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BIOACTIVE SUBSTANCES AND THEIR POTENTIAL THERAPEUTIC PROPERTIES OF SORGHUM [SORGHUM BICOLOR (L) MOENCH]

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Abstract: Sorghum is one of the important dryland C4 crops mainly grown in sub-saharan Africa and Asia and serves as a source of food, feed, fodder and biofuel. It is the main source of dry season ration to the livestock and provides nutrition and security for livelihood of poor farmers in rural areas. Sorghum can withstand drought and comes up well in adverse climatic conditions and hence, it is considered as an important climate resilient crop. Sorghum possesses diverse phytochemicals especially in the bran layer which represents a promising opportunity to be exploited as a functional food and to reduce the risk of non – communicable chronic diseases. Owing to high phenolic content and gluten free nature of sorghum and low glycemic index the grains are increasingly utilized for human consumption realising concept of eat right food for good health. Red sorghum bran is known as a rich source of

anthocyanins. The bran and flour extracts analysis using various colorimetric methods including HPLC (High Pressure Liquid Chromatography) found with presence of several phenolic compounds like anthocyanins particularly 3-deoxyanthocyanin that includes apigeninidin and luteolinidin, condensed tannin, ferulic acid etc., which provide the therapeutic properties of antioxidant, anti-cancer, anti-diabetic, prevention of cardiovascular diseases. The data collected from recent studies indicate that these molecules have a promising future as natural biofood agents for the treatment of various diseases, and this is particularly due to their strong antioxidant properties, in addition to modulating the intestinal microbiota. This review is made to understand the bioactive substances present in sorghum grains, their extraction and their potential therapeutic effects.



Keywords: Sorghum, Gluten free, 3-deoxyanthocyanin, Antioxidant, anti-cancer, cardiovascular disease, therapeutic effects, intestinal microbiota.

I. INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is one of the important dryland crops and is the fifth most important cereal crop in world after rice, wheat, corn and barley (Rao et al., 2013). The origin and early domestication of sorghum is believed to have taken place in northeastern Africa or at the Egyptian-Sudanese border around 5,000–8,000 years ago (Mann et al. 1983). The secondary centre of origin of sorghum is considered to be Indian sub-continent, with evidence for early cereal cultivation discovered at an archaeological site in western parts of Rojdi (Saurashtra) dating back to about 4,500 years (Vavilov 1992; Damania 2002). It is grown in 42 m ha in 98 countries including Africa, Asia, Oceania, and the Americas. Nigeria, India, USA, Mexico, Sudan, China and Argentina are the major producers (ICRISAT).

In India, several processed and value-added ready-to-eat or ready-to-cook food preparations such as cookies, breads, bars, cakes and pancakes, deep-fried food products, flakes, or healthy and convenient products such as multigrain flour, suji have been developed for easy cooking (Dayakar Rao et al. 2014). Moreover, extrusion products for ready-to-eat foods like breakfast cereals, pasta, and vermicelli noodles have been made from sorghum grains (Malleshi, 2015). The grain is mainly consumed in the form of unleavened flat bread or roti or bhakri prepared from the flour. (Hariprasanna et al., 2016). In China, distilled beverages such as Maotai and Erguotou has sorghum as its main ingredient. In many countries, especially in Africa, sorghum is being used as a substitute for other grains in the production of gluten-free beer Thin or thick fermented or unfermented porridge is mainly consumed in Africa (Hariprasanna et al., 2016). In India, sorghum is utilized in the preparation of many traditional daily foods using powder like grain for cooking and bakery products such as bread, cakes, and biscuits. The grain is mainly consumed in the form of unleavened flat bread or roti or bhakri prepared from the flour. (Hariprasanna et al., 2016). Globally, sorghum production was 62 million tonnes in 2020. United States of America stands first in total production with 9.4 million tonnes (15%) followed by Nigeria, Ethiopia, Sudan. India ranks fifth in total sorghum production with 4.7 million tonnes. (USDA, 2020). In India, sorghum production gets decreased after the 'green revolution' possibly due to expanded cultivation of maize for feed and other industries, but it is now reclaiming the momentum owing to numerous health and nutritional benefits (Prasad et al., 2015).

Sorghum grain is composed of pericarp, testa, endosperm and germ from the outside to the inner surface of grain. Testa is located between pericarp and endosperm, which is

unique in sorghum grains and distinct from other cereal grains. After 10 days of anthesis, the testa developed into two layers, the outermost and innermost layers are pigmented and non-pigmented respectively but the walls contained ferulic acid. Pigmented material was deposited differently in the two sorghum types; type II sorghum pigments were deposited in the vesicles, whereas in type III sorghum pigments are deposited along the cell walls of the integument. The method of pigment deposition may reflect the difficulty in extraction of procyanidins (tannins) for nutraceutical use (Earp et al., 2004).

The gluten-free nature of sorghum shows its great potential as an alternative cereal grain for human consumption by eliminating the risk of celiac disease and also for celiac patients. Apart from gluten – free nature, sorghum grain possesses bioactive phenolic compounds, such as phenolic acids, flavonoids (Shenet al., 2018). Flavonoids can be further divided into flavanone, flavonol, anthocyanins, and condensed tannins, known as proanthocyanidins in sorghum (Xuet al., 2021). Sorghum can be used as a substitute for conventional cereals due to its high phenolics, fibre, and carotenoids, etc., (Anunciacao et al., 2017).

Phenolic compounds are quality-grade markers for the preparation of several foods because of enzyme inhibitory activities, colour, or antioxidant activities. Large inter-varietal differences in contents of phenolic compounds and their antioxidant activities among sorghum varieties existed for utilization. Moreover, some red sorghum varieties have higher antioxidant activities than the most important sources of natural antioxidants (Dicko et al., 2006). These bioactive compounds have been linked with multiple health benefits, including cholesterol-lowering, antioxidants, slow digestibility, anti-inflammatory, and anti-carcinogenic properties (Birt et al., 2001). A few sorghum-based products including coarse semolina upma, fine semolina upma, flakes poha and pasta have glycemic index of less than 55 which is lower than their respective wheat/rice-based foods- helps decreasing postprandial blood glucose levels (Prasad et al., 2015), plays a protective role against the development of metabolic disease (Zhang et al., 2006; Du et al., 2008; Nilsson et al., 2008) and breast cancer (Sieriet al., 2007).

Thus, sorghum cereal shows a high nutritional value and, about 75% of polysaccharides, with the highest concentration of resistant starch, followed by soluble (about 25%) and insoluble (about 90%) fibers. This cereal consists of around 15% of protein, with emphasis on kafirins, the main prolamins (about 79%) and, it has about 3 g/100 g of lipids, containing a high concentration of polyunsaturated fatty acids, around 88%, especially linoleic acid (approximately 51%). It also has a high content of monounsaturated fatty acid, especially oleic acid (about 42%). The lipid composition of sorghum may justify the benefits in controlling dyslipidemia, and may contribute to cardioprotective effects (Martino et al., 2012; Cardoso et al., 2017). Sorghum is a source of phosphorus, potassium, zinc



and fat-soluble vitamins (D, E and K), some B vitamins (thiamine, riboflavin and pyridoxine) (Cardoso et al., 2017; Li et al., 2022). In addition, in vivo and in vitro studies have shown an increase in the richness and diversity of the intestinal bacterial community, which can positively modulate the microbiota by phenolic compounds, fat-soluble compounds, fibers and resistant starch from sorghum (Martínez et al., 2010; Cardoso et al., 2017; Sousa et al., 2019; Ashley et al., 2019). Polyphenolic substances have high antioxidant activity in the control of diseases associated with oxidative stress, in addition to offering antiproliferative, antimicrobial, antiobesity and antidiabetogenic properties (Espitia-Hernández et al., 2020)(Figure.1; Table.1).

Therapeutic Properties:

Sorghum grains are unique, in that they contain diverse phytochemicals, particularly polyphenols, which are known to significantly impact human health. Presence of high levels of phenolic compounds viz., luteolinidin, apigeninidin, and 3-deoxyanthocyanins improves antioxidant activity of sorghum flours (Suet al., 2019). Besides, phytochemicals are mostly concentrated in the bran fraction; and are reported to be beneficial in the prevention of metabolic syndromes such as type 2 diabetes, obesity, hypertension and certain cancers (Althwabet al., 2015 and Awika et al., 2018). The intake of phenolic compounds is thought to have more health benefits such as reducing oxidative stress that promotes curing of number of chronic diseases such as diabetes, cancer, cardiovascular diseases, cataract and inflammation and providing anti-inflammatory, anti-carcinogenic properties (Sosa et al., 2013). Sorghum has most diverse types and amounts of the major polyphenols (Awika et al., 2018), that are synthetically modified and directly provided by diet would have the ability to scavenge free radicals and modulate various signalling pathways relevant to disease prevention (Girard and Awika, 2018) and may thus decrease noxious effects due to oxidative stress (Meo et al., 2013).

Most of the flavonoids and other therapeutic metabolites are more abundant in dark red pericarp than in light red and white pericarp accessions (Ramalingam et al., 2021). Red sorghum bran is known as a rich source for anthocyanins. The contents of anthocyanins appeared to be associated with the sorghum pericarp color, but a diversity of anthocyanin contents was present among and between the phenotypic pericarp colors (Davis et al., 2019). The antioxidant activity of the red sorghum bran was directly related to the total anthocyanin found in red sorghum bran (Devi et al., 2011). Anthocyanins are reported to have some therapeutic benefits including vasoprotective and anti-inflammatory properties (Tsuda et al., 2002) and anticancer (Zhao et al., 2004) as well as hypoglycemic effects (Tsuda et al., 2003). Sorghum brans had 3-4 times higher anthocyanin contents than the whole grains. Black sorghum had highest anthocyanin

content (average = 10.1 mg/g in bran) followed by brown and red sorghum brans of 2.8 – 4.3 mg/g (Awika et al., 2004). All of the sorghum grains contained high contents of phenolic acids, especially ferulic acid. It is therefore important to consider the genotype while selecting sorghums for human food and animal feed so as to obtain maximum energy and protein availability and they can be used as high antioxidant value-added health foods having nutraceutical or pharmaceutical applications (Bhukya et al., 2020).

Grain colour and Phenolic compounds:

Sorghum grains come in a wide variety of color such as red, yellow, pearly white black, dark red and reddish brown. At least 10 pair of genes that affect sorghum grain color have been identified. The pericarp color is determined by the genes: $R\_Y\_I\_S\_B1\_B2$. The genes P_Q and $Tp\_$ affect the expression of this basic pool of genes. The phenotypic pigments of sorghum pericarp and endosperm are determined by genotypic factors (Suet al., 2017; Salunkhe et al., 1990). The genes RR and YY determine the grain color and its appearance. $R\_Y\_$ red epicarp, R_yy white epicarp, $rrY\_$ lemon yellow epicarp, $rryy$ white epicarp. These genes interact epistatically to produce the observed colors (Rooney et al., 2009). Red pericarp turn black when maturing in the presence of sunlight and thus are termed as “black” sorghum (Dykes et al., 2005). The color of testa layer is dependent on the $TpTp$, $tptp$ genes. The dominant $TpTp$ presents a brown pigmentation and the recessive ($tptp$) a purple pigmentation. The pigmentation is determined by $B1B1$ and $B2B2$ genes. $B1\_B2$ pigmented testa, the interactions $B1\_b2b2$, $b1b1B2\_$ and $b1b1b2b2$ express non pigmented testa.

The presence of tannin in testa layer is positively correlated with the presence of dominant genes $B1B2$. The pigmentation in testa layer produced by these genes is the effect of the condensed phenols. The amount of tannin is determined by the presence of intensifier ($I\_$) and spreader genes ($S\_$), interacting with genes $B1\_B2$. $Tp\_$, $tptp$ and homozygous recessive gene zz controls the thickness of pericarp. Genes P_Q that affect plant color, in general, are correlated with glume color but some plants have a glume color different from the plant color (Rooney et al., 2005).

Not present in other major cereals, such as rice, wheat, and maize, condensed tannins (proanthocyanidins) in the pigmented testa of some sorghum cultivars have been shown to promote human health because of their high antioxidant capacity and ability to fight obesity through reduced digestion. Combining quantitative trait locus mapping, meta-quantitative trait locus fine-mapping, and association mapping, reported that the nucleotide polymorphisms in the $Tan1$ gene, coding a WD40 protein, control the tannin biosynthesis in sorghum. Wu et al., 2012 reported that sequence annotation indicated that Sb04g031730.1 encodes a WD40 protein; thus, it was designated as Tannin1 ($Tan1$).



A major type of pigments responsible for the colours as well as the health benefits of the cereals are anthocyanins (Zhu, 2018). Understanding the anthocyanin composition in diverse coloured cereals can be critical for food processing and human nutrition (Collison et al., 2015). Purple maize kernel tends to have a higher anthocyanin content including CGE (cyanidin 3-glucoside equivalent), than blue and red samples (Collison et al., 2015). Cyanidin 3- glucoside and peonidin 3-glucoside appeared to be the major anthocyanins in the kernels of diverse rice genotypes (Zhu et al., 2017; Hao et al., 2015). Like the other cereals, in barley most of the anthocyanins concentrate in the bran of the kernels (Lee et al., 2013). Pigmented sorghum bran has high levels of unique 3-deoxyanthocyanidins, which are stable to change in pH and have a good potential as natural food pigments (Awika et al., 2004).

Maize samples contained anthocyanin in both the pericarp and aleurone layer. Total anthocyanin content among samples ranged from 54 mg to 115 mg/100 g of sample (Moreno et al., 2005). The most abundant phenolic compound in whole maize grain is ferulic acid (4-hydroxy-3-methoxycinnamic). Its content in maize is around 216-3400 mg/100g or 2.16 – 34 mg/g of maize (Zhao & Moghadasian, 2008). Maize possess anthocyanin, ferulic acid, phytosterols etc., with anti-oxidant, anti- diabetic, anti- atherogenic, low LDL cholesterol (Roufet al., 2016). Though Maize possesses phytochemicals, it was lower in content than that of sorghum grains. This shows sorghum has more nutraceutical or therapeutic benefits than Maize. But these purple/ dark red/ purple genotypes are rarely available in genetic resources and yet to be brought to cultivation in such fully cross-pollinated crops.

Table.1. Bioactive compounds of sorghum;

S. No	Biochemicals	Bioactive principles	Reference
1.	Extractable phenolic acids 15–1650 µg/g	Mostly caffeoyl glycerides; some feruloyl and coumaroyl esters. The synthesis of Monocaffeoylmonofatty acyl glycerols could improve the antioxidant properties of Caffeic acid.	(Awika and Rooney, 2004, Chiremba et al., 2012) Weng et al., 2020
2.	Bound Phenolic acids 430–1200 µg/g	Mostly ferulic acid derivatives Anti-inflammatory, antioxidant, antimicrobial activity, anticancer, and antidiabetic effect and free radical scavenger.	(Awika and Rooney, 2004, Chiremba et al., 2012) Zdunska et al., 2018
3.	Flavonoids (3-deoxy) anthocyanin 200–4500 µg/g	Exclusively 3-deoxyanthocyanins Antioxidant, anti-inflammatory, anti-proliferative, anti-diabetic, and anti-atherogenic activities	(Awika et al., 2004) Xu et al., 2021
4.	Flavones 20–390 µg/g	Apigenin, luteolin, and their glycosides Antioxidant, anti-inflammatory, anti-proliferative, anti-diabetic, and anti-atherogenic activities	(Dykes et al., 2011, Yang et al., 2015) Xu et al., 2021
5.	Flavanones 0–2000 µg/g	Naringenin, eriodictyol, and their O-glycosides in white, red, and lemon-yellow varieties	(Dykes et al., 2009, Dykes et al., 2011, Yang et al., 2015)
6.	Flavan-3-ols 0–33,000 µg/g	Present in some varieties, primarily B-type linkages, highly polymerized (mDP 20)	(Awika et al., 2003, Girard et al., 2018)



Assessment of sorghum phenolic compounds:

Total phenolic content (TPC) can be determined using Folin-Ciocalteu method (Liu et al., 2018; Devi et al., 2012; Shen et al., 2018). By Folin-Ciocalteu method, several scientists have reported varied range of total phenolic content of sorghum grains. Shen et al., 2018 reported that, the TPC showed obvious variations among different varieties ranging from 174.40 ± 4.09 to 1238.83 ± 31.67 mg GAE/100 g grain. The presence of TPC in most sorghum whole grain is $0.46 \sim 20$ mg GAE/g. The content of TPC reported in sorghum bran varies even more, ranging from $0.18 \sim 70$ mg GAE/g (Li et al., 2021). Red sorghum bran contained a higher amount of total phenols i.e., 33.18 mg/g. TPC content in sorghum grains with red pericarp is 3.38 ± 0.20 mg/g GAE (Khoddami, et al., 2015). Apart from sorghum, total phenols in raw samples of pearl millet ranged from $268.5 - 420$ mg/100g of DW and $247.5 - 335$ mg/100g of DW in cooked recipes (Nambiar et al., 2012). TPC showed a continuous growth from 242 ± 24 to 304 ± 10 mg GAE/100g DW during the maize kernel development (Zhang et al., 2020). Chandrasekara et al., (2012) reported that the dehulled grains of foxtail millet and pearl millet has a total phenolic content of 3.80 μ mol FAE/g and 8.50 μ mol FAE/g, respectively. Derivative compounds of phenolic acid were assayed using High Performance Liquid Chromatography (HPLC) (Mizziet al., 2020; Liu et al., 2018).

Antioxidant potential can be determined through 1,1-diphenyl-2-picrylhydrazyl (DPPH); radical scavenging test (Liu et al., 2018); **ABTS** [2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)] and FRAP (Ferric reducing antioxidant power) (Kumari et al., 2021). The antioxidant capacity of free fractions among all varieties of sorghum showed significant differences ($p < 0.05$), ranged from 0.90 ± 0.01 to 18.43 ± 0.88 mg/g VcE (DW) and 2.02 ± 0.05 to 11.66 ± 0.39 mg/g VcE (DW) by the DPPH and FRAP assay, respectively (Shen et al., 2018). In the DPPH assay, highest antioxidant activity was exhibited by genotype IS13116 at 21.02 ± 5.17 mg/g TE and the lowest by QL12HT at 0.33 ± 0.10 mg/g TE. (Rao et al., 2018). Genotype SSG 59-3 ($20.55 \pm 0.11a$, $45.66 \pm 0.23b$, $15.34 \pm 0.10c$) had higher antioxidant activity evaluated by DPPH, ABTS and FRAP assay (Puniaet al., 2021). The DPPH activity of dehulled pearl millet flour was found to be 13.8 μ mol FAE/g whereas foxtail millet flour showed 6.77 μ mol FAE/g in a study conducted by Chandrasekara et al., 2012.

Condensed tannins in sorghum and millet flour and bran samples were extracted with vanillin-HCl (Ahmad et al., 2018). The standard used for condensed tannin determination is catechin. The condensed tannin content in different types of sorghum brans was recorded lowest for fine sorghum bran (172.38 mg/100g) and highest for coarse sorghum bran (179.77 mg/100 g) (Ahmad et al., 2018). The

red variety of sorghum had higher tannin content: condensed tannin, 0.035 g/100 g DM and hydrolysable tannin 0.094 g/100 g DM than the white variety found with condensed tannin 0.016 g/100 g DM and hydrolysable tannin 0.062 g/100 g DM (Ojediran et al., 2018). HHB-223 cultivar depicted the highest amount of condensed tannin contents (138.45 mg CE/100 g DWB).

Total flavonoid contents (TFC) can be determined by aluminum chloride method (Chang et al., 2002; Derby et al., 2016; Ofosu et al., 2020). Flavonoids constituted the main phenolic class in the free phenolic fraction. TFC in decorticated sorghum grain varieties ranged from 110.3 to 126.5 mg catechin equivalent/100 g, DW (Ofosu et al., 2020). TFC of eight sorghum grains ranged from 11.72 ± 1.69 to 61.10 ± 5.46 mg RE/100 g grain (total, DW) (Shen et al., 2018). White maize had a lower total flavonoid content (248.64 mg CE/kg d.m.) than those of red and dark red ones (267.58 and 270.54 mg CE/kg d.m., respectively) as well as light and dark blue maize (337.51 and 307.42 mg CE/kg d.m., respectively) (Zilicet al., 2012).

Anthocyanin extraction can be of Two types; conventional and microwave assisted extraction (MAE); Microwave Assisted Extraction resulted in significantly improved extraction efficiency (yield and speed) of 3-DXA from sorghum compared to conventional acidified methanol extraction. 1 min of MAE yields $1570\text{--}2230$ μ g/g of 3-DXA which is similar to or higher than the 2 h of conventional extraction of 1520 μ g/g. (Herrman et al., 2020). Devi et al., 2012 reported that the amount of anthocyanin obtained through acidified methanol extract of red sorghum bran polyphenols showed 4.7 mg/g, which is greater to 1.95 and 1 mg/g obtained through methanol and acetone extract, respectively. Similarly, the highest total flavonoids (143 mg/g) and total phenolic contents (0.93 mg/g) were obtained in acidified methanol extracts than methanol and acetone extracts. UV-Vis spectrophotometer can be used in the quantification of Total Anthocyanin Content (TAC). Brewing sorghum grains and seed-reserved sorghum grains both had high levels of apigeninidin, with the total content ranging from 2.89 ± 0.12 to 4.77 ± 0.29 mg/100g grain (DW) (Shen et al., 2018).

Anthocyanins were characterized using HPLC (Perkin Elmer, USA) outfitted with UV-Vis detector, column C18 thermostated at 40°C . (Ahmad et al., 2018). The presence of methylated 3-deoxyanthocyanidins in red sorghum bran was identified for the first time using LCMS-Liquid Chromatography Mass Spectrometry (Suganyadevi et al., 2021).

With all the references quoted above, sorghum is considered to have diverse phytochemicals compared to maize, pearl millet, etc., and hence found to have comparatively more therapeutic effects. Sorghum and millets have widest varieties of phenolic acids. Flavonoids are located in the



pericarp of all cereals. Thus far, sorghum has the widest variety of flavonoids reported by Awika et al., 2005. Sorghum and pearl millet has nutrient content better than or similar to rice and wheat since there is a considerable variation in sorghum for levels of proteins, lysine, lipids, carbohydrates, fiber, calcium, phosphorus, iron, thiamine, and niacin (Kumar et al, 2016).

Potential therapeutic effects of grain sorghum:

Anti-cancer

Consumption of sorghum-based food could reduce the risk of certain types of cancer, which may be due to the high concentration of proanthocyanidins in it (Awika & Rooney, 2004). Red and brown pericarp genotypes had higher total proanthocyanidin and total phenolic content, therefore, resulting in overall high antioxidant activities (Punia et al., 2021). Currently, sorghum is the only known natural food source of the 3-deoxyanthocyanin (3-DXA) (Awika et al., 2004) and its unique properties may extend their biochemical activity as well. Shih et al., (2007) recently demonstrated that the major sorghum 3-DXA aglycons, apigeninidin and luteolinidin, were more cytotoxic to human cancer cells than their anthocyanidin analogues, cyanidin and pelargonidin. The concentrations of the sorghum-specific 3-deoxyanthocyanidins luteolinidin and apigeninidin were higher in red sorghum (Punia et al., 2021). More of the 3-deoxyanthocyanidins apigeninidin and luteolinidin are associated with more anticancer activity. Furthermore, luteolinidin suppressed colon cancer stem cells proliferation more than apigeninidin (Massey et al., 2014).

All of the sorghum extracts due to the presence of due to apigeninidin and luteolinidin showed relatively strong antiproliferative activity against the HT-29 colon cancer cells and the antioxidant activity of anthocyanin from red sorghum bran also showed moderate cytotoxic activity against HT 29 and HEP G2 cell lines. So the anthocyanins extracted from easily available red sorghum bran would be a valuable source for antioxidant and antiproliferative activity in food industry (Devi et al., 2010).

The dietary consumption of high phenolic sorghum bran offers an alternative and safe colon cancer preventive measure that targets multiple molecular pathways (Lee et al., 2021). The PI3K (Phosphoinositide 3-kinase)/protein kinase B (AKT) pathway plays an important role in cancer cell proliferation, survival, motility, and metabolism signalling, and is one of the most frequently deregulated pathways in colon cancer (Zhang et al., 2011). Dietary compounds that inhibit the activation of PI3K/AKT have been proposed as potential anti-cancer preventives (Suvarna et al., 2017). Human colon cancer cells were pre-treated with sorghum bran extracts (HP, SC and Sumac), followed by co-treated with IGF-1, using LY294002 (a selective inhibitor of PI3K) as a positive control. This results in

marked induction of phospho-AKT in the IGF-1 (insulin-like growth factor-1)-treated colon cancer cells.

Cytotoxic studies of sorghum anthocyanins extracted from red sorghum bran, showed 84.09% of inhibition in the proliferation of human breast cancer cell line MCF 7. The sorghum 3-deoxyanthocyanins induced apoptosis in MCF 7 was mediated by stimulation of the p⁵³ gene and down regulation of the bcl 2 gene (Suganyadevi et al., 2013). Sorghum tannins have been shown to inhibit aromatase, which is an enzyme involved in breast cancer and thus prevent the formation of undesirable cancer growth stimulus (Hargrove et al., 2011);

Treatment of Hepatocarcinoma (HepG2) with the extracted phenolics resulted in reduction in cell numbers. The underlying mechanisms were further examined using the highest phenolic content and the lowest IC₅₀, resulting in a non-cytotoxic decrease in cell number that was significantly correlated with increased cell cycle arrest at G2/M and apoptotic cells in both HepG2 and Caco-2 cells. Taken together, these results indicated, for the first time, that inhibition of either HepG2 or adenocarcinoma Caco-2 cell growth by phenolic extracts was due to cytostatic and apoptotic but not cytotoxic mechanisms, suggesting some specialty sorghums are a valuable, functional food, providing sustainable phenolics for potential cancer prevention (Chen et al., 2021).

Pre-treatment of Ovarian cancer (OVCA) with ethanol extract of sorghum grains led to chemosensitization and the proliferation and the colony formation of OVCA cells were reduced by 14.7 to 44.6% and 36.4 to 40.1, respectively (Diet al., 2016).

Anti-diabetic

The prevalence of obesity and diabetes is a global health problem which has received increased attention over the past two decades (Bagheri et al., 2018). Due to the side effects of anti-diabetic medications involving the use of insulin and oral hypoglycemic agents, there is an increasing demand by patients to use natural products exhibiting anti-diabetic activity (Chung et al., 2011). The starches and sugars in sorghum are released more slowly than in other cereals and hence it could be beneficial to diabetics (Ratnavathi et al., 2020).

Ferulic acid which is one of the most abundant phenolic acids in sorghum grains have a protective and therapeutic effect on diabetic nephropathy by reducing oxidative stress and inflammation (Choi et al., 2011). The benefit of flavonoid intake is in the sense of reducing the amount of Reactive Oxygen Species-ROS, with a direct effect on pro-oxidant enzymes or enzymes that have antidiabetic action (Habtemariam and Varghese, 2014).

The anti-diabetic effect of grain sorghum extract was due to its ability to inhibit the hepatic gluconeogenesis enzymes, which was similar to that of anti-diabetic drugs (Kim & Park, 2012) - The hypoglycemic effect of sorghum extract



was related to hepatic gluconeogenesis but not the glucose uptake of skeletal muscle, and the effect was similar to that of anti-diabetic medication. Grain sorghum phenols have also been shown to inhibit hepatic gluconeogenesis enzymes thereby promoting endogenous insulin sensitivity. Procyanidin A2, a condensed tannin as a potent anti-diabetic agent that exhibits significant glucose-6-phosphatase inhibitory activities and down regulated mRNA level in diabetic mice as well as increases glucose uptake in hepatocytes and myoblast cells (Sheikh et al., 2019).

Sorghum extracts inhibited *in vitro* activity of enzymes like human pancreatic and salivary α -amylase (Kim et al., 2011). Thus, the inhibition of digestive enzymes, to prevent glucose digestion, may be the first step in anti-diabetic mechanism of sorghum phenolics on human (Links et al., 2015). Understanding of the glycaemic index (GI) and glycaemic load (GL) of staples can help in choosing suitable foods for the prevention and control of diabetes (Prasad et al., 2015). The four key compounds (β -sitosterol, campesterol, propyleneglycolmonoleate, and 25-Oxo-27-norcholesterol) detected in Sorghum bicolor might ameliorate Type 2 Diabetes mellitus severity by activating the PPAR signalling pathway (Oh et al., 2020). Results demonstrate that a SE appeared to have an antidiabetic effect and may have exerted its therapeutic effects through peroxisome proliferator-activated receptor- γ (PPAR- γ) overexpression in mice fed a High Fat diet (Park et al., 2012). Sorghum bicolor and its flavonoid rich extracts could be considered as supplemental and or functional foods having beneficial effects against blood coagulation-induced ischemia, possibly thromboembolism disease, as well as diabetes (Nguyen et al., 2014).

Celiac disease

Celiac disease (CD) is an autoimmune condition characterized by a specific serological and histological profile triggered by gluten ingestion in genetically predisposed individuals (Fasano et al., 2012). Currently, the only treatment for celiac disease is a life-long, strict gluten-free diet leading to improvement in quality of life, ameliorating symptoms, and preventing the occurrence of refractory celiac disease (Cai et al., 2019). *In silico* analysis of the recently published sorghum genome predicts that sorghum does not contain peptides that are toxic for celiac patients. Molecular evidence for the absence of toxic gliadin-like peptides in sorghum, confirming that sorghum can be definitively considered safe for consumption by people with celiac disease (Pontieri et al., 2013). Replacing routine wheat chapathi with jowar roti can help manage celiac. Wheat products and some other grains like barley and rye produce certain toxic metabolites which are responsible for symptoms of this disease.

Obesity

In vivo studies reported that sorghum administration increased satiety, decreased adiposity, waist circumference and body fat percentage (Stefoska-Needham et al., 2016; Anunciação et al., 2018). Other studies have also shown that components present in sorghum, such as tannin, can influence the reduction of body weight by modifying the starch, making it resistant, which makes its digestibility difficult. This fact occurs by inhibiting digestive enzymes such as amylase, protease and lipase, leading to beneficial effects for obesity, a chronic non-communicable disease exponentially growing in the world (Barros et al., 2012; Sánchez-Zapata et al., 2015). Fermentation of resistant polysaccharides in the intestinal microbiota may also be associated with obesity control, due to production of SCFA involved in the decrease of lipogenesis (Li et al., 2017). For example, SCFA acetate, a small metabolite related to the decrease in the accumulation of abdominal fat (Yamashita et al., 2009), butyrate and propionate with activation of intestinal gluconeogenesis, through the regulation of food intake, promoting homeostasis in lipid and glucose metabolism (Kasubuchi et al; 2015; Dugas et al., 2018).

Cardiovascular disease

High cholesterol and Obesity, along with some unhealthy life style habits like consuming alcohol, cigarette smoking, hypertension, lack of sleep etc., are the major cause of cardiovascular disease. Atherosclerosis is the dominant cause of cardiovascular disease (CVD) including myocardial infarction (MI), heart failure, stroke and claudication (Frostegard, 2013). Sorghum kafirin extract had a good antioxidant potential as evidences in both *in vitro* and *in vivo* studies. The *in vivo* study confirmed that sorghum kafirin reduced the total cholesterol (TC) levels and increased the high density lipoprotein cholesterol (HDL-C) levels in hyperlipidemic rats, suggesting that sorghum kafirin fraction can potentially reduce the risk of cardiovascular disease

(Cruz et al., 2015). Secretion of the pro-atherogenic cytokine tumor necrosis factor- α induces the expression of endothelial adhesion molecules, such as P-selectin, vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1), which mediate attachment of circulating monocytes and lymphocytes. It is reported that the extract significantly decreased the expression of VCAM-1, ICAM-1 and the pro-inflammatory factor cyclooxygenase-2 *in vitro*. Meanwhile, the extract significantly increased the expression of the anti-atherogenic factor heme oxygenase-1 (Ham et al., 2019).

Dyslipidemia is an established risk factor for cardiovascular disease (CVD). Dyslipidemia may be defined as increased levels of serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), or decreased serum high-density lipoprotein cholesterol (HDL-C) concentration (Kumari et al., 2021); all these can be resulted from the action of phytosterols, polycosanols and



phenolic compounds, that regulate absorption, excretion and synthesis of cholesterol (Birhanu, 2021). The benefit of raising HDL-C, consequently reduce Cardio Vascular events in appropriate patients (Sirtoriet al., 2022). Lipid fraction may also affect cholesterol absorption by altering the gut microbiota. The addition of sorghum lipid fraction to the diet of hamsters increased HDL-c (High density lipoprotein cholesterol). HDL reduces atherosclerosis by multiple mechanisms, leading to a reduced risk of cardiovascular disease, and HDL-C, as a metric of HDL quantity, is inversely associated with cardiovascular disease, independent of LDL-C (Ito et al., 2020). Sorghum Distillers Dried Grains with Soluble (DDGS) lipid extract lowers plasma and liver cholesterol concentrations similarly to grain sorghum lipid extract from whole kernels. DDGS has been widely used for its high nutritional content and low production cost in the feed industry (Marie et al., 2021). Mechanisms by which this occurs are due, at least in part, to increased faecal neutral sterol excretion (i.e., decreased cholesterol absorption) (Hoi et al., 2009). Oral administration of SE inhibits hepatic cholesterol biosynthesis that significantly reduces serum or liver cholesterol concentrations by, particularly through a decrease in 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), sterol regulatory elementary binding protein 2 (SREBP2) or fatty acid synthase (FAS), and an increase in active AMP-activated protein kinase (AMPK) expression in high-cholesterol diet-fed mice (Kim et al., 2015).

Intestinal Microbiota Modification Caused by Sorghum

A potential nutritional strategy is presented by the chemical composition and bioactive compounds of sorghum for host intestinal and metabolic homeostasis. These compounds are responsible for increasing concentrations of short-chain fatty acids (SCFA), which are energy sources for intestinal cells, besides adjusting the intestinal epithelium and tight junctions; thus contributing to greater diversity of the bacterial community and maintenance of intestinal health (Woting et al., 2014; Ritchie et al., 2015). Studies have shown *Prevotella* genus dominance in a high-fiber sorghum cereal diet (Chen et al., 2017). Moreover the relative abundance of *Bacteroidetes* and an increase in the genus *Roseburia*, producer of butyrate, which is most responsible for the supply of energy to colonocytes were observed (Ashley et al., 2019). Another study pointed out that cereal-based diets, the consumption of cereal fibers, including sorghum, increased SCFA production and increased relative abundance of bacterial families *Bacteroidaceae*, *Bifidobacteriaceae*, *Lactobacillaceae*, *Prevotellaceae*, *Ruminococcaceae* and *Veillonellaceae* (Gamage et al., 2017). Therefore, dietary fibers may interfere with intestinal mucosal lining integrity of the gut and its immune and

inflammatory responses associated with immune tolerance to the microbiota (Ashley et al., 2019; Risdon et al., 2021). In this context, fibers impact the composition and functionality of bacterial communities, indicating as a biotherapeutic intestinal immune system factor. Since it participates in the lymphoid tissue modulation and maturation, in addition to stimulating cytokines and chemokines production of associated with intestinal microbiome homeostasis (Risdon et al., 2021).

As for the resistant sorghum starch, administration in obese rats revealed an increase in *Bifidobacterium* and *Lactobacillus* populations, with a reduction in *Enterobacteriaceae* (Shen et al., 2015). Furthermore, the lipid content may also show an impact at intestinal microbiota composition, increasing *Bifidobacterium* spp concentrations, as described by Martínez et al. (2009). In addition, dietary phenolic compounds, tannins and anthocyanins effects on the increase of *Bifidobacterium* spp and *Lactobacillus* spp and, *Propionibacterium* spp, *Bacteroides* spp, *Salmonella typhimurium*, *Clostridium* spp, *Escherichia coli* and *Streptococcus mutans* decrease (Cardoso et al., 2017)

II. CONCLUSION:

The phenolic compounds in sorghum grain, in terms of the extraction method, profile and biological functions are reviewed for knowledge dissemination of utilization. Sorghum contains diverse bioactive substances with potential therapeutic effects including anti-cancerous activity of 3-deoxyanthocyanin; anti-diabetic and prevention of cardiovascular diseases. Gluten – free nature of sorghum makes it a highly preferred cereal for human consumption especially for celiac patients. Sorghum Kafirin is considered to be a main component to prevent cardiovascular diseases because of its ability in reducing total cholesterol level and increasing high density lipoprotein cholesterol. Furthermore, functional compounds in sorghum can promote the health of the intestinal bacterial community by modulating the microbiota. However, because of tannins and other phenolics present in sorghum it has high potential as a nutraceutical and functional ingredient for use in food processing.

Currently, there is rapid population growth throughout the world, so the need for nutritious and eat right foods that have the capacity to improve the quality of life is very important including affordability to low-income people across world. Because of nutritional deficiencies and health problems prevalence, a good alternative would be the incorporation of sorghum and its co-products in human diets. Hence, diverse sorghum based traditional foods as well as value added or an innovative bakery product for human consumption has to be launched in the market.

Figure.1 Red Sorghum Genotypes in Southern India serving as Traditional Food crop





III. REFERENCES

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