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A Comprehensive Evaluation of Total Phenolics, Flavonoids Content and In-Vitro Antioxidant Capacity of Selected 18 Cereal Crops

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ABSTRACT

The anti-oxidant activity of extracts from 18 cereal crops were evaluated for their phenolic (TPH), flavonoids (TF) and antioxidant activity (AOX). Antioxidant activity mg/100g was evaluated using the in-vitro assay viz ferric reducing antioxidant activity power (FRAP), cupric antioxidant activity (CUPRAC) and 2,2-diphenyl picryl hydrazyl (DPPH) Large significant variation was observed ($p<0.05$) amongst cereal and between their varieties and content ranged from beans displayed significantly ($p<0.05$) higher phenolics and flavonoids content than cereals, millets. Black gram followed by finger millet, pearl millet, kidney bean and black soyabean had the highest TPH ranging from 488.41, 394.4, 254.30 and 229.90 GAE/100g respectively. TF content ranged from 8.84-116.89 mg CE/100G. AOX in FRAP, DPPH and CUPRAC assays ranged from 2.31-8.29, 6.10-15.03 and 0.65-4.68 µmol TE/g. The results indicate that cereals, millet and beans containing high phenolics may provide a source of dietary antioxidants.

Key words: *Phenolics, Flavonoids, antioxidant activity, Cereals, Beans, Millets*

INTRODUCTION

Cereals, millets and beans are sources of phenolic compounds and other bioactive compounds and they also food containing complex carbohydrates with higher levels of dietary fiber which they are health beneficial for human being. Such as they health promoting properties that whole grain consumption helps lower risk of cardiovascular disease, ischemic, stroke, type II diabetes, metabolic syndrome and gastrointestinal cancer due to presence of phenolic compounds. In cereal grains, these compounds are located mainly in the pericarp and they can be concentrated by decorticating the grain to produce bran, which can be incorporated into a food product (i.e., breads, cookies and tortillas) with increase dietary fiber levels and nutraceutical properties. Cereals (millets & sorghum) can grow and give higher and more stable grain yields in the regions that are characterized by low rainfall or drought, high temperature and low soil fertility.

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In other words, they perform well under poor soil and growth conditions. The environment and climate in India are characterized by such conditions, and these cereals are adapted in India. In Africa, India and China, sorghum grain comes third among cereals for human consumption, super seeded only by rice and wheat. Cereals and millets are the most commonly consumed food items in India. They contain a wide range of phenolics which are good sources of natural antioxidants. Studies report that methanolic extracts from red sorghum showed higher antioxidant activity and contain higher polyphenolic levels compared to rice, foxtail millet, pros millet and barley. Bran, a by-product of milling has antioxidant potential due to phenolic acids such as p-coumaric acid and vanillic acids that are concentrated in the bran portion of cereal kernels. Antioxidant activity of five bran extracts exhibited appreciable levels of total phenolics, flavonoids and DPPH radical scavenging activities. Processing, such as soaking and roasting, have been shown to influence total phenolic, flavonoid and antioxidant contents in selected dry beans. Raw kodo millet and finger millet have higher DPPH radical scavenging activities. However, cooking of these millets by roasting or boiling reduced their antioxidant activity.

MATERIALS AND METHODS

Materials

Samples of 18 cereals were taken from local market and grains of sorghum cultivars were obtained from Directorate of sorghum Research, Hyderabad and rest was collected from the IARI experimental fields.

Methods

Grains flour was taken for analysis. Some samples of course cereals, millets and pulse were taken from the local market and rest was collected from the IARI experimental fields. For each sample three replicates were taken. Each replicate comprised of 50gm from which was homogenized in a waring blender and from 2-3 g of aliquot was taken for sample preparation. by the method of Zhishen *et al*[6]., and results were expressed as catechin equivalents/100 ml. Antioxidant activity was measured using three in-vitro assays; namely, ferric-reducing antioxidant power (FRAP) Benzie and Strain[3], cupric-reducing antioxidant capacity (CUPRAC) and 2,2-diphenyl-1-picryl hydrazyl (DPPH)[1] (Apak et al. 2008). Results were expressed as µmol Trolox/g (TE/g).

Determination of phenolic content

Total polyphenolic content was estimated spectrophotometrically Folin ciocealteau reagent (FCR) as described by Singleton *et al*[5]., using gallic acid as a standard. To the 10 gm of the suitably diluted sample extract, 2.9 ml of deionized water and 0.5 ml of Folin ciocealteau reagent and 2.0 ml of 20% Na_2CO_3 solution was added. The mixture was allowed to stand for 90 min and absorption was measured at 760 nm against water as a blank. The amount of total phenolics was expressed as gallic acid equivalent (GAE, mg gallic acid/g flour) through caliberation of Gallic acid. Two parallel determinations of each sample were performed and average values were calculated.

Total flavonoids

Total flavonoids were measured by a colorimetric assay developed by Zhishen *et al*[6]. A 1 ml sample extract was added to a 10 ml volumetric flask containing 4ml of distilled water. A 0.3 ml portion of 5% $NaNO_2$ was added to this mixture and allowed to stand for 5 min at room temperature. A 0.3ml portion of 10% $AlCl_3.6H_2O$ was added and the mixture was allowed to stand for 6 min at room temperature. Two millilitres of 1N NaOH was added and the solution was diluted to the desired volume (10 ml) with distilled water. The absorbance of the solution versus a blank at 510 nm was measured immediately. The results were expressed as catechin equivalents (CE) using a standard curve (absorbance versus concentration) prepared.

Ferric reducing antioxidant power (FRAP)

FRAP assay developed initially to measure ferric reducing ability of blood plasma[3] has now been widely employed in a variety of plant and food samples. The FRAP assay also takes advantage of the electron transfer

reactions, wherein a ferric salt, Fe $(TPTZ)_2$ III, is used as an oxidant under acidic conditions, pH 3.6. FRAP assay was performed according to the procedure described by Benzie and Strain[3] with some modifications. FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known concentration. Aqueous solutions of known ferrous ion concentration in the range 100 – 1000 µl ($FeSO_4.7H_2O$) were employed for calibration. Working reagent was prepared freshly each day by mixing 300 mM acetate buffer, pH 3.6, 10mM TPTZ in 40 mM HCl and 20mM $FeCl_3$ in the ratio 10:1:1 (v:v:v). Briefly 3 ml of the FRAP reagent was mixed with 100 µl of sample extract in a test tube and vortexed. Absorbance readings were recorded after 4 min of sample reagent mixing at a wavelength of 593 nm.

Cupric reducing antioxidant capacity (CUPRAC)

CUPRAC stands for 'cupric reducing antioxidant capacity'. This method recently developed by Apak *et al*[1]., measures the copper (II) or cupric ion reducing ability of polyphenols. This is a simple and widely applicable antioxidant capacity index for dietary polyphenols, vitamins C and E. It makes use of the copper (II)-neocuproine [Cu (II)-Nc] reagent as the chromogenic oxidizing agent.

The method comprises mixing of the antioxidant solution with a copper (II) chloride solution, a neocuproine alcoholic solution, and an ammonium aqueous buffer at pH 7 and subsequent measurement of the developed absorbance at 450 nm after 30 min. Normal sample measurement is as follows:

To a test tube were added 1 mL each of copper (II) chloride solution (10^{-2}M), Neocuproine solution (Nc) of 7.5 x 10^{-3}M, and ammonium acetate (NH_4Ac) buffer (pH 7) solutions. Antioxidant sample (or standard) solution (x mL) and H_20 (1.1- x mL) were added to the initial mixture so as to make the final volume 4.1 mL. The tubes were stoppered and after one hour, the absorbance at 450 nm was recorded against a reagent blank. The standard calibration curve of each antioxidant compound was constructed in this manner as absorbance versus concentration. The molar absorptivity of the CUPRAC method for each antioxidant was found from the slope of the calibration line concerned and the antioxidant activity was expressed as µmol Trolox g-1.

2, 2-Diphenyl-1-picrylhydrazyl radical scavenging activity

The antiradical capacity of the sample extracts was estimated. DPPH is one of a few stable and commercially available organic nitrogen radicals and has UV-Vis absorption maxima at 515 nm. On reduction the colour solution fades and the reaction progress is monitored with a spectrophotometer.

50 grams of sample was thoroughly crushed and homogenized in mortar pestle with 10 ml of 50% methanol. The extract was centrifuged at 10000 *g* for 15 min at 4oC. Following centrifugation the pellet was washed with 20 mL of methanol and the resulting supernatant was combined with the initial extract. Triplicate supernatant extractions were made for each sample. The methanolic extract volume was reduced in the evaporator to 20 mL. An aliquot (100 µL) of methanolic extract of the sample was added to 3.9 mL of DPPH (0.025 g L-1) in methanol. Absorbance of the samples were measured using a spectrophotometer at different time intervals until the reaction reached a plateau (time at steady time) at 515 nm against methanol without DPPH as the blank reference. Antioxidant activity was expressed in terms of EC_{50} value, which is defined as the amount of sample necessary to decrease the initial DPPH concentration by 50%.

RESULTS AND DISCUSSION

Determination of total phenolic, total flavonoids and antioxidant activity

The total phenolic content of juice (80% ethanol extract) was estimated spectrophotometrically using Folin–Ciocalteu reagent as described by Singleton *et al*[5]., and results expressed as gallic acid equivalents (mg Gallic acid equivalents (GAE)/100 ml).

Total flavonoids were estimated by the method of Zhishen *et al*[6]., and results were expressed as catechin equivalents/100 ml. Antioxidant activity was measured using three in-vitro assays; namely, ferric-reducing antioxidant power (FRAP) Benzie and Strain[3], cupric-reducing antioxidant capacity (CUPRAC) and 2,2-diphenyl-1-picryl hydrazyl (DPPH)[1]. Results were expressed as µmol Trolox/g (TE/g).

Table 1: Total phenolic and flavonoids contents in selected 18 cereals

S.No.	Cereals	Scientific name	Family	Part analyzed	Phenolic content (mg GAE/100 g)	Flavonoids content (mg CE/100 g)
1	*Finger millet (Local variety)*	*Eleusine coracana (L.)*	*Poaceae*	Grain (Flour)	394.41±62.50	22.82±0.77
2	*Finger millet (GBU-67)*	-	-	-	199.13±54.71	61.84±12.47
3	*Finger millet (GBU-48)*	-	-	-	265.50±75.42	56.25±14.15
4	*Finger millet (GBU-45)*	-	-	-	231.20±51.64	93.12±28.40
5	*Finger millet (L-5)*	-	-	-	284.10±75.92	116.89±30.75
6	Pearl millet	-	-	-	268.43±31.25	9.60±1.64
7	Sorghum (CSV18VR)	*Sorghum bicolor* (L.)	*Poaceae*	-	189.90±19.81	36.98±15.37
8	Sorghum (CSV14VR)	-	-	-	188.71±18.97	8.78±1.00
9	Sorghum (M35-1)	-	-	-	156.00±27.51	7.41±0.80
10	Sorghum (Phule Yashoda)	-	-	-	81.38±27.50	8.8±1.55
11	Barley	*Hordeum vulgare* (L.)	*Poaceae*	-	211.28±11.23	2.62±0.90
12	Oat	*Avena sativa* (L.)	-	-	180.28±24.55	16.86±2.13
13	Red Rice	*Oryza punctata* (K.)	-	-	224.93±15.83	19.07±2.32
14	Black gram	*Vigna mungo* (L.)	*Fabacee*	-	488.41±48.03	21.44±3.79
15	Kidney bean	*Phaseolus vulgaris* (L.)	-	-	254.30±22.48	16.76±3.92
16	Soybean (White)	*Glycine max* (L.)	-	-	241.08±32.23	10.53±1.94
17	Soyabean (White)	-	-	-	141.48±25.25	23.47±7.44
18	Soyabean (Black)	-	-	-	229.70±28.63	30.88±10.35

TP[1] (Total phenolic), TF[2] (Total flavonoids)

Table 2: Total antioxidant activity in selected 18 cereals

S.No.	Cereals	Scientific name	Family	Part analyzed	FRAP (µmol TE/g)	DPPH (µmol TE/g)	CUPRAC (µmol TE/g)
1	Finger millet (Local)	*Eleusinecoracana (L.)*	*Poaceae*	Grain (Flour)	7.08±1.03	6.31±1.31	4.07±2.63
2	Finger millet (GBU-67)	-	-	-	3.07±0.78	8.92±1.2	2.44±0.62
3	Finger millet (GBU-48)	-	-	-	4.13±0.31	12.95±0.7	3.19±0.14
4	Finger millet (GBU-45)	-	-	-	3.80±0.50	11.84±0.5	2.73±0.25
5	Finger millet (L-5)	-	-	-	4.30±0.31	13.67±	3.19±
6	Pearl millet	*Pennisetum glaucum* (L)	*Poaceae*	-	3.41±0.35	9.32±1.81	3.34±2.24
7	Sorghum (CSV18VR)	*Sorghum bicolor* (L.)	-	-	2.32±0.32	9.55±1.10	1.24±1.06
8	Sorghum (CSV14VR)	-	-	-	3.23±0.52	6.27±0.42	2.99±1.17
9	Sorghum (M35-1)	-	-	-	3.32±0.29	8.45±1.17	3.65±2.53
10	Sorghum Phule Yashoda	-	-	-	1.66±0.32	13.66±0.74	0.65±1.34
11	Barley	*Hordeum vulgare* (L.)	*Poaceae*	-	3.25±0.75	6.1±1.31	1.51±1.20
12	Oat	*Avena sativa* (L.)	-	-	3.27±0.52	15.03±0.80	3.43±1.69
13	Red Rice	*Oryza punctata* (K.)	-	-	8.29±3.65	8.73±1.03	2.86±2.21
14	Black gram	*Vigna mungo* (L.)	*Fabaceae*	-	9.60±5.33	11.08±1.58	3.45±2.56
15	Kidney bean	*Phaseolus vulgaris* (L.)	-	-	7.07±0.76	13.37±0.48	4.68±1.30
16	White soybean	*Glycine max* (L.)	-	-	4.37±0.45	7.70±1.78	1.99±2.00
17	Soya bean (White)	-	-	-	2.53±0.37	10.12±0.98	1.50±0.47
18	Soya bean (Black)	-	-	-	4.42±0.80	9.02±2.69	3.83±4.31

FRAP[1] (Ferric Reducing Antioxidant Power, DPPH[2] (2-DPPH, 2, 2-Diphenyl-1picrylhydrazyl),), CUPRAC[3] (Cupric Reducing Antioxidant Power).

The results of TPH, TF and AOX, in 18 cereal crops are presented in (Table 1 and 2) The group constituted, five finger millet cultivars, four sorghum cultivars, three soyabean, pearl millet, barley, oats, red rice, black gram and kidney bean. It was observed that coarse cereals, millets and beans (kidney and soyabean) had significantly ($p<0.05$) higher content than fruits and vegetables. Black gram followed by finger millet, pearl millet, kidney bean and black soyabean had the highest content ranging from 488.41, 394.4, 254.30 and 229.90 mg GAE/100g respectively. TF content ranged from 8.84- 116.89 mg CE/100g. AOX in FRAP, DPPH and CUPRAC assays ranged from 2.31- 8.29, 6.10 -15.03 and 0.65-4.68 µmol TE/g. On the whole, finger millet (Ragi) and black soyabean scored over the rest cereals and beans in the group.

Finger millet (Eleusine coracana) or ragi, one of the important minor cereals is a rich source of several phytochemicals and among them polyphenols are the most important because of their nutraceutical potentials. The seed coat-rich fraction (SCF) of the millet contains over 70% of polyphenols of the millet[2]. Brown seeds exhibited superior proanthocyanidin contents. Bean seeds consisting of greater amounts of anthocyanins exhibited superior antioxidant activity. The nutritional data obtained suggest that the selected grain, particularly sorghum, is promising as healthy food. The five common bean species exhibited variations in anthocyanins, proanthocyanidin and antioxidant activity. The highest contents of total phenolics were found in Serbian cultivar 1511 and Chinese cultivar LN92-7369, which also displayed the highest total antioxidant activity due to anthocyanin[4]. Cereals are valuable for their phytochemicals, which are mainly in the bran and germ, and include anthocyanins, phytosterols, policosanols, carotenoids, and tocopherols1. Phytochemicals can affect the colour, flavour and texture of cereal products, with concomitant variations in the antioxidant properties. Genotypes poor in phenolics also showed low levels of DPPH-radical scavenging activity. The results suggested that besides protein and oil contents, the phenolic contents should be also considered as an important characteristic feature of soybean seeds.

CONCLUSION

Course cereals are rich source of nutritional and functional quality due presence of anthocyanin beta carotenoids and these cereals containing high phenolics may provide a source of dietary antioxidants. Those are responsible for reducing the cardiovascular disease and cancer.

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