Int J Pharm Bio Sci 2013 July; 4(3): (B) 659 - 667

Research Article **BioChemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

EVALUATION OF *IN VITRO* ANTIOXIDANT PROFILE OF SELECTED CEREALS

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ABSTRACT

The antioxidant activities, total phenolic content (TPC) and total flavonoid content (TFC) of seven selected cereals including Wheat (*Triticum aestivum* var WH-147), Rice (*Oryza sativa* var Ganga Kaveri), Basmati rice (*Oryza sativa* var Pusa-1121), Maize (*Zea mays* var K-65), Pearl millet (*Pennisetum americanum* var Banjara Gold), Barley (*Hordeum vulgare* var Ratna) and Sorghum (*Sorghum bicolor* var MFSH-4) were determined. The TPC was determined according to the folin-ciocalteu method and TFC by spectrophotometric method. TPC and TFC varied from 53.5 to 211.2 mg/100g and 0.28 to 0.65 mg QE/g respectively. Antioxidant activity of methanolic extract of cereals was evaluated according to the DPPH radical scavenging activity. Antioxidant activity, reducing capacity and Ferric reducing antioxidant potential (FRAP) ranged from 63.9 to 73 %, 18.6 to 62 AAE/g and 1.70 to 3.70 mmoL of Fe equi/g of flour respectively.

KEYWORDS: **Polyphenol; Flavonoid; Antioxidant activities; Ferric reducing antioxidant potential**

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INTRODUCTION

Accumulating evidence on the human health benefits provided by cereal based food consumption has led to an increased focus on the phytochemical content of different grains and grain varieties. Nutritional benefits are particularly enhanced when cereals and pseudocereals are used in food preparation as whole grains and or whole flours. These cereals are major source of protein, carbohydrate and calorie in the diets of large segment of population[1]. Natural polyphenols exert their beneficial health effects by their antioxidant activity[2]. These compounds are also capable to remove free radicals, chelate metal catalysts, activate antioxidative enzymes, reduce alpha tocopherol radicals and inhibit oxidases[3, 4]. The natural antioxidants in cereals may function as reducing agents, free radical scavengers, singlet oxygen quencher and potential complexer of prooxidants[5]. The reducing power is also an indicator of antioxidant activity. Several studies involving whole grain intakes have shown a consistent protective role of whole grain consumption in reduced risk of cancer, coronary heart disease and diabetes[6, 7, 8]. Flavonoids have generated interest because of their broad human health promoting effects, most of which are related to their antioxidant properties[9] and to synergistic effects with other antioxidants[10]. The antioxidant mechanism of flavonoids, may also result from the interactions between flavonoids and metal ions especially iron and copper[11]. Phytic acid (Myoinositol hexa phosphate) is a major phosphorus storage form, which is present in plants and its salt is known as phytate. Apart from its antinutrient activity, phytic acid also exhibit antioxidant activity, because it chelates metal ions such as iron and copper and in this way it prevents various oxidative processes[12]. Phytic acid also regulates various cellular functions such as DNA repair, chromatin remodeling, endocytosis, nuclear messenger for RNA export and hormone signaling process[13]. Therefore, the aim of this study was to determine the antioxidant activities of different cereals.

MATERIALS AND METHODS

Mateirals

Seven different types of cereals were procured from Krishi Sewa Kendra, Allahabad. These varieties includes Wheat (*Triticum aestivum* var WH-147), Rice (*Oryza sativa* var Ganga Kaveri), Basmati rice (*Oryza sativa* var Pusa-1121), Maize (*Zea mays* var K-65), Pearl millet (*Pennisetum americanum* var Banjara Gold), Barley (*Hordeum vulgare* var Ratna) and Sorghum (*Sorghum bicolor* var MFSH-4). The samples were cleaned by hand to remove dirt, grit and broken grains and then packed in air tight plastic containers. Gallic acid, folin-ciocalteu phenol reagent, quercetin and ascorbic acid were procured from Merck, India. DPPH (2, 2- diphenyl-1-picryl hydrazyl) was purchased from HIMEDIA, India.

Analytical methods

Total polyphenolic (TPC) content

The total polyphenolic content of the aqueous methanolic extract of raw cereal samples was determined according to the 'Folin ciocalteu method'[14]**.** One ml- aliquot of the sample extract was taken in a test tube. There after 5 ml. of diluted folin ciocalteu reagent (FCR) (1:10 with distilled water) and 4 ml sodium carbonate solution (7.5 %, w/v) were added sequentially to each tube. Soon after mixing, the test tubes were placed in the dark for 60 minutes at room temperature and the absorbance was monitored by UV-VIS spectrophotometer (model-Evolution600) at 765 nm against blank as standard. A standard curve was prepared with "Gallic acid" and results were expressed in terms of mg/100g of polyphenol present in the sample. Samples were analyzed in triplicates and mean was calculated.

Total flavonoids content (TFC)

The total flavonoids content was determined as previously described by Boetang et al[15]. Flour of selected grains (5.0 g) was extracted with 50 ml chilled aqueous ethanol (80:20, v/v) at room

temperature for 2 hrs. Diluted extract (2.0 ml) was added to 150 µl of sodium nitrite (5 %, w/v), the mixture was allowed to stand for 5 min. Further, 150 µl of 10 % aluminium chloride was added and the mixture was allowed to stand for 10 min. After that, 1.0 ml of 1M sodium hydroxide and 1.2 ml of distilled water was added and the solution was mixed well. The absorbance was measured immediately at 510 nm using a spectrophotometer. Quercetin was used for the standard curve construction (0.05 to 0.5 mg/ml). The results were expressed as mg Quercetin Equivalent (QE)/g of flour.

Phytic acid content

Phytic acid content in selected cereals varieties was determined according to the method of Wheeler and Ferrel[16]. The phytic acid was extracted with trichloroacetic acid and 3.2 N nitric acid. The iron content of the precipitate was determined colorimetrically and the phytate phosphorous content calculated from this value assuming a constant 4 Fe: 6 P molecular ratios in the precipitate. Ferric nitrate was used as standard. The phytic acid content was expressed as 'Phytate-P mg/100g'of sample.

DPPH radical scavenging activity

The DPPH (2,2-diphenyl 1-pycril hydrazil) radical scavenging activity of selected cereals extracts was measured according to the method given by Sanja et al[17] with slight modification. Accordingly, as per method 150 µl of DPPH solution (4.3 mg in 3.3ml. of methanol) was mixed with 3 ml acidified methanolic extract of selected cereals varieties. The mixture was shaken and decrease in absorbance was measured at 515 nm with the help of UV/VIS spectrophotometer after 15 minutes incubation at room temperature. DPPH solution was used as control. The percent (%) antiradical activity was calculated by following
formula:- % Antiradical activity = (Control absorbance - sample absorbance / Control absorbance) ×100

Reducing capacity (RC)

The reducing power was measured as described by Sharma and Gujral[18] with slight modification. Cereal flour (1.0 g) was extracted with 80 % methanol (3.0 ml) for 2 h. The extract was mixed with phosphate buffer (2.5 ml, 0.2 mol/L, pH 6.6) and 2.5 ml potassium ferricyanide (1.0 %) was added followed by incubation at 50° C. Then 2.5 ml trichloroacetic acid solution (10%) was added to mixture, which was then centrifuged at 10,000 *g* for 10 min. The upper layer of solution (2.5 ml) was mixed with 2.5 ml deionized water and 1.0 ml ferric chloride (0.1%). The absorbance of the mixture was measured at 700 nm. The results were reported as µmol ascorbic acid equivalent (AAE)/g of flour using standard curve of ascorbic acid.

Determination of ferric reducing antioxidant power (FRAP)

The FRAP was performed according to methods described by Sutharut and Sudarat[19]. The 200 µL of methanolic extract of each sample was mixed with 1.3 mL of the FRAP reagent. FRAP reagent consisted of 0.3 M acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3$ in a ratio of 10:1:1 (v/v/v). After 30min of incubation at 37°C, absorption was measured at 595 using a spectrophotometer. The absorbance changes in the test mixture were compared to those obtained from standard mixture of ferrous sulphate ($FeSO_4$. $7H_2O$) (0.1 mmol/L – 1.0 mmol/L). FRAP values, expressed as mmol of Fe(II) equivalent per g flour.

Statistical analysis

All tests were conducted in triplicate. Data are reported as mean ± SE. Analysis of variance and significant difference among means were tested by one-way ANOVA using SPSS software (version 12.0 for Windows). The pearson correlation analysis was also performed by SPSS 12.0.

RESULTS

Total Phenolic Content (TPC)

The total phenolic content of the methanolic extracts of cereals was expressed in terms of

mg/100g. The TPC content of selected cereals varied between 53.5 to 211.2 mg/100g (Table 1). Barley showed the highest (211.2 mg/100g) and rice showed lowest TPC content (53.5 mg/100g) among selected cereals.

Table 1

Total polyphenol, total flavonoids and phytic acid content of different cereal samples.

Cereals	TPC(mg/100g)	TFC(mg QE/g)	Phytic acid (mg/100g)
Wheat	148.0± 0.21^{a}	0.34±0.01^{ab}	207.0±2.07^{e}
Rice	53.5± 0.31^{b}	0.28±0.01^{a}	65.0±2.07^{a}
Basmati rice	70.0±0.15^{c}	0.30±0.01^{a}	101.0±1.73^{b}
Maize	127.6±0.11^{d}	0.40±0.01^{b}	228.0±2.07^{f}
Pearl millet	195.3±0.38^{e}	0.55±0.02^{c}	162.3±3.37^{d}
Barley	211.2±0.40^{f}	0.32±0.00^{a}	152.3±1.44^{c}
Sorghum	128.9±0.40^{g}	0.65±0.01^{d}	300.0±1.15^{h}

TPC, total polyphenol content; TFC, total flavonoids content; QE, quercetin equivalent.Values are mean ± SE (n=3). Means within a column with the same superscript letter are not significantly different (p≤0.01).

Total flavonoids content

The total flavonoid content varied significantly among each cereal types (p≤0.01). The TFC ranged from 0.28 (in rice) to 0.65 mg QE/g (in sorghum) (Table.1). The TFC of rice (0.28 mg QE/g), basmati rice (0.30 mg QE/g) and barley (0.32 mg QE/g) were non significantly different (p≤0.01).

Phytic acid content

Phytic acid content varied among different cereals (Table.1). The highest phytic acid content was observed in sorghum (300 mg/100g) followed by maize, wheat, pearl millet, barley, basmati rice and rice.

DPPH radical scavenging activity

The DPPH radical scavenging activity of acidified methanolic cereal extract was determined and results are presented in Table 2. All the studied cereal samples were found to possess higher radical scavenging activity. The decreasing order of DPPH radical scavenging activity is as follows: Sorghum> Rice> Basmati rice> Maize> Pearl millet >Wheat> Barley.

Reducing capacity

The values of reducing capacity were presented in Table 2. The values of reducing capacity for seven selected cereal varieties were ranged from 19.0 (in rice) to 62.0 µmol AAE/g of flour. Pearl millet showed strongest reducing capacity (60 µmol AAE/g), followed by Barley (57 µmol AAE/g) and Maize (47 µmol AAE/g) respectively.

FRAP

The ferric reducing antioxidant power of selected cereal samples varied between 1.70 to 3.70 mmol of Fe (II) equi./ g (Table. 2). Barley showed the highest (3.70 mmol of Fe (II) equi./ g) and basmati rice (1.70 Fe (II) equi./ g) showed lowest ferric reducing antioxidant power among selected cereals. The correlation of TPC, TFC and phytic acid with different parameters of antioxidant activity is presented in table 3.

Table 2
Antioxidant activity of selected cereal samples.

Cereals	DPPH radical scavenging activity (%)	RC (AAE/g of flour)	FRAP mmol of Fe(II) equi./ g
Wheat	64.6±0.38^{a}	18.6±0.87^{a}	2.68±0.19^{b}
Rice	72.4±0.46^{c}	20.0±1.15^{a}	1.73±0.11^{a}
Basmati rice	72.2±0.06^{c}	20.0±0.57^{a}	1.70±0.04^{a}
Maize	69.0±0.57^{b}	47.3±1.44^{b}	2.68±0.14^{b}
Pearl millet	68.1±0.64^{b}	62.0±1.73^{d}	3.16±0.16^{bc}
Barley	63.9±0.46^{a}	57.0±0.57^{c}	3.70±0.26^{c}
Sorghum	73.0±0.59^{c}	46.0±1.15^{b}	3.62±0.15^{c}

AAE,ascorbic acid equivalent. Values are mean ± SE (n=3). Means within a column with the same superscript letter are not significantly different (p≤0.01).

DISCUSSION

This study was designed to determine the complete profile of phytochemicals in grains and their relationship and contribution to the total antioxidant activities. Total phenol content by FCR and *in vitro* antioxidant capacity assays such as the DPPH (which was used in this study); represent convenient methods for the identification of potential sources of antioxidant compounds[20]. Phenolics are considered as a major group of compounds that contribute to the antioxidant activities of grains. Sorghum and Barley are important food grains reported to contain significant quantities of phenolic compounds[21]. In present study the TPC of cereals and millets ranged from 53.5 (rice) to 211.2 (barley) mg/100g. These values are in agreement with literature reports on the phenolic content of plant foods[22, 23]. The values of TPC reported by Yadav et al[24] in Chickpea were also with this range (145mg/100g). Several studies have investigated phenolic composition of different cereals[25, 26] and these studies demonstrated that most of the phenolics in grain were in the bound form, with the free phenolic contributing 18-23 % of the total in the different phenotypes. We found a negative correlation (r = -0.852) (Table. 3) between TPC and antioxidant activity of cereals, these result appeared to suggest that TPC might not contribute significantly to the antioxidant activity[27]. Variable results have been reported on the relation between phenolic content and antioxidant activity of different plant materials[28]. Some authors found a correlation between the TPC and antioxidant activity (DPPH assay) [25, 29]; however, Zielinski and Kozlowska[30] found no correlation between TPC and antioxidant activity of water extracts of buckwheat and rye. The lack of correlation between TPC and antioxidant activity could be explained by the fact that folin-ciocalteu method measures other constituent rather than phenolics. The FCR is reported to detect all phenolic compounds present in extract including those in extractable proteins[31], since other compounds found to be responsible for the antioxidant activity[32]. In our study we found good correlation between TPC and reducing capacity (Table. 3). In reducing power method ferric –ferricyanide complex is reduced to the ferrous form depending on the presence of antioxidant[33]. Choi et al[34] found highly significant correlation between TPC and reducing power of methanolic extract of red sorghum and black rice (r=0.907, p≤0.05).

Table 3
Correlation between total polyphenol content, total flavonoid content and antioxidant activities.

	TPC	TFC	Phytic acid
DPPH radical scavanging activity	-0.768	0.255*	0.004
Reducing capacity	0.786*	0.579*	--
FRAP	0.849*	0.632*	--

**** Significant at 5 % probability level.***

Flavonoids play a very protective role by reducing the risk for cancer and cardiovascular diseases[35]. According to Shoba et al[36] flavonoids act as potential and therapeutic agents to treat polycystic kidney disease. The flavonoid content in millet was found to be better than cereals. There are limited researches available on flavonoid content of cereals. The antioxidant mechanism of flavonoids, may results from the interaction between flavonoids and metal ions especially iron and copper[11]. Significant correlation (r=0.257, p≤0.05) were observed between TF and DPPH radical scavenging activity (Table. 3). This means that most of the flavonoids estimated by the aluminium chloride method have antioxidant activity and are sensitive to the DPPH test[37]. Indeed Chang et al[38] showed that aluminium chloride method is specific only for flavones and flavonols which are known to be present in fruits and to possess antioxidant activities[39]. In our study a linear correlation (r = 0.578, p≤0.05) was found between total flavonoids content and reducing capacity of the methanolic extract of selected cereals. Sharma and Gujral[18] also found a significant (p≤0.05) positive correlation (r= 0.720) between total flavonoid content and reducing power of different varieties of barley. While after roasting this correlation disappeared. This could be attributed to increase in reducing power and decrease in total flavonoid content and or formation of maillard reaction products after roasting of barley, further research should be conducted to find the processing effect on the total flavonoid and antioxidant activities of cereal grains.

The calculated data (Table. 4) showed that the TPC and TFC was best correlated with the FRAP [r = 0.849 (p≤0.05) and r = 0.632 (p≤0.05) respectively]. Therefore, FRAP can be used, and could replace the other methods, such as NO• and CUPRAC[40, 41]. Our result showed parity with Ryan et al[42] who reported significant (p≤0.01) positive correlation (r = 0.710) between the FRAP values and polyphenol contents of the oat based breakfast cereal samples. Antioxidants are often added to foods to prevent oxidation. However, the commonly used synthetic antioxidant such as butylated hydroxyanisole (BHA) and butylated hydroxyl toluene (BHT) are restricted by legislative rules because they are suspected to have some toxic effects and as possible carcinogens[43]. Therefore there has been a considerable interest by the industry and a growing trend in consumer preferences for natural antioxidants over synthetic compounds[44].

CONCLUSION

The results from this study highlight the importance of total phenol content, total flavonoid content and antioxidant capacities of some selected cereals from India, as well as indicating the important need for further investigations into the identification of phenolics and flavonoids and in vivo antioxidant activities in cereals.

ACKNOWLEDGEMENT

We are thankful to Prof G K Rai, Director IPS, University of Allahabad for providing all necessary facilities for this research work. Financial support given in the form of UGC-JRF Scholarship is also duly acknowledged to carry out this study.

Conflict of interest
Conflict of interest declared none.

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