EFFECT OF SPENT LUBRICATING OIL ON THE TOTAL ANTIOXIDANT PROPERTIES, TOTAL FLAVINOID CONTENT AND TOTAL PHENOLIC CONTENT OF SORGHUM BICOLOR SEEDLINGS AFTER 14 DAYS OF GERMINATION

BY

IBUDE IREDIA

(BMS1304542)

A PROJECT SUBMITTED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE, B.Sc. (HONS) MEDICAL BIOCHEMISTRY, OF THE UNIVERSITY OF BENIN, BENIN CITY.

OCTOBER, 2023

CERTIFICATION

We the undersigned hereby certify that IBUDE IREDIA (BMS1304542) carried out this research in the Department of Medical Biochemistry, University of Benin, Benin city and thereby approve same as adequate in scope and quality for the award of Bachelor of Science Degree (B.Sc) in Medical Biochemistry.

Signed

....

Dr. Aguebor-Ogie N. Bobby **(Date)**

(Project Supervisor)

....

Dr. Fidelis E. Olumese **(Date)**

(Head of Department)

....

External Examiner **(Date)**

DEDICATION

This project is dedicated to Almighty God, the giver of life who has made it possible to complete my Bachelor of Science Degree (B.Sc) program in the Department of Medical Biochemistry and my entire family.

ACKNOWLEDGEMENT

My gratitude goes for Almighty God for his grace in all my endeavors, unto him is all the glory. My sincere appreciation goes to my amiable supervisor Dr. Aguebor-Ogie Bobby who also doubles as my course adviser alongside with the head of department Dr. Fidelise Olumese and other lecturers in the Medical Biochemistry department.

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ABSTRACT

The present study investigated the influence of spent lubricating oil (SLO) and its various fractions on the Total Antioxidant Potential (TAP), Total Phenolic Content (TPC), and Total Flavonoid Content (TFC) in Sorghum bicolor. The research aimed to understand the potential ecological and physiological implications of SLO contamination in agricultural soils. Four extracts were analyzed: water extract, water insoluble fraction extract, water-soluble fraction extract, and whole spent lubricating oil extract. Each extract exhibited distinct effects on the biochemical parameters measured. The results are presented in terms of milligrams of Gallic Acid Equivalent (GAE) per gram for TAP, milligrams of GAE per gram for TPC, and milligrams of Quercetin Equivalent (QE) per gram for TFC. The water extract displayed a TAP value of 5.00 mg GAE/g, which was significantly lower than the other extracts, indicating reduced antioxidant potential. In contrast, the water insoluble fraction extract exhibited the highest TAP value of 15.00 mg GAE/g, suggesting that this fraction possessed the most potent antioxidant properties. These findings highlight the varying impacts of SLO and its fractions on TAP, TPC, and TFC in Sorghum bicolor. The differences in these parameters among the extracts indicate that SLO contamination can have a multifaceted effect on the antioxidant and phenolic composition of this important agricultural crop. Understanding these effects is crucial for mitigating the potential harm to both the environment and human health, and for developing strategies to ensure the sustainability of Sorghum bicolor cultivation in contaminated soil.

CHAPTER ONE

1.0 INTRODUCTION

Soil is the most valuable and non-renewable component of farming which requires proper maintenance for environmental sustainability. Sustainable use of this natural resource on which agriculture depends is absolutely necessary for agricultural productivity. Soil pollution by crude oil and petroleum products such as fuel oils, spent engine and diesel fuels are presently a menace in Nigeria, particularly in urban areas (Adenipekun *et al*., 2009, Onwuka *et al*., 2012). Spent engine oil is a waste lubricating oil collected from automobile workshops, garages and industrial sources like hydraulics oil, turbine oil, process oil and metal working fluids (Osuagwu *et al*., 2017). Spent engine oil affects the physical, chemical and biological properties of the soil and hence, it is affecting the growth, development, productivity and yield of plants (Swapna *et al*., 2021). Continuous increase in the number of cars and automobile users in Nigeria and the ways of disposal of this oil are of major concern to the scientists, agriculturists, environmentalists, and other stake holders. In the recent time, many scientists (Ahamefule *et al*., 2017; Ikhajiagbe *et al*., 2017; Oluwanisola and AbdulRahaman 2018) have reported on the implications of the uncontrolled, improper and indiscriminate disposal of spent engine oils on the soil Bioaccumulation of hydrocarbons in plant appears to be related to the lipid content of the plant tissue, the greater the hydrocarbon accumulation. Plants may be killed by oil pollution or suffer reduced growth and reproductive rates due to combination of physical coating, altered soil chemistry and toxic effects of crude oil components Sorghum is the fifth most important cereal crop in the world after wheat, rice, corn and barley. Sorghum out-performs other cereals under various environmental stresses and is thus generally more economical to produce. More than 35% of sorghum is grown directly for human consumption. The rest is used primarily for animal feed and alcohol and industrial products. Sorghum contains various phytochemicals (including phenolic compounds, plant sterols and policosanols) that are secondary plant metabolites or integral cellular components. Phenols help in the natural defense of plants against pests and diseases, while the plant sterols and policosanols are mostly components of wax and plant oils. The phytochemicals have gained increased interest due to their antioxidant activity, cholesterol lowering properties and other potential health benefits. The phenols in sorghums fall under two major categories; phenolic acids and flavonoids. The phenolic acids are benzoic or cinnamic acid derivatives (Dykes *et al*., 2006), whereas the flavonoids include tannins and anthocyanins as the most important constituents isolated from sorghum to date (Krueger *et al*., 2003). Sorghum phytosterols are similar in composition to those from corn and contain mostly free sterols or stanols and their fatty acid/ferulate esters (Singh *et al*., 2003).

1.1 AIM OF STUDY

In this connection, this present study has been undertaken to determine the effect of soil contamination with petrol and spent oil on the growth of guinea corn.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 ENVIRONMENTAL POLLLUTANTS

Crude oil pollutants are highly stable compounds persisting in the environment for a long time before they can be broken down (Kumar *et al.,* 2023). They accumulate in tissues of most fauna and flora life, poisoning and causing a wide range of toxic effect on them. Oil pollutants include aviation fuel, engine oil, petrol, spent oil, and diesel. The extent to which the environment is polluted by these oils depends on the frequency and severity of the spills in a given area (Lecklin *et al.,* 2011). Oil pollution is associated with highly toxic heavy metals such as cadmium, zinc, lead, chromium, nickel, and manganese with great negative effects on plants (Nagajyoti *et al.,* 2010).

Different heavy metals at optimal concentrations have been shown to inhibit various metabolic processes in plants resulting in their reduced growth and development (Goyal, 2020). Plants may be killed by oil pollution or suffer reduced growth and reproductive rates due to a combination of physical coating, altered soil chemistry, and toxic effects of crude oil components (Onen, 2023). Noticeable forms of stunted growth and chlorosis in plants and also marked anatomical change in plant tissues are responses to heavy metal toxicity (Reichman, 2002).

Oil spillage on land retards vegetative growth for a period of time and in extreme cases, leads to the destruction of vegetation. It also increases potential for hazards and renders the soil unfit for cultivation (Komolafe *et al.,* 2015). Any hydrocarbon solvent is liable to penetrate into plants through lipophilic surface, roots and once inside, it dissolves the cell membranes and cause loss of cell sap and in some cases, accumulates in the tissue of fruits of crops.

Bioaccumulation of hydrocarbons in plant appears to be related to the lipid content of the plant tissue, the greater the hydrocarbon accumulation (Studabaker *et al.,* 2012). The contamination of soil environment can undoubtedly limit its protective function, upset metabolic activity, unfavorably affect its physicochemical characteristics, reduce fertility, and negatively influence plant production (Murphy, 2013). The above features are very much influenced by anthropogenic factors, which include the contamination of soil with petroleum-derived products (Wyszkowski and Ziółkowska, 2008). Changes in some soil properties resulting from contamination with petroleum-derived substances, and particularly those related to physicochemical composition, bring about some changes in the biological composition of soil which in consequence, can lead to water and oxygen deficits, as well as to a shortage of available forms of nitrogen and phosphorus (Aislabie *et al.,* 2013).

Since contamination of soil with refinery products deteriorates its biochemical and physiochemical properties, it also limits the growth and development of plants, whose nutritive and technological value can be low and often questionable (Allan *et al., 2021*). In this connection, this present study has been undertaken to determine the effect of soil contamination with petrol and spent oil on the growth of guinea corn.

2.1.1 SPENT LUBRICATING OIL

Spent engine oil is a waste lubricating oil collected from automobile workshops, garages and industrial sources like hydraulics oil, turbine oil, process oil and metal working fluids (Osuagwu *et al*., 2017). Spent engine oil affects the physical, chemical and biological properties of the soil and hence, it is affecting the growth, development, productivity and yield of plants (Swapna *et al*., 2021). Continuous increase in the number of cars and automobile users in Nigeria and the ways of disposal of this oil are of major concern to the scientists, agriculturists, environmentalists, and other stake holders. In the recent time, many scientists have reported on the implications of the uncontrolled, improper and indiscriminate disposal of spent engine oils on the soil. Many authors have investigated and reported the effects of petroleum oil and spent engine oil on soil and plant noted that petroleum oil in soil have deleterious effects on biological, chemical and physical properties of the soil depending on the dose, type of the oil and other factors. Spent auto-engine oil is known to contain increased amounts of heavy metals compared to the unused oil. observed that soil contamination with spent engine oil can alter the physio-chemical properties of the soil and degrade its capacity to provide suitable habitat for plants' growth in such soil.

2.2 WHITE SORGHUM

Sorghum or broomcorn is a genus of about 25 species of flowering plants in the grass family (Poaceae). Some of these species are grown as cereals for human consumption, in pastures for animals as fodder, and as bristles for brooms (Tonapi *et al.,* 2022) Sorghum grain is a nutritious food rich in protein, dietary fiber, B vitamins, and minerals. Figures 2.1 and 2.2 shows the leaves and seeds of white sorghum.

Sorghum is either cultivated in warm climates worldwide or naturalized in open plains. In 2021, world production of sorghum was 61 million tonnes, with the United States as the leading grower.

FIG 2.1 showing the leaves of white sorghum

FIG 2.2 seeds of white sorghum

2.3 TAXONOMY

Sorghum is in the grass family, Poaceae, in the subfamily Panicoideae, in the tribe Andropogoneae – the same as maize (*Zea mayz*), big bluestem (*Andropogon gerardi*), and sugarcane (*Saccharum spp*.). Genetics and genomics

Agrobacterium transformation can be used on this genus, (Guo *et al*.,2019) as shown in a 2018 report of such a transformation system. A 2013 study developed and validated an SNP array for molecular breeding.

2.4 DISTRIBUTION AND HABITAT

Seventeen of the 25 species are native to Australia, with the range of some extending to Africa, Asia, Mesoamerica, and certain islands in the Indian and Pacific Oceans (Sorenson *et al*., 2005).

2.5 PRODUCTION AND TOXICITY

in 2021, world production of sorghum was 61 million tones, led by the United States with 19% of the total. India, Ethiopia, and Mexico were secondary producers. In the early stages of plant growth, some sorghum species may contain levels of hydrogen cyanide, hordenine, and nitrates lethal to grazing animals. Plants stressed by drought or heat can also contain toxic levels of cyanide and nitrates at later stages in growth (Gleadow *et al*., 2006).

2.6 USES

2.6.1 NUTRITION

Sorghum grain is 72% carbohydrates including 7% dietary fiber, 11% protein, 3% fat, and 12% water. In a reference amount of 100 grams (3.5 oz) (Tonapi *et al.,* 2022), sorghum grain supplies

79 calories and rich contents (20% or more of the Daily Value, DV) of several B vitamins and dietary minerals

The grain is edible and nutritious. It can be eaten raw when young and milky, but has to be boiled or ground into flour when mature.

2.6.2 CULTIVATION

Sorghum cultivation has been linked by archeological research to ancient Sudan around 6,000 to 7,000 BP. One species, S. bicolor, native to Africa with many cultivated forms, is a common crop worldwide, used for food (in the form of grain or sorghum syrup), animal fodder, the production of alcoholic beverages, and biofuels (Popp *et al.*, 2016).

In Nigeria, the pulverized red leaf-sheaths of sorghum have been used to dye leather, and in Algeria, sorghum has been used to dye wool.

Most varieties of sorghum are drought- and heat-tolerant, nitrogen-efficient, and are grown particularly in arid and semi-arid regions where the grain is one of the staples for poor and rural people (Dorwad 2012). These varieties are forage in many tropical regions. S. bicolor is a food crop in Africa, Central America, and South Asia, and is the fifth most common cereal crop grown in the world.

2.6.3 ROLE IN GLOBAL ECONOMY

Global demand for sorghum increased dramatically between 2013 and 2015, when China began purchasing US sorghum crops to use as livestock feed as a substitute for domestically grown maize (Popescu *et al.*, 2014). China purchased around $1 billion worth of American sorghum per year until April 2018, when China imposed retaliatory duties on American sorghum as part of the trade war between the two countries.

2.7 ANTIOXIDANT PROPERTIES OF SORGHUM PHENOLS AND THEIR BIOAVAILABILITY

Currently antioxidant activity is the most common in vitro parameter used to assess or predict potential benefits of plant phytochemical compounds. However, correlations between in vitro antioxidant activity and actual health benefits are unknown. Such in vitro antioxidant data ignore other potentially beneficial or harmful effects of phytochemicals like modification of enzyme activity and/or cell signaling pathways. For example, vitamin C and E, and the carotenoids, which were previously recognized only for their antioxidant characteristics, were shown to induce other biological responses (Astley, 2003). Antioxidant activity data are also hard to compare since there are no standardized methods; the methods currently used do not always agree in terms of ranking samples for antioxidant efficacy. Additionally, measured antioxidant activity in vitro tells us nothing about release and uptake of the compounds, as well as their distribution and metabolism in the body. However, antioxidant activity data still provide useful information for screening plant materials and products with desirable compounds and properties that can be used for further biological testing. In sorghum, phenol content correlates most strongly with antioxidant activity measured by various methods indicating the phenols are largely responsible for the activity (Awika, 2003).

2.7.1 TANNINS IN SORGHUM

Tannins are the most uniquely important phytochemical components of sorghum since they possess properties that produce obvious and significant effects in animals, and have also been associated with various positive and negative impacts on human health (Aerts *et al*., 2018). These aspects and their relevance are discussed in later sections of the review.

Production and genetics of tannin sorghums

Even though tannins are commonly associated with sorghums, more than 99% of sorghum currently produced in the US is tannin-free. Decades of breeding efforts to eliminate tannins from sorghum were motivated mostly by the reduced feed value of the tannin sorghums. Tannins bind to and reduce digestibility of various food/feed nutrients, thus negatively affecting productivity of livestock. Current non-tannin sorghums grown for livestock feed in the US have virtually the same energy profile as corn. The limited quantities of tannin sorghums grown in the US are mostly identity preserved seed stock lines (Wu *et al*., 2012).

Tannins are present in sorghums with a pigmented testa (classified as type II and III sorghums). These sorghums have dominant $B_{1\text{and}}B_2$genes. The B_1 and B_2 genes control the presence or absence of the pigmented testa layer (Earp *et al*., 2004). Both genes must be dominant for a pigmented testa to develop. When the S gene (spreader gene) is dominant concurrently with the dominant B_1 and B_2 genes, pericarp color becomes phenotypically brown (Earp *et al*., 2004). The sorghums with the dominant S gene generally contain tannins that are more easily extractable than the ones with the recessive gene. Such sorghums (with dominant S gene) also produce greater anti-nutritional effects in animals (Hodges *et al*., 2021). Since the pericarp color and secondary plant color of sorghum is genetically controlled, it is possible to develop different combinations of pericarp and plant color with and without the pigmented testa and spreader genes, which opens the possibility of significantly different levels and combinations of phenolic compounds.

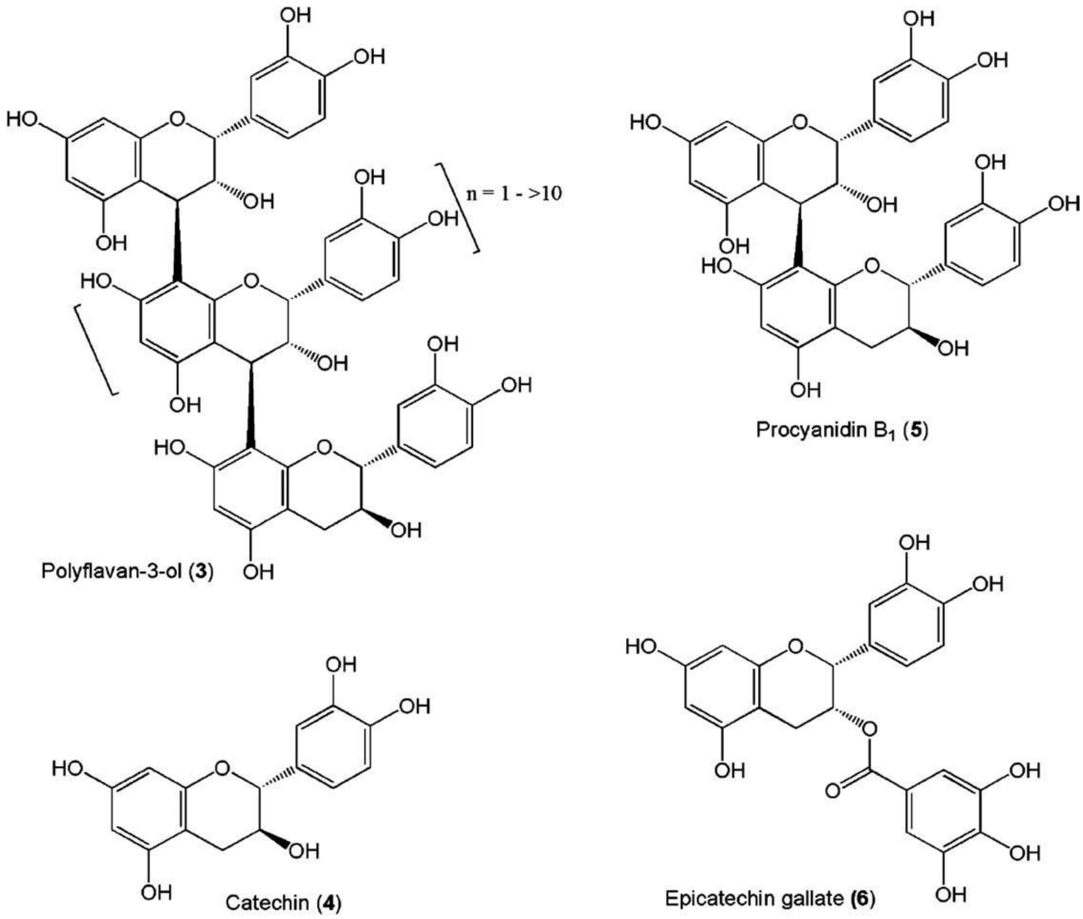
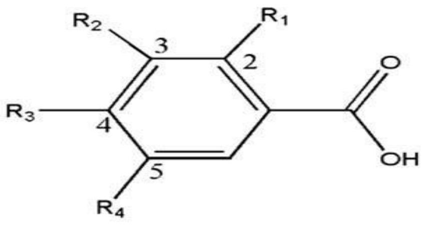


Fig. 2.3. The proanthocyanidins most commonly reported in sorghum (Awika, 2003).

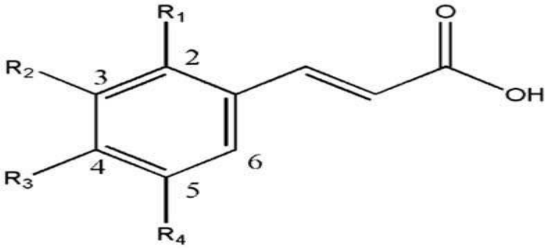
2.7.2 PHENOLIC ACIDS OF SORGHUM

The phenolic acids (PA) of sorghum largely exist as benzoic (11–16) or cinnamic (17–21) acid derivatives (fig 2.4). As in other cereals, the sorghum phenolic acids are mostly concentrated in the bran (outer covering of grain). The phenolic acids exist mostly in bound forms (esterified to cell wall polymers), with ferulic acid being the most abundant bound PA in sorghum and other cereals (Adom and Liu, 2002).



Benzoic acids (**11-16**)

Gallic acid (**11**): $R_1 = H$, $R_2 = R_3 = R_4 = OH$
Gentisic acid (**12**): $R_1 = R_4 = OH$, $R_2 = R_3 = H$
Salicylic acid (**13**): $R_1 = OH$, $R_2 = R_3 = R_4 = H$
p-hydroxybenzoic acid (**14**): $R_1 = R_2 = R_4 = H$, $R_3 = OH$
Syringic (**15**): $R_1 = H$, $R_2 = R_4 = OCH_3$, $R_3 = OH$
Protocatechuic(**16**): $R_1 = R_4 = H$, $R_2 = R_3 = OH$



Cinnamic acids (**17-21**)

Caffeic acid (**17**): $R_1 = R_4 = H$, $R_2 = R_3 = OH$
Ferulic acid (**18**): $R_1 = R_4 = H$, $R_2 = OCH_3$, $R_3 = OH$
o-coumaric acid (**19**): $R_1 = OH$, $R_2 = R_3 = R_4 = H$
p-coumaric acid (**20**): $R_1 = R_2 = R_4 = H$, $R_3 = OH$
Sinapic (**21**): $R_1 = H$, $R_2 = R_4 = OCH_3$, $R_3 = OH$

Fig 2.4 showing the various types of phenolic acid in S.bicolor

Several other PA have been identified in sorghum including syringic, protocatechuic, caffeic, p-coumaric, and sinapic as the more abundant (Rao *et al*., 2018). The Phenolic acids like other phenols are thought to help in plant defense against pests and pathogens. The PA show good antioxidant activity in vitro and thus may contribute significantly to the health benefits associated with whole grain consumption.

2.7.3 ANTHOCYANINS

Anthocyanins were reported to have low absorption compared to other flavanoids (Wu *et al*., 2002). However significant absorption of these compounds was demonstrated (Shinde *et al.,* 2022). Most of these data were obtained for fruit anthocyanins which are thought to contribute significantly to the health benefits of fruit consumption. We have not found any work reporting bioavailability of the 3-deoxyanthocyanidins commonly found in sorghum. This is probably because these relatively rare anthocyanins were not considered to be of economic interest. The high antioxidant capacity of black sorghums and their brans were correlated with their anthocyanin

Hence, anthocyanins may contribute significantly to any potential health benefits of these sorghums. (Boveris *et al*. 2001) demonstrated that a 3-deoxyanthocyanidin (apigeninidin) isolated from white sorghum had strong dose-dependent quenching ability against ascorbyl and lipid radicals. Anthocyanins from fruits have been shown to possess several therapeutic benefits, including vasoprotective and anti-inflammatory properties (Alam *et al*., 2021), anti-cancer and chemoprotective properties, as well as anti-neoplastic properties (Shinde *et al.,* 2022).

2.8 SORGHUM AND CARDIOVASCULAR DISEASE

Cardiovascular disease (CVD) is the number one killer in the USA (Sistino, 2003). Various epidemiological data indicate that whole grain consumption significantly lowers mortality from CVD (Kushi *et al*., 1999; Slavin *et al*., 2000; Anderson, 2003). The phytosterols are believed to contribute to beneficial effects. Other components of the whole grains, including polyphenols and fiber, also play a role in CVD prevention. For example, a cholesterol-lowering effect of tea and grape catechins and tannins is widely report (Tebib *et al*., 1997; Santos-Buelga and Scalbert, 2000). We did not find any study that directly assesses effects of sorghum polyphenols on cholesterol.

Benefits of sorghum to cardiovascular health may not be limited to positive effects on cholesterol. Lee and Pan (2003) demonstrated that dietary tannin-sorghum distillery residues inhibited 63–97% of hemoglobin-catalyzed oxidation of linoleic acid in cultured mullet fish compared to soybean (13%) and rice bran (78%). The authors also found that the sorghum residues significantly improved blood-thinning and erythrocyte membrane integrity of the fish blood cells during winter, thus maintaining normal blood fluidity and preventing RBC hemolysis induced by H_2O_2. They attributed the prevention of RBC hemolysis to the antioxidant activity of the tannins and other polyphenols present in the sorghum residue. Such protective effect on RBC was previously demonstrated for tea and red wine polyphenols. The blood thinning effect is also in agreement with epidemiological data that suggest that polyphenols from tea may reduce risk of stroke (Zaragoza *et al*., 2011).

In general, several epidemiological studies show that red wine and tea consumption correlate with reduced risk of CVD (Poirier *et al.,* 2006). The effects are largely attributed to the tannins and other polyphenols in these food products. There is no epidemiological data on tannin sorghum consumption and CVH in humans. This may partly be due to the fact that in the regions with high human sorghum consumption, CVD is not regarded as a major problem since there are other diseases that are of bigger concern. Such a study is important to help validate some of the evidence reported for in vitro and controlled animal studies, and determine the role sorghum may play in fighting CVD. The presence of unique polyphenols in sorghum (discussed earlier) is likely to produce positive benefits.

2.9 EFFECTS OF WHITE SOGHURM ON ANIMAL WEIGHT GAIN

Depend on levels fed as well as animal species. Al-Mamary *et al*., (2001) found addition of 1.4% catechin equivalents (CE) sorghum to rabbit diet had no effect on growth rate and weight gain, whereas at a CE of 3.5%, there was a marked decrease in live weight gain and feed conversion ratio. Cousins *et al*. (1981) reported a 10% reduction in feed efficiency (relative to corn) when high tannin (3.1– 3.4% CE) sorghums were fed to pigs. A low tannin sorghum (0.8% CE) had similar feed efficiency as corn. Lizardo *et al*. (1995) also observed a 10% reduction in weight gain when pigs were fed tannin-sorghum diet compared to corn. Broiler chicks fed low tannin sorghums (0.6–0.9% CE) had similar weight gain and feed efficiency compared to corn. However, these authors observed an average of 50% reduction in weight gain when high tannin sorghums (2.7–3.5% CE) were fed to the broiler chicks. Such data suggest that there is a threshold for the inhibitory effects of sorghum tannins, and that animal species are affected differently (Awika *et al*., 2004).

It is essential to assess the possibility of using this information to fight obesity in humans. Grain-based foods (breakfast cereals, bread, cookies, extruded snacks, etc.) are common and widely consumed in many parts of the world. High tannin sorghum grains and their milled fractions (bran) can find use as parts of ingredients in such foods. Brans from high tannin sorghums were incorporated in bread and cookies at up to 15% and 30%, respectively, without significant differences in texture or flavor profiles compared to wholewheat products (Awika *et al.,* 2004). Cereal-based foods are a viable means of fighting obesity since they are consumed more consistently than most other foods and contribute a significant portion of the daily calorie intake.

However, to use tannin sorghums in human diet to help fight obesity, the following need to be determined:

1. Whether the effects observed in animals are reproducible in humans.
2. The levels of tannins in sorghum necessary to produce desired effects.
3. Potential side effects of using tannin sorghums and their fractions at such levels, e.g., effects on availability of other essential micronutrients, especially divalent minerals like iron (which is chelated by tannins), and how the effects can be overcome.
4. How various food processing conditions affect potential activity of the tannins.

2.10 WHITE SORGHUM PHYTOCHEMICALS AND HUMAN HEALTH

The most effective (cost or otherwise) solutions to many major human health conditions lie in the natural components of foods we eat, rather than expensive medical intervention. The challenge is to find and incorporate a balance of the functional ingredients in everyday foods at adequate levels. Sorghum, which is currently underutilized, is definitely worth attention as a source of health-promoting phytochemicals for such foods. Some potential applications of sorghum include:

1. Direct food use. Whole sorghum grain can be used in baked, extruded and other cereal-based products (bread, cookies, expanded snacks, pasta, breakfast cereals, etc.) as partial or complete substitutes for other cereals. The sorghum brans can be used to fortify bread, cookies and other snacks, to improve the phytonutrient content, as well as dietary fiber and sensory properties. We previously demonstrated that pigmented sorghum brans in combination with other ingredients produce acceptable quality (Kean *et al.*, 2011), dark-colored baked goods that

appeal to consumers due to their natural ‘healthy’ appearance (no need for artificial darkening, e.g., as caramel is used to darken rye bread).

2. Extraction of active components for commercial use. Most phytochemicals in sorghum are concentrated in bran fractions. These fractions are easily separated from sorghum by decortication and can then be used to extract the various phytochemicals for dietary supplementation, food quality improvement or therapeutic applications. For example, sorghum anthocyanins, which were reported as more stable than the fruit anthocyanins, can be used as natural food colors with functional properties. The phytosterols and policosanons are found in sorghum bran and spent distiller’s grain in relatively large quantities; these sorghum fractions may provide a low-cost source of these valuable compounds. Sorghum tannins can be extracted for use as antioxidant supplements as well as anti-caloric agents for obese individuals (Awika *et al*., 2004). Other phenolic antioxidants from sorghum can also be used as natural food preservatives, antioxidant supplements and therapeutic agents, among other uses.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Chemicals and Reagents

Sodium nitrite, Sodium hydroxide, Aluminium chloride, Quercetin, Folin-Ciocalteu reagent (FCR), Sodium carbonate, Gallic acid, Sodium phosphate, Ammonium molybdate, Sulfuric acid, Citric ethanol, Normal saline (0.9% Nacl), spent lubricating oil.

3.1.2 Equipmemt

The apparatus used in the experiment include Analytical weighing balance, Reagent bottles, Aluminum foil, Magnetic stirrer, Mortar and pestle, Hand gloves, Kitchen towel, Paper tapes, Measuring cylinder, Micro pipette, Beakers – 25ml, 50ml, 100ml and 500ml (Pyrex, England), Test tubes and Racks, Separating funnel, Retort stand, Conical flask, Cotton wool, Ruler, Thread, Dissecting set, Plastic cans, Petri dishes, 10ltr jerrican, Distilled water, spent lubricating oil and a 5ltr Transparent Plastic Bucket.

3.2 METHODS

3.2.1 Experimental Protocol

A cup of white sorghum seeds was soaked in a clean bowl with distill water for 24 hours. Grains that floated were sieved out and others that submerged were planted in a cotton wool soil base, which was treated with various fractions of the spent lubricating oil according to the groupings, which are:

i. Control group
ii. Water insoluble fraction (WISF) group
iii. Water soluble fraction (WSF) group
iv. Whole spent lubricating oil (WSLO) group.

48 seeds were planted in the four different groups with each group containing twelve seeds. After 14 days of planting, the stem and roots were harvested by using sickles and then homogenized. The mixture of the stems and roots homogenates were extracted into different samples bottles based on the groups by using 3ml of Normal (0.9% Nacl) for each sample. The samples were then centrifuged at 4000rpm for 5 minutes and decanted into plain bottles and kept for future use.

3.2.2 Total Antioxidant Power (TAP) Estimation

Total Antioxidant Power (TAP) was determined by the procedure of Otitolaiye *et al. (*2023). The antioxidants present in the extracts reduce the phosphomolybdate (VI) ion to a green phosphomolybdate (V) ion. This assay is also called phosphomolybdate (Sasikumar and Kalaisezhiyen, 2014) and in this case, the reactions of sodium phosphate and ammonium molybdate in the presence of H_2SO_4 produce the free radical (phosphomolybdate ion). First, equal amounts of 4 mM ($(NH_4)_2MoO_4$), 28 mM Na_3PO_4, and 0.6 M H_2SO_4 were added to produce the standard phosphomolybdate reagent solution. The standard used for this assay was (gallic acid) and was prepared in concentrations of 10, 25, 50, 100, 200, 300, 400, and 500µg/mL while 500µg/mL of extracts were utilized. To 1mL of extract or gallic acid reference was added 2 mL of the standard phosphomolybdate solution. Then, it was placed in a water bath at 95°C for 90 min. A subtle green colour developed after it was allowed to cool to ambient temperature. After determining the absorbance at 695 nm, the overall antioxidant strength was shown as the corresponding amount of gallic acid (mgGAE/g) in one gram of the extracts.Gallic acid (mgGAE/g) in one gram of the extracts.

Procedure

	Blank	**Test**	**Standard**
Standard (Gallic acid)	-	-	1ml
Distill water	1ml	-	-
Extract	-	1ml	-
Standard reagent solution (Na_3PO_4 + H_2SO_4 + $(NH_4)_2MoO_4$)	2ml	2ml	2ml

Incubated in a water bath at 95°C for 90 min.

Allowed to cool to 25°C

Read absorbance at 695nm

3.2.3 Phytochemicals Contents Estimation

3.2.3a Total Flavonoid Content (TFC)

The flavonoid concentration in this assay was estimated using the aluminum chloride colorimetric method. The standard quercetin (1mg/mL) was prepared in a range of concentrations, such as 10, 50, 75, 100 and 150 µg/mL. To 1 mL of the extract, or quercetin was added 0.3 mL of sodium nitrite (5%). After covering, this solution was incubated for 5 min at ambient temperature. Thereafter, 10% aluminum chloride (0.3 mL) was introduced, stirred, and the solution was once more allowed to stand for 5 min at ambient temperature. The solution was then combined with 2mL of 1M NaOH and left to stand at room temperature for an additional 10 min. The flavonoid concentration was quantified as milligrams of quercetin equivalent per gram of extract (mgQE/g) after the absorbance was measured at 510 nm.

Procedure

	Blank	**Test**	**Standard**
Standard (Quercetin)	-	-	1ml
Distill water	1ml	-	-
Extract	-	1ml	-
5% Sodium Nitrite	0.3ml	0.3ml	0.3ml
Covered and incubated for 5mins @ room temperature			
10% Aluminium chloride	0.3ml	0.3ml	0.3ml
Mixed and incubated for 5mins @ room temperature			
1M NaoH	2ml	2ml	2ml
Incubated for 10mins @ room temperature			
Absorbance was read at 510nm			

3.2.3b Total Phenolic Content (TPC) Estimation

The phenolic content of the extracts was ascertained using the Folin-Ciocalteu reagent (FCR) method. As described by Roy *et al*. (2018).The Folin-Ciocalteu reagent (FCR) assay's principle is that phenolic in the extracts reduce FCR, which causes the development of a blue colour that gets darker as phenolic concentration increases. One and a half (1.5) mL of 10% FCR reagent was put to a test tube already holding 200µL of extract/standard. The solution was covered and put in a dark cupboard at ambient temperature for 5 min. After that, 1.5mL of 5% Na_2CO_3 was introduced and thoroughly mixed. This was again covered and left to stand in a dark place for 2 hr at room temperature. The absorbance was later read at 750nm. Gallic acid (1mg/mL) was prepared at different concentrations of 5, 10, 25, 50, 75, 100, 150, and 200µg/mL, to plot the standard curve. The equivalent amount of gallic acid, in milligrams (mgGAE/g), was used to determine the amount of phenol in the extracts.

Procedure

	Blank	**Test**	**Standard**
Standard (Gallic acid)	-	-	200µL
Distill water	200µL	-	-
Extract	-	200µL	-
10% Folin-Ciocalteu reagent	1.5ml	1.5ml	1.5ml
Allowed to stand in the dark @ room temperature for 5min			
5% Na_2CO_3	1.5ml	1.5ml	1.5ml

CHAPTER FOUR

RESULTS

Table 4.1. Effect of Spent Lubricating Oil (SLO) and its Fractions on TAP, TPC and TFC of *S. Bicolor.*

Parameter	Water extract	Water insoluble fraction extract	Water soluble fraction extract	Whole spent lubricating oil extract
TAP (mgGAE/g)	5.00	15.00	12.00	4.00
TPC (mgGAEg)	28.00	32.00	20.00	24.00
TFC (mgQE/g)	4.00	6.00	5.00	4.00

QE-Quercetin Equivalent; GAE-Gallic Acid Equivalent. From the table (Table 4.1) above, the total antioxidant power (TAP) of *S. bicolor* was determined using gallic acid as standard. It was discovered that the water insoluble fraction (WISF) extract (15.00 mgGAE/g) had a higher TAP than the water-soluble fraction (WSF) extract (12.00 mgGAE/g) and the whole spent lubricating oil (WSLO) extract (4.00 mgGAE/g).

CHAPTER FIVE

DISCUSSION

Spent lubricating oil disposal is a significant environmental issue (Botas *et al.,* 2017). Finding alternative uses for spent oil can mitigate the negative environmental impact. The quality of agricultural products, such as white sorghum, is a vital aspect of food safety and nutrition. Assessing the impact of spent oil contamination is essential for consumer safety. The Total Antioxidant Potential (TAP), Total Phenolic Content (TPC), and Total Flavonoid Content (TFC) are critical indicators of the nutritional value and health benefits of foods (Bibi *et al.,* 2022). Understanding how spent oil affects these parameters in sorghum can provide insights into the potential health risks associated with consumption. White sorghum samples can be treated with varying concentrations of spent oil, representing different levels of contamination. TAP, TPC, and TFC can be measured using established laboratory techniques, such as Folin-Ciocalteu method for TPC, and colorimetric assays for TFC. It was discovered that spent oil had a huge impact on the total phenolic, antioxidant and flavonoid activity of the plant and this correlates with previous studies.

CONCLUISON

The reckless dumping of used engine oil results in soil becoming inhospitable for plant growth, leading to detrimental consequences for phytochemical and antioxidant characteristics as shown in this study for *Sorghum bicolor*. This presents a severe risk to both ecosystems and human well-being. It is of utmost importance to enhance public awareness concerning responsible disposal methods, as this is essential for the protection of the environment and the promotion of robust crop cultivation, ultimately securing a sustainable future

REFERENCES

Adom, K.K. and Liu, R.H. (2002). Antioxidant activity of grains. *Journal of agricultural and food chemistry*. *50*(21): 6182-6187.

Aerts, R., Honnay, O., and Van Nieuwenhuyse, A. (2018). Biodiversity and human health: mechanisms and evidence of the positive health effects of diversity in nature and green spaces. *British medical bulletin*, *127*(1): 5-22.

Aislabie, J., Deslippe, J.R. and Dymond, J. (2013). Soil microbes and their contribution to soil services. *Ecosystem services in New Zealand–conditions and trends. Manaaki Whenua Press, Lincoln, New Zealand*. 1(12): 143-161.

Alam, M. A., Islam, P., Subhan, N., Rahman, M. M., Khan, F., Burrows, G. E., ... and Sarker, S. D. (2021). Potential health benefits of anthocyanins in oxidative stress related disorders. *Phytochemistry reviews*, *20*(4): 705-749.

Allan, J.D., Castillo, M.M. and Capps, K.A. (2021). *Stream ecology: structure and function of running waters*. Springer Nature.

Awika, J. M., and Rooney, L. W. (2004). Sorghum phytochemicals and their potential impact on human health. *Phytochemistry*, *65*(9): 1199-1221.

Ayandele, A. (2012). *isolation, characterisation and biodegradation ability of bacteria isolated from soil contaminated with hydrocarbons*

Bibi, N., Shah, M.H., Khan, N., Al-Hashimi, A., Elshikh, M.S., Iqbal, A., Ahmad, S. and Abbasi, A.M., (2022). Variations in total phenolic, total flavonoid contents, and free radicals' scavenging potential of onion varieties planted under diverse environmental conditions. *Plants 11*(7): 950.

Botas, J. A., Moreno, J., Espada, J. J., Serrano, D. P., and Dufour, J. (2017). Recycling of used lubricating oil: Evaluation of environmental and energy performance by LCA. *Resources, Conservation and Recycling 125*: 315-323.

Charlton, N.C., Mastyugin, M., Török, B. and Török, M.0. (2023). Structural features of small molecule antioxidants and strategic modifications to improve potential bioactivity. *Molecules*. 28(3): 1057.

Chavan, U.D., 2020. *Sorghum Medicinal Food (Medicinal and Industrial Perspective)*. Scientific Publishers. 1(12): 143-161.

Deshpande, S.S., Cheryan, M., Salunkhe, D.K. and Luh, B.S. (1986). Tannin analysis of food products. *Critical Reviews in Food Science and Nutrition*. *24*(4): 401-449.

Dorward, Andrew. "The short-and medium-term impacts of rises in staple food prices." *Food security* 4 (2012): 633-645.

Duodu, K.G. and Awika, J.M. (2019). Phytochemical-related health-promoting attributes of sorghum and millets. In *Sorghum and millets* (pp. 225-258). AACC International Press.

Dykes, L. and Rooney, L.W. (2006). Sorghum and millet phenols and antioxidants. *Journal of cereal science*. *44*(3): 236-251.

Earp, C. F., McDonough, C. M., and Rooney, L. W. (2004). Microscopy of pericarp development in the caryopsis of Sorghum bicolor (L.) Moench. *Journal of Cereal Science*, *39*(1): 21-27.

Gleadow, R. M., Ottman, M. J., Kimball, B. A., Wall, G. W., Pinter Jr, P. J., LaMorte, R. L., and Leavitt, S. W. (2016). Drought-induced changes in nitrogen partitioning between cyanide and nitrate in leaves and stems of sorghum grown at elevated CO2 are age dependent. *Field Crops Research*, *185*: 97-102.

Goyal, D., Yadav, A., Prasad, M., Singh, T.B., Shrivastav, P., Ali, A., Dantu, P.K. and Mishra, S. (2020). Effect of heavy metals on plant growth: an overview. *Contaminants in agriculture: sources, impacts and management*. 79-101.

Gu, L., House, S. E., Rooney, L. W., and Prior, R. L. (2008). Sorghum extrusion increases bioavailability of catechins in weanling pigs. *Journal of Agricultural and Food Chemistry*, *56*(4):1283-1288.

Gujer, R., Magnolato, D. and Self, R. (1986). Glucosylated flavonoids and other phenolic compounds from sorghum. *Phytochemistry*. *25*(6): 1431-1436.

Guo, M., Ye, J., Gao, D., Xu, N. and Yang, J. (2019). Agrobacterium-mediated horizontal gene transfer: Mechanism, biotechnological application, potential risk and forestalling strategy. *Biotechnology advances*. *37*(1): 259-270.

Hodges, H. E., Walker, H. J., Cowieson, A. J., Falconer, R. J., and Cameron, D. D. (2021). Latent anti-nutrients and unintentional breeding consequences in Australian Sorghum bicolor varieties. *Frontiers in Plant Science*, *12*: 242-256.

Kean, E.G., Bordenave, N., Ejeta, G., Hamaker, B.R. and Ferruzzi, M.G., 2011. Carotenoid bioaccessibility from whole grain and decorticated yellow endosperm sorghum porridge. *Journal of Cereal Science*, *54*(3):450-459.

Komolafe, R.J., Akinola, O.M. and Agbolade, O.J. (2015). Effect of petrol and spent oil on the growth of Guinea Corn (Sorghum bicolor L.). *International Journal of Plant Biology*. *6*(1): 5883.

Kuhnert, S., Lehmann, L. and Winterhalter, P. (2015). Rapid characterisation of grape seed extracts by a novel HPLC method on a diol stationary phase. *Journal of Functional Foods*, *15*: 225-232.

Kumar, M., Bolan, S., Padhye, L.P., Konarova, M., Foong, S.Y., Lam, S.S., Wagland, S., Cao, R., Li, Y., Batalha, N. and Ahmed, M. (2023). Retrieving back plastic wastes for conversion to value added petrochemicals: opportunities, challenges and outlooks. *Applied Energy*. *345*: 121307.

Lazarus, S.A., Adamson, G.E., Hammerstone, J.F. and Schmitz, H.H. (1999). High-performance liquid chromatography/mass spectrometry analysis of proanthocyanidins in foods and beverages. *Journal of Agricultural and Food Chemistry*. *47*(9): 3693-3701.

Lecklin, T., Ryömä, R. and Kuikka, S. (2011). A Bayesian network for analyzing biological acute and long-term impacts of an oil spill in the Gulf of Finland. *Marine Pollution Bulletin*. *62*(12): 2822-2835.

Lee, S.M. and PAN, B.S. (2003). Effect of dietary sorghum distillery residue on hematological characteristics of cultured grey mullet (Mugil cephalus)—an animal model for prescreening antioxidant and blood thinning activities. *Journal of Food Biochemistry*. *27*(1): 1-18.

Li, L., and Sun, B. (2019). Grape and wine polymeric polyphenols: Their importance in enology. *Critical reviews in food science and nutrition*. *59*(4): 563-579.

Malabadi, R.B., Kolkar, K.P. and Chalannavar, R. (2022). Sweet Sorghum for Biofuel energy: Grain sorghum for Food and Fodder-Phytochemistry and Health benefits. *International Journal of Innovation Scientific Research and Review*. *4*(9): 3305-3323.

Mathanghi, S.K. (2012). Nutraceutical properties of great millet-Sorghum vulgare. *Int J Food Agric Veter Sci*. *2*(2): 40-45.

Mohapatra, D., Tripathi, M.K. and Deshpande, S. (2017). *Sorghum fermentation for nutritional improvement*. *59*(4): 3-9.

Murphy, C.W. (2013). *Modeling the Environmental Impacts of Cellulosic Biofuel Production in Life Cycle and Spatial Frameworks*. University of California, Davis.

Nagajyoti, P.C., Lee, K.D. and Sreekanth, T.V.M. (2010). Heavy metals, occurrence and toxicity for plants: a review. *Environmental chemistry letters*. *8*: 199-216.

Onen, H., Luzala, M.M., Kigozi, S., Sikumbili, R.M., Muanga, C.J.K., Zola, E.N., Wendji, S.N., Buya, A.B., Balciunaitiene, A., Viškelis, J. and Kaddumukasa, M.A. (2023). Mosquito-Borne Diseases and Their Control Strategies: An Overview Focused on Green Synthesized Plant-Based Metallic Nanoparticles. *Insects*. *14*(3): 221.

Osuagwu, A.N., Ndubuisi, P. and Okoro, C.K. (2017). Effect of spent engine oil contaminated soil on Arachis hypogea (L.), Zea mays (L.) and Vigna unguiculata (L.) walp. *Indian Journal of Advance Agricultural Research*. (*5*): 76-81.

Piatkowski and B.T., (2020). *From Genes to Traits and Ecosystems: Evolutionary Ecology of Sphagnum (Peat Moss)*. (*2*): 6-8.

Poirier, P., Giles, T. D., Bray, G. A., Hong, Y., Stern, J. S., Pi-Sunyer, F. X., and Eckel, R. H. (2006). Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss, *Physical Activity, and Metabolism. Circulation*, *113*(6): 898-918.

Pontieri, P., Di Maro, A., Tamburino, R., De Stefano, M., Tilley, M., Bean, S.R., Roemer, E., De Vita, P., Alitano, P., Del Giudice, L. and Massardo, D.R. (2010). Chemical composition of selected food-grade sorghum varieties grown under typical mediterranean conditions. *Maydica*. *55*(2): 139.

Popp, J., Harangi-Rákos, M., Gabnai, Z., Balogh, P., Antal, G., and Bai, A. (2016). Biofuels and their co-products as livestock feed: global economic and environmental implications. *Molecules*, *21*(3): 285.

Prior, R.L. and Gu, L. (2005). Occurrence and biological significance of proanthocyanidins in the American diet. *Phytochemistry*. *66*(18): 2264-2280.

Rao, S., Santhakumar, A. B., Chinkwo, K. A., Wu, G., Johnson, S. K., and Blanchard, C. L. (2018). Characterization of phenolic compounds and antioxidant activity in sorghum grains. *Journal of Cereal Science*, *84*: 103-111.

Ratnavathi, C.V. and Tonapi, V.A. (2020). Functional characteristics and nutraceuticals of grain sorghum. *Sorghum in the 21st Century: Food–Fodder–Feed–Fuel for a Rapidly Changing World*. 23:839-858.

Reichman, S.M. (2002). *The responses of plants to metal toxicity: A review forusing on copper, manganese and zinc* (Vol. 14). Melbourne: Australian Minerals and Energy Environment Foundation 14:81-85.

Riethmuller, A., Voglmayr, H., Goker, M., Weiß, M. and Oberwinkler, F. (2002). Phylogenetic relationships of the downy mildews (Peronosporales) and related groups based on nuclear large subunit ribosomal DNA sequences. *Mycologia*. *94*(5): 834-849.

Rios, L.Y., Bennett, R.N., Lazarus, S.A., Rémésy, C., Scalbert, A. and Williamson, G. (2002). Cocoa procyanidins are stable during gastric transit in humans. *The American journal of clinical nutrition*. *76*(5): 1106-1110.

Sarma, B., Gogoi, L., Gogoi, N. and Kataki, R. (2022). Crop Plants Under Metal Stress and Its Remediation. In *Plant Stress: Challenges and Management in the New Decade* 42(8): 57-71

Sharma, K., Kumar, V., Kaur, J., Tanwar, B., Goyal, A., Sharma, R., ... and Kumar, A. (2021). Health effects, sources, utilization and safety of tannins: A critical review. *Toxin Reviews*, *40*(4): 432-444.

Shinde, E.M., Gajmal, D.B. and Giri, S.A. (2022). Health and immunity boosting sorghum properties and its applications in food industry: A review. *Journal of Current Research in Food Science*. *3*(2): 104-108.

Sorenson, J. L. (2005). Ancient voyages across the ocean to America: from "impossible" to "certain". *Journal of Book of Mormon Studies*, *14*(1): 3.

Studabaker, W.B., Krupa, S., Jayanty, R.K.M. and Raymer, J.H. (2012). Measurement of polynuclear aromatic hydrocarbons (PAHs) in epiphytic lichens for receptor modeling in the Athabasca Oil Sands Region (AOSR): A pilot study. *Developments in Environmental Science*. *11*: 391-425.

Swapna, A.A., Vijayammal, R. and Radha, D.S. (2021). Effects Of Spent Engine Oil on Soil Characteristics And Selected Phytochemicals In Amaranthus Hybridus *14*(1): 3.

Taylor, J. R., and Duodu, K. G. (2022). Resistant-Type Starch in Sorghum Foods—Factors Involved and Health Implications. *Starch-Stärke*, *185*: 9-10

Tonapi, V.A., Ganapathy, K.N., Hariprasanna, K., Bhat, B.V., Amasiddha, B., Avinash, S. and Deepika, C. (2022). Small Millets Breeding. In *Fundamentals of Field Crop Breeding 15*: 449-497.

Truong, H.H., Neilson, K.A., McInerney, B.V., Khoddami, A., Roberts, T.H., Cadogan, D.J., Liu, S.Y. and Selle, P.H. (2016). Comparative performance of broiler chickens offered nutritionally equivalent diets based on six diverse, 'tannin-free'sorghum varieties with quantified concentrations of phenolic compounds, kafirin, and phytate. *Animal Production Science*. *57*(5): 828-838.

Van Hung, P. (2016). Phenolic compounds of cereals and their antioxidant capacity. *Critical reviews in food science and nutrition*. *56*(1): 25-35.

Wu, Y., Li, X., Xiang, W., Zhu, C., Lin, Z., Wu, Y., ... and Yu, J. (2012). Presence of tannins in sorghum grains is conditioned by different natural alleles of Tannin1. *Proceedings of the National Academy of Sciences*, *109*(26):10281-10286.

Wyszkowski, M. and Ziółkowska, A. (2008). Effect of petrol and diesel oil on content of organic carbon and mineral components in soil. *American-Eurasian Journal of Sustainable Agriculture*. *2*(1): 54-60.

Zaragoza, C., Gomez-Guerrero, C., Martin-Ventura, J. L., Blanco-Colio, L., Lavin, B., Mallavia, B., ... and Egido, J. (2011). Animal models of cardiovascular diseases. *BioMed Research International*, *84*: 10-11.