International Journal of Bioassays
ISSN: 2278-778X
www.ijbio.com
OPEN ACCESS

Original Research Article

ASSAY OF *IN VITRO* FREE RADICAL SCAVENGERS AND THEIR ANTIOXIDANT ACTIVITY OF SORGHUM (*SORGHUM BICOLOR* (L.) MOENCH)

Bangaru Naidu Thaddi* **and Sarada Mani Nallamilli**
Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, India.

Received for publication: August 27, 2014; **Revised**: September 04, 2014; **Accepted**: September 17, 2014

Abstract: Sorghum is a one of the leading cereal crop, among the cereals Sorghum contains unique variety of bioactive compounds which affect the classification, colour, appearance, nutrition quality, and functionality of the grain. The present study deals with six different genotypes of Sorghum including three without pigmented testa (IS 3477, IS 33095, IS 7005), three pigmented testa (IS 7155, IS 2898, IS 1202) for their evolution of bioactive components and *in vitro* free radical scavenging of antioxidant activity. After the process of soxhlation, an obtained methanolic extract from the six genotypes of Sorghum, they were analyzed for their total phenolics and capacity of *in vitro* free radical assay included Superoxide radical, 1, 1-Diphenyl -2- pycrilhydrazyl (DPPH), Hydroxyl radical, and Lipid peroxidation. Here we have estimated the free radical scavenger's order an increased level concentrations of percentage inhibition (10-1280 µg/ mL). The maximum IC_{50} values were favoured to pigmented testa genotypes of Sorghum. These various antioxidant activities were compared to excellent standard antioxidant like Ascorbic acid. The scavenging effects of all grain extracts on the DPPH radical were greater than that of compared with other free radicals. The pigmented testa genotypes exhibited the highest antioxidant capacities than non-pigmented testa genotypes and thus could be potential rich sources of natural antioxidants.

Key Words: Sorghum cultivars, DPPH, Superoxide radical, Hydroxyl radical, Lipid peroxidation.

INTRODUCTION

Sorghum is a 5th most important cereal poor man's crop native to Africa, because of it's originated in the semi-arid tropics and is generally heat and drought tolerant, where this condition is very high. Sorghum is also grown in Asia and United States, throughout the world Sorghum production above 41 million hectors [1]. Now a day's polyphenols, tannins, carotenoids, and flavonoids generally regarded as a plant secondary bioactive compounds which are contain very high level in Sorghum [2]. It has single most characteristic among cereals, because of their seed coat testa of pigmented grains [3, 4]. Recent years, these bioactive compounds play a crucial role in human diet due to their antioxidant activity and preventing human illness [5].

Free radicals are natural by-products of human metabolism. These are charged molecules which attack cells, breaking cellular membranes and reacting with the nucleic acids, proteins, and enzymes present in the cells. These attacks by free radicals, collectively known as oxidative stress, are capable of causing cells to lose their structure, function and eventually result in cell dysfunction. They are continuously produced by our body's use of oxygen, such as in respiration and some cell-mediated immune functions. Free radicals are also generated through environmental pollutants, cigarette smoke, automobile exhaust, radiation, air-pollution, pesticides, etc. [6]. Normally, there is a balance between the quantity of free radicals generated in the body and the antioxidant defence systems which scavenge/quench these free radicals preventing them from causing deleterious effects in the body [7]. The antioxidant defence systems in the body can only protect the body when the quantity of free radicals is within the normal physiological level. But when this balance is shifted towards more free radicals, increasing their burden in the body either due to environmental conditions or infections, it leads to oxidative stress [8]. While the concept of developing drugs from plants used in indigenous medical system is ancient, the link between indigenous use and subsequent scientifically derived biomedical use is not always clear. In some cases the relationship is much more complex [9].

The role of free radicals has been implicated in the causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, aging, arthritis, diabetes etc. [10] and the compounds that scavenge free radicals have great potential in ameliorating these disease processes [11]. Antioxidants have the ability to scavenge free radicals, and thus may protect the body from oxidative and free radical damage which are implicated in the development of some cancers, degenerative diseases such as atherosclerosis, coronary heart disease and the aging process [12]. There have been suggestions that antioxidants could be of potential benefit in individuals infected by the human immunodeficiency virus (HIV) [13, 14]. There is increasing evidence that some bioactive compounds in the diet possess the ideal structure for free radical scavenging activities and some have been found to be more effective *in vitro* on molar basis, than other antioxidants like vitamin E and C [15].

***Corresponding Author:**
Dr. Bangaru Naidu Thaddi,
Department of Botany,
Andhra University, Visakhapatnam-530003,
Andhra Pradesh, India.

A previous report on free radical scavenging activity and their antioxidant activity has been made in Sorghum whole grain as used by different organic solvents [16, 17, 18]. The use of specific genotypes of Sorghum and its products of methodologies are very confined, but there is no routinely on effect of genotypes on its antioxidant activity in Sorghum by using greater than free radical scavenging increased level of percentage tests. The antioxidant capacities of plant extracts largely depend on the composition of the extracts and conditions of the test system. The antioxidant capacities are influenced by many factors, which cannot be fully described with one single method. Therefore, it is necessary to perform more than one type of antioxidant activity capacity measurement to take into account the various mechanisms of antioxidant action. In this study, different concentrations of methanolic extracts of six Sorghum genotypes were evaluated for their antioxidant capacities using DPPH, Superoxide radical, Hydroxyl radical, and Lipid peroxidation.

MATERIALS AND METHODS

Sorghum seed sample extraction

Methanol extraction: The raw seeds were finely powdered by using electric grinder and their extraction was done by Soxhlet extractor using aqueous methanol (80%) as solvent. The aqueous methanol extract were redissloved in methanol for assessment of *in vitro* free radical scavenging activity and quantification of total phenolic contents.

Quantification of total phenolic constituents

Determination of total phenolics content: The content of total phenolics in extracts was determined by a modified Folin–Ciocalteu method [19]. Briefly, 100 µl of each extract were shaken for 1 min with 500 µl of Folin–Ciocalteu reagent and 6 mL of distilled water. After the mixture was shaken, 2 mL of 15% Na_2CO_3 were added and the mixture was shaken once again for 5 min. Finally, the solution was brought upto 10 mL by adding distilled water. After 2 hours, the absorbance was read on the UV/visible spectrophotometer at 750 nm (25°C) using glass cuvettes against a blank (100 µl of distilled water instead test samples). The TPC was assessed by plotting the gallic acid calibration curve (from 1 to 1500 µg/mL) and expressed as milligrams of gallic acid equivalents (GAE) per gram of dried extract.

Antioxidant and free radical scavenging assay of Sorghum

Antioxidant activity was determined by using the Superoxide radical, 1, 1-Diphenyl-2-picrylhydrazyl (DPPH), Hydroxyl radical, and Lipid peroxidation.

Determination of 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity: DPPH radical has been widely used in assessment of radical scavenging activity because of its ease and convenience. An aliquot of 3 mL of 0.004% DPPH solution in methanol and 0.1mL of plant extract at various concentrations were mixed. The mixer was shaken vigorously and allotted to reach a steady state at room temperature for 30min, decolonization of DPPH was determined by measuring the absorbance at 517nm. A control was prepared using 0.1mL of responsive vehicle in the place of plant extract / Ascorbic acid [20]. The percentage inhibition of DPPH by the extracts was determined by comparing the absorbance values of the control and the experimental tubes.

Determination of Superoxide radical Scavenging Activity

Riboflavin Photo reduction Method: Superoxide radical scavenging activity of the extract was determined by the method of Mecord and Fridovich [21], which depends on light induced superoxide generation by riboflavin and the corresponding reduction of NBT. The assay mixture contained different concentrations of the extracts and EDTA (6 µM containing 3 µg NaCN), NBT (50 µM), Riboflavin (2 µM) and phosphate buffer (58 µM, pH 7.8) to give a total volume of 3mL. The tubes were uniformly illuminated for 15 minutes and there after the optical density (O.D) was measured at 560 nm. The percentage inhibition by the extracts of superoxide production was evaluated comparing the absorbance values of control and experimental tubes as calculated DPPH radical assay.

Determination of Hydroxyl radical Scavenging Activity

Deoxyribose degradation method: Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the Fe^{2+} /EDTA /H_2O_2 system (Fenton reaction). The hydroxyl radical attacks deoxyribose which eventually results in thiobarbutric acid reacting substances (TBARS) formation. The reaction mixture contains deoxy ribose (2.8 mM) ferrous sulphate (10 mM), EDTA (10 mM), H_2O_2 (1.0 mM), phosphate buffer (0.1 mM, pH 7.4) and various dilutions of the extracts. The reaction was incubated for 4 hours at 37°C. Deoxyribose degradation was measured as TBA reacting substances by the method of Ohkawa [22] and the percentage of inhibition was calculated from the control when no test compound was added. The percentage inhibition of hydroxyl radicals by the extracts was determined by comparing the absorbance values of the control and the experimental tubes as calculated DPPH radical assay.

Determination of Lipid peroxidation inhibition activity

Induction by $Fe2^+$/Ascorbate system: Inhibition of lipid peroxidation was determined by the

thiobarbutiric acid method of Ohkawa [22]. Reaction mixture (0.5 mL) containing Rat liver homogenate (0.1 mL, 25% w/v) in Tris HCl buffer (40 mM, pH 7.0), KCl (30 mM), Ascorbic acid (0.06 mM) and ferrous ion (0.16 mM) and various concentrations of the extracts were incubated for 1 hour at 30°C. The reaction mixture (0.4 mL) was treated with sodium dodecyl sulphate (SDS, 0.2 Ml, 8.1%), Thiobarbutric acid (TBA, 1.5 mL, 0.8%) and Acetic acid (1.5 mL, 20% pH 3.5). The total volume was then made upto 4 mL by adding distilled water and kept in oil bath at 100°C for 1 hour. After the mixture had been cooled, 1mL distilled water and 5mL of butanol pyridine mixture (15:1 v/v) was added. Following vigorous shaking, the tubes were centrifuged and the absorbance of the organic layer containing the chromophore was read at 532 nm. The percentage inhibition of lipid peroxidation by the extract was determined comparing the absorbance values of the control and the experimental tubes as calculated DPPH radical assay.

Calculation of 50% Inhibition Concentration (IC_{50} values)

The optical density obtained with each concentration of extracts and Ascorbic acid was plotted on a graph taking concentrations on X-axis and percentage inhibition on Y-axis. The graph was extra plotted to find the concentration needed for 50% inhibition.

Statistical analysis

All values are expressed as means ± standard error for three replicates. Two way - ANOVA was used to determine significant differences in total phenols, antioxidant activities among Sorghum types including DPPH, Superoxide, Hydroxyl and Lipid peroxidation antioxidant activity. P values were considered significant when less than 0.05. All statistical analyses were done using the statistical software SPSS

RESULTS

Quantification of total Phenolics contents

The total phenolic contents of Sorghum grains were determined by the Folin-Ciocalteu method and the grains showed high amount of phenolic contents in all genotypes. The polyphenolic contents in the methanolic extracts were expressed as mg of Gallic acid equivalents (GAE) per 100 gm of sample. A wide variation was observed in the total polyphenol content among the six Sorghum genotypes. The total polyphenolic contents of the samples ranged from 40.33 ± 0.98 to 96.24 ± 1.97 mg GAE/100 gm. Among all the samples, pigmented Sorghum genotypes IS 2898, IS 7155, IS 1202 exhibited high amount of total polyphenols i.e., 96.24 ± 1.97 mg/100 gm, 78.22 ± 1.69 mg/100 gm, 69.25 ± 1.35 mg/100 gm whereas non pigmented genotypes IS 3477, IS 33095, IS 7005 exhibited 61.22 ± 1.12 mg/100 gm, 56.12 ± 1.52 mg/100 gm, 40.33 ± 0.98 mg/100 gm respectively. The total phenols were significantly affected by Sorghum types. The Sorghum types with pigmented testa layers had higher levels of total phenols. The total phenol levels differed significantly ($p<0.05$) between the Sorghum types, with pigmented testa Sorghum having the highest, followed by IS 2898 and the lastly IS 7005.

1, 1-Diphenyl-2-picryl hydrazyl (DPPH)

The DPPH scavenging activity is unique radical activity because of it is a quick and reliable parameter to assess the *in vitro* antioxidant activity of plant extracts. In the present study, DPPH test to find out the ability of a compound to act as donor for hydrogen atoms or electrons was measured spectrophotometrically. Fig. 1 shows the scavenging activities of DPPH exerted by methanol extract of Sorghum as well as Ascorbic acid. The scavenging effect of extracts in the range 10–1280 µg/mL on the DPPH radical increased with an increasing level of concentration in Sorghum seed extract. At the concentration of 1280 µg/mL exhibited the highest percentage activity in pigmented genotypes IS 2898 (94.00%), IS 7155 (93.75%), IS 1202 (90.99%) while low activity in non-pigmented genotypes IS 3477 (88.36%), IS 33095 (74.35%), IS 7005 (72.71%) was shown in (Table 1). Among these genotypes, the pigmented variety IS 2898 exhibited high percentage activity whereas non-pigmented variety IS 7005 showed low percentage activity. The mean of IC50 values for DPPH radical by methanol extracts of Sorghum showed in the six genotypes was lowest in IS 2898 (36 µg/mL), IS 7155 (26 µg/mL), IS 1202 (56 µg/mL) highest in IS 3477 (146 µg/mL), IS 33095 (285 µg/mL), IS 7005 (281 µg/mL) was shown in (Fig. 5).

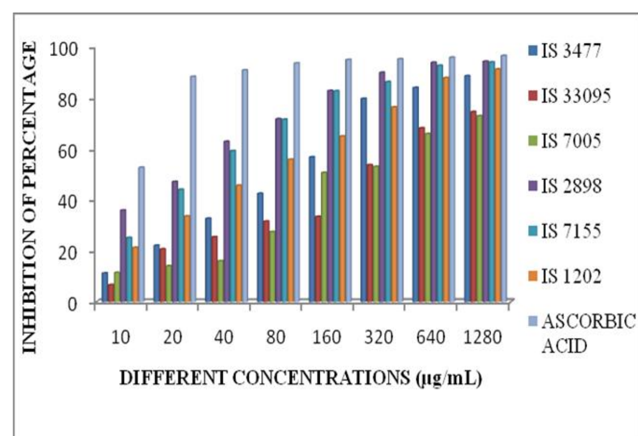

Figure 1: *In Vitro* Concentration Dependent Inhibition of DPPH Radical by methanolic extract of Sorghum and Ascorbic Acid

Superoxide radical

In the present study, methanolic extract of Sorghum was found to possess concentration

dependent scavenging activity on Superoxide radicals is generated by photoreduction of riboflavin. The concentration dependent percentage inhibition of superoxide radical activity in methanolic extract of Sorghum and Ascorbic acid was given in (Table 2) and shown in (Fig.2). The inhibition produced by the different concentrations of the methanolic extract were measured that ranged from 10–1280 µg/mL in the reaction mixture. At the 1280 µg/mL, the highest activity was showed in pigmented genotypes IS 2898 (91.21%), IS 7155 (80.30%), IS 1202 (85.25%) lowest activity in IS 3477 (77.20%), IS 33095 (70.13%), IS 7005 (67.93%). The pigmented variety IS 2898 exhibited high percentage activity, non-pigmented variety IS 7005 had showed low percentage activity.

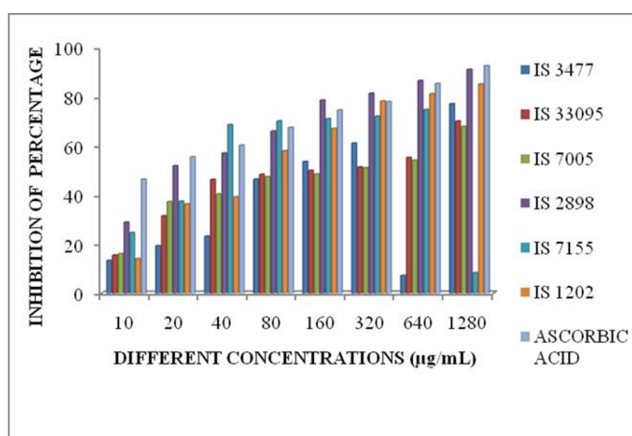

Figure 2: *In Vitro* concentration dependent inhibition of Superoxide radical by methanolic extract of Sorghum and Ascorbic acid.

In this study, between the pigmented and non-pigmented genotypes of Sorghum gave the mean of IC50 values exhibited at different microgram concentration when the percentage of superoxide radial extraplotted to graph. The quantity of microgram values of the six Sorghum genotypes i.e., IS 2898 (18 µg/mL), IS 7155 (28 µg/mL), IS 1202 (51 µg/mL), IS 3477 (145 µg/mL), IS 33095 (160 µg/mL) and IS 7005 (300 µg/mL) were shown in (Fig. 5).

Hydroxyl radical

In the present study, the methanolic extract of Sorghum was found to possess concentration dependent scavenging activity on hydroxyl radicals generated by Fenton reaction. The concentration dependent percent inhibition of hydroxyl radical activity by methanolic extract of Sorghum and Ascorbic acid were given in (Table 3) (Fig. 3). The increasing level of concentrations of pigmented genotypes exhibit more activity in IS 2898 (84.88%), IS 7155 (80.86%), IS 1202 (78.11%) than non-pigmented genotypes IS 3477 (73.42%), IS 33095 (71.31%), IS 7005 (62.21%) at 1280 µg/mL concentration. Among the six genotypes, pigmented genotypes given low level of IC50 values IS 2898 (55 µg/mL), IS 7155 (78 µg/mL), IS 1202 (160 µg/mL) better than non-pigmented genotypes IS 3477 (292 µg/mL), IS 33095 (302 µg/mL), IS 7005 (590 µg/mL) was shown in (Fig. 5).

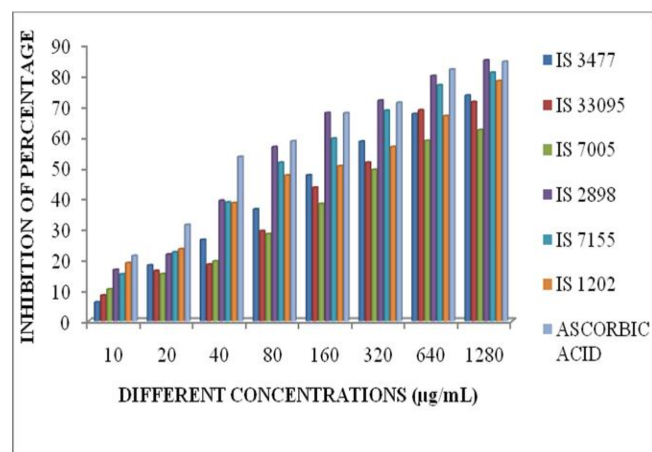

Figure 3: *In Vitro* concentration dependent inhibition of Hydroxyl radical by methanolic extract of *Sorghum* and Ascorbic acid

Lipid peroxidation

Lipid peroxidation, which involves a series of free radical mediated chain reaction processes, is also associated with several types of biological damage. Therefore much attention has been focused on the use of natural antioxidants to inhibit lipid peroxidation and to protect from damage due to free radicals.

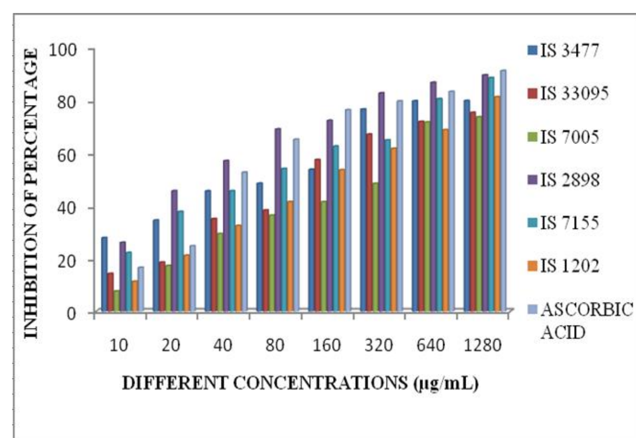

Figure 4: *In Vitro* Concentration Dependent Inhibition of Lipid Peroxidation Radical by methanolic extract of Sorghum and Ascorbic Acid

In the present study, methanolic extracts of Sorghum was found to inhibit the lipid peroxidation generation induced by Fe^{2+}/ascorbate on rat liver homogenate in a concentration dependent manner. The concentration dependent lipid peroxidation inhibition activity by methanolic extracts of Sorghum and Ascorbic acid were given in (Table 4) and shown in (Fig. 4). These results indicated that all the Sorghum seed extracts showed excellent lipid peroxidation

scavenging activity at a concentration 10-1280 µg/mL in the reaction mixture and increased steadily with the increased concentration. The percentage activity at 1280 µg/mL in pigmented genotypes IS 2898, IS 7155 IS 1202 produced 89.47%, 88.44%, 81.18% while non-pigmented genotypes IS 3477, IS 33095, IS 7005 was found 79.77%, 75.25%, 73.59% respectively. The mean of IC50 values were found at very low microgram quantity in pigmented genotypes, IS 2898 (32 µg/mL), IS 7155 (108 µg/mL), IS 1202 (68 µg/mL) than non-pigmented genotypes IS 3477 (100 µg/mL), IS 33095 (146 µg/mL), IS 7005 (350 µg/mL) were given in (Fig. 5).

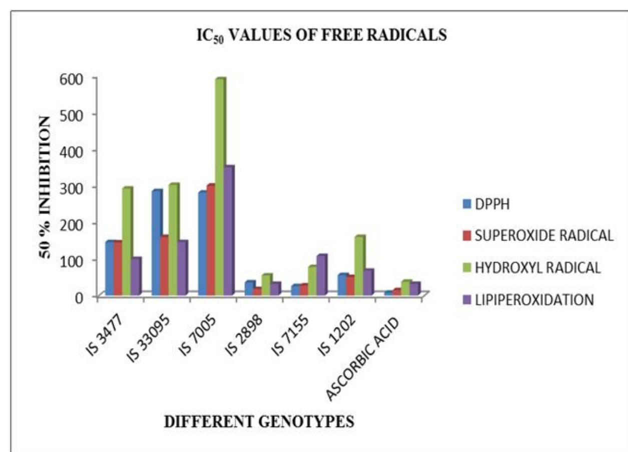

Figure 5: 50% Inhibition concentrations (IC_{50} values) of methanolic extract of Sorghum and Ascorbic acid.

In the present study, we observed the percentage inhibition of DPPH, Superoxide, Hydroxyl and Lipid peroxidation increased with the increasing concentration of methanolic seed extract of all six genotypes of Sorghum and maximum percentage of inhibition was observed at 320, 640, 1280 µg/mL concentrations. The methanolic extract of Sorghum produced a dose component inhibition of free radical generation of DPPH, Superoxide anion, Hydroxyl radical and Lipid peroxides in *in vitro*. The 50% inhibitory concentration (IC50) of DPPH, Superoxide radical, Hydroxyl radical and Lipid peroxidation were clear to assay of the amongst radicals in *in vitro*. Among these genotypes tested against the four radicals the pigmented genotypes of IC50 values showed below 50 µg/mL concentrations than non-pigmented genotypes were shown in (Fig.5). It means a lower concentrations of IC50 values indicated a higher antioxidant activity. In the present study, the results clearly indicated the free radical scavenging activity of methanolic extract of Sorghum in *in vitro* and this activity compared with the well-known antioxidant Ascorbic acid as standard, but all the genotypes showed lowest percentage activity when compare with standard at the all concentrations.

DISCUSSION

Reactive oxygen species such as Superoxide, Hydroxyl and Lipid peroxidation radicals have been widely implicated in the pathology of several diseases. Removal of these reactive oxygen species or suppression of their generation is an efficient way in controlling these diseases. Antioxidant actions might be exerted by inhibiting generation of reactive oxygen species and reactive nitrogen species, or by directly scavenging free radicals or by raising the levels of endogenous antioxidant defenses. In the present investigation, superoxide, or hydroxyl, or DPPH radical scavenging and lipid peroxidation inhibition activities was conducted on Sorghum seed extract. The results of previous studies with different antioxidant assay on Sorghum methanol-water extract suggest that the Sorghum brans had 3 to 5 times antioxidant activity in the grains [23, 24].

In the present study, we conducted on different radical activity including which one of the DPPH radical is a stable free radical, which has been widely used to evaluate the free radical scavenging effects of natural antioxidants [20]. In this assay Sorghum methanolic extract reduced the DPPH and by the odd electron of the DPPH radical becomes paired off. Because of the odd electron DPPH radical is having the maximum absorbance at 517 nm after getting the hydrogen by Sorghum extract, the absorbance was reduced at 517 nm [25, 26]. The results showed that the Sorghum methanolic extract was exhibited significant DPPH radical scavenging property at all concentrations were used. In this assay the methanol extract exhibited effectively antioxidant property and IC50 values in pigmented genotypes, which was nearly with L-ascorbic acid, a well-known antioxidant**.** In the current study, similarly reported that tannin Sorghums, Red Swazi, NS 5511 and Framida, had significantly higher ABTS and DPPH antioxidant activity when compared to Sorghums without pigmented testa layer, Macia and NK 283 [27]. Our studies further corroborate the findings of several workers [23, 28, 29, 30, 31] Who reported that various Sorghum products possess high antioxidant activity.

Superoxide radical anion plays an important role in the formation of more reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radical, and singlet oxygen, which include oxidative damage in lipids, proteins, and DNA [32]. Therefore studying the scavenging activity of Sorghum seed extract on superoxide radical is one of most important ways of clarifying the mechanism of antioxidant activity. The radical activity results compared with previous studies on Sorghum. They studied on using aqueous acetone extract, but in our study using methanol as extract solvent [17].

Table 1: *In vitro* concentration dependent inhibition of DPPH radical by methanolic extract of Sorghum / Ascorbic acid

Sorghum Genotypes	Percentage inhibition of DPPH radical Concentration of methanolic extract/Ascorbic acid in micrograms (µg/mL)							
	10 µg	20 µg	40 µg	80 µg	160 µg	320 µg	640 µg	1280 µg
IS 3477	11.28 ± 0.33	22.05 ± 1.21	32.61 ± 1.02	42.36 ± 1.91	56.64 ± 1.94	79.50±1.56	83.77±0.82	88.36 ± 0.88
IS 33095	6.61 ± 0.95	20.62 ± 0.89	25.36 ± 0.79	31.48 ± 0.67	33.33 ± 0.57	53.56 ± 0.59	67.95±0.64	74.35 ± 0.49
IS 7005	11.39 ± 0.8	14.02 ± 0.91	15.95 ± 0.89	27.37 ± 1.29	30.51 ± 0.98	52.87 ± 1.52	65.71±1.82	72.71 ± 0.69
IS 2898	35.80 ± 0.98	46.99 ± 0.69	62.70 ± 0.92	71.56 ± 0.54	82.56 ± 1.23	89.69 ± 1.50	93.64 ± 1.22	94.00 ± 1.25
IS 7155	25.08 ± 0.91	43.92 ± 0.83	59.03 ± 0.81	71.35 ± 0.78	82.45 ± 0.68	86.05 ± 0.59	92.45±0.49	93.75 ±0.45
IS 1202	21.20 ± 0.15	33.49 ± 1.99	45.50 ± 0.87	55.66 ± 1.66	64.77 ± 1.54	76.14 ± 0.58	87.62 ± 0.66	90.99 ± 1.56
Ascorbic Acid	52.56 ± 0.23	88.08± 1.89	90.63 ± 1.45	93.36 ± 0.92	94.74 ± 1.26	94.98 ± 0.89	95.60 ± 1.20	96.30 ± 1.55

Table 2: In vitro concentration dependent inhibition of Superoxide radical by methanolic extract of Sorghum/Ascorbic acid

Sorghum Genotypes	Percentage inhibition of Superoxide radical Concentration of methanolic extract/Ascorbic acid in micrograms (µg/mL)							
	10 µg	20 µg	40 µg	80 µg	160 µg	320 µg	640 µg	1280 µg
IS 3477	13.34±0.56	19.32±1.03	23.23±1.55	46.44 ± 1.90	53.59 ± 1.28	61.13 ± 1.58	70.20 ± 0.94	77.20 ± 1.98
IS 33095	15.58 ± 0.33	31.50 ± 1.65	46.34 ± 0.84	48.42 ± 1.56	50.05 ± 1.09	51.40 ± 0.85	55.29 ± 1.84	70.13 ± 1.62
IS 7005	16.09 ± 0.49	37.28 ± 0.44	40.39 ± 1.99	47.42 ± 1.05	48.52 ± 0.88	51.10 ± 1.45	54.15 ± 0.92	67.93 ± 1.06
IS 2898	28.90± 0.92	51.91±0.65	57.06± 0.99	66.06±1.33	78.66±1.16	81.45±1.22	86.63±1.85	91.21± 0.62
IS 7155	24.67± 0.69	37.45±0.66	68.65± 0.95	70.19± 1.58	71.08± 2.22	72.09±1.29	74.80± 1.87	80.30± 0.91
IS 1202	14.06± 0.22	36.33± 1.63	39.25± 0.54	58.05± 0.35	67.10± 0.33	78.29± 0.21	81.25± 0.66	85.25± 0.65
Ascorbic Acid	46.43± 0.25	55.57± 0.65	60.35± 0.52	67.52± 0.81	74.66± 0.84	78.04± 0.92	85.43± 0.90	92.67± 0.61

Table 3: *In vitro* concentration dependent inhibition of Hydroxyl radical by methanolic extract of Sorghum/Ascorbic acid

Sorghum Genotypes	Percentage inhibition of Hydroxyl radical Concentration of methanolic extract/Ascorbic acid in micrograms (µg/mL)							
	10 µg	20 µg	40 µg	80 µg	160 µg	320 µg	640 µg	1280 µg
IS 3477	6.13±0.35	18.14± 0.92	26.44± 1.85	36.41± 2.01	47.47± 0.82	58.44± 0.84	67.40±1.33	73.42±1.00
IS 33095	8.35±0.19	16.35± 0.55	18.41± 0.21	29.31± 2.22	43.44± 0.85	51.58± 0.66	68.66± 1.23	71.31±1.82
IS 7005	10.33±0.55	15.31± 0.85	19.46± 0.85	28.32± 1.08	38.16± 0.24	49.22± 0.54	58.66±1.52	62.21±0.91
IS 2898	16.66±0.98	21.67± 0.33	39.22± 1.05	56.66± 1.92	67.71± 0.86	71.78± 0.21	79.77±1.85	84.88±1.06
IS 7155	15.22±0.36	22.44± 0.88	38.66± 1.33	51.56± 1.65	59.37± 0.91	68.48± 0.84	76.77±0.94	80.86±1.65
IS 1202	18.88±0.66	23.43± 0.75	38.41± 1.09	47.41± 0.94	50.44± 0.61	56.66± 0.66	66.73±0.65	78.11±0.91
Ascorbic Acid	21.22±0.69	31.32± 1.99	53.47± 1.66	58.56± 0.94	67.68± 0.65	71.09± 0.24	81.89±0.59	84.41±0.98

Table 4: *In vitro* concentration dependent inhibition of Lipid peroxidation by methanolic extract of Sorghum/Ascorbic acid

Sorghum Genotypes	Percentage inhibition of Lipid peroxidation Concentration of methanolic extract/Ascorbic acid in micrograms (µg/mL)							
	10 µg	20 µg	40 µg	80 µg	160 µg	320 µg	640 µg	1280 µg
IS 3477	27.85 ±0.22	34.58 ±.0.65	45.55 ± 0.98	48.45 ± 0.26	53.72 ±0.28	76.52 ±0.25	79.66 ±0.89	79.77 ±1.24
IS 33095	14.27± 0.55	18.57 ± 1.99	35.04 ± 1.95	38.31 ± 1.95	57.43 ±0.88	67.03 ±0.99	71.86±0.85	75.25 ±0.3.1
IS 7005	7.66 ±0.62	17.30 ± 0.32	29.39 ± 0.91	36.33 ± 0.44	41.44 ±0.17	48.41± 0.42	71.62 ±0.34	73.59 ±0.25
IS 2898	26.06 ±0.30	45.60 ±1.22	56.99 ± 0.61	68.99 ± 0.22	72.26 ±0.58	82.13 ±0.36	86.66 ±0.64	89.47 ±0.28
IS 7155	22.22 ±0.99	37.74 ±1.95	45.61 ± 0.34	54.00 ± 0.36	62.51 ±0.59	64.82 ±0.64	80.48 ±0.61	88.44 ±0.94
IS 1202	11.33 ±0.26	21.19 ±1.61	32.44 ± 0.59	41.48 ±0.89	53.56 ±0.94	61.66 ±0.61	68.71 ±0.98	81.18 ±0.24
Ascorbic Acid	16.58 ±0.95	24.72 ±1.35	52.64 ± 0.66	65.09 ±0.30	76.25 ±0.36	79.59 ±0.52	83.19 ±0.27	91.10 ±0.31

In order to determine if the extracts were capable of reducing *in vitro* oxidative stress, lipid peroxidation was assessed by means of an assay that determines the production of malondialdehyde and related compounds in rat liver homogenates. Malondialdehyde (MDA) is one of the major degradation products of lipid peroxidation, which has been extensively studied and measured as a marker for oxidative stress [33]. Thiobarbituricacid reactive species (TBARS) are produced as by-products of lipid peroxidation that occurs in the hydrophobic core of biological membranes [34]. Therefore lipid peroxidation has been identified as one of the basic reactions involved in oxygen free radical induced cellular damages [35] and plays an important role in the pathogenesis of several diseases. The methanol extract of Sorghum prominently inhibited the formation of MDA and exhibited significant antilipid peroxidative effect. The previous study was conducted on this radical in Sorghum by using acetone as solvent extract [17]

Among the ROS, the hydroxyl radicals are the most reactive and predominant radicals generated endogenously during aerobic metabolism. Due to the high reactivity, the radicals have a very short biological half-life. The generated hydroxyl radicals initiate the lipid peroxidation process and/or propagate the chain

process via decomposition of lipid hydroperoxides [36]. A single hydroxyl radical can result in the formation of many molecules of lipid hydroperoxides in the cell membrane, which may severely, disrupts its function, and lead to cell death. The effect of the plant extracts on the inhibition of free radical mediated DNA sugar damage was assessed by means of the iron (II)-dependent DNA damage assay. The Fenton reaction generates hydroxyl radicals (OH^-), which degrade DNA deoxyribose, using Fe^{2+} salts as an important catalytic component [37]. Oxygen radicals may attack DNA either at the sugar or the base, giving rise to a large number of products. Attack at a sugar ultimately leads to sugar fragmentation, base loss and strand break with a terminal fragmented sugar residue [38]. Addition of low concentrations of transition metal ions such as iron to DNA causes degradation of the sugar into malondialdehyde and other related compounds which form a chromogen with thiobarbituric acid (TBARS). The methanol extract of Sorghum significantly scavenged on the hydroxyl radical generated through the Fenton reaction. This study was agreed with previous reports was done on Sorghum [17, 18].

CONCLUSION

In conclusion, Sorghum is a one of the most cultivated cereal crop throughout the world. We have used pigmented and non-pigmented testa genotypes by using methanol as solvent extract for their evaluating anti-radical activity. Among the genotypes, pigmented testa genotypes exhibited more antioxidant activity when compared with non-pigmented genotypes. Our results suggest that a diet rich in Sorghum may be useful in combating diseases in which free radical production plays a key role.

ACKNOWLEDGMENT

The authors wish to acknowledge the Head, Department of Botany, Andhra University, and Visakhapatnam for their given financial support as UGC (BSR) SAP-RFSMS.

REFERENCES

1. FAO (Food & Agricultural Organization), FAOSTAT, 2010.
2. Thaddi BN, Nallamilli MN, Estimation of total bioactive compounds in pigmented and non-pigmented genotypes of Sorghum (*Sorghum bicolor* (L.) Moench), Int J Adv Res Sci Technol, 2014, 3, 86-92.
3. Beta T, Rooney LW, Marovastanga, LT, and Taylor, JRN, Phenolic compounds and kernel characteristics of Zimbabwean Sorghums. J Agri Food Chem, 1999, 79:1003-1010.
4. Boren B, and Waniska, RD, Sorghum seed color as an indicator of tannin content. J Applied Poultry Research. 1992, 1,117-121.
5. Hu FB, Dietary pattern analysis: a new directional in nutritional epidemiology. Current opinion in Lipidology, 2002, 13, 3-9.
6. Li Y, and Trush MA, Reactive oxygen dependent DNA damage resulting from the oxidation of phenolic compounds by a copper redox cycle, Cancer Res, 1994, 54, 1895s–1898s.
7. Nose K, Role of reactive oxygen species in regulation of physiological functions, Biol. Pharmaceut. Bull, 2000, 22, 897–903.
8. Finkel T, and Holbrook NJ, Oxidants, oxidative stress and biology of ageing. Nature, 2000, 408, 239–247.
9. Heinrich M, and Gibbons S, Ethno pharmacology in drug discovery: an analysis of its role and potential contributions J Pharm Pharmacol, 2001, 53, 425–432.
10. Halliwell B, Gutteridge JM, The importance of free radicals and catalytic metal ions in human disease. Mol. Aspects. Med, 1985, 8, 89-93.
11. Wilson RL, Iron, Zink, Free radicals and oxygen in tissue disorders and cancer control. Ciba found Symp, 1976, 51, 331-354.
12. Chung KT, Wong TY, Huang YW, Lin Y, Tannins and human health: a review. Critical Reviews in Food Science and Nutrition. 1998, 38, 421–464.
13. Liang B, Larson DF, and Watson RR, Oxidation and nutritional deficiencies.in AIDS: Promotion of immune dysfunction for cardiac toxicity? Nutritional Research, 1998, 18, 417-431.
14. Sepulveda RT, and Watson RR, Treatment of antioxidant deficiencies in AIDS patients. Nutritional Res, 2002, 22, 27-37.
15. Rice-Evans CA, Miller NJ, and Paganga G, Antioxidant properties of phenolic compounds. Trends in Plant Sci, 1997, 2, 152-159.
16. Awika JM, and Rooney LW, Sorghum phytochemicals and their potential aspects on human health. Phytochem, 2004, 65, 1199 – 1221.
17. Naidu BT, Mani NS, and Varaprasad B, Antioxidant and Free radical Scavenging activity of *Sorghum bicolor* (L.) Moench. J Pharmacy Res, 2009, 2 (10), 1659-1662.
18. Kamath VG, Chandrasekhar A, Rajini PS, Antiradical properties of Sorghum (Sorghum bicolor L. Moench) flour extracts. J Cereal Sci, 2004, 40, 283-288.

19. Singleton VL, and Rossi JA, Colorimetry of total phenols withn phosphomolybdic phosphotungstic acid reagents, Am. J Enol Vitic, 1999, 16, 144-158.

20. Blois MS, Antioxidant determinations by the use of a stable free radical. Nature, 2002, 26:1199–1200.

21. MeCord. J, Fridovich I, Superoxide dismutase: an enzymatic function for erthrocupein (hemocuprein). J Biol Chem, 1969, 244, 6049-6052.

22. Okhawa H, Oishi N, Yagi K, Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. Anal Biochem, 1979, 95, 351-358.

23. Awika JM, Antioxidant properties of Sorghum. Ph.D. Dissertation. Texas A&M University: College Station, TX, 2003.

24. Dykes L, Flavonoid composition and antioxidant activity of pigmented Sorghums of varying genotypes. Ph.D. Dissertation. Texas A&M University: College Station, TX, 2008.

25. Porto CD, Calligaris S, Cellotti E, Nicoli MC, Antiradical properties of commercial cognacs assessed by the DPPH test. J Agric Food Chem, 2000, 48, 4241-4245.

26. Cotelle N, Bernier JL, Catteau JP, Pommery J. Wallet, J.C. Gaydou EM, Antioxidant properties of hydroxyl flavones Free Radical Biol. Med, 1996, 20:35-43.

27. Dlamini NR, Taylor JRN, and Rooney LW, The effect of Sorghum type on the antioxidant properties of African Sorghum-based food. Food Chem, 2007, 105, 1412-1419.

28. Dykes L, Rooney LW, Waniska RD, and Rooney WL, Phenolic compounds and antioxidant activity of Sorghum grains of varying genotypes. J Agric Food Chem, 2005, 53, 6813-6818.

29. Nomusa RN, Effects of Sorghum type and processing on the antioxidant properties of Sorghum (*Sorghum bicolor* (L.) Moench) based foods. Ph.D. Dissertation. Texas A&M University: College Station, TX, 2007.

30. Choi Y, Heon-Sang J, Lee J, Antioxidant activity of methanolic extracts from some grains consumed in Korea. Food Chem, 2007, 13, 130-138.

31. Sikwese FE, and Duodu KG, Antioxidant effect of a crude phenolic extract from Sorghum bran in sunflower oil in the presence of ferric ions, Food Chem, 2007, 104, 324-331.

32. Janero DR, Malondialdehyde and thiobarbituric acid reactivity as diagonostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic Biol Med, 1990, 9, 515–540.

33. Fraga C, Leibovitz B, Tappel A, Halogenated compounds as inducers of lipid peroxidation in tissue slices. Free Radical Res, 1987, 3, 119–123.

34. Halliwell B, Aruoma OI, DNA damage by oxygen derived species: its mechanism and measurement in mammalian systems. FEBS Lett, 1991, 281, 9–19.

35. Wickens AP, Aging and the free radical theory. Respiration Physiol, 2001, 128, 379–391.

36. Halliwell B, Gutteridge J, Formation of a thiobarbituric acid-reactive substance from deoxyribose in the presence of iron salts. The role of superoxide and hydroxyl radicals. FEBS Lett, 1981, 128, 347–352.

37. Imlay J, and Linn S, (DNA damage and oxygen radical toxicity, Science, 1988, 240, 1302–1309.

38. Pietta PG, Flavonoids as antioxidants, J Nat Prod, 2000, 63, 1035–1042.

Source of support: UGC (BSR) SAP-RFSMS
Conflict of interest: None Declared