2008) followed by the qualitative and quantitative measurements.

Although some data have been documented on the proximate composition and phytochemicals of few local rice cultivars but mostly this information did not cover the detailed antioxidant attributes of majority of the commonly grown rice varieties of Pakistan. In the present study, phenolic contents and antioxidant properties of commonly cultivated varieties of Pakistani rice were evaluated through extraction by different solvents with the main objective to recover optimum yields of potent antioxidant components.

MATERIALS AND METHODS

Collection of rice (*Oryza sativa* L.) samples: Samples of 10 selected varieties namely Basmati Super, Basmati 515, Basmati 198, Basmati 385, Basmati 2000, Basmati 370, Basmati Pak, KS 133, KSK 282 and Irri-6 of paddy rice (*Oryza sativa* L.), were procured/collected from Rice Research Centre, Kalashahkako, Lahore, Pakistan. For each variety 5 kg sample was obtained.

Pretreatment of samples: Rice samples were air-dried at ambient temperature to remove the excess of moisture. The dried rice was subjected to husk removal using a Satake Rice Huller (Model THO35A), yielding brown rice. The weight of husk fractions was calculated. A portion of the brown rice (unpolished rice) samples was kept as such for phenolics analysis. The brown rice were ground to fine powder, and then heated at 110^{o}C for 15 min to stop the activity of lipase enzyme (Juliano, 1985). The processed ground material was tightly packed in polyethylene bags and stored at 4^{o}C in a freezer until used for analysis.

Antioxidant Activity

Antioxidants extraction: Extraction of ground rice materials for antioxidant components was made by using orbital shaker employing six different solvent systems namely 100% methanol, 80% methanol (80:20 methanol/water v/v), 100% ethanol, 80% ethanol (80: 20 ethanol/water v/v) and 100% isopropanol and 80% isopropanol (80: 20 isopropanol/water v/v) (Hu *et al.*, 1996; Duvernay *et al.*, 2005).

Briefly, 20 g powdered each of rice materials was extracted separately with 100 mL of solvent such as methanol (100%, 80%), ethanol (100%, 80%) and isopropanol (100%, 80%) for 24 h using an Orbital Shaker (Gallenkamp, UK) at 40^{0}C. The extracts were filtered and the residues re-extracted twice with the same fresh solvents. The three extractions were pooled. The excess of the solvent was evaporated using rotary vacuum evaporator N–N Series, Eyela (Tokyo, Japan). The crude concentrated extracts were flushed with nitrogen, weighed and stored at -4^{0}C until used for further analyses.

Total phenolics content: Total phenolic contents of extracts from the tested varieties of rice were determined according to the method described by Singleton *et al.* (1999) using gallic acid as a standard. A 0.1 mL of the extract solution (1.0 mg/mL) was taken into a test tube and into it added 2 mL of 7.5% Na_2CO_3 and mixed the test tube contents for 3 min on vortex mixer (HeidolphReax Top D-91128 Schwabach, Germany). After adding 1.0 mL of 10% Folin-Ciocalteau reagent, the volume was made up to 10mL with distilled water and the final mixture allowed to stand for 30 min for completion of the reaction. The absorbance of the finally obtained reaction mixture was taken at 760 nm using UV-Visible spectrophotometer. For each sample, three measurements were made and the results were then expressed as "g" gallic acid equivalent (GAE) per kg of the extract, according to the standard calibration curve plotted using series of gallic acid standard solutions.

DPPH radical scavenging assay: The rice extracts were also tested for DPPH radical scavenging activity by the method of Chotimarkorn *et al.* (2008). A portion (0.1 mL) of the extract solution (1.0 mg/mL) was taken in a Pyrex test tube. To this, added 4.0 mL of pure methanol and 1.0 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution in methanol (1.0 mM). The mixture was kept at ambient conditions for at least 30 min prior to measurement of the absorbance. Absorbance was measured at 517 nm using spectrophotometer. Butylated hydroxy toluene (BHT) was used as the reference standard for comparison. All measurements were taken in triplicates.

Reducing power: The reducing power of the extracts was determined according to the modified method of Yen *et al.* (1993). To 2.5 mL of rice extract (1.0 mg/mL), 2.5 mL of 2M sodium phosphate buffer (pH 6.6) and 5.0 mL of 1% potassium ferricyanide were sequentially added and mixed well. The mixture was then incubated at 50^{0}C for 30 min, added 5.0 mL of trichloroacetic acid (10%) and centrifuged at 10,000 *g* for 10 min. A 5.0 mL portion of the upper layer (supernatant) was mixed with 5.0 mL distilled water and then added 1.0 mL ferric chloride (1.0%). The absorbance was recorded at 700 nm using a UV-Vis spectrophotometer. For comparison purposes, butylated hydroxy toluene (BHT) was used as reference standard. All the measurements were made in triplicate and the results averaged.

Metal chelating activity: Ferrous ion-chelating activity of rice extracts was determined according to the modified method of Decker and Welch (1990) and Shimada *et al.* (1992). A 2.0 mL portion of the extract solution (1.0 mg/mL) was taken into a test tube and to this added 1.0 mL of $FeCl_2$ (2 mM). After thorough mixing, 0.2 mL 5 mM ferrozine solution was added and the mixture mixed well again and kept at room temperature for 10 min for uniform conditions. The absorbance of the final reaction mixture was measured at 562 nm indicating the intensity of ferrozine-Fe^{2+} complex formed. All the samples were analyzed in triplicate. The metal chelating activity was calculated with reference to disodium ethylene diamine tetracetate (Na_2EDTA) and the results expressed as EDTA equivalent (EDTA, g/100 g).

Statistical analysis: Three different samples of rice for each variety were assayed. Each sample was analyzed individually in triplicate and data reported as mean (n = 3 x 3) ± SD (n = 3 x 3). ANOVA was used to determine the differences considering a level of significance at less than 5% ($P< 0.05$) by using the statistical software Co-Stat (Stat Soft Inc., Tulsa, Oklahoma, USA).

RESULTS AND DISCUSSION

Solvent extraction technique has got worth of applicability with in many fields of universal importance because of its suitability as a selective process of separation for health and nutrition, foods and some other substances of vital use. The extraction yields from brown rice, revealed that solvents have a significant effect on the recovery of extractable components. The extracts yield from tested varieties of rice ranged from 2.17-2.47 g/100 g in 100% methanol and 2.75-3.33 g/100 g in 80% methanol followed by 1.56-1.81 and 1.97-2.37 g/100 g in 100% and 80% ethanol, respectively (Table I). In isopropanol (100 & 80%) extraction yield varied from 2.66 to 3.22 g/100 g and 2.73 to 3.45 g/100 g of dry mass of brown rice. Overall, 80% isopropanol as a solvent was established to be the most effective solvent towards the extraction of brown rice for extractable components.

Wu *et al.* (1994) extracted wild rice with methanol, ethanol, and ethyl acetate and reported the extraction yields as 1.0-3.9%, which is similar to the extraction yield obtained in the present study. In another study, Devi and Arumughan (2007) reported extract yields from rice in the range of 1.0% to 4.9% in line with our present data.

The results shown in Table II indicated that total phenolic contents of rice of different varieties with methanol (100% & 80%) ranged from 130.2-201.8 mg GAE/kg in Irri-6 and Basmati Super and 166.8-275.0 mg GAE/kg in Basmati 198 and Basmati Pak using ethanol (100% & 80%). TPC were noted to be minimum in Basmati 198 (130.5-147.9 mg GAE/kg) and maximum in Basmati Pak (196.3-(224.8 mg GAE/kg). The maximum TPC extracted in 100% isopropanol ranged from 203.8-293.5 mg GAE/kg in irri-6 and Basmati Pak (Table II). Hodzic *et al.* (2009) reported the amounts of total phenolic compounds ranging from 295-2035 mg GAE/kg at 20ºC and 429-3065 mg GAE/kg at 40ºC in different extracts from rye, oats, barley, corn, wheat, and rice. Literature revealed that the contents of total phenolics extracted with different solvents in brown rice are higher than those in white rice **(**Hodzic *et al.*, 2009).

The scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) radical is commonly employed for the assessment of antioxidant activity of plant materials. DPPH$^{\bullet}$ radical scavenging activity (IC_{50} value) of extracts obtained from different rice by using six solvent systems, ranged from 6.26 mg/mL (Basmati 515) to 2.22 mg/mL (Basmati 2000) (Table III). Of the different solvents extracts tested, 80% methanol and 80% isopropanol extracts offered the best results for DPPH radical scavenging activity (IC_{50} value 2.22 mg/mL) while 100% ethanol the poorest (6.26 mg/mL). In the present study, the observed order of DPPH radical scavenging activity (IC_{50} value) among rice varieties in relation to extraction solvent was as follow: isopropanol (80%) >methanol (80%) > isopropanol (100%) > methanol (100%) > ethanol (80%) > ethanol (100). The superior DPPH radical scavenging activity of 80% isopropanol and 80% methanol extracts may be linked to their efficacy for extractable antioxidant components (Shon *et al*., 2004).

Over all, the present results indicated that aqueous methanol and isopropanol extracts exhibited better free radical scavenging activity (IC_{50} value). Attributable to polar nature of natural antioxidants, the antioxidant activity generally increases in those extracts, which are prepared in polar solvents. It is widely accepted that with increasing the concentration of phenolic compounds or the degree of hydroxylation of phenolic compounds, DPPH radical scavenging activity also increases, thus correlating directly to the antioxidant efficacy of a typical plant material (Roginsky & Lissi, 2005). As DPPH radicals are very sensitive to the presence of hydrogen donors, the whole system operates at very low concentration; hence, a large number of samples can be tested in a short time (Cheng *et al*., 2006).

In the present study, the antioxidant potential of the selected varieties of rice was also evaluated using reducing power assay (Yen *et al*., 2000). The results of reducing power of different rice extracts showing the effects of six extraction solvents: methanol (80%, 100%), ethanol (80%, 100%) and isopropanol (80%, 100%) are shown in Fig. 1. The reducing powers of the rice extracts (1.0 mg/mL) tested ranged from 0.22 (Basmati 370) to 0.95 (KSK 133). The results revealed that 80% isopropanol and 100% isopropanol extracts had the highest values of reducing powers (0.95) while 100% ethanol extract the lowest (0.22).

Generally, 80% isopropanol extracts (1.0 mg/mL), were found to be most potent reductants followed by 100% isopropanol > 80% methanol > 100% methanol > 80% ethanol > 100% ethanol (Fig. 1). Order of reducing powers with regard to the varieties tested was Basmati 370 > KSK 133 > KS 282 > Irri-6 > Basmati 515 > Basmati 198 >Basmati 385 > Basmati 2000 > Basmati Pak > Basmati Super. Nam *et al.* (2006) investigated rice cultivars for reducing powers and reported that pigmented rice have greater potential than that of white rice. It has been examined by Nam *et al.* (2006) and Rao *et al.* (2010) that reducing power of rice extracts increased in a concentration dependent manner. Iqbal *et al.* (2005) measured the reducing potential of methanolic extracts of different rice bran and established an increasing trend for reducing powers with increasing the concentration.

Iron chelating activity can also be reasonably correlated with antioxidant properties as iron complexion could provide defense against oxidative harm (Yoshimura *et al.*, 1997). In the present work, the metal chelating activity

Table I: Extract Yield (g/100 g) of Antioxidant Components from Various Varieties of Rice (*Oryza sativa* L.) Using different Solvents

Varieties	Methanol		Ethanol		Isopropanol	
	100%	80%	100%	80%	100%	80%
Basmati Super	$2.32 \pm 0.10_{b}^{bcd}$	$2.86 \pm 0.10_{a}^{fg}$	$1.66 \pm 0.13_{d}^{bcd}$	$2.10 \pm 0.10_{c}^{bcd}$	$2.81 \pm 0.19_{a}^{cde}$	$2.88 \pm 0.17_{a}^{cd}$
Basmati 515	$2.17 \pm 0.15_{b}^{d}$	$3.02 \pm 0.10_{a}^{def}$	$1.57 \pm 0.10_{d}^{cd}$	$1.97 \pm 0.10_{c}^{d}$	$2.96 \pm 0.10_{ab}^{bc}$	$3.04 \pm 0.11_{a}^{bc}$
Basmati 198	$2.46 \pm 0.10_{b}^{ab}$	$2.75 \pm 0.20_{ab}^{g}$	$1.81 \pm 0.18_{d}^{ab}$	$2.24 \pm 0.15_{b}^{ab}$	$3.00 \pm 0.15_{a}^{b}$	$3.08 \pm 0.10_{a}^{b}$
Basmati 385	$2.39 \pm 0.20_{a}^{bc}$	$3.33 \pm 0.10_{a}^{ab}$	$1.73 \pm 0.14_{d}^{abc}$	$2.17 \pm 0.15_{b}^{bc}$	$3.21 \pm 0.11_{a}^{a}$	$3.29 \pm 0.20_{a}^{a}$
Basmati 2000	$2.47 \pm 0.14_{ab}^{ab}$	$3.15 \pm 0.10_{a}^{a}$	$1.56 \pm 0.15_{d}^{d}$	$2.25 \pm 0.13_{b}^{ab}$	$2.66 \pm 0.10_{ab}^{e}$	$2.73 \pm 0.10_{ab}^{d}$
Irri – 6	$2.40 \pm 0.12_{ab}^{bc}$	$3.21 \pm 0.2_{a}^{bc}$	$1.80 \pm 0.10_{d}^{ab}$	$2.18 \pm 0.19_{c}^{bc}$	$2.66 \pm 0.15_{bc}^{de}$	$2.73 \pm 0.21_{b}^{d}$
KSK 133	$2.18 \pm 0.10_{bc}^{d}$	$3.16 \pm 0.2_{a}^{bcd}$	$1.65 \pm 0.10_{d}^{bcd}$	$1.98 \pm 0.20_{cd}^{d}$	$2.80 \pm 0.20_{ab}^{cde}$	$2.88 \pm 0.16_{ab}^{cd}$
KS 282	$2.26 \pm 0.18_{b}^{cd}$	$3.14 \pm 0.10_{a}^{cde}$	$1.73 \pm 0.20_{d}^{abc}$	$2.05 \pm 0.10_{bc}^{cd}$	$2.83 \pm 0.10_{ab}^{cd}$	$2.90 \pm 0.10_{ab}^{cd}$
Basmati 370	$2.61 \pm 0.20_{b}^{a}$	$3.25 \pm 0.13_{a}^{bc}$	$1.87 \pm 0.10_{c}^{a}$	$2.37 \pm 0.10_{bc}^{a}$	$3.22 \pm 0.15_{a}^{a}$	$3.45 \pm 0.20_{a}^{a}$
Basmati Pak	$2.47 \pm 0.15_{b}^{ab}$	$2.98 \pm 0.10_{ab}^{de}$	$1.81 \pm 0.10_{c}^{ab}$	$2.24 \pm 0.15_{bc}^{ab}$	$2.95 \pm 0.10_{ab}^{bc}$	$3.03 \pm 0.10_{a}^{bc}$

Table II: Total Phenolic Contents (mg GAE /kg) of Various Varieties of Rice (*Oryza sativa* L.) Extracted Using Different Solvents

Varieties	Methanol		Ethanol		Isopropanol	
	100%	80%	100%	80%	100%	80%
Basmati Super	$201.8 \pm 8.3_{ab}^{a}$	$204.6 \pm 8.4_{ab}^{cd}$	$143.5 \pm 5.5_{c}^{de}$	$152.5 \pm 4.3_{c}^{f}$	$221.0 \pm 11.9_{a}^{ef}$	$222.2 \pm 10.8_{a}^{ef}$
Basmati 515	$163.2 \pm 7.6_{bc}^{de}$	$197.7 \pm 7.8_{b}^{de}$	$133.4 \pm 4.6_{c}^{def}$	$149.2 \pm 3.8_{bc}^{f}$	$278.3 \pm 12.7_{a}^{ab}$	$279.9 \pm 11.1_{a}^{ab}$
Basmati 198	$158.3 \pm 6.9_{bc}^{e}$	$166.8 \pm 6.7_{b}^{g}$	$130.5 \pm 4.7_{c}^{f}$	$147.9 \pm 4.8_{bc}^{f}$	$236.3 \pm 10.8_{a}^{de}$	$237.5 \pm 9.9_{a}^{de}$
Basmati 385	$183.8 \pm 8.2_{ab}^{bc}$	$180.5 \pm 7.6_{ab}^{fg}$	$145.3 \pm 3.6_{c}^{d}$	$149.1 \pm 5.1_{c}^{f}$	$252.5 \pm 12.1a^{cd}$	$253.7 \pm 11.0_{a}^{cd}$
Basmati 2000	$166.6 \pm 6.6_{b}^{de}$	$213.2 \pm 9.8_{a}^{c}$	$135.2 \pm 5.7_{bc}^{ef}$	$162.1 \pm 4.5_{b}^{e}$	$208.9 \pm 10.4_{a}^{f}$	$210.1 \pm 8.8_{a}^{f}$
Irri 6	$130.2 \pm 5.2_{c}^{f}$	$189.9 \pm 8.9_{ab}^{ef}$	$172.1 \pm 5.4_{b}^{c}$	$192.4 \pm 5.1_{ab}^{c}$	$203.8 \pm 9.9_{ab}^{f}$	$225.4 \pm 9.7_{a}^{ef}$
KSK 133	$180.7 \pm 7.2_{b}^{bc}$	$185.7 \pm 6.4_{b}^{ef}$	$184.9 \pm 4.6_{b}^{b}$	$179.0 \pm 4.3_{b}^{d}$	$263.8 \pm 13.6_{a}^{bc}$	$265.0 \pm 11.3_{a}^{bc}$
KS 282	$185.9 \pm 8.0_{b}^{b}$	$258.7 \pm 10.5_{a}^{b}$	$188.1 \pm 5.8_{b}^{b}$	$203.2 \pm 5.9_{ab}^{b}$	$222.6 \pm 12.0_{a}^{ef}$	$223.8 \pm 10.4_{a}^{ef}$
Basmati 370	$172.1 \pm 7.9_{c}^{cd}$	$218.3 \pm 9.8_{bc}^{c}$	$186.2 \pm 4.7_{c}^{b}$	$218.6 \pm 6.3_{b}^{a}$	$281.6 \pm 12.1_{a}^{a}$	$284.8 \pm 11.6_{a}^{a}$
Basmati Pak	$199.6 \pm 8.4_{b}^{a}$	$275.0 \pm 7.4_{a}^{a}$	$196.3 \pm 5.3_{b}^{a}$	$224.8 \pm 7.3_{ab}^{a}$	$293.5 \pm 13.0_{a}^{ab}$	$293.2 \pm 10.5_{a}^{a}$

Table III: DPPH Radical Scavenging Activity (IC_{50} mg/mL) of Extracts of Various Varieties of Rice (*Oryza sativa* L.)Produced by Different Solvents

Varieties	Methanol		Ethanol		Isopropanol	
	100%	80%	100%	80%	100%	80%
Basmati Super	$4.69 \pm 0.22_{b}^{ab}$	$3.77 \pm 0.11c^{b}$	$5.39 \pm 0.25_{a}^{bcd}$	$4.79 \pm 0.13_{b}^{b}$	$3.89 \pm 0.13_{c}^{b}$	$3.48 \pm 0.16_{c}^{b}$
Basmati 515	$4.49 \pm 0.20_{b}^{bc}$	3.04 ± 0.13^{d}	$6.26 \pm 0.23_{a}^{a}$	$4.47 \pm 0.16_{b}^{c}$	$3.75 \pm 0.10_{c}^{b}$	$3.42 \pm 0.15_{c}^{b}$
Basmati 198	$4.76 \pm 0.18_{b}^{ab}$	$4.63 \pm 0.15_{b}^{a}$	$5.09 \pm 0.21_{a}^{d}$	$5.41 \pm 0.21_{b}^{a}$	$4.37 \pm 0.22_{c}^{a}$	$3.88 \pm 0.16_{c}^{a}$
Basmati 385	$4.51 \pm 0.15_{b}^{bc}$	$3.27 \pm 0.13_{c}^{c}$	$5.20 \pm 0.19_{a}^{cd}$	$4.63 \pm 0.11_{b}^{bc}$	$3.31 \pm 0.19_{c}^{c}$	$3.01 \pm 0.13_{c}^{c}$
Basmati 2000	$4.99 \pm 0.16_{b}^{a}$	$3.01 \pm 0.12_{c}^{d}$	$6.19 \pm 0.20_{a}^{a}$	$4.58 \pm 0.10_{b}^{bc}$	$2.44 \pm 0.07_{c}^{e}$	$2.22 \pm 0.11_{d}^{e}$
Irri 6	$4.16 \pm 0.17_{b}^{d}$	$2.71 \pm 0.09_{c}^{e}$	$5.60 \pm 0.24_{a}^{b}$	$4.64 \pm 0.21_{b}^{bc}$	$3.23 \pm 0.08_{c}^{c}$	$2.71 \pm 0.12_{d}^{d}$
KSK 133	$4.22 \pm 0.23_{b}^{cd}$	$2.64 \pm 0.10_{c}^{ef}$	$5.56 \pm 0.18_{a}^{b}$	$4.55 \pm 0.18_{b}^{bc}$	$2.94 \pm 0.08_{c}^{d}$	$2.67 \pm 0.14_{d}^{d}$
KS 282	$4.15 \pm 0.18_{b}^{d}$	$2.69 \pm 0.09_{c}^{e}$	$5.47 \pm 0.15_{a}^{bc}$	$4.49 \pm 0.17_{b}^{c}$	$2.55 \pm 0.06_{c}^{e}$	$2.32 \pm 0.11_{d}^{e}$
Basmati 370	$3.59 \pm 0.12_{b}^{e}$	$2.48 \pm 0.08_{c}^{fg}$	$5.45 \pm 0.24_{a}^{bc}$	$5.17 \pm 0.14_{b}^{a}$	$2.99 \pm 0.10_{c}^{d}$	$2.72 \pm 0.10_{d}^{d}$
Basmati Pak	$4.51 \pm 0.13_{b}^{bc}$	$2.34 \pm 0.10_{c}^{h}$	$5.13 \pm 0.16_{a}^{cd}$	$5.15 \pm 0.20_{b}^{a}$	$2.96 \pm 0.11_{c}^{d}$	$2.69 \pm 0.10_{d}^{d}$

Values are mean ± SD for three samples of each variety, analyzed individually in triplicate (n = 3x3)
Means with different superscript letters with in the same column indicate significant differences ($p < 0.05$) among varieties tested
Means with different subscript letters with in the same row indicate significant differences ($p < 0.05$) among solvents used
IC_{50} Value of BHT = 0.03 mg/mL

of selected varieties of rice was determined against Fe^{2+} and reported as EDTA equivalents. Mostly, a considerable ($p<0.05$) variation was observed for metal chelating activity among the tested varieties. Metal chelating activities (EDTA Eq.) of rice extracts (1.0 mg/mL), obtained using different solvents are shown in Fig. 2. As expected, the highest results of metal chelating activity were observed for 80% isopropanol and 100% isopropanol extracts and the lowest for 100% ethanol extracts. The order of metal chelating activity of rice extracts with regard to the solvent was found to be 80% methanol extract > 80% isopropanol extract > 100% isopropanol extract> 100% methanol extract > 80% ethanol extract > 100% ethanol extract; while on varieties basis Basmati Pak has stronger antioxidant potential than Basmati 515 followed by KSK 133, Basmati 385, Basmati 370, Basmati 198, Basmati 2000 Basmati Super and KS 282 (Fig. 2).

CONCLUSION

Overall, it has been observed that aqueous organic mixtures of solvents, especially, isopropanol and methanol are more efficient towards higher recovery of rice antioxidant components including the phenolics, free radical scavengers and metal chelators. The present data reveal that Pakistani rice varieties are not only famous for their unique

Fig. 1: Reducing Power of Extracts of Various Varieties of Rice (*Oryza sativa* L.) Produced by Different Solvents
M. Methanol, E. Ethanol, Iso. Isopropanol
Bas Basmati

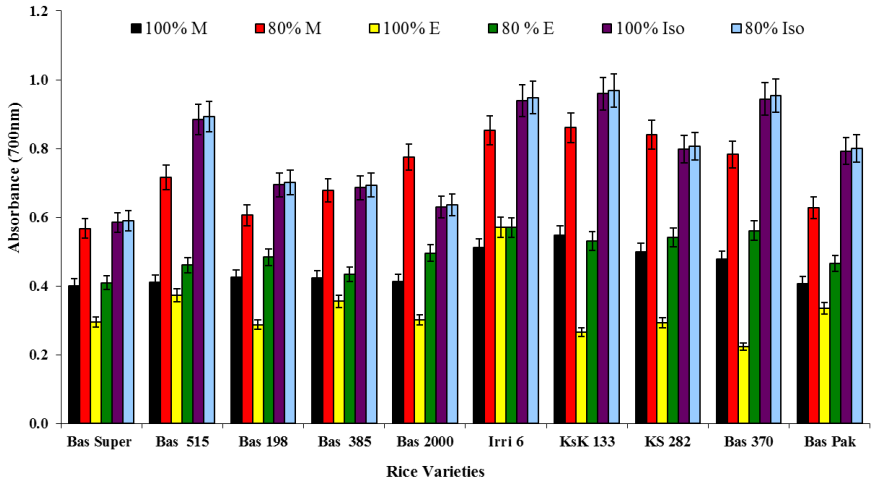
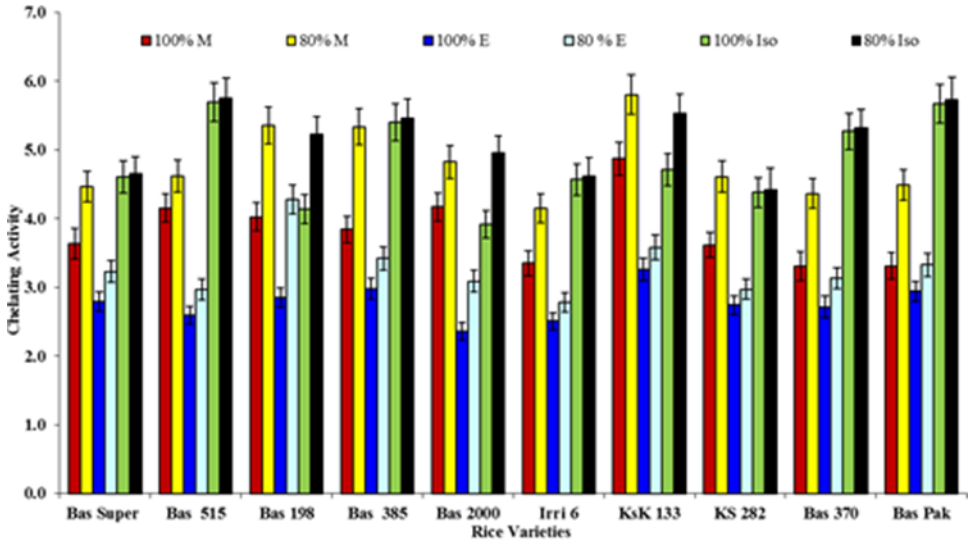


Fig. 2: Metal Chelating Activity (EDTA Eq. g/100 g) of Extracts ofVarious Varieties of Rice (*Oryza sativa* L.) Produced by Different Solvents
M. Methanol, E. Ethanol, Iso. Isopropanol
Bas Basmati



aroma but also possess considerable amount of total phenolics and good antioxidant activity. These results could create new opportunities for rice growers and eventually commercial rice producers, to nourish the production of rice enriched with enhanced levels of natural antioxidants and phenolics. Local rice varieties, in particular, the Basmati cultivars, rich in potent phenolics and antioxidants, may be explored for functional food applications leading to value addition.

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