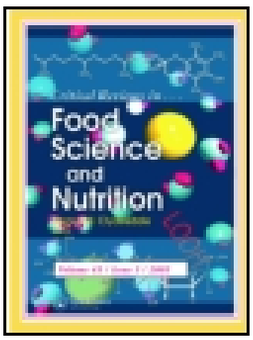

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Grain and sweet sorghum (*Sorghum bicolor* L. Moench) serves as a novel source of bioactive compounds for human health

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Abstract

Grain sorghum is an important staple food crop grown globally while sweet sorghum is increasingly considered as a promising biofuel feedstock. Biofuels are the major economic products from the processing of large quantities of biomass, which is currently being utilized to make value-added products in the biorefinery approach. To date, these value-added products are typically commodity chemicals and waste materials used in agriculture. However, there are opportunities to generate high value bioactive compounds from sorghum grain and biomass. Chronic diseases, such as cancers, are the top causes for morbidity and mortality in developed nations and are promoted by inflammation and oxidative stress. Globally, colorectal cancer results in approximately one-half million deaths annually. It is estimated that as much as 80% of colorectal cancer cases can be attributed to environmental and dietary factors. The sorghum grain and ligno-cellulosic biomass generated for biofuel production has been reported to be high in

bioactive compounds, including phenolic acids and flavonoids, with antioxidant and anti-inflammatory properties. This review focuses on the bioactive compounds of grain and sweet sorghum (*Sorghum bicolor* L. Moench), for their anti-inflammatory, antioxidant, anti-colon cancer and immune modulator functions. The review summarizes previous efforts to identify and quantify bioactive compounds in sorghum and documents their anti-cancer biological activities. Finally, this review discusses bioactive compound extraction methodologies and technologies as well as considerations for incorporating these technologies into current biorefining practices.

Highlights

- **Sorghum is a rich source of several bioactive compounds like phenolic acids, flavonoids, and stilbenoids possessing antioxidant, anti-inflammatory and anti-proliferative activity.**
- **3-deoxyanthocyanidins (3-DXA) are novel anthocyanidins present in different parts of sorghum with putative anti-cancer properties against human colon cancer stem cells *in vitro*.**
- **Unexplored opportunity exists in grain sorghum based floor mills and sweet sorghum-based biorefineries to extract bioactive extracts/compounds on commercial scale.**

1. Introduction

The cultivars of sorghum are C4, graminaceous, drought-resistant plants with a growing season of approximately four months. Sorghum is the fifth most important crop globally, in terms of area and production. It is grown in over 44.96 m ha in about 111 countries with a cumulative grain production of 68.94 million tonnes (http://www.fao.org/faostat/en/#data/QC). Global sorghum economy falls broadly in two production and utilization systems. The first system is characterized by low-input, extensive production systems in developing regions of Africa and Asia besides parts of Latin America that grows sorghum in over 39 m ha with a productivity of less than 1 t ha^{-1} where sorghum is primarily used as a food crop and stover is fed to livestock. In contrast, the developed world has intensive and commercial system that produces 40% of the global sorghum grain, which is primarily used for feeding the livestock, and industrial uses besides international trade.

There is a large and growing pressure on the finite supply of fossil fuels due to the increasing demand for oil for energy and chemicals (Uggetti et al., 2014). Furthermore, the use of fossil fuels for energy is a major contributor to climate change (Cherubini, 2010). For this reason, there is a need for alternative strategies to reduce oil dependence (Uggetti et al., 2014). Biorefining is a strategy, similar to oil refining, by which biomass is utilized for the production of biofuels, energy and derived by-products (Thomsen, 2005). This could be accomplished in a comprehensive fashion that concurrently maximizes economic value and reduces waste (Thomsen, 2005). There have been numerous proposed byproducts, including the generation of steam power from combustion; the creation of fiber products and wood plastic composites (biopolymers); the fermentation of biomass into monomers and commodity chemicals; and

nanomaterial production from cellulose and lignin (Cherubini, 2010; Yu et al., 2012). Most of the biorefineries are concentrated in the developed world. The biorefineries primarily focus on biofuel production from corn as in USA (231 biorefineries with a cumulative capacity of 15815 million gallons) while European Union uses sugarbeet and mustard as major biofuel feedstocks (311 biorefineries). However, Brazil has pioneered in sugarcane-based ethanol production in 390 biorefineries (Barros, 2016; Slette and Aradhey, 2016). In developing countries such as India and Vietnam power producing biorefineries utilize agricultural residues and forest produce. The modern biorefineries are diversifying their product portfolio by producing high value products (e.g., butanediol, succinate and synthetic starch etc.) and materials (e.g., carbon fibers, thermoplastics and paper etc.), thereby enhancing their economic viability. Both the sweet sorghum and biomass sorghum (*Sorghum bicolor* L. Moench) are being increasingly considered as valuable feedstocks for increasing the product portfolio of modern biorefineries intended to enhance their sustainability and profitability.

Although there is a considerable variation in phenotype, both grain and sweet sorghums belong to *S. bicolor* (Rao et al., 2016; Zheng et al., 2011). There are varieties of grain sorghum with notable differences in phenotype. Two such examples are colored sorghum and bird-resistant grain sorghum. Interestingly, the accumulation of tannins and anthocyanidins, bioactive phenolic compounds, are responsible for these phenotypic differences in bird-resistant and colored varieties, respectively (Parbhoo et al., 1995; Polycarpe et al., 2011; Reed et al., 1987). In contrast to grain sorghum, sweet sorghums have a tendency to accumulate fermentable sugars in the stalk and, as a result, have taller, juicier and thicker stalks when compared to grain sorghums (Rao et al., 2015; Rao et al., 2009; Ritter et al., 2007). In the recent past sorghum, in particular

sweet sorghum and biomass sorghum are used as feedstock for producing biofuels, power, materials and industrial products in biorefineries (Rao et al., 2016). For the purposes of this review, studies involving both grain and sweet sorghum cultivars were included.

Growing evidence suggests that the bioactive compounds found in grain and sweet sorghum varieties have demonstrated anti-inflammatory, antioxidant and anti-cancer activity (collectively referred to here as bioactivity). This may explain why sorghum has been used in traditional medicine cultures for the treatment of various diseases, including cancer (Akande et al., 2010). Bioactive compounds result from complex biosynthetic pathways and have various functions, including pathogen defense, communication, allelopathy, and protection from environmental stresses (e.g., ultraviolet light, predation and excess temperature) (Rao et al., 2009, Rao et al., 2016). Recent advances have expanded our capacity to remove these compounds from the plant matrix to create enriched extracts. The extraction of bioactive compounds from sweet sorghum biomass can be achieved through several methods, including liquid-liquid extraction, microwave-assisted extraction, ultrasound-assisted extraction, enzyme-assisted extraction, and supercritical fluid extraction. However, care needs to be taken with the processing and extraction of plant matter for bioactive compounds, as we have shown that processing of potatoes reduces the efficacy of phenolic-rich extracts against colorectal cancer cells *in vitro* (Madiwale et al., 2011). This also includes the need to optimize extraction parameters to minimize losses in bioactive compounds and bioactivity. For example, it has been shown that the yield of silymarin through subcritical water extraction of *Silybummarianum* seeds is substantially altered by the time and temperature of the extraction (Duan et al., 2009).

A major contributor of phenolic-rich extracts are grape seeds, fruit peels, and buckwheat hulls in industrial agriculture (Balasundram et al., 2006). These phenolic-rich extracts are used primarily as alternatives to synthetic antioxidants to prevent oxidation of foodstuffs (e.g., meats and oils) and as functional food additives (Balasundram et al., 2006). In 2007, the latest year of reliable market data, the global market for antioxidants used for food production was 787 million USD and projected to grow by 8% by 2010 (Anon, 2011). Phenolic extracts from various sources are also marketed to consumers as health-promoting dietary supplements and as functional food additives (e.g., grape seed extract). Recently, growing evidence supports the use of phenolic extracts as human health-promoting anti-inflammatory and antioxidant agents (Pan et al., 2009; Vanamala et al., 2008). It is now becoming clear that these phenolic bioactive compounds with health-benefiting properties are partially responsible for the epidemiological findings reporting negative correlations between diets rich in fruits and vegetables and chronic disease (Chang et al., 2011; Kris-Etherton et al., 2002). Indeed, many chronic diseases, such as cardiovascular disease, Alzheimer's disease, type 2 diabetes and certain cancers, have etiologies deeply rooted in inflammation and oxidative stress (Berg and Scherer, 2005; Reuter et al., 2010; Ye et al., 2010). The extraction of phenolic bioactive compounds for the prevention or treatment of chronic diseases may represent a novel by-product to add value to biorefining.

Globally, colorectal cancer is the third leading cause of cancer related deaths in males and females (American Cancer Society, 2016). It is estimated that 80% of all colorectal cancer cases can be associated with environmental and dietary factors, and that diets rich in fruits and vegetables negatively correlate with incidence (Reddy et al., 2003). Non-steroidal anti-inflammatory drugs (NSAID), such as Sulindac, have long been used in the prevention and

treatment of colorectal cancer, pointing to the importance of inflammation and oxidative stress in the etiology of the disease (Baron and Sandler, 2000). In recent decades, a growing understanding of the anti-inflammatory and antioxidant properties of phenolic bioactive compounds have led to an increase in the number of clinical trials investigating their efficacy in colorectal cancer treatment and prevention (e.g., resveratrol and curcumin) (Ruhul et al., 2009). As chronic diseases continue to increase, due in part to diets poor in bioactive compounds, alternative strategies for augmenting levels of health-promoting plant compounds will be increasingly important. This review discusses the unique opportunity in biorefining to capture the bioactive compounds inherent in the biomass used for fuel production, which have significant bearing on the whole value chain viability particularly when energy costs are low. The review uses sorghum as a model feedstock containing compounds with bioactivity in various models of colorectal cancer. This article summarizes previous efforts to identify and quantify bioactive compounds in sorghum, and documents their anti-inflammatory and antioxidant biological activities. This review also discusses bioactive compound extraction methods and technologies that can be incorporated into current biorefining practices intended to produce biofuels and chemicals.

2. Phenolic compounds

Bioactive compounds are broadly organized into classes based on chemical structure. These include phenolics, carotenoids, and glucosinolates. Of these three classes, phenolics are the most abundant and are found ubiquitously throughout the plant kingdom (Dai and Mumper, 2010). These compounds are synthesized by the phenylpropanoid pathway and include phenolic acids, flavonoids, stilbenoids and lignins (Dai and Mumper, 2010).

Efforts have been made to quantify total phenolics (TP) and antioxidant activity (AOA) from different components of sorghum (Table 1). The levels of TP and AOA in sorghum range widely and depend on the component of sorghum extracted, as well as, genotype or variety. The grain of grain sorghums has been the most studied component with respect to TP and AOA. TP values have been reported to range from 2 to 9 mg gallic acid equivalents per g of extracted material (mg $GAEg^{-1}$, Folin-Ciocalteu assay) (Dykes et al., 2005) and AOA values from 5 to 85 µmol Trolox equivalents per g of extracted material (µmolTE g^{-1}, 2,2-diphenyl-1-picrylhydrazyl [DPPH] assay) (Awika et al., 2009; Dykes et al., 2005) in the grain. Phenolics seem to be more concentrated in the bran, which makes up the hard outer layer of the grain, containing up to 782 µmol TEg^{-1} (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonate) radical cation [ABTS] assay) (Awika et al., 2003b). Other parts of the sorghum plant, including the leaves and stalk, have been reported to contain 11.7 and 1.0 mg $GAEg^{-1}$ of total phenolics, respectively (Ring et al., 1988). We have shown the TP of the dermal layer of to contain 1.0 mg $GAEg^{-1}$ (Massey et al., 2014; Massey et al., 2016).

Genotypic and tissue variability was observed for TP and AOA. For example, colored sorghums have increased TP and AOA when compared to other genotypes. The grain of colored sorghum contains much higher TP and AOA with reported values as high as 23 mg GAE g^{-1} and 147 µmol TE g^{-1} (DPPH), respectively. The leaf sheaths of colored sorghum have been reported to contain as much as 135 mg $GAEg^{-1}$ (Polycarpe et al., 2011). This may be due to the presence of anthocyanins, which are concentrated in the leaf sheaths of these varieties of sorghums. These findings reflect the high variation of TP and AOA inherent in the different varieties and components of sorghum, which may affect related bioactivities.

2.1 Phenolic acids

Phenolic acids represent the simplest phenolic compounds, containing a single aromatic ring with hydroxyl and methoxyl substituted groups, and are derivatives of cinnamic acid and hydroxybenzoic acid (Sikwese, 2005). They are spread broadly throughout the plant kingdom and are estimated to contribute to one-third of the total phenolic compounds consumed in the diet (Yang et al., 2001). The major phenolic acids identified in sorghum grain include protocatechuic, gentisic, caffeic, cinnamic, ferulic, sinapic, salicylic and *p*-coumaric acid (Table 2) (Waniska et al., 1989). Ferulic and *p*-coumaric acids have been reported in the sorghum stalks (Billa et al., 1997). Vanillic, *p*-hydroxybenzoic and gallic acids have also been identified in the sorghum grain (Bröhan et al., 2011). *p*-coumaric, *o*-coumaric, and *p*-hydroxybenzoic acids have been identified in the leaf sheaths and leaves of sorghum varieties (Polycarpe et al., 2011). In the leaves, these compounds have been reported to inhibit *Locustamigratoria* feeding (Woodhead and Cooper-Driver, 1979), indicating the importance of these compounds to plant defense against insects.

2.2 Flavonoids

Flavonoids are the second major class of sorghum phenolics and represent the largest class of phenolics in the plant kingdom. Flavonoids can be divided into six major subclasses based on C-ring substitutions: flavanones, flavonols, flavones, catechins, anthocyanidins, and isoflavones (Ross and Kasum, 2002). Anthocyanidins, flavones, flavanones, and flavonols have been previously identified in the sorghum (Table 3). Apigenin, luteolin, and tricin are flavones identified in the grain and the stem of sorghum (Bröhan et al., 2011; Kwon and Kim, 2003; Rey et al., 1993). While certain flavonoids are seemingly ubiquitous throughout the sorghum plant,

the flavanones naringenin and eriodictyol have only been identified in the grain (Dykes et al., 2009). We have previously shown the presence of naringenin in the stalk of the Dale sorghum variety (Massey et al., 2014). Kaempferol 3-rutinoside-7-glucuronide, (Nip and Burns, 1969) taxifolin and related glycosides, (Gujer et al., 1986) quercetin glycosides, (Kwon and Kim, 2003) catechins, (Awika et al., 2003a) epicatecin (Awika et al., 2003a) and procyanidins (Awika et al., 2003a) have also been reported in sorghum grain. In addition, we have identified apigenin, luteolin, naringenin, naringeninglucoside, eriodictyolglucoside, taxifolin, and catechin by analyzing LCMS mass spectra of ethanolic extracts in sorghum seed head, and stalk dermal layer.

2.2.1 Sorghum 3-Deoxyanthocyanidins

The 3-deoxyanthocyanidins (3-DXA) are anthocyanidins that lack a hydroxyl group on the 3-carbon (Fig. 1). The synthesis of 3-DXAs (Fig. 2) starts with the phenylpropanoid pathway converting phenylalanine to 4-coumaryol-CoA with phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H) and 4-coumarate:CoA ligase (4CL). Chalcone synthase (CHS) then catalyzes the condensation of 4-coumaroyl with three molecules of malonyl-CoA forming naringeninchalcone. Chalcone-flavanoneisomerase (CHI) catalyzes the stereo-specific isomerization of naringeninchalcone to naringenin. Under conditions suitable for 3-DXA formation, naringenin is converted to apiferol by the actions of dihydroflavonolreductase (DFR). The subsequent actions leading to the formation of the 3-DXA apigeninidin are currently unknown. Naringenin can also be converted to eriodictyol by the actions of flavonoid 3’ hydroxylase (F3’H) and similarly converted to the 3-DXA luteolinidin by the aforementioned enzymatic steps involving DFR and some other uncharacterized step. When compared to their 3-

hydroxylated counterparts, these compounds are more stable in acidic solutions (Awika et al., 2004; Polycarpe et al., 2011). This stability makes 3-DXAs an attractive source of natural water-soluble pigments for the food industry. Apigeninidin, luteolinidin, and their methoxylated counterparts, are uncommon in higher plants and are unique to sorghum (Polycarpe et al., 2011). These compounds are ubiquitous in the sorghum plant and have been identified in the glume, grain, leaf and leaf sheath (Bröhan et al., 2011; Njongmeta, 2009). Based on LCMS relative abundances, we have observed that apigeninidin and luteolinidin were present in quantities of approximately 10- and 100-fold more in the seed head than the stalk dermal layer of sweet sorghum, respectively. The presence of unique 3-DXAs suggests that sorghum is a unique reservoir of health benefiting compounds.

2.3 Stilbenoids

Stilbenes are 1,2-diarylethenes and are synthesized from cinnamic acid derivatives (Cassidy et al., 2000). In sorghum, the synthesis of stilbenes are controlled by the stilbene synthase gene *sbSTS1* (Yu et al., 2008). The stilbenoid s*trans*-piceid and *trans*-resveratrol have been reported in the grain of red sorghum at an amount of 0.4-1.0 mg kg^{-1} and 0.2 mg kg^{-1}, respectively (Bröhan et al., 2011). Yields of stilbenes in sorghum may be increased through fungal infection. Indeed, during fungal pathogen infection *sbSTS1* expression was higher (Yu et al., 2008). In infected sorghum seedlings, *trans*-piceid accumulated up to 35 mg kg^{-1}, suggesting that stilbene levels can be enhanced (Yu et al., 2008). However, as an intentional fungal infection would decrease yield, other options should be explored. Some varieties of sorghum resistant to certain fungal infections showed a greater induction of *sbSTS1* activity upon infections (Sharma et al., 2013), indicating that variation in genotype could be selected for through breeding

programs to augment levels of stilbene synthesis. Alternatively, genetic engineering could be used to over express this *sbSTS1,* resulting in greater accumulations of stilbenes.

Collectively, the existent data on sorghum phenolics demonstrates that the sorghum plant contains a wide variety of phenolic compounds including phenolic acids, stilbenoids, and a myriad of flavonoids. These compounds are ubiquitous in the plant with varying content, which is dependent on the part of the plant and genotype. This variation in phenolic compound expression may facilitate the breeding of sorghum varieties aimed to enhance expression of bioactive compounds.

2.4 Polyamines

Amines are nitrogenous bases of low molecular weight that play important role in plant metabolism and physiology, which can be classified as can be classified as biogenic amines and polyamines. The radical-scavenging properties of polyamines can protect membranes from lipid peroxidation and other oxidative stresses besides playing role in the regulation of inflammation, food allergy prevention and anti-glycation. The total amines ranged from 5.8 to 41.4 mg/100 g, and the polyamines represented 60--100% of the total. The predominant amines in sorghum are spermine, spermidine, putrescine and cadaverine (Paiva et al., 2015).

3. Methods for the extraction of bioactive compounds

3.1 General considerations and precautions

It is essential to have a comprehensive outlook taking into account several considerations and precautions when developing protocols for the extraction and isolation of desired bioactive compounds from plant matrices. The intended use of the compound(s) of interest is an important determinant in developing method for extraction. In the context of this review, the extracted

compounds are intended for the promotion of human health. For this purpose, extraction methodologies should accomplish the following: 1) maintain the integrity of the bioactive constituent(s); 2) ensure the safety of the end product for human consumption; and 3) standardize the extract to ensure product consistency and efficacy.

The chemical properties of the desired compound, particularly those that are different from the other compounds in the mixture, are important determinants of any extraction procedure. The polarity of the compound(s) is an important determinant of solubility, impacting the need for solvent selection and optimization (Dai and Mumper, 2010). Solubility can also be affected by physical isolation of the compound, which prevents interaction with extraction solvent. For example, a compound may be chemically bound to the matrix versus adsorbed to the surface of the matrix (Mustafa and Turner, 2011). In these cases, it may be important to disrupt the matrix chemically or physically to assist in the extraction process. In some cases, including phenolic acids, the compound(s) may be rendered insoluble due to covalent bonding to plant wall constituents. Extraction can only be achieved if this bond is cleaved, typically by some form of hydrolysis (e.g., enzymatic, acid, or alkaline). Other properties to consider are pH stability and sensitivity to temperature. These factors can influence the rate at which bioactive compounds degrade. For example, anthocyanins are more stable at pH values < 2, where they form the stable flavylium ion. At higher pH values, anthocyanins form less stable isoforms and may degrade to form chalcones (He and Giusti, 2010). Although there is a dearth of information pertaining to this subject with respect to biorefining, investigations over the effects of food processing on phenolic compounds may provide some insight. For example, heating of elder berries to 95°C for 3 hr resulted in 50% anthocyanin losses (Patras et al., 2010).

There may be a need to ensure either an extract's purity or the absence of some undesirable substance. For example, pesticides use on sweet sorghum crops may vary, and pesticides may become concentrated throughout the extraction procedure. On the other hand, separation techniques may be necessary to improve the purity of the extract. This may include crude separations (e.g., removal of organic acids and sugars) to enrich phenolic extracts. Alternatively, it may be that the final desired extract is of a single compound or class of compound, which would require a sequence of separation/extraction technologies. It is also important to standardize extracts to some desired target compound to ensure that a desired level of active constituent is present or that the extract has a desired level of antioxidant or biological activity.

Of course, the extraction methodology utilized must balance these factors with economic feasibility. This will depend on factors like the economic value of the bioactive compound(s), yield of the bioactive compound(s), fixed cost of the extraction equipment, and running costs of the extraction. Bioactive extraction methodologies include liquid-liquid extraction, pressurized liquid extraction, enzyme-assisted extraction, microwave-assisted extraction, ultrasound-assisted extraction, and supercritical fluid extraction. More detailed explanations of the bioactive compound extraction methodologies can be found in several reviews (Dai and Mumper, 2010; Garcia-Salas et al., 2010; Pereira and Meireles, 2009; Soni et al., 2012; Turkmen et al., 2006).

3.2. Extraction methods

Liquid-liquid extraction is one of simplest and least expensive methods for extracting bioactive compounds. This method involves the use of organic and inorganic solvents for the extraction of bioactive compounds (Garcia-Salas et al., 2010). Depending on the application, disadvantages

for this technology may include the loss of bioactive compound integrity, lack of specificity for solute, toxicity and volatile nature of most organic solvents (Dai and Mumper, 2010; Garcia-Salas et al., 2010). Extractions using this method usually involve large volumes of solvent and require strategies to purify the solute and to remove, capture, and reuse solvents (Dai and Mumper, 2010). Solvent selection is an important determinant of extraction yield and bioactivity. We have shown that while aqueous acetone extraction of biorefined sweet sorghum stalk byproduct yielded more total phenolics, aqueous ethanol extracts had higher anti-proliferative bioactivity in HCT116 colon cancer cells (Massey et al., 2014). This finding suggests that extractions need to be optimized not only for yield, but also for bioactivity. Pressurized liquid extraction is a variant of liquid-liquid extraction (Mustafa and Turner, 2011). In this method, temperatures and pressures higher than in standard liquid-liquid extraction can be used to enhance diffusion rate, solubility, and mass transfer properties of the solvent (Mustafa and Turner, 2011). Pressurized extractions are faster and use less solvent; however, they require more equipment cost, and higher temperature may degrade labile compounds.

Different types of liquid-liquid extractions have been designed to enhance the extraction process. These include enzyme-assisted extractions, microwave-assisted extractions and ultrasound-assisted extractions and have been reviewed in detail (Puri et al., 2012; Soni et al., 2012). Enzyme-assisted extractions utilize enzymatic pretreatments to hydrolyze cell wall constituents enhancing extraction efficiency by partially breaking down the physical barrier that is the plant cell wall (Puri et al., 2012). Microwave-assisted extractions involve the application of microwave energy to superheat water contained in the plant matrix, causing disruption facilitating compound desorption (Soni et al., 2012). Microwave-assisted extractions have been

used to extract bound phenolic acids in sorghum bran (Chiremba et al., 2012). Similarly, ultrasound-assisted extraction involves the application of high frequency sound waves to physically disrupt the matrix, enhancing compound recovery (Soni et al., 2012).

Supercritical fluid extraction makes use of a supercritical fluid to separate compounds from complex matrices. Pure CO_2 is the most commonly used solvent, however, ethanol can be used as an adjunct to extract increasingly polar compounds (Pereira and Meireles, 2009). The most attractive aspect of this technology is the ability to manipulate extraction conditions (temperature, pressure, and co-solvent) to facilitate extraction of the compounds of interest (Pereira and Meireles, 2009). Other advantages include the absence of toxic substances in extraction and that the integrity of the bioactive constituent is not compromised during extraction. However, the main disadvantage of using this technology is the high cost of the equipment (Pereira and Meireles, 2009). Additional equipment expenses may be offset by highly valuable extracts. Given the need for natural sources of stable water-soluble pigments for the food industry, extractions yielding large quantities of highly pure 3-DXAs may prove to be economically feasible for supercritical fluid extraction.

4. Colorectal cancer and sorghum phenolics

4.1. Colorectal cancer

Colorectal cancer (CRC) is the second most common cancer in females and the third most common cancer in males worldwide (Jemal et al., 2011). In addition to environmental factors, chronic inflammatory states of the colon result in the promotion of colitis-associated colon cancer (CAC) (Reddy et al., 2003; Terzić et al., 2010). In both CRC and CAC, inflammation plays key roles in cancer progression. The process by which normal colonic

epithelium progresses to colon cancer is multi-step, whereby cells accumulate successive genetic alterations, establish clones and gain a proliferative advantage (Worthley and Whitehall, 2007).

The traditional CRC pathway accounts for 70 to 85% of all cancers and starts with loss of the tumor suppressor gene adenomatous polyposis coli (APC) function, resulting in cytosolic accumulation and nuclear translocation of the transcription factor β-catenin (Worthley and Whitehall, 2007). Aberrant β-catenin signaling occurs in almost all cases and drives colorectal carcinogenesis (Bienz and Clevers, 2000; Firestein et al., 2008). Loss of APC is then followed by mutation of K-ras, loss of the chromosomal region18q, and finally loss of p53 via loss at the chromosomal region 17p (Worthley and Whitehall, 2007). The critical tumor suppressor gene, p53,is responsible for cell cycle arrest and apoptosis (Vousden and Lu, 2002). In most cases, mutation in p53 marks the transition from non-invasive to invasive colon cancer (Worthley and Whitehall, 2007). Furthermore, p53 inactivation alters responses to conventional fluorouracil-based chemotherapies making it harder to effectively eliminate cancerous cells with chemotherapy (Longley et al., 2003).

In contrast, the development of CAC begins with chronic inflammatory conditions, such as inflammatory bowel disease (Terzić et al., 2010). Chronic colitis leads to increased oxidative stress and inflammatory cytokine expression, resulting in CAC initiation events (Terzić et al., 2010). For example, damage of cellular DNA can result in the activation of oncogenes. Sustained mitogenic inflammatory signals could sustain such oncogenic effects by suppressing apoptosis and elevating cell proliferation (Terzić et al., 2010). For example, enhanced nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and Akt inflammatory signaling

pathways results in β-catenin activation mimicking early events in sporadic CRC (Terzić et al., 2010).

In both sporadic CRC and CAC, increased genomic instability arising from various mechanisms, results in the increased incidence in tumor promoting mutations (Terzić et al., 2010). Another common trait between CRC and CAC is the elevation in inducible cyclooxygenase-2 (COX-2) expression, resulting in elevated cell proliferation and suppressed apoptosis (Terzić et al., 2010). Yet another example of the strong similarity between CRC and CAC is seen in murine models of Western-style diet-induced CRC. In this model, a Western diet alone is sufficient to induce oxidative stress, inflammation, and immune dysfunction, and leads to sporadic tumor formation in mice (Newmark et al., 2009).

Recent advancements in our understanding of CRC have highlighted the importance of stem cells in the etiology of the disease. The cancer stem cell theory suggests that a small number of cancer stem cells exist at the heart of most tumors and drive the proliferation, renewal, and metastasis of cancers (Jordan et al., 2006; Li and Neaves, 2006). Cancer stem cells are aberrant forms of stem cells that are characterized by the ability to develop into any cell in the overall tumor population due to self-renewal, symmetric and asymmetric division, multipotency, and the proliferative ability to drive continued expansion of the population of malignant cells (Jordan et al., 2006). Interestingly, these are characteristics shared with normal stem cells (Jordan et al., 2006). However, cancer stem cell have acquired mutations that allow for malignant growth independent of the stem cell niche, highlighting the importance of niche-related regulation of proliferation and differentiation (Li and Neaves, 2006). Furthermore, these

cells are resistant to chemotherapy and radiation therapy, making them harder to target with conventional anti-cancer treatments (Wicha et al., 2006).

The colon stem cell niche is primarily regulated by Wnt signals secreted by surrounding fibroblasts forming the base of the colonic crypt (Vaiopoulos et al., 2012). Under normal physiologic conditions, Wnt signals act to maintain the colonic stem cell reservoir by controlling the rate of stem cell proliferation and self-renewal. Daughter cells are known as progenitor cells and move out of the stem cell niche. The progenitor cells then proliferate throughout the next one-third of the crypt, where a decrease in Wnt signaling is associated with differentiation of progenitor cells into differentiated colonocytes (Vaiopoulos et al., 2012). Colon carcinogenesis is thought to be initiated by dysfunctional stem cell renewal pathways (Kasdagly et al., 2014; Wicha et al., 2006). Once transformed, CCSC evade colonocyte growth regulation by mutations in the Wnt/β-catenin signaling pathway resulting in the cytosolic accumulation and nuclear translocation of β-catenin, a downstream effector of Wnt pathway, promoting colon cell proliferation, suppressed apoptosis, and suppressed cell differentiation (Vaiopoulos et al., 2012), ultimately leading to colon tumor formation.

4.2. Phenolics as tumor suppressors

Emerging evidence highlights the importance of targeting key pathways involved in CCSC proliferation and apoptosis (e.g., Wnt/β-catenin signaling and p53) for prevention and treatment of colon cancer. Investigations into the bioactive constituents of sorghum have revealed that these compounds, acting individually and in combination, are effective in targeting many pathways related to CRC across various models (Tables 4 and 5).

Elevated cell proliferation and suppressed apoptosis are two well-recognized hallmarks of cancer and contribute to tumor progression (Hanahan and Weinberg, 2011). There are numerous studies citing anti-proliferative and pro-apoptotic effects of sorghum extracts and related bioactive compounds in numerous cancer cell lines. Vanillic, protocatechuic, *p*-coumaric and ferulic acids have all shown anti-proliferative and/or pro-apoptotic activities in colon cancer cell lines (Table 4). The 3-DXAs apigeninidin and luteolinidin as well as extracts rich in sorghum 3-DXAs suppressed proliferation of HT29 colon cancer cells (Table 5). These studies are supported by tumor xenograft mouse model consuming 10 mg of grain extract from grain sorghum per kg of body weight. Administration of this extract for four weeks significantly and markedly reduced tumor growth, elevated tumor cell death and suppressed metastasis (Park et al., 2012). Thus, there is growing evidence in support of sorghum and sorghum-related compounds having *in vitro* and *in vivo* anti-colon cancer activity.

Aberrant Wnt/β-catenin signaling is found in almost all colon cancers, including CRC, CAC, and familial forms of the disease. Sorghum related compounds have been shown to directly suppress this important colon cancer related pathway. For example, Quercetin, a flavonoid found in sorghum, inhibited β-catenin signaling in SW480 colon cancer cells (Park et al., 2005). Quercetin and luteolin inhibited cellular proliferation in colon cancer cells with mutated K-ras (Xavier et al., 2009). We have demonstrated that extracts from the dermal layer and seed head of sweet sorghum concurrently suppressed proliferation (Fig. 3A), elevated apoptosis (Fig. 3B), and suppressed levels of β-catenin and downstream targets (cMyc and survivin) in human colon cancer stem cells (Fig. 3C; Massey et al., 2016). Furthermore, this activity was only slightly diminished in cell lines without p53 (CCSC p53 shRNA), indicating a

partial dependence on p53 for bioactivity. This is particularly important as loss of p53 results in chemotherapy-resistance and marks the transition from noninvasive to invasive disease. In azoxymethane (AOM; a colon-specific carcinogen)-treated F344 rats, ferulic and protocatechuic acid were shown to inhibit the colorectal cancer biomarker aberrant crypt foci (ACF) (Kawabata et al., 2000; Kawamori et al., 1994). In AOM-treated Sprague-Dawley rats, apigenin and naringenin decreased the number of high multiplicity ACF (HMACF) (Leonardi et al., 2010). The evidence presented here suggests that sorghum extracts and associated phenolic compounds have strong *in vitro* and *in vivo* anti-proliferative and pro-apoptotic activities at various stages of colorectal cancer.

The role of inflammation and oxidative stress is important to both CAC and CRC. Sorghum extracts and sorghum related compounds have demonstrated numerous anti-inflammatory and antioxidant activities. Many compounds have shown anti-inflammatory activities in immune or even the colon cancer cell cultures. For example, protocatechuic acid decreased inflammation in lipopolysaccharide (LPS)-stimulated macrophages (RAW 264.7 cells) and in 12-O-tetradecanoyl-phorbol-13-acetate (TPA)-treated BALB/c mice (Min et al., 2010). Taxifolin suppressed inflammation in primary murine dendritic cells by decreasing levels of TNF-α (Balkwill and Mantovani, 2012; Kim et al., 2008). Tumor necrosis factor alpha (TNF-α) signaling plays a critical role in tumor development in models of gastric and colon cancers (Balkwill and Mantovani, 2012). Ferulic acid suppressed COX-2, an inducible form of cyclooxygenase which is over expressed during chronic inflammation, expression in HT29 cells (Ferguson et al., 2005). Indeed, increased COX-2 expression is observed in colon cancer and COX-2 levels increase with disease progression. Furthermore, there is strong evidence

supporting the preventive effects of NSAIDs and bioactive compounds targeting COX-2 in cancers of the gastrointestinal tract (Gupta and DuBois, 2001; Murakami and Ohigashi, 2007). In the early stages of CAC, lamina propria macrophages are primarily responsible for most of the tumor promoting inflammatory cytokines (Terzić et al., 2010). Luteolin, apigenin, naringenin, and quercetin have all demonstrated anti-inflammatory activity in RAW 264.7 macrophage cells (Hu and Kitts, 2004; Liang et al., 1999; Lyu and Park, 2005; Wang and Mazza, 2002). Interleukin-6 (IL-6) is a proinflammatory cytokine and has been shown to promote colon cancer growth and survival as well as angiogenesis (Terzić et al., 2010). In a murine DSS-induced colitis model, naringenin was shown to ameliorate colitis by suppressing interferon-γ and IL-6 production (Azuma et al., 2013). This evidence suggests that sorghum related phenolics could aid in countering growing epidemic of chronic inflammation- promoted diseases such as inflammatory bowel disease (IBD), CRC and type 2 diabetes.

Chronic inflammation results in increased levels of oxidative stress, which can lead to damage of cellular DNA forming new genetic mutations. Conversely, elevated oxidative stress can result in inflammatory responses. Bioactive compounds of sorghum may act as direct antioxidants or increase levels of antioxidant defense enzymes to protect cells from oxidative stress. Taxifolin was shown to induce detoxifying enzymes in HCT116 cells (Lee et al., 2007). Cinnamic acid elevated levels of the antioxidant enzyme glutathione-S-transferase and decreased DNA damage in the colonic mucosa of rats (Guglielmi et al., 2003). Results from our lab have demonstrated that feeding of 1% dermal layer phenolic extract to mice suppressed oxidative stress in a high-fat diet induced obesity model as measured by plasma 8-isoprostane (Reddivari

et al., 2016). These and similar findings support the use of sorghum bioactive compounds as a source of antioxidant compounds for human health.

In summation, the totality of the information presented here strongly supports sorghum as a source of phenolic bioactive compounds with anti-colon cancer activity. These compounds and extracts have been shown to inhibit events related to the initiation, promotion, and progression of colon and other cancers. Sorghum phenolic compounds, acting individually or in complex extracts, have demonstrated *in vitro* and *in vivo* antioxidant, anti-inflammatory, anti-proliferative, pro-apoptotic, and anti-metastasis activities. These studies are constricted to cell culture and rodent models and there is a little information available the concentration of the various sorghum polyphenols (individually and/or together) required in the diet to be effective against chronic diseases and whether obtaining those levels is practical via dietary means. To address these questions, it is critical to understand the bioavailability of plant polyphenols. In order to exert bioactivity at the site of action, dietary polyphenols undergo absorption, distribution and metabolism to tissues subsequent to excretion. Tissue levels of bioactive polyphenols are influenced by many factors such as food matrices, concentration in the diet, metabolic enzyme genotype, age, and gender as well as health status. Recent evidence suggests that proportion of ingested polyphenols delivered to tissues is typically low in mammals (Maša et al., 2016). One school of thought holds that polyphenol bioavailability must be increased through chemical modification (Maša et al., 2016) or utilize low doses of different polyphenols to achieve synergy (Martin, 2016). Another school of thought holds that even though polyphenol parent compound bioavailability is low, the polyphenols affect the systemic parameters via their gut bacterial metabolites (Marín et al., 2015; Stevens and Maier, 2016; Park et al., 2005) Emerging evidence

suggests that due to poor polyphenol bioavailability, polyphenol concentration in the colon is greater than the systemic levels and the polyphenols in the colon undergo gut bacterial metabolism and the resulting daughter compounds are more potent than the parent compounds and are highly bioavailable (Marín et al., 2015). Thus, there is a critical need for human/animal studies that systematically examining the physiological processes and/or gut bacterial metabolism governing polyphenol bioavailability. It will be of utmost importance in the near future to study extracts or individual bioactive compounds of sweet sorghum biomass in pre-clinical and clinical studies of colon cancer to elucidate effective dose, efficacy and mechanisms of action.

5. Conclusion and future directions

In the past one to two decades, the study and increased understanding of sorghum bioactive compounds have led to the discovery of unique 3-DXAs with putative anti-inflammatory and anti-cancer activity. These compounds may also serve as much needed stable and natural water-soluble pigments for the food industry. In addition, sorghum genotypes contain numerous other bioactive compounds, including phenolic acids, flavonoids, and stilbenoids. Phenolic-rich sorghum extracts have proven to have *in vitro* and *in vivo* anti-cancer, antioxidant, and anti-inflammatory activity. Sorghum bioactive compounds show great promise for the prevention and possible treatment for human colon cancer. However, additional studies elucidating the anti-cancer mechanisms of sorghum phenolics using pre-clinical and clinical models of colorectal cancer are prerequisite for this to become a reality. The use of selective breeding programs to enhance the expression of desired sweet sorghum bioactive compounds is another area of future research and will be important for enhancing biorefining. Indeed, this has already been

demonstrated in mutagenesis breeding of the sweet sorghum Della variety for enhanced accumulation of valuable 3-DXAs (Petti et al., 2014). Similar studies, screening sorghum biomass for anti-cancer, anti-inflammatory and/or antioxidant activity, may also prove useful in the development of varieties with enhanced bioactivity. In addition to serving as a feedstock for biofuel production, sugar-rich sweet sorghum and starchy grain sorghums may also be an invaluable reservoir for bioactive compounds to aid in the fight against the growing epidemic of chronic diseases globally.

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Table 1. Comparisons of reported total phenolic and antioxidant activity in grain and sweet sorghums.

Sorghum	Component	Method	Concentration (μ mol TE g^{-1} or mg GAE g^{-1})
Grain sorghums	bran	ABTS	28-786 (Awika et al., 2003a)
	grain	ABTS	10-170 (Dykes et al., 2005)
			125-562 (Awika et al., 2009)
			6-226 (Awikaet al., 2003a)
		Folin-Ciocalteu	2-9 (Dykes et al., 2005)
			4.8-22.5 (Awika et al., 2004)
			8-30 (Awika et al., 2009)
			9-23 (Bröhan et al., 2011)
			10.6-17.5 (Kobue-Lekalake, Taylor, and de Kock, 2007)
	leaf	Folin-Ciocalteu	3.4-11.7 (Ring et al., 1988)
	leaf sheath	ABTS	3760-5580[a] (Polycarpe Kayodé et al., 2011)
		Folin-Ciocalteu	65-135 (Polycarpe Kayodé et al., 2011)
	stalk	Folin-Ciocalteu	0.4-1.0 (Ring et al., 1988)
Sweet sorghums	dermal	ABTS	9.2-26.0
		Folin-Ciocalteu	0.8-2.4
	leaf	ABTS	17
		Folin-Ciocalteu	4.5
	pith	ABTS	5.2-10.0 (Massey et al., 2014)
		Folin-Ciocalteu	0.9-1.3 (Massey et al., 2014)
	seed head	ABTS	50.3-114.7
		Folin-Ciocalteu	2.3-8.9

[a]Measurement made on a dry matter basis. TE = Trolox equivalents; GAE = gallic acid equivalents.

Table 2. Reported phenolic acids in sorghum

Class	Compound	Source	Concentration (mg kg^{-1})
Phenolic acids	protocatechuic acid	grain	7-141 (Bröhan et al., 2011)
	p-hydroxybenzoic acid	grain	15-34 (Bröhan et al., 2011)
		leaf	reported (Woodhead and Cooper-Driver, 1979)
	vanillic acid	grain	8-51 (Bröhan et al., 2011)
		leaf	reported (Woodhead and Cooper-Driver, 1979)
	p-coumaric acid	Grain leaf sheath	86-232 (Bröhan et al., 2011)512-834 (Polycarpe Kayodé et al., 2011)
		leaf	reported (Woodhead and Cooper-Driver, 1979)
		pith	13 907[a] (Billa et al., 1997)
		bark	19 893[a] (Billa et al., 1997)
	o-coumaric acid	leaf	reported (Woodhead and Cooper-Driver, 1979)
	ferulic acid	grain	105-343 (Bröhan et al., 2011)
		leaf	reported (Woodhead and Cooper-Driver, 1979)
		pith	6466[a] (Billa et al., 1997)
		bark	8446[a] (Billa et al., 1997)
	gallic acid	grain	20-46 (Bröhan et al., 2011)
	gentisic	leaf	reported (Woodhead and Cooper-Driver, 1979)
	caffeic acid	grain	26-52 (Bröhan et al., 2011)
	cinnamic acid	grain	5-20 (Bröhan et al., 2011)
	hydroxybenzoic acid	leaf sheath	381-1555 (Polycarpe Kayodé et al., 2011)
	salicylic	grain	reported (Waniska et al., 1989)
	syringic	grain	reported (Waniska et al., 1989)
	sinapic	grain	reported (Waniska et al., 1989)

[a]Values are represented in g kg^{-1} of cell wall residue

Table 3. Reported flavonoids in *Sorghum bicolor.*

Class	Compound	Source	Concentration (mg kg^{-1})
Anthocyanidins	apigeninidin	glume	100-7000 (Njongmeta, 2009)
		grain	300-1000 (Bröhan et al., 2011)
		leaf	0-1300 (Njongmeta, 2009)
		leaf sheath	14 720-45 760 (Polycarpe Kayodé et al., 2011)
	luteolinidin	glume	0-1000 (Njongmeta, 2009)
		grain	trace-1500 (Bröhan et al., 2011)
		leaf	0-500 (Njongmeta, 2010)
		leaf sheath	430-1660 (Polycarpe Kayodé et al., 2011)
	7-methoxyapigenindin	glume	0-2000 (Njongmeta, 2009)
		grain	0.4-137.4 (Bröhan et al., 2011)
		leaf	0-350 (Njongmeta, 2009)
		leaf sheath	0-2500 (Njongmeta, 2009)
	5-methoxyluteolinidin	glume	0-300 (Njongmeta, 2009)
		grain	0.3-153.5 (Bröhan et al., 2011)
		leaf	0-200 (Njongmeta, 2009)
		leaf sheath	0-4500 (Njongmeta, 2009)
	malvidin	leaf sheath	570-1030 (Polycarpe Kayodé et al., 2011)
Flavones	apigenin	grain	2.8-203.7 (Dykes et al., 2009)
		stem	reported (Rey et al., 1993)
	luteolin	grain	2.6-182.2 (Dykes et al., 2009)
		stem	reported (Rey et al., 1993)
	tricin	stem	reported (Kwon and Kim, 2003)
Flavanones	naringenin	grain	5.6-48.4 (Dykes et al., 2009)
	eriodictyol	grain	5.6-12.9 (Dykes et al., 2009)
	eriodictyol 5-glucoside	grain	reported (Dykes and Rooney, 2006)
Flavonols	kaempferol 3-rutinoside-7-glucuronide	grain	reported (Nip and Burns, 1969)
	quercetin 3,4'-dimethyl ether		
		stem	reported (Kwon and Kim, 2003)
Dihydroflavonols[b]	taxifolin	grain	reported (Gujer et al., 1986)
	taxifolin 7-glucoside	grain	reported (Gujer et al., 1986)
Flavan-3-ols[b]	catechin	grain	10-180 (Awika et al., 2003a)
	epicatechin	grain	10-180 (Awika et al., 2003a)
	procyanidins	grain	1300 -- 22000 (Awikaet al., 2003a)
Stilbenes	*trans*-resveratrol	grain	reported (Bröhan et al., 2011)
	trans-piceid	grain	reported (Bröhan et al., 2011)

Reported, references which only reported presence of compound.

Table 4. Bioactivity of phenolic acid compounds identified in *Sorghum bicolor*.

Class	Compound	Model	Biological activity
Phenolic acids	protocatechuic acid	HepG2 liver cancer cells	↑ apoptosis through JNK and p38 stimulation (Yip et al., 2006)
		RAW 264.7 macrophages	↓ TNF-α; ↓ IL-1β (Min et al., 2010)
		TPA treated BALB/c mice	↓ TNF-α; ↓ COX-2 (Min et al., 2010)
		T47D breast cancer cells	↓ proliferation; ↑ apoptosis (Kampa et al., 2004)
		AOM treated F344 rats	↓ ACF formation
	vanillic acid	HT29 colon cancer cells	↓ proliferation; ↑ apoptosis (International et al., 2009)
		NIH/3T3	↓ proliferation; ↑ apoptosis (International et al., 2009)
		RAW 264.7 macrophages	↓ COX-2
	p-coumaric acid	HT-29 colon cancer cells	↓ proliferation (Ferguson et al., 2005)
		EMT-6 breast cancer cells	↓ proliferation (Ferguson et al., 2005)
		SW620 colon cancer cells	↓ proliferation (Ferguson et al., 2005)
		LOVO colon cancer cells	↓ proliferation (Ferguson et al., 2005)
	ferulic acid	HT29 colon cancer cells	↓ proliferation; ↓ genotoxicity;
			↓ COX-2 (Ferguson et al., 2005)
		T47D breast cancer cells	↓ proliferation; ↑ apoptosis (Kampa et al., 2004)
		Nicotine treated Wistar rats	↓ COX-2; ↓ NF-κB (Sudheer et al., 2008)
		AOM treated F344 rats	↓ ACF formation (Kawabata et al., 2000)
	caffeic acid	T47D breast cancer cells	↓ proliferation; ↑ apoptosis (Kampa et al., 2004)
		TPA treated Swiss rats	↓ TNF-α; ↓ lipid peroxidation (Khan et al., 2012)
	cinnamic acid	F344 rats colonic mucosa	↓ genotoxicity; ↑ glutathione-S-transferase (Guglielmi et al., 2003)
		Caco-2 colon cancer cells	↓ proliferation (Ekmekcioglu et al., 1998)

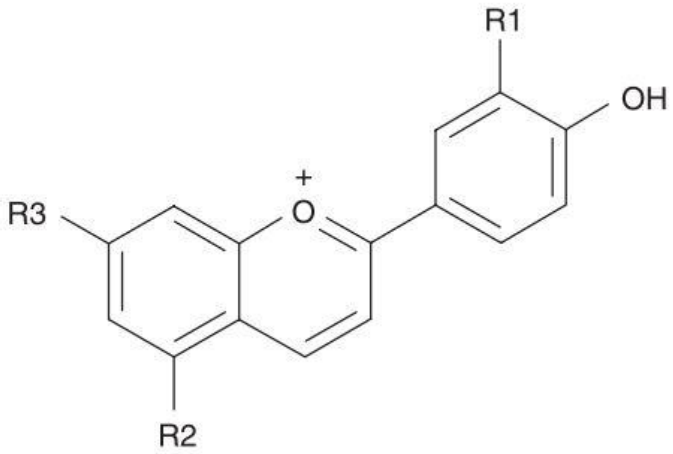
AOM = Azoxymethane; JNK = c-Jun N-terminal kinase TNF-α = Tumor necrosis factor-α; IL = Interleukin; COX-2 = cyclooxygenase-2; ACF = Aberrant crypt foci

Table 5. Bioactivity of flavonoid compounds identified in *Sorghum bicolor*.

Class	Compound	Model	Biological activity
Anthocyan-idins	flavonoid rich sorghum extract	murine xenograft breast cancer model	↓ tumor growth; ↓ metastasis(Park et al., 2012)
	3-DXA rich sorghum extract	HT29 colon cancer cells	
		Hepa 1c1c7	↓ proliferation (Yang et al.,2009)
			↑ phase II enzymes (Yang et al., 2009)
	luteolinidin	HL60 leukemia cells	↓ proliferation (Shih et al., 2007)
		HT29 colon cancer cells	↓ proliferation (Yang et al., 2009)
	apigeninidin	HL60 leukemia cells	↑ apoptosis; ↑ Bak; ↑ Bax (Woo et al., 2012; Yang et al., 2009)
		HT29 colon cancer cells	↓ proliferation (Yang et al., 2009)
Flavones	apigenin	SW480, HT29, Caco-2 colon cancer cells	G2/M cell-cycle arrest
		SW480, DLD1, LS174T colon cancer cells	
		Orthotopic murine colon cancer model	↓ migration distance; ↓ invasive cell count (Chunhua et al., 2013)
		Hep3B liver cancer cells	↓ tumor growth; ↓ metastasis (Chunhua et al., 2013)
		RAW 264.7 macrophages	
			↓ VEGF expression (Osada et al., 2004)
			↓ COX-2; ↓ iNOS; ↓ Nf-κB (Liang et al., 1999)
		AOM treated Sprague-Dawley rats	↓ HMACF (Leonardi et al., 2010)
	luteolin	HCT15 and CO115 colon cancer cells	↑ apoptosis; ↓ proliferation (Xavier et al., 2009)
		JB6 P+ mouse epidermal cells	
			↓ NF-κB; ↓ Src kinase; ↓ PKCε;
		SKH-1 hairless mouse	↓ COX-2 (Byun et al., 2010)
		RAW 264.7 macrophages	↓ UVB induced skin cancer (Byun et al., 2010)
			↓ COX-2; ↓ iNOS (Hu and Kitts, 2004)
Flavanones	naringenin	ddY mice implanted with S-180 sarcoma cells	↓ tumor growth (Kanno et al., 2005)
		AOM treated Sprague-Dawley rats	
		DSS induced colitis BALB/c mice	↓ HMACF (Leonardi et al., 2010)
		RAW 264.7 macrophages	↓ IFN-γ; ↓ IL-6 (Azuma et al., 2013)
			↓ TNF-α (Lyu and Park, 2005)
	eriodictyol	JB6 Cl41 cells	↓ RSK2; ↓ EGF-induced cell transformation (Liu et al., 2011)
Flavonols	kaempferol	A549 lung cancer cells	↓ proliferation; ↑ apoptosis via MEK-MAPK (Nguyen et al., 2003)
			↑ apoptosis via p53 (Li et al., 2009)
		HCT116 colon cancer cells	
	quercetin	HCC1937 breast cancer cells	↓ proliferation; ↓ PI3K-Akt/PKB pathway (Gulati et al., 2006)
			↑ apoptosis (Psahoulia et al., 2007)
		HT29, SW60, and Caco-2 colon cancer cells	
		HCT15 and CO115 colon cancer cells	↑ apoptosis; ↓ proliferation (Xavier et al., 2009)
		SW480 colon cancer cells	
		RAW 264.7 macrophages	↓ β-catenin signaling (Park et al., 2005)
			↓ TNF-α (Wang and Mazza, 2002)
	taxifolin	HCT116 colon cancer cells	↑ detoxifying enzymes by activating antioxidant response element (Lee et al., 2007)

			↓ TNF-α; ↓ IL-12 (Kim et al., 2008)
		LPS treated primary murine dendritic cells	
Flavan-3-ols	catechin	PANC-1 pancreatic cancer cells	↑ apoptosis (McMillan et al., 2007)
		MIAPACA pancreatic cancer cells	↑ apoptosis (McMillan et al., 2007)
Stilbenes	*trans*-resveratrol	F344 rats	↓ HMACF (Tessitore et al., 2000)
		C57BL/6NIA mice	↓ IGF-1; ↓ insulin (Baur et al., 2006)
		HCT116 colon cancer cells	↑ apoptosis; ↑ caspases 2,9,8, and 3 (Mohan et al., 2006)

AOM = Azoxymethane; DSS = Dextran sodium sulfate; COX-2 = Cyclooxygenase-2; iNOS = Inducible nitric oxide synthase; NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells; HMACF = High multiplicity aberrant crypt foci; UVB = Ultraviolet light B; IFN-γ = Interferon-γ; IL = Interleukin; EGF = Epidermal growth factor; PI3K = phosphoinositied-3 kinase; IGF-1 = Insulin-like growth factor-1



Apigeninidin: R1 = H; R2 = OH; R3 = OH
Apigeninidin 5-glucoside: R1 = H; R2 = OGlc; R3 = OH
5-Methoxyapigeninidin: R1 = H; R2 = OCH_3; R3 = OH
7-Methoxyapigeninidin: R1 = H; R2 = OH; R3 = OCH_3
7-Methoxyapigeninidin 5-glucoside: R1 = H; R2 = OGlc; R3 = OCH_3
Luteolinidin: R1 = OH; R2 = OH; R3 = OH
Luteolinidin 5-glucoside: R1 = OH; R2 = OGlc; R3 = OH
5-Methoxyluteolinidin: R1 = OH; R2 = OCH_3; R3 = OH
5-Methoxyluteolinidin 7-glucoside: R1 = OH; R2 = OCH_3; R3 = OGlc
7-Methoxyluteolinidin: R1 = OH; R2 = OH; R3 = OCH_3

Fig. 1. Structures of 3-deoxyanthocyanidins identified in sorghum (Dykes and Rooney, 2006)

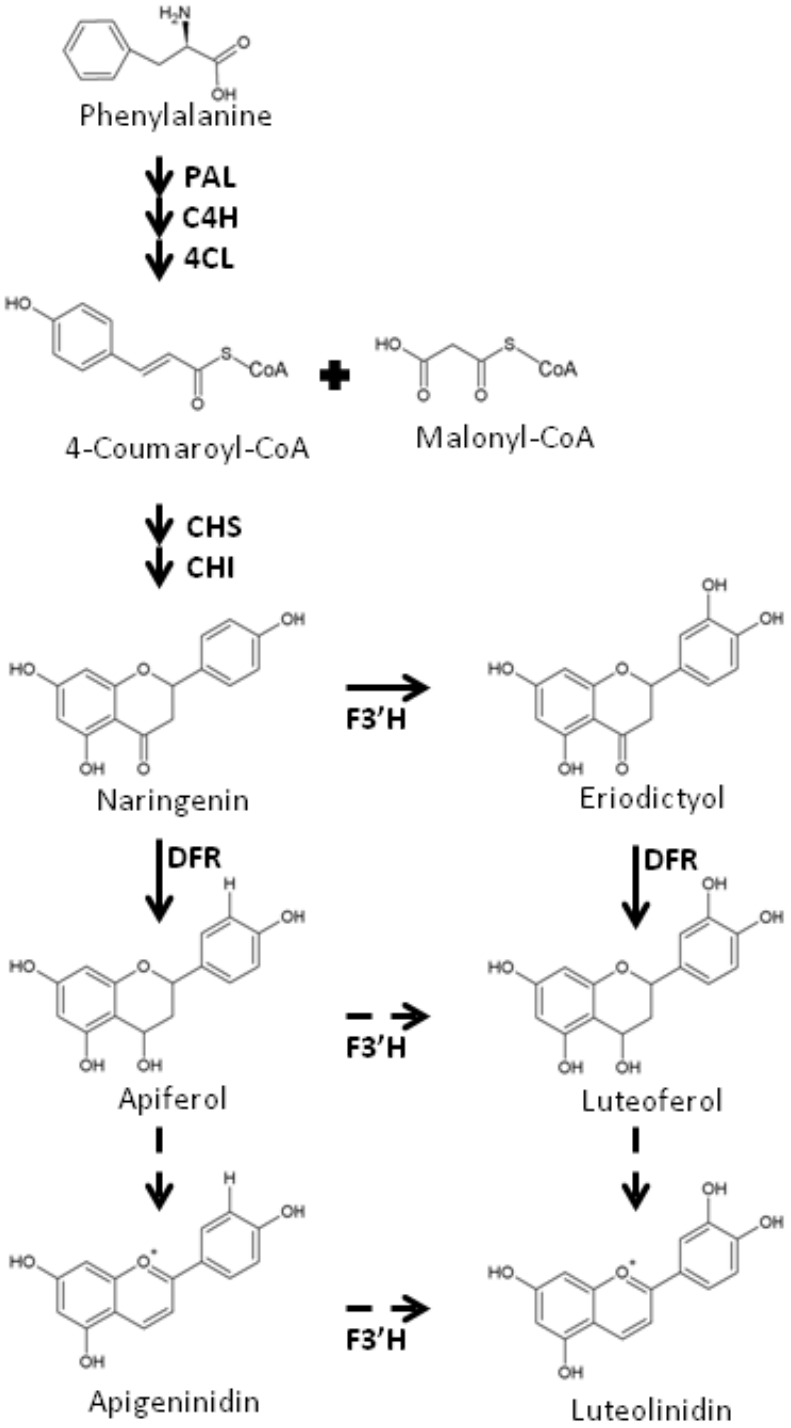


Fig. 2. Similar to the formation of most flavonoids, the biosynthesis of 3-deoxyanthocyanidins (3-DXAs) starts with the phenylpropanoid pathway. This pathway converts phenylalanine to 4-coumaryol-CoA with phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H) and 4-coumarate:CoA ligase (4CL). Chalcone synthase (CHS) then catalyzes the condensation of 4-coumaroyl with three molecules of malonyl-CoA forming naringenin chalcone (not depicted). Chalcone-flavanone isomerase (CHI) catalyzes the stereo-specific isomerization of naringenin chalcone to naringenin. Under conditions suitable for 3-DXA formation, naringenin is converted to apiferol by the actions of dihydroflavonol reductase (DFR). The subsequent actions leading to the formation of the 3-DXA apigeninidin are currently unknown. Naringenin can also be converted to eriodictyol by the actions of flavonoid 3' hydroxylase (F3'H) and similarly converted to the 3-DXA luteolinidin by the aforementioned enzymatic steps involving DFR and some other uncharacterized step.

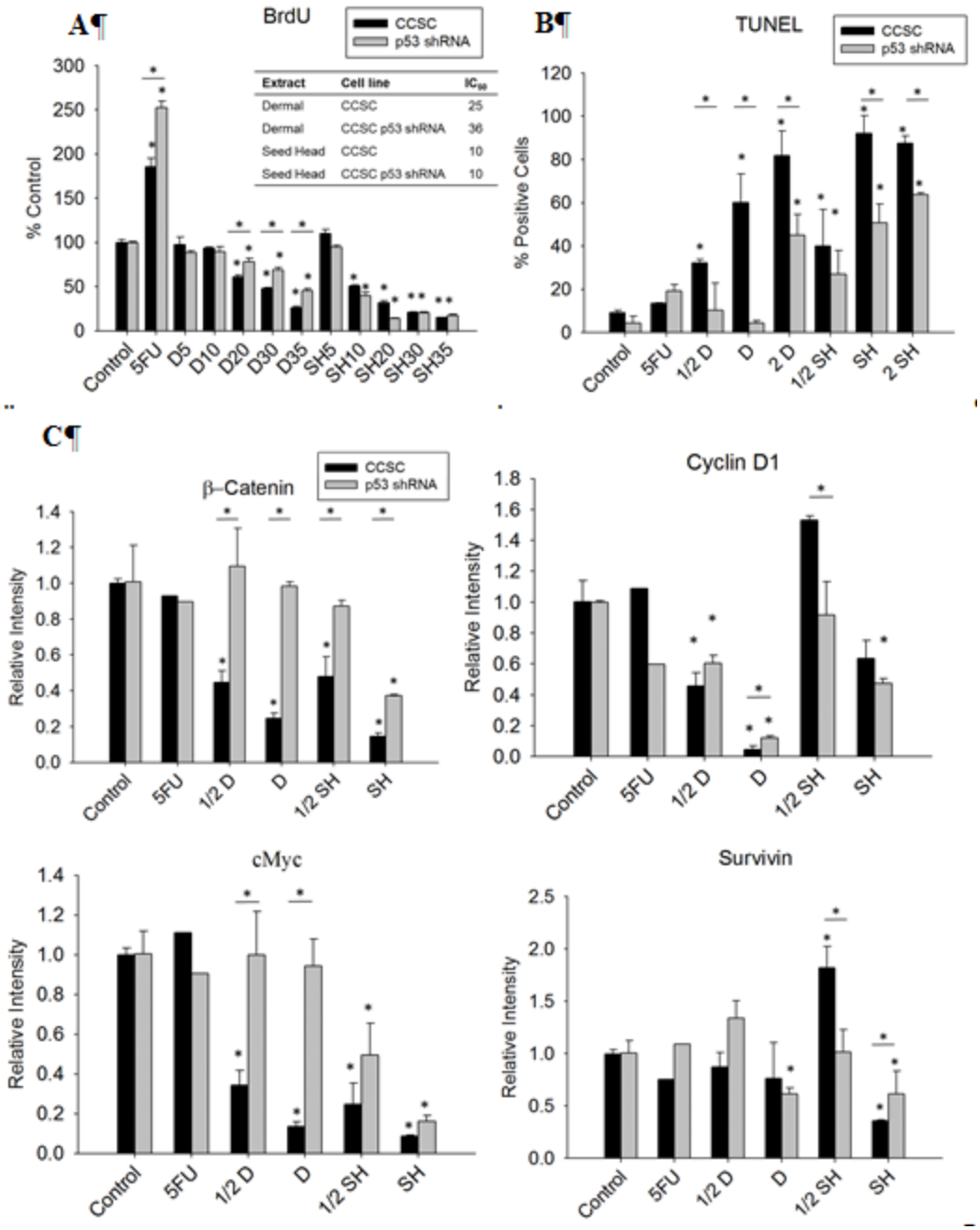


Fig. 3. Effects of sweet sorghum dermal layer and seed head extracts on proliferation, apoptosis, and levels of β-catenin and β-catenin targets cMyc, cyclin D1, and survivin in human colon cancer stem cells (CCSC) and CCSC expressing p53 shRNA (CCSC p53 shRNA). **(A)** The

antiproliferative activity of select dermal layer and seed head extracts and calculated IC_{50} values were determined by BrdU assay after 24 h treatment with extracts. The antiproliferative activity of dermal layer is partially p53-dependent while that of seed head is p53-independent. Data are presented as percent of the solvent control (% Control) as means (n = 3) ± standard error. **(B)** The proapoptotic activity of select dermal layer and seed head extracts were determined by Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. Results from TUNEL assay experiments are presented as apoptotic index (% Positive cells) as means (n = 2) ± standard deviation. **(C)** Dermal layer and seed head extracts suppressed expression of β-catenin, cyclin D1, cMyc and survivin as measured by densitometry analysis of western blots. Densitometry analysis was used to quantify these proteins relative to β-actin with control lanes set to one. Data are presented as means (n = 2) ± standard deviation. * Indicates significant differences ($P < 0.05$). Control (C) = 80% ethanol used for solvent control, D = Dermal layer extract, SH = Seed head extract, 5FU = 5-fluorouracil (18 μg mL^{-1}). Dermal layer and seed head treatments were dosed based on IC_{50} values in μg GAE mL^{-1}. The coefficient preceding the treatment represents dose (i.e. 1/2 is equivalent to 1/2 of the IC_{50} value).

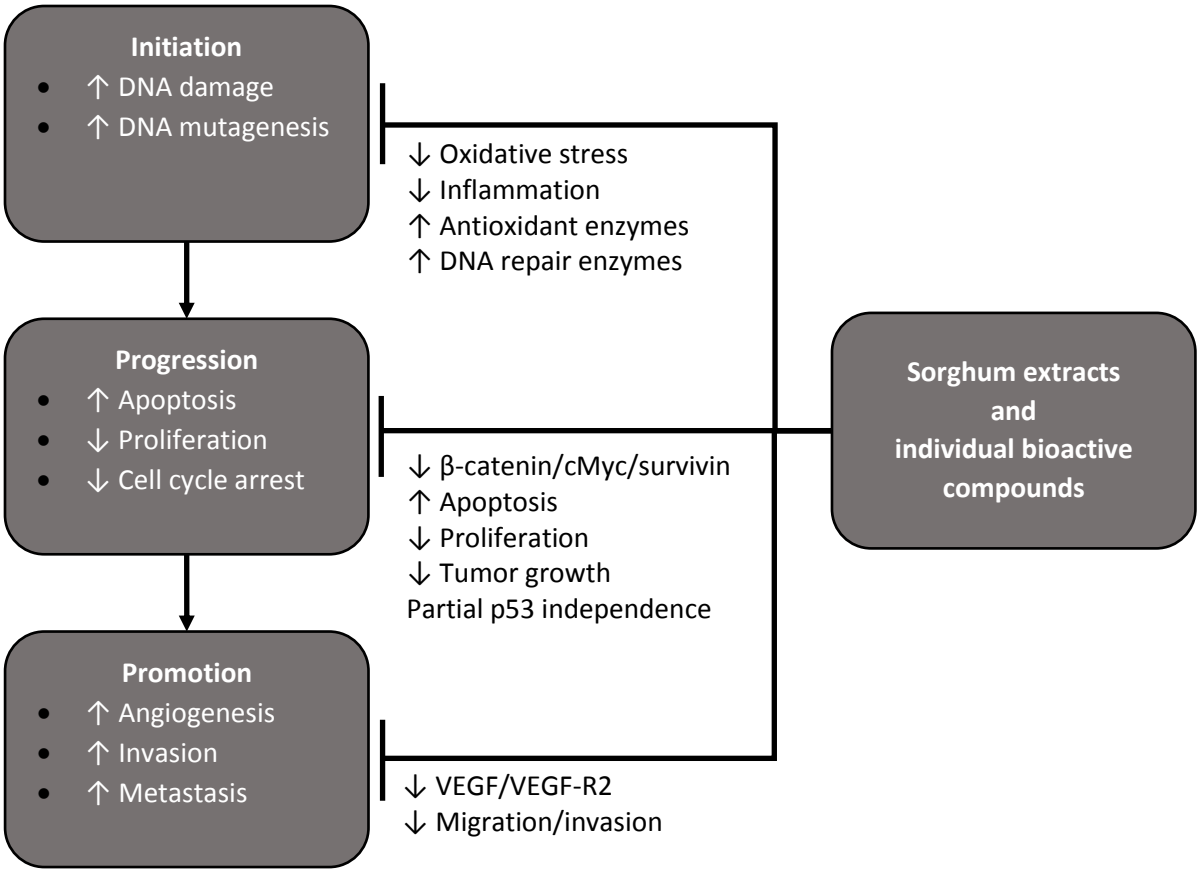


Fig. 4. The possible anticolon cancer mechanism of sorghum extracts and identified individual bioactive compounds.