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RESEARCH ARTICLE

Comparative studies on raw and cooked extracts of *Sorghum* Cultivars for their Bio-active Constituents.

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Abstract

Studies on phytochemicals in Cereals are important because of their significant role in health benefits. Polyphenolic compounds are the major naturally occurring phytochemicals in *Sorghum* grains. In this study, methanolic extracts from non-pigmented grains of six genotypes (HC-260, M-35-1, CSV-15, CSV-17, CSV-20, and CSV-22) were analyzed for total phenolic content, total antioxidant capacity, total flavonoid and total carotenoid content and effect of cooking on them was depicted. Out of the six genotypes, CSV-15 showed good profile of biochemical constitution. Our studies are helpful for the *Sorghum* breeders in selection of genotypes as parents with good biochemical profile. The summary of our reports revealed that the effect of cooking on reduction of concentration of phenolics for genotype CSV-20 was less compared to other genotypes.

Introduction

Sorghum is an important food source in Africa and Asia and is widely grown in the southern United States as a cattle feed. In India, *Sorghum* was grown in 7381700 Ha that yielded 9487 Hg/Ha and recorded as top producer of *Sorghum* in the world. *Sorghum* ranks fifth in India for commodity value (FAO STAT 2011). As a cereal grain, it is humanity's principle source of calories and protein along with other cereals. Normally, cereal grains contain 10-15% water, 8-14% protein, 70-75% carbohydrates and 2-7% fat as well as variety of minerals and vitamins. Among the cereals, *Sorghum* has the highest content of phenolic compounds comprising of approximately 6% w/w that includes all classes of phenolic compounds such as, phenolic acids, flavonoids, antioxidants, carotenoids and condensed tannins (seeds with pigmented testa). *Sorghum* is consumed in different forms. The grain can be roasted (Dogget 1988), can be boiled whole (Ensminger *et al.*, 1991), popped, milled into flour and used for making bread, soft and thick porridges (Ensminger *et al.*, 1983). It is also used for production of alcoholic beverages and other non fermented beverages. *Sorghum* can also be processed for starch and oil (Dogget, 1988). This study was aimed at identifying *Sorghum* bioactive components comprising antioxidant activity and checking their food functionality because the potential use of the antioxidant constituents of *Sorghum* for the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists and food manufacturers as consumers move toward functional food in their diet. *Sorghum* varieties have unique compositions of 3-deoxy flavonoids (Awika and Rooney 2004, Hwang *et al.*, 2004) and other components that are different from those found in other cereal grains (Mohammad *et al.*, 2009). Compound which contains a benzene ring with one or more hydroxyl groups is a phenolic compound. They are well known for the electron donating property. Generally these are bound to the Cell wall. They are also one of the most effective antioxidative constituents in plant foods including fruits, vegetables and grains (Velioglu *et al.*, 1998). Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (Velioglu *et al*., 1998). Antioxidant activity may come from the presence of secondary metabolites such as volatile oils, carotenoids and vitamins (Javanmardi *et al.*, 2003). Typical phenolics that possess antioxidant activity have been characterized as phenolic acids and flavonoids (Kahkonen *et al.*, 1999). Hence, it is important to quantify

polyphenolic contents and to assess their contribution for human nutrition. Moreover, studies on *Sorghum* shown that it has antioxidant activity (Choi *et al.,* 2006), anti-carcinogenic effects (Ha *et al.,* 1998), DPPH radical scavenging activity and anti-mutagenic affects (Kwak *et al.,* 2004) and can reduce the risk of cardio-vascular disease (Cho *et al.,* 2000). Importance of phenolics in *Sorghum* by protecting against stress was also reported (Dicko *et al*., 2006).

Cooking generally reduce the *in vitro* protein digestibility of the *Sorghum* significantly ($p<0.05$) (Awadelkareem *et al*., 2009). Our results conformed previous studies that cooking reduced phenolic levels.

Materials and methods

2.1 Seed material:

Grains of six genotypes of *Sorghum* HC260, M-35-1, CSV-15, CSV-17, CSV-20, and CSV-22 were provided by Directorate of *Sorghum* Research, Hyderabad.

2.2 Extraction:

The raw seeds were finely powdered by using electric grinder and their extraction was done by Soxhlet extractor using methanol as solvent. After extraction, the solvent was removed in a condenser and dry extracts were stored in plastic bottles. For cooking process, the seeds were soaked overnight with sterile distilled water. Next day the soaked seeds were cooked by autoclaving and then dried in oven. The seeds thus dried were homogenized and using methanol as a solvent, extraction was done using Soxhlet extractor (Mubarak A.E 2005).

2.3 Determination of Total Phenolic Content:

The total phenolic content of the methanol seed extracts of six genotypes of *Sorghum* was measured using Folin-Ciocalteu reagent by the method followed by Singleton *et al*. (1999), with some modifications. 0.5 ml of plant extract (1 mg/ml) was mixed with 1.5 ml (1:10 v/v diluted with distilled water) Folin-Ciocalteau's reagent and allowed to stand for 22°C for 5 min. Then 2 ml of sodium carbonate (Na_2CO_3, 7.5%, w/v) was added and the mixtures were allowed to stand for another 90 min and kept in the dark with intermittent shaking. Then the absorbance of the blue colour was measured at 725 nm using spectrophotometer. Gallic acid was used for constructing the standard curve (Fig.1) (50-500 mg/100ml, $Y= 0.001X$; $R^2= 0.993$). The total phenolic contents in the methanolic extracts of raw and cooked grains of six genotypes were expressed as milligram Gallic acid equivalents per gram of dry weight (mg CE/g) of seed and represented in Fig. 2.

2.4 Determination of Total Antioxidant Capacity:

The total anti oxidant capacity was evaluated by following the method of Prieto *et al* (1999). An aliquot of 0.1 ml of sample solution / ascorbic acid equivalent to 500mg was combined with 1ml of the reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4Mm sodium molybdate). 0.1ml of methanol was used as blank. The tubes were capped and incubated in a boiling water bath at 95^oC for 90 minutes. After the samples were cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695nm against blank. The anti-oxidant activity was expressed as equivalents of ascorbic acid (mg/g) using an ascorbic acid standard curve (Fig. 3) (25 to 125 µg/ml; $Y= 0.014X- 0.063$; $R^2= 0.979$).

2.5 Determination of Total Flavonoid Content:

Aluminium chloride colorimetric method (Woisky, R. and Salatino, A. 1998) was used with some modifications to determine flavonoid content. 1ml of extract was mixed with 3 ml of methanol, 0.2 ml of 10% aluminium chloride, 0.2 ml of 1M potassium acetate and 5.6 ml of distilled water and maintained at room temperature for 30 minutes. The absorbance was measured at 420 nm. Quercetin was used as standard (1mg/ml). Flavonoid contents were determined from the standard curve and were expressed as mg Quercetin equivalent /gm of seed (Fig. 4).

2.6 Determination of Total Carotenoids Content:

Total carotenoids can be extracted in petroleum ether and estimated in UV-visible spectrophotometer at 450 nm. 0.5 gm of seed was homogenized and saponified for about 30 minutes in a water bath at 60°C with a specific volume of 12 % alcoholic KOH. The saponified extract was transferred into a separating funnel containing 10-15 ml of petroleum ether (40-60°C) and mixed well. The lower aqueous phase was transferred to another separating funnel and the upper petroleum ether containing carotenoid pigment was collected separately. The extraction was repeated until the aqueous phase was colorless. A small quantity of anhydrous sodium sulphate was

added to the petroleum ether extract, to remove the turbidity. Absorbance of the extract was measured at 450 nm using a spectrophotometer with petroleum ether as blank (Zakaria *et al*., 1979).

Results

3.1 Total phenol content:

The total phenolic content of the six genotypes ranged between 91.2±0.05 to 242.93±1.47 mg GAE/gm for the raw extract and 60.9±0.03 to 172.33±0.24 mgGAE/gm for the cooked extracts (Table 1). The highest phenolic content was observed for the cultivar CSV-15 of 242.93±1.47mg/gm but cooking drastically reduced its content to 117.03±0.84 mg/gm (Fig 2). Among the cooked seed extracts, CSV-20 showed highest TPC content of 172.33±0.24mg/gm against the raw extract (229.53±0.53mg/gm) as shown in Fig. 2. The genotype HC-260 reported 96.67±0.17 mg/gm and 60.9±0.03 mg/gm for raw and cooked extracts respectively. The genotype M-35-1 reported 91.2±0.05 mg/gm for raw and 85.05±0.21mg/gm for cooked extracts. CSV-17 reported 143.79±0.09 mg/gm for the raw and 133.2±0.2 mg/gm for the cooked extracts. The genotype CSV 22 reported 122.97±0.57 mg/gm for the raw and 101.8±0.31mg/gm for the cooked extracts.

3.2 Total Antioxidant capacity:

The total antioxidant capacities for the six genotypes ranged between 6.2±0.15mg/gm to 21.9±0.23mg/gm. CSV-15 reported highest antioxidant capacity 21.9±0.23mg/gm followed by CSV-20 18.9±0.09mg/gm for the raw seed flour extracts (Table. 1). But cooked extract of CSV-20 showed higher levels of antioxidant capacity 16.33±0.06mg/gm compared to CSV-15 (8.53±0.18mg/gm). Genotype HC-260 reported 6.56±0.08mg/gm and 5.8±0.17mg/gm for the raw and cooked extracts respectively. M-35-1 reported 8.2±0.15mg/gm for the raw and 6.53±0.11mg/gm for the cooked extract. Genotype CSV-17 reported 17.2±0.11mg/gm for the raw and 12.2±0.15mg/gm for the cooked extract. CSV-22 showed 6.2±0.15mg/gm for the raw and 6.2±0.15mg.gm for the cooked extracts.

3.3 Flavonoid content:

The highest flavonoid content was observed for the genotype CSV-15 (28.96±0.13 mg/gm) for the raw and 20.91±0.01mg/gm for the cooked extracts that was cited in Table 1. The order of total flavonoids of remaining five genotypes was as follows: CSV-20(19.24±0.03mg/gm), CSV-17(15.19±0.09mg/gm), CSV-22(11.54±0.13mg/gm), HC260 (11.17±0.09mg/gm), M-35-1(9.06±0.03mg/gm) for the raw extracts and CSV-20(17.18±0.16mg/gm), CSV-17(13.75±0.09mg/gm), CSV-22(9.68±0.01mg/gm), M-35-1(8.00±0.05mg/gm), and HC260 (7.73±0.03mg/gm) for the cooked extracts.

3.4 Total Carotenoid content:

The carotenoid contents in grains were expressed in mg β-carotene equivalents per gm of seed. The highest Carotenoid content was observed for the genotype CSV-20 (23.75±0.07 mg/gm) for the raw seed bran extract and 19.49±0.09 mg/gm for the cooked extract (Fig. 5). Other genotypes comparatively reported very lower contents as quoted in Table 1. The genotype HC-260 reported 4.08±0.05mg/gm and 2.92±0.03mg/gm for the raw and cooked extracts respectively. The genotype M-35-1 reported 2.14±0.02 mg/gm for raw and 1.69±0.02mg/gm for cooked extracts. CSV-17 reported 1.21±0.01mg/gm for the raw and1.02±0.01mg/gm for the cooked extracts. The genotype CSV 22 reported 1.20±0.02mg/gm for the raw and 0.94±0.02 mg/gm for the cooked extracts. While the genotype CSV-15 that reported high profile of TPC, TAC, and TFC reported only 1.78±0.05mg/gm for the raw and 1.09±0.02mg/gm for the cooked extracts. Total Carotenoid content in grains was relatively lower in all the tested samples than polyphenolic and flavonoid contents (Table 1).

Figures:

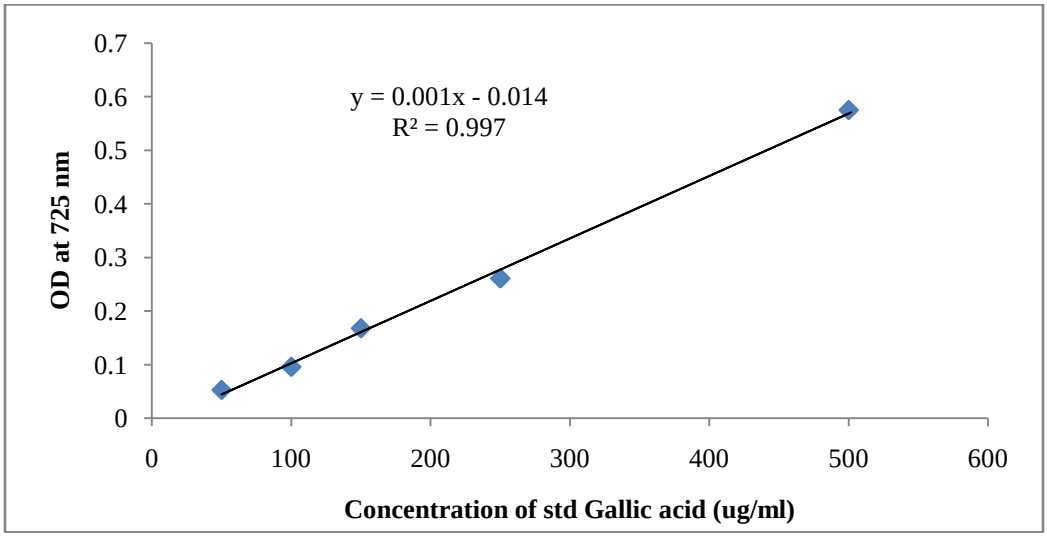

Fig.1. Calibration curve for estimation of Total Phenolic Content.

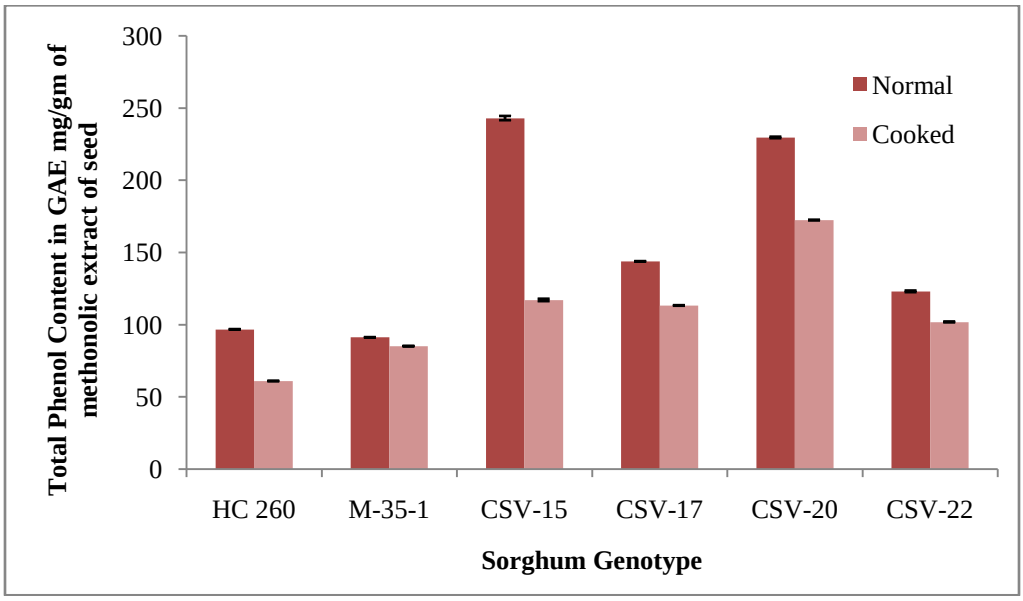

Fig.2. Total Phenolic Content of *Sorghum* genotypes.

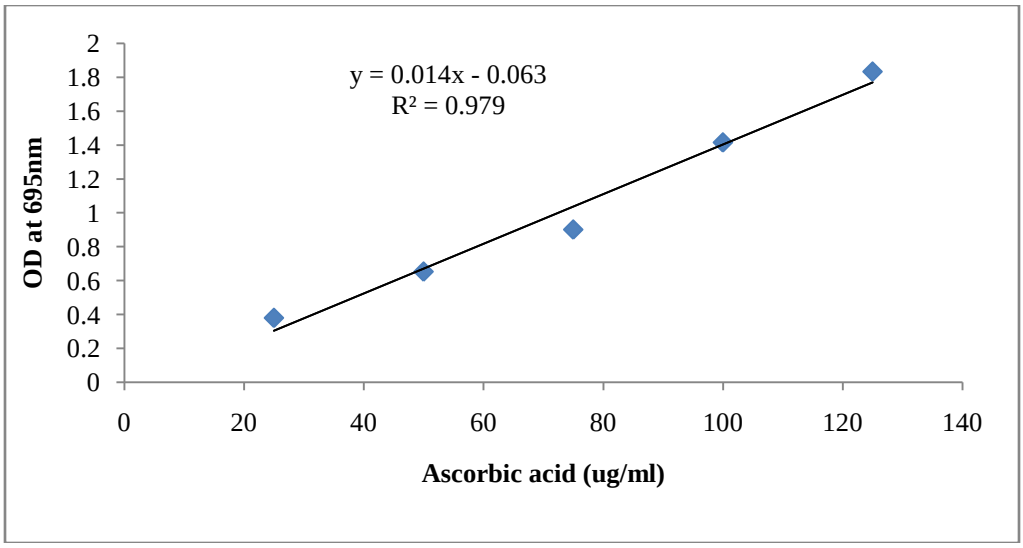

Fig.3. Calibration curve for estimation of Total Antioxidant Capacity.

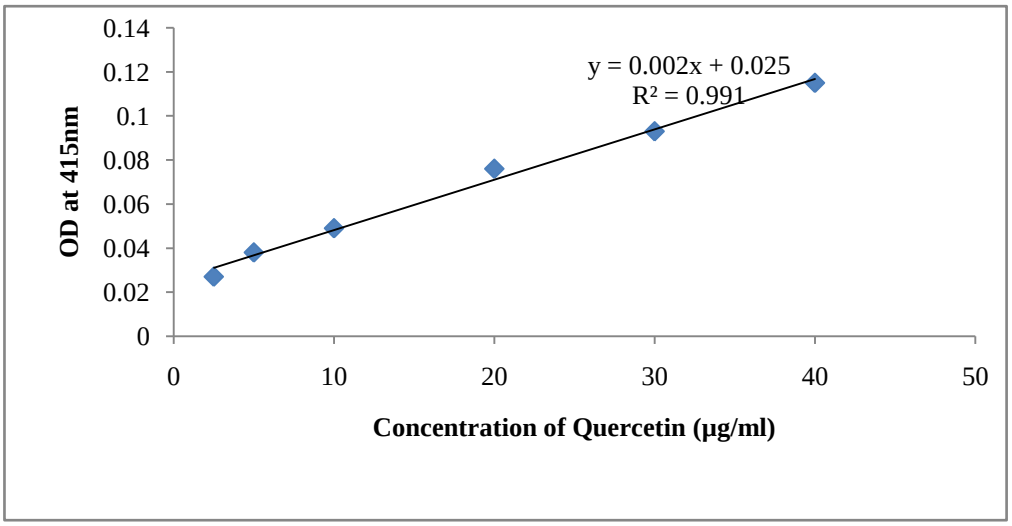

Fig.4. Calibration curve for estimation of Total Flavonoid Content.

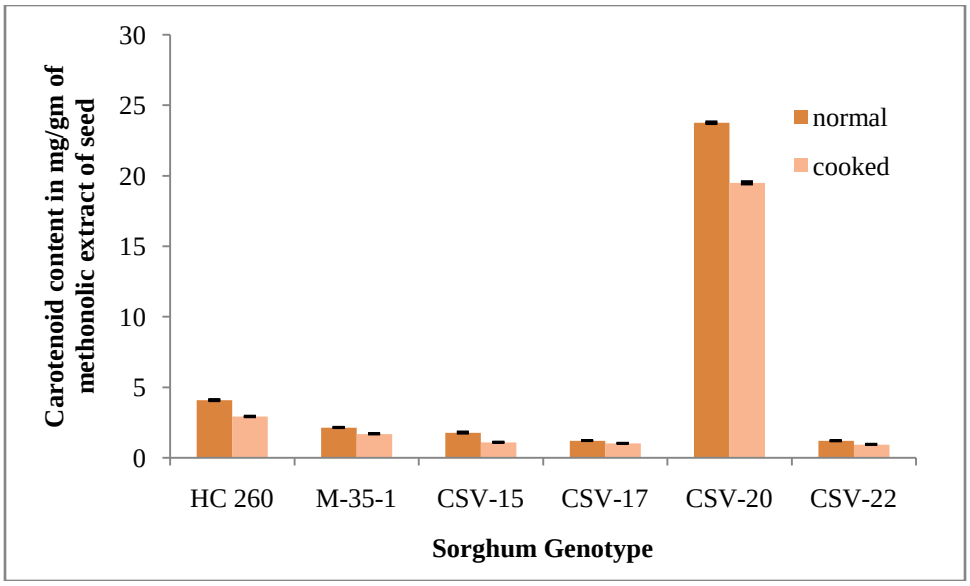

Fig 5: Total Carotenoid content of *Sorghum* genotypes.

Genotype Raw extract	Total phenolic content in GAE mg/gm	Total Anti oxidant Capacity in Ascorbic acid equivalents in mg/gm	Total Flavonoids in Quecertin equivalents in mg/gm	Total Carotenoids in mg/gm
HC 260	96.67±0.17	6.56±0.08	11.17±0.09	4.08±0.05
M-35-1	91.2±0.05	8.2±0.15	9.06±0.03	2.14±0.02
CSV-15	242.93±1.47	21.9±0.23	28.96±0.13	1.78±0.05
CSV-17	143.79±0.09	17.2±0.11	15.19±0.09	1.21±0.01
CSV-20	229.53±0.53	18.9±0.09	19.24±0.03	23.75±0.07
CSV-22	122.97±0.57	6.2±0.15	11.54±0.13	1.20±0.02
Cooked extract				
HC 260	60.9±0.03	5.8±0.17	7.73±0.04	2.92±0.03
M-35-1	85.05±0.21	6.53±0.11	8.00±0.05	1.69±0.02
CSV-15	117.03±0.84	8.53±0.18	20.91±0.01	1.09±0.02
CSV-17	133.2±0.2	12.2±0.15	13.75±0.09	1.02±0.01
CSV-20	172.33±0.24	16.33±0.06	17.18±0.16	19.49±0.09
CSV-22	101.8±0.31	5.56±0.08	9.68±0.01	0.94±0.02

Table 1: Quantification of bio active constituents of raw and cooked methanolic extracts of genotypes of *Sorghum*.

Discussion

The significant importance of *Sorghum* grain is its potentiality as a source of bioactive component. Among the cereals, *Sorghum* and Barley are the two important food grains reported to contain significant quantities of phenolic compounds (Dicko *et al*., 2002). Therefore, studies to identify varieties of *Sorghum* with bioactive constituents of particular interest are very essential. Varieties containing high levels of phenolic acids would be ideal to isolate these bioactive components because of their antioxidant activity that also functions as anti-bacterial agents.

Among phenolic compounds of *Sorghum*, compounds with specific interest are pro-anthocyanins and flavan-4-ols (Dicko *et al*., 2006) because of their importance in human nutrition. These phenolic compounds can be regarded as desirable components of human food because of their antioxidant activity and neutraceutical importance (Awika and Rooney 2004; Parr and Bolwell 2000; Santos-Buelga and Scalbert 2000). The overview of literature in the recent past on studies related to bioactive constituents of *Sorghum* revealed acidified methanolic/methanolic extracts of seed brans to be effective (Oki et al., 2002, Zielinski and Kozlowska 2000). Therefore, methanol was selected as an extraction solvent in our study. In 1983, Hahn *et al*., separated free bound phenolic acids of *Sorghum* by Reverse phase HPLC. In 1989, Waniska *et al*., partitioned *Sorghum* phenolic acids and reported that seeds without pigmented testa contain lowest amount of phenolic acids. Awika *et al.,* (2005) analyzed the total phenolic content for whole grain and bran sample of two Malawian *Sorghum*, phatafuli (A bran colour condensed tannin variety) and shabalala (A white colour condensed tannin variety). The phatafuli variety had higher total phenols and antioxidant activity than the shabalala variety. Javanmardi *et al.,* 2003 examined antioxidant and total phenolic content of Iranian *Oscimum* accessions and obtained a significant correlation between them. Mohammad *et al*., 2009 reported polyphenol of pigmented *Sorghum* lines as potential anti bacterial agents. Effect of germination and cooking on the content of phenols in cereal grains (*Sorghum*, Millet and Leguminous pulses) were done by Towo *et al*., 2003. They reported reduced levels of total phenols for cooked grains than germinated. Dri *et al*., 2012 also evaluated the effect of cooking on antioxidant properties of three African whole grains of *Sorghum*. They reported a negative effect of cooking on total phenolic levels. Dykes *et al*., 2013 evaluated phenols and their anti-oxidant activity of black *Sorghum* hybrids in comparison to its lines. They reported the lower levels of 3-deoxyanthocyanidins, flavan-4-ols, and flavones in hybrids than corresponding lines. They also reported enhanced antioxidant activity of black *Sorghum* hybrids because of presence of tannins. Barros *et al*., 2013 developed accelerated solvent extraction (ASE) method to enhance concentration of phenolics from *Sorghum* brans. They compared effect of conventional/ traditional and ASE extracts at different temperatures for total phenol content and antioxidant capacity of tannin *Sorghum* bran that reported that ASE to be an efficient method that resulted 12% more antioxidants than traditional methods. Antioxidants in grains are difficult to extract due to their solubility of active compounds (Miller *et al.,* 2000). The objective of our research is to investigate the profile of bio-active constituents of methanolic extracts of raw and cooked *Sorghum* grains.

Statistical analysis: All the results were reported as mean±standarderror. Correlation between Total Phenolic Content and Total antioxidant capacity of *Sorghum* genotypes had a correlation coefficient of R^2=0.9 that was significant.

Conclusion

Cooking is the common food processing for the whole cereal grains everywhere in the world. Bioactive compounds in plants can be defined as secondary plant metabolites that have important functions with desirable dietary qualities. The paucity of information available on studies and effect of cooking on phenolic profiles about cultivars of *Sorghum* attained incipiency. Our report provides the profile of bioactive components of *Sorghum* genotypes and suggests the selection of specific cultivars with better phenolic profile for consumption. The summary of our reports revealed, genotype CSV-20 showed good profile for the cooked extracts. Although CSV-15 showed higher profile overall for the raw extracts, cooking drastically reduced its contents. Consuming Food grains with health benefits are very essential. Proper extraction of the phytochemicals from *Sorghum* grains and further research on their bio activity helps to combat lifestyle diseases. Our studies are useful for the *Sorghum* breeders in selecting genotypes with good biochemical profile as elite parental lines. The essence of Scientific researches and its achievements really succeed when their application reach to a common man.

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