ANTIOXIDANTS, FREE RADICALS SCAVENGER AND LIPID PEROXIDATION ACTIVITIES OF WHOLE FINGER MILLET (*Eleusine coracana* (L.)) GRAINS

BY

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DEDICATION

This project is dedicated to the glory of El-Shadai, Jehovah Elyon, the Most Merciful and Most Gracious, for His mercies endures forever and in Him I trust.

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I am eternally grateful to the Almighty God for His kindness, love and grace for the way He shaped the course of my life and for His help to complete this programme.

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I say a big thanks you, God bless you and He will continue to uphold you in every areas of your life. To my younger ones, Damilola Adeyemo, Faith Adeyemo, Precious Adeyemo, Praise Adeyemo I love you and stay blessed.

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ABSTRACT

Eleusine coracana also known as finger millet is a common cereal crops or grains for fodder and human food. It is considered as one of nature's most potent antioxidant. In this study, a comparative evaluation of the in vitro radical scavenging, lipid peroxidation and antioxidant properties of the crude methanolic(MeOH) and ethanolic(EtOH) extract of finger millet were investigated. Weighed amounts (10g) of the powdered material of E.coracana was extracted by cold maceration in 80% methanol and 80% ethanol for 24hours to obtain the crude MeOH and EtOH. The MeOH and EtOH extract was evaporated to dryness in vacou at 45°C in a Rotary evaporator to obtain the resinous solid crude MeOH and EtOH. Stock solution of both the MeOH extract was prepared in methanol while that of EtOH was prepared in ethanol. Both test solution were then subjected to two free- radical scavenging and two antioxidant assays (2, 2-dipenyl-2-picrylhydrazyl hydrate (DPPH), nitric oxide radical and hydrogen peroxide and total antioxidant capacity. In addition, the total flavonoid content and total phenolic content of the crude MeOH and EtOH extract were determined using the Folin-Ciocalteau and the Aluminium chloride method respectively. The results showed that the finger millet grain contain a high antioxidant activities and can also inhibit a high percentage of their free radicals been produced when the plant is under oxidative stress especially when using ethanol as the extraction solvent compared to methanol. The results also showed that ethanol extracted more than 50% of the antioxidants including total flavonoids, total phenolics and total antioxidant capacity (TAC). For extraction of the total flavonoid content using methanol, at different absorbance of 0.449, 0.499, 0.483 against 13.693, 15.399,14.853mg QUE/g sample respectively gives a mean of 14.648 and standard deviation of 0.712 compared to the ethanol extract of absorbance 1.168,

1.129, 1.203 against 38.232, 36.901, 39.427mg QUE/g sample respectively also gives a mean of 38.187 and standard deviation of 1.032. The ethanol inhibit more than 50% of the free radicals than using the methanol. It can therefore be concluded from the study that E. coracana possess phytoconstituents which account for its antioxidant properties which can also help to reverse auto-oxidative stress in organism.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Eleusine coracana is an annual plant widely grown as a cereal in the arid areas of Africa and Asia. Earliest records of its cultivation in India show that it was cultivated in the Hallur region of Karnataka in the later Iron Age. It remains one of the main ingredients of the staple diet in Karnataka.It is commonly known as finger millet, African finger millet, red millet, caracan millet koracan, and ragi Kannada. *E. coracana* is native to the Ethiopian Highlands. It is very adaptable to higher elevations and is grown in the Himalaya up to 2,300 metres in elevation. Finger Millet is a group of highly variable small-seeded grasses, widely grown around the world as cereal crops or grains for fodder and human food. Millets are important crops in the semiarid tropics of Asia and Africa (especially in India, Nigeria, and Niger), with 97% of millet production in developing countries. The crop is favored due to its productivity and short growing season under dry, high-temperature conditions. The most widely grown millet is pearl millet, which is an important crop in India and parts of Africa. Finger millet, proso millet, and foxtail millet are also important crop species. In the developed world, millets are less important. For example, in the United States, only proso millet is significant, and it is mostly grown for bird seed (Lihui *et al.,* 2005).

It belong to the group of cereals, grass family *Poaceae*, which includes wheat, rice, barley, oats, rye, maize, sorghum and millets as major grain crops in the world market. Millets are small seeded cereals that are cultivated as subsistence crops mainly in semiarid and tropical

regions in Asia and Africa. Pearl millet is the most widely grown millet type followed by foxtail, proso, and finger millets. A wide variety of traditional foods and beverages is produced in countries where millets are grown for consumption. Millet foods produced from meal or flour include flat bread (fermented or unfermented), couscous and porridges, in addition to snack foods, such as 'Halepe' in Sri Lanka, prepared with finger millet flour.

The waste fractions of dehulled grains may serve as a potential source of natural antioxidants. In cereal grains, polyphenols and phytates are mainly concentrated in the pericarp, seed coat and aleurone layer (Awika *et al.,* 2005; McDonough *et al.,* 1986). Dehulling and decortication decrease polyphenolic and phytic acid contents of pearl millet (Monawar, 1983). On the other hand, dehulling increases starch content and *in vitro* protein digestibility of pearl and finger millets (Almeida-Dominguez *et al.,* 1993). In the process of dehulling, the outermost pericarp is separated from the grain. The successive steps may detach outer layers of the grain, depending on the time and degree of abrasion used which is commonly referred to as decortication.

Millets are prepared for consumption after submission to a wide range of thermal and hydrothermal treatments. Several studies on cereals have reported that thermal treatments may reduce or increase the phenolic content and their antioxidant activities, depending on severity of heat treatment, time of exposure and the type of cereal tested (Hegde and Chandra, 2005, Towo *et al.,* 2003; Zielinski *et al.,* 2006). However, the available information is limited to only a few millet species. The effects of different processing conditions such as malting, fermentation, and germination on phenolic content and antioxidant activities of millet have been examined and reported (Rao and Muralikrishna, 2002; Sripriya *et al.,* 1996). Malting of finger millet changes

the composition of free and bound phenolic acids contents (Rao and Muralikrishna, 2002). According to Sripriya *et al.* (1996), fermentation and germination of finger millet decreased their DPPH radical quenching ability compared to the raw finger millet. Shobana and Malleshi (2007) showed that hydrothermal treatment and decortication of finger millets reduced polyphenolic content by 14% and 74%, respectively. Furthermore, Towo *et. al.* (2003) reported that hydrothermal treatment of finger millet reduced the total phenolic content (TPC) by 1.7 times compared to that of the raw grain. Processing may increase the bioavailability of bioactive compounds in grains; however processing may decrease their levels (Slavin *et al.,* 2001). The available literature on the effects of millet processing on levels of phenolic compounds is scarce.

Previous studies have demonstrated that millet whole grains are rich sources of phenolic compounds (Chandrasekara and Shahidi, 2010; Chandrasekara and Shahidi, 2011). The presence of phenolic acids as well as flavonoids has been reported in millets. Depending on the species of millet and the form of phenolic compounds present in the grain, the contents of hydroxycinnamic acids, hydroxybenzoic acids and flavonoids differ (Chandrasekara and Shahidi, 2011).

In general, phenolics are not distributed evenly in the grain. Thus, processing of cereals may exert a marked effect on their antioxidant activity (Shahidi, 2009). Several studies have demonstrated that the outmost layers of the grains possess a high phenolic content and antioxidant activity (Garcia-Conesa *et al.,* 1997; Liyana-Pathirana and Shahidi, 2007; Madhujith *et al.,* 2006; Zhou *et al.,* 2004). However, the concentration of antioxidants present in the grains and their antioxidant activities may vary depending on the species, cultivar, and growing location and environmental conditions, among others (Adom *et al.,* 2003; Bonoli *et al.,* 2004; Zielinski and Kozlowska, 2000).

Phenolics are notable among bioactive phytochemicals in foods and in supplements for their role in disease risk reduction and improving health and wellness. Cereal-based foods are viable vehicles for bioactive compounds due to their widespread consumption as staple foods by much of the world's population. There is limited information on the phenolic content and antioxidant activities of different species of millet hulls and grains.

Finger millet has the highest calcium content among all the food grains, but it is not highly assimilable. The protein content in millet is very close to that of wheat; both provide about 11% protein by weight, on a dry matter basis. (Ian J *et al.*, 2013) Millets are rich in B vitamins (especially niacin, B_6 and folic acid), calcium, iron, potassium, magnesium, and zinc. Millets contain no gluten, so they are not suitable for raised bread. When combined with wheat (or xanthan gum for those who have celiac disease) they can be used for raised bread. Alone, they are suited for flatbread. As none of the millets are closely related to wheat, they are appropriate foods for those with celiac disease or other forms of allergies/intolerance of wheat. However, millets are also a mild thyroid peroxidase inhibitor and probably should not be consumed in great quantities by those with thyroid disease. (Gary, 1983). It is another cereal grain popularly used in rural areas and by poor people to consume as a staple in the form of *roti*.

Per capita consumption of millets as food varies in different parts of the world. It is highest in western Africa. In the Sahel region, millet is estimated to account for about 35 percent of total cereal food consumption in Burkina Faso, Chad and the Gambia. In Mali and Senegal, millets constitute roughly 40 percent of total cereal food consumption per capita, while in Niger and arid Namibia it is over 65 percent. Other countries in Africa where millets are a significant food source include Ethiopia, Nigeria and Uganda. Millet is also an important food item for the

population living in the drier parts of many other countries, especially in eastern and central Africa, and in the northern coastal countries of western Africa. In developing countries outside Africa, millet has local significance as a food in parts of some countries, such as China, India, Burma and North Korea. (Basavaraj *et al.*,2010).

It is now well established that oxidative stress is a major risk factor for the development of several diseases including atherosclerosis,cardiovascular disease, and cancer. Oxidative stress is the condition in which there is an imbalance between the concentrations of reactive oxygen species (ROS) and physiological antioxidants, resulting in the oxidative damage to many biomolecules within the cell. A wide variety of the free radical scavenging molecules has been found in plants, including flavonoids, anthocyanins, carotenoids and vitamins. These endogenous metabolites rich in the antioxidants activities, called natural antioxidants may function as singlet- and triplet-oxygen quenchers, peroxide decomposer, enzyme inhibitors or synagists (Cao *et al*; 1996; Choi *et. al*; 2002). Recent studies have shown that the antioxidant properties of plants correlate with their oxidative stress defences which mainly interfere with free radicals or scavenging ROS (Malencic *et al.,* 2000).

1.2 AIMS AND OBJECTIVES

The objectives of this study is to

- To determine the antioxidant constituents of the whole grain Finger millet using two extraction solvents.
- To investigate the free radicals scavenging activities of the finger millet.

Phenolics are notable among bioactive phytochemicals in foods and in supplements for their role in disease risk reduction and improving health and wellness. Cereal-based foods are viable vehicles for bioactive compounds due to their widespread consumption as staple foods by much of the world's population. There is limited information on the phenolic content and antioxidant activities of different species of millet hulls and grains.

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1.3 LITERATURE REVIEW

ELEUSINE CORACANA

Among the minor millet, finger millet stands unique because of its superior nutritional properties. It is a near spherical small seeded caryopsis that is widely used in india as a food crop. It is an annual robust grass that grows to a height of 40-100cm. it is mainly grown in semi-arid tropics and sub tropics of the world under rain fed conditions. It belongs to the genus *Eleusine* in the tribe Eragrostideae. *Eleusine coracana* species are the most cultivated species whereas *Eleusine africana* and *Eleusine indica* species have remained as wild grass. It is believed that millet is originated in the Uganda region of Africa and was transported to India in the pre-Aryan period (1500BC). In Sanskirt literature, it is referred to as Nruteya-kondaka which means 'dancing grain'. Even today, in Uganda, numerous tribal rituals and religious ceremonies are associated with finger millet (Rachie and Peters, 1977).

1.3.1 MORPHOLOGICAL FEATURES OF FINGER MILLET KERNEL

Finger millet grain are smaller in size with 1.2-1.8mm diameter and 1000 kernel weight ranges from 2.0 to 3.5g. The colour of the seed coat of the millet varies from dark red to purple but brick red is the most common colour. A few varieties of finger millet with a white seed coat have also been released but they have not become productive and popular mostly because of their poor keeping qualities and bland taste (Mahudeshwaran *et al.*1966; Mallanna and Rajasekhara 1969). The seed coat, embryo and endosperm form main structural or botanical tissues of the millet kernel. The endosperm and the seed coat accounting for about 85% and 13% of the seed mass, respectively whereas the embryo forms only 1.2% of it. The kernel consists of

a single aleurone layer that completely encircles the endosperm. The aleurone cells are rectangular with thick cell walls and contain considerable proportion of seed protein, oil, minerals and enzymes. The peripheral a swell as the corneous and floury endosperm area are embedded in the protein matrix and the protein bodies are distributed throughout the matrix. The starch granules are spherical in the floury area and become progressively polygonal in the corneous and peripheral endosperm. The entire seed is edible.

1.3.2 NUTRIENT COMPOSITION OF FINGER MILLET

Finger millet is a good source of dietary carbohydrates. Free sugars(1-2%) starch(75-80%) and non-starch polysaccharides(NSP;15-20%) form the main constituents of finger millet carbohydrates(Malleshi 2008) amylose to amylopectin ratio in the millet is normally 20:80 and there are no reports of very low amylose (<15%) or very high amylose (>30%) millet cultivars. The slow digestibility of the millet based diets normally could be attributed to the intrinsic hypoglycaemic characteristics of its starch, as well as to the high proportion of NSP contents.

The millet contains about 6-8% protein. Varietal as well as the agronomical conditions of its cultivation influences the protein and other nutrient content (Hulse *et al.*,1980). The sulphur-based amino acid (0.35mg/g protein) content of the millet protein is much higher compared to the other cereal proteins and happens to be a good source of tryptophan also. Prolamins are the major fractions of the millet protein. Similar to other cereal proteins lysine, one of the essential amino acids,forms of limiting amino acid of its protein. The leucine/isoleucine quotient of the millet protein being about two is almost equivalent to rice and wheat. Albumins and globulins constitute 8-15% whereas prolamins and glutelin like proteins form 12-28% of the proteins in the millet (Hulse *et al.,* 1980).

The millet is a poor source of lipids (1-2%). Oleic (49%), linoleic (25%) and palmitic (25%) acids are predominated fatty acids. Major components of the free lipids are triglycerides (Mahadevappa and Raina 1978). Finger millet is exceptionally rich in calcium (300-400mg%) besides, it is also a good sources of many other micronutrients such as iron, magnesium, zinc, chromium, iodine and thiamine. The millet is also a very good source of phytochemicals such as dietary fibre, polyphenols and phytate (Hulse *et al.,* 1980). The whole grain millet is edible and the traditional foods are generally prepared from the whole meal. This indicates that, the millet phytochemicals including polyphenols are edible and do not cause any adversities on the human health.

On the other hand, some of the known health benefits associated with the millet, such as its hypoglycemic (Lakshmi kumara and Sumathi 2002), anti-glycating (Hedge *et al.,* 2002), hypocholestrolemic (Kurup and Krishonmurthy 1993) and also anti-ulcerative properties (Tovey 1994) besides the excellent storage quality of the millet (Iyengar *et al.,* 1945) could be attributed to a large extent to its polyphenols have received a considerable interest in view of their antioxidant and other nutraceutical properties.

1.3.3 ANTIOXIDANT PROPERTIES OF MILLET

The antioxidant properties of the millet polyphenols have received the attention of many researches. Sripriya *et al,.*(1996) investigated the radicals activities according to the DPPH radical quenching ability of finger millet was 94%, whereas its germinated and fermented counterparts showed only 22 and 25%, on the other hand germination followed by fermentation showed only 10% quenching this showed that these kinds of processing of the millet reduces its free radical quenching capacity. The major antioxidant principles identified by them was

catechin. The antioxidant activity of free phenolics acids were higher compared to that of bound phenolics acids. They reported an increase in the antioxidant activity coefficient from 770 to 1686 in the case of free phenolic acids and a decrease from 570 to 448 upon 96h of germination in bound phenolic acids. They compared various naturally occurring phenolic acids such as caffeic, coumaric, ferulic, gallic, gentistic, protocatechuic, syringic and vanillic acids with the synthetic antioxidants such as BHA and BHT, and it was observed that, the antioxidant activity of the millet polyphenols were slightly lower than the synthetic antioxidant compounds.

Cinnamic acid deratives such as ferulic,caffeic and coumaric acid exhibit antioxidant activities (AACs) to higher extent than their corresponding benoic acid deratives namely, vanillic, protacatechuic and p- hydroxyl benzoic acid respectively. Among the cinnamic acid derivateds,caffeic and ferulic acids were found to be stronger than coumaric acid. Gallic acid, which contains three 'OH' groups, is stronger than protocatechuic acid,gentisic and syringic acids with respect to the antioxidant properties. Finger millet polyphenol exhibit the antioxidative properties effectively on superoxide, hydroxyl and nitric oxide radicals also (Bindu and Malleshi,2003). Asharami *et al.,*(2005), have shown that the millet contain 199±77ug/100g, 4±1mg% and 15.3±3.5TE/g for carotenoids,vitamin E and the total antioxidant activity respectively. They have identified the isomers of these and reported that the antioxidant activity of whole meal of finger millet is considerably higher than other millet. Varsha *et al.,* (2008) determined the antioxidant activity of the polyphenol extracts from finger millet seed coat and the whole meal and reported that the seed coat extract exhibits about 5times higher activity compares to whole meal assayed in terms of reducing power assay and the beta carotene bleaching method.

1.4 REACTIVE OXYGEN SPECIES (ROS)

Reactive Oxygen Species (ROS) is a phrase used to describe a number of reactive molecules and free radicals derived from molecular oxygen. The production of oxygen based radicals is the bane to all aerobic species. These molecules, produced as by products during the mitochondrial electron transport of aerobic respiration or by oxido-reductase enzymes and metal catalysed oxidation, have the potential to cause a number of deleterious events. It was originally thought that only phagocytic cells were responsible for ROS production as their part in host cell defense mechanisms. Recent work has demonstrated that ROS have a role in cell signalling, including; apoptosis; gene expression; and the activation of cell signalling cascades (Hancock *et al.*, (2001). It should be noted that ROS can serve as both intra- and intercellular messengers. In addition ROS are formed as necessary intermediates of metal catalysed oxidation reactions. Atomic oxygen has two unpaired electrons in separate orbits in its outer electron shell. This electron structure makes oxygen susceptible to radical formation. The sequential reduction of oxygen through the addition of electrons leads to the formation of a number of ROS including: superoxide; hydrogen peroxide; hydroxyl radical; hydroxyl ion; and nitric oxide.

Due to its radicals character, superoxide a reactive oxygen species (ROS),in addition to mitochondrial respiratory chain there are other endogenous sources of superoxide production. ROS are produced by a variety of oxidative enzymes present in cells such as xanthine oxidase. Under normal physiological conditions, xanthine oxidase acts as a dehydrogenase that removes oxygen from xanthine or from hypoxanthine and attaches it to NAD, thereby generating NADH. However, under certain conditions such as inhibition of blood flow to a tissue, xanthine dehydrogenase is converted to ROS – producing oxidase form. Alcohol consumption may also

promote conversion of xanthine dehydrogenase to xanthine oxidase (Sultatos. 1988), which can generate ROS, thereby enhancing oxidative stress.

ROS production is also beneficial and essential to organisms because it plays a crucial role in destroying pathogens (Rosen *et al.,* 1995). Macrophages and neutrophils contain a group of enzymes called NADPH oxidase complex, which when activated generate superoxide radicals and hydrogen peroxide. Hydrogen peroxide then reacts with chloride ion present in the cell to produce hypochlorite (the active ingredient in bleach) which in turn destroys pathogens. The NADPH oxidase complex and resulting ROS production are important in body's defence against all kinds of diseases as in evident in patient with a condition called chronic granulomatous disease in which ROS production by NADPH oxidase is reduced. Patients with condition are highly sensitive to infections and usually die at early age.

In addition, there are a few sources of superoxide, especially cigarette smoke which is packed with free radicals and reactive oxygen species. However, most environmental pollutants except cigarette smoke and possibly ozone layer depletion do not contribute to the amount of free radicals we are exposed to.

1.4.1 REACTIVE NITROGEN SPECIES (RNS)

Reactive nitrogen species such as nitric oxide and its products nitrite (NO_2^-),nitrite(NO_3),peroxynitrile ($ONOO^-$) and 3-nitrotyrosine have been shown to have direct role in cellular signalling,vasodilation and immune response. Nitric oxide is produced within cells by action of a group of enzyme called nitric oxide synthase, inducible nitric oxide synthase and endothelial nitric oxide synthase. While nitric oxide is relatively not a reactive radical, it can form other reactive intermediates which could have effects on protein function.

In 1987, nitric oxide was discovered to be produced in biological tissue by nitric oxide synthase which act as a catalyst that convert L-arginine to nitric oxide and L-citrulline. Mural *et al.,* (1977) reveals that nitric oxide has ability to dilate blood vessels and relax growth muscle tissue. Three pharmacologists were awarded Nobel Prize in physiology and medicine for discovering that nitric oxide is a signalling molecule in the cardiovascular system. This discovery led to the importance nitric oxide plays in cellular signalling molecule, vasodilation and immune response.

Nitric oxide is an uncharged lipophilic molecule that contains a single unpaired electron which causes it to be reactive with other molecules such as oxygen, glutathione and super oxide radical therefore nitric oxide could function as electron donor (oxidant) and electron acceptor(antioxidant).

1.5 BIOCHEMICAL FUNCTIONS OF ANTIOXIDANTS

The term "phytochemicals" or "plant chemicals" refers to every naturally occurring chemical substance present in plants, which also has a potential for antioxidant activity. Antioxidants play an important role in the body's defense system against reactive oxygen species (ROS), which are the harmful byproducts generated during normal cell aerobic respiration (Hampsch-Woodill *et al.,*2002). In foods, antioxidants prevent undesirable changes in flavor and nutritional quality of a product (Zielinski *et al.,* 2000). Several methods have been developed to measure "antioxidant activity". Commonly used assays are reducing power assay (RPA), ferric reducing antioxidant power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity. There are two basic categories of antioxidants, namely, natural and synthetic. Natural antioxidants are phenolic compounds (flavonoids, phenolic acids), nitrogen

compounds (alkaloids, chlorophyll derivatives, amino acids, and amines), carotenoids and ascorbic acid. Butylatedhydroxyanisole (BHA) and butylatedhydroxytoluene (BHT) are commonly used synthetic antioxidants that have been in use since the beginning of this century. Restrictions on the use of these compounds, however, are being imposed because of their carcinogenicity (Velioglu *et al.,*1998). Thus, natural antioxidants have gained considerable interest in recent years.

Cereals and millets are the most commonly consumed food items in India. They contain a wide range of phenolics which are good sources of natural antioxidants. Studies report that methanolic extracts from red sorghum showed higher antioxidant activity and contain higher polyphenolic levels compared to rice, foxtail millet, prosomillet and barley (Choi *et al.,*2007). Bran, a byproduct of milling has antioxidant potential due to phenolic acids such as *p*-coumaric acid and vanillic acids that are concentrated in the bran portion of cereal kernels. Antioxidant activity of five bran extracts exhibited appreciable levels of total phenolics, flavonoids and DPPH radical scavenging activities (Iqbal *et al.,*2007). Processing, such as soaking and roasting, have been shown to influence total phenolic, flavonoid and antioxidant contents in selected dry beans. Raw kodo millet and finger millet have higher DPPH radical scavenging activities. However, cooking of these millets by roasting or boiling reduced their antioxidant activity. (Hegde *et al.,*2005) Millets contain phytic acid, tannins, and phenols which can contribute to antioxidant activity, important in health, ageing and metabolic diseases. To obtain the biggest benefit from antioxidants, they are eaten raw or lightly steamed. Some classes are:

Beta carotene and other carotenoids- apricots, asparagus, broccoli, cantaloupe, carrot, corns, green pepper, mangoes, peaches, watermelon, tomatoes, pumpkin, etc. Vitamin C- berries,

broccoli, Brussels, sprouts, cantaloupe, grapefruit, honeydew, kiwi, papaya, strawberry, mangoes, oranges, tomatoes etc.

Vitamin E- broccoli, carrots, chards, mustard and turnip greens, mangoes, nuts, papaya, pumpkins, red pepper, sunflower seeds, spinach etc.

Vitamins are not the only antioxidants in food. Other antioxidants that may help boost immunity include zinc which is found in oysters, red meat, poultry, beans, nuts, seafoods, whole grains, dairy products etc.

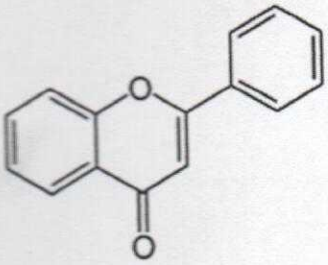
1.5.1 Phenols

Phenols are class of chemicals compounds consisting of a hydroxyl group bonded directly to an aromatic hydrocarbon. Phenolic compounds are important class of phytochemicals in plant food sources. Phytochemicals are often responsible for the bright red colour in berries and vegetables (Atkins, 2000; Escarpa and Genzales, 2000). Phenolic phytochemicals can be grouped into polyphenols and flavonoids.

1.5.2 Flavonoids

Flavonoids, formally referred to as vitamin P are polyphenolics compounds that are ubiquitinous in plants. The compound possess a common phenylbenzopyrone structure and are categorized according to the saturation level and opening of the central pyran ring, mainly into flavones, flavonols, isoflavones, flavonones and flavonoids. They are the most abundant group of polyphenola (Madeson *et al.*, 2000).

They are present in practically all dietary plants like fruits and vegetables. Flavonoids are found in several medicinal plants and herbal remedies. They possess biological activities which include those that might be able to influence processes that are deregulated during cancer development. They include anti-allergic, anti-inflammatory, antioxidant, anti-mutagenic and anti-carcinogenic activities.



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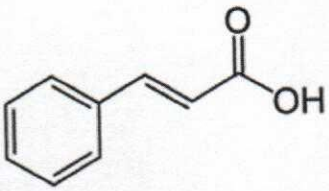

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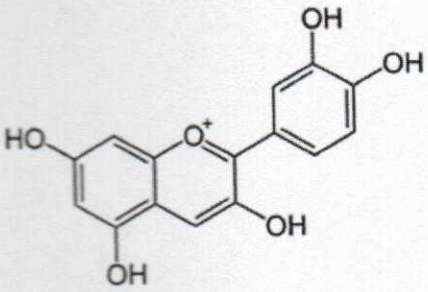

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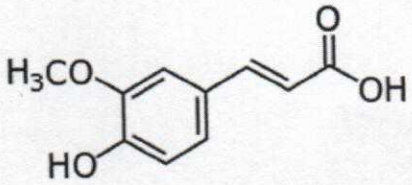
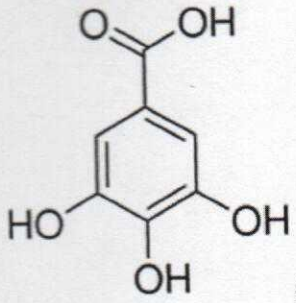
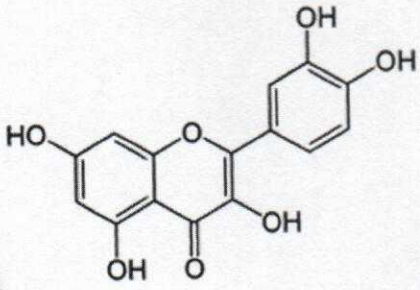


ferulic acid



gallic acid



quercetin

Major antioxidant polyphenols (Laguerre *et al.*,2007)

1.5.3 Vitamins

Vitamin E

Vitamin E is an example of fat soluble vitamin,other fat soluble vitamins are vitamin A, D and K. it consist of eight naturally occurring tocopherols which alpha tocopherol is the most active. It is an antioxidant that stops production of ROS formed when fat undergoes oxidation.Vegetable oils are rich sources of vitamin E, whereas liver and eggs contain vitamin E in moderate amount. Its requirement increases as the intake of polyunsaturated fatty acid increases. Asides antioxidant function, Vitamin E also have enzymatic activities, gene expression and neurological functions (Rahimi *et al.*,2007).

Vitamin C

Vitamin C is a water soluble vitamin which indicates that the body doesn't store it. It is obtained from foods which include citrus fruits, broccoli and tomatoes. Its active form is ascorbic acid and it functions as a reducing agent in several reactions. It also functions as coenzyme in hydroxylation reaction. Vitamin C is the strongest antioxidant (Rahimi *et al.*,2005). Consumption of diets rich in vitamin C is associated with a decreased incidence of some chronic diseases which include cardiovascular diseases and some cancers.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 SEED COLLECTION

Finger millet seeds were purchased in an open market of Lafia market, Nasarawa State, Nigeria. And the seeds were identified by the supervisor.

2.2 SEED EXTRACTION

The plant was extracted using cold maceration method. Ten (10g) of the fine powder was put into 250ml conical flask followed by addition of 200ml of 80% methanol and ethanol. The mixture was placed in a magnetic stirrer and stirred for 24hours. The mixture was allowed to settle thereafter and filtered using a whatman No. 1 filter paper to obtain the filtrate. The filtrate was evaporated under reduced pressure at $45^{O}C$ in a rotary evaporator while the crude extract was obtained. The concentration of the stock solution of ethanol and methanol extract used was 0.1mg/ml.

2.3 DETERMINATION OF ANTIOXIDANTS ACTIVITIES OF FINGER MILLET

2.3.1 Total Phenol Content`

The method of determining the total phenolic content was described by Singleton and Rossi, 1965 as described by Gulcin *et al.,* (2003) using the folinciocalteu's phenol reagent which is an oxidizing reagent.

To a mixture of 0.1ml of the seed sample, 0.9ml of water was added, 0.2ml of folin-ciocalteu's phenol reagent and the resulting mixture voltexed. After five minutes of standing, 1.0ml of 7% (w/w) Na_2CO_3 solution then added and the solution was then distilled to 2.5ml before incubated for 90min at room temperature. The absorbance against a negative control containing 1ml of

water in place of the sample was then taken at 750nm. The standard used was the Gallic acid at 0.1mg/ml in order to determine Gallic Acid Equivalent (GAE) of sample, after preparing a calibration curve. Distilled water was used as blank.

2.3.2 Total Flavonoid Content

Standard quercetin with varying concentration 0.1, 0.2, 0.3, 0.4 and 0.5mg/ml was used as standard in comparison to the sample extract. This was carried out based on the aluminium chloride colorimetric assay method according to Zhilen *et al.,* (1999) as described by Miliauskas *et al.,* (2004).

About 0.1ml of extract/standard was added 0.4ml of distilled water. This was followed by 0.1ml of 5% sodium nitrite. After 5minutes, 0.1ml of 10% Aluminum Chloride and 0.2ml of sodium hydroxide was added and the volume was made up to 2.5ml with distilled water. The absorbance at 510nm was measured against the blank. The total flavonoid content of the plant, expressed as mg quercetin equivalents per gram of the plant extract is calculated as:

X = Total content of flavonoid compound in quercetin equivalent

q= concentration of quercetin established from the standard curve

V= volume of extract (ml)

w= weight of the crude methanolic extract obtained.

2.3.3 Total Antioxidant Capacity (TAC)

This method is based on the reduction of Molybdenum (VI) to Molybdenum (V) by the extract and the subsequent formation of a green phosphate/Molybdenum (V) complex at an acidic pH (Prieto *et al.,* 1998).

Exactly 0.1ml of the oil extracts or standard solutions of ascorbic acid (20, 40, 60, 80, 100µg/ml) was added 1ml of the reagent solution which consisted of 0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate. The tubes containing the reacting mixture were incubated in a water bath at 95°C for 90mins. The mixture was then allowed to stand and cool to room temperature and the absorbance measured at 695nm against a blank which consisted of the reacting mixture containing distilled water in place of the extract. The antioxidant activities of the extracts were expressed as an ascorbic acid equivalent.

2.4 FREE RADICAL SCAVENGING ACTIVITIES OF FINGER MILLET

2.4.1 DPPH Assay

The radical scavenging ability of the oil was determined using the stable radical DPPH(2,2-diphenyl-1-picrylhydrazyl hydrate) as described by Brand-Williams *et al.,*(1995). The reaction of DPPH with an antioxidant compound which can donate hydrogen, leads to its reduction (Blois, 1958). The change in colour from deep violet to light yellow was measured spectrophotometrically at 517nm.

One ml of different concentrations (10, 5, 2.5, 1.25, 0.625, 0.3125mg/ml) of the oil extract or standard (vitamin C) in a test tube was added 1ml of 0.3mM DPPH in methanol. The mixture was mixed and incubated in the dark for 30mins after which the absorbance was read at 517nm against a DPPH control containing only 1ml methanol in place of the extract.

The percent of inhibition was calculated in following way:

$I\% = [(A_{blank}-A_{sample})/A_{blank}] \times 100$

WhereA_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration.

2.4.2 Nitric Oxide (NO) Radical

The inhibition nitric oxide radical activity of the extract was carried out according to the method of Green *et. al.* (1982) as described by Marcocci *et al.*, (1994).Nitric oxide, generated from sodium nitroprusside in aqueous solution at physiological pH, interacts with oxygen to produce nitrite ions which was measured by Griess reaction.

The reaction mixture, containing 0.1ml of different concentrations (10, 5, 2.5, 1.25, 0.625, 0.3125mg/ml) of the oil extract and 0.9ml of sodium nitroprusside (2.5mM) in phosphate buffer saline was incubated under illumination for 150minutes. After incubation, 0.5ml of 1% sulphanilamide in 5% phosphoric acid was added and incubated in the dark for 10min., followed by addition of 0.5ml 0.1% NED (N-1-napthylethylenediamine dihydrochloride). The absorbance of the chromophore formed was measured at 546nm (Marcocci *et al.,* 1994). The percentage inhibition of nitric oxide radical formation was calculated as expressed above in DPPH radical scavenging assay.

2.4.3 Hydrogen Peroxide (H_2O_2) Radical

A solution of hydrogen peroxide (20mM) was prepared in phosphate buffered saline (PBS pH 7.4). Various concentrations of 1ml of the extracts or standards in methanol were added to 2ml of hydrogen peroxide solutions in PBS. The absorbance was measured at 230 nm, after 10 min

against a blank solution that contained extracts in PBS without hydrogen peroxide (Jayaprakah *et al.*, 2004).

CHAPTER THREE

3.0 RESULTS

3.1 ANTIOXIDANT ACTIVITIES OF FINGER MILLET

3.1.1 Total Flavonoids:

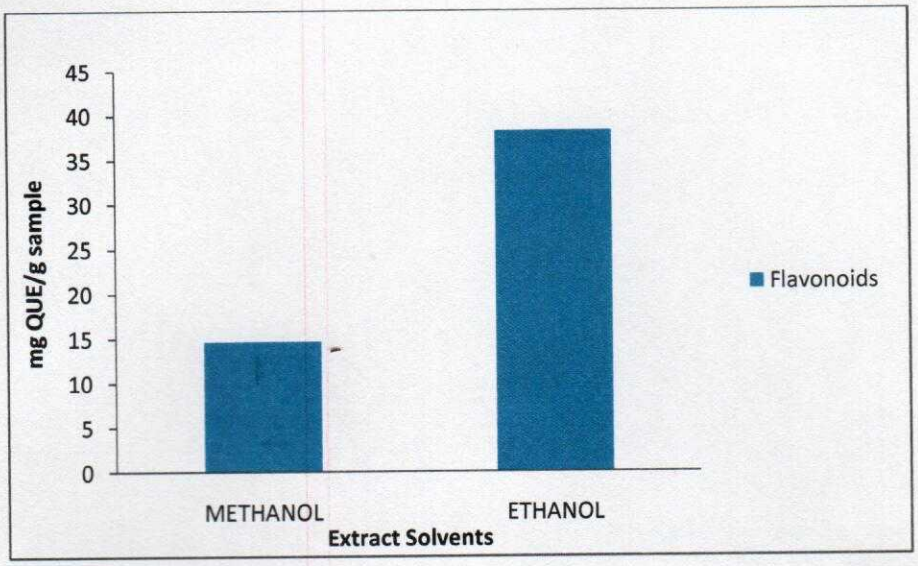


Figure1: Total Flavonoids contents of Finger millet

The result in fig 1 above showed that ethanol extract more of the whole finger millet grain than the methanol. The ethanol extract 14.648g against the quercetin standard while ethanol extracted 38.187g of the total flavonoid content of the finger millet grain. It means that the ethanol solvent extracted about 63% total flavonoid than the methanol.

3.1.2 Total Phenolics:

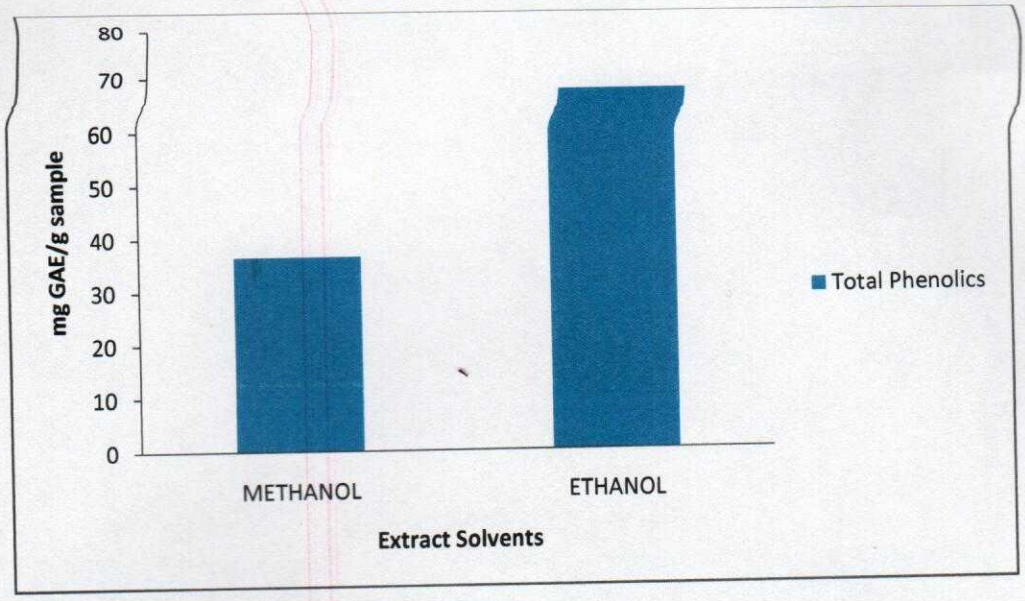


Figure 2: **Total phenolics contents of Finger millet**

The above result showed that the extraction of the total phenolics of whole finger millet grain was greatly favoured by ethanol rather than methanol. The methanol extracted 34.918g against the gallic acid equivalent standard while ethanol extracted 67.095g which was about 46% extracted more than methanol.

3.1.3 Total Antioxidant Capacity (TAC):

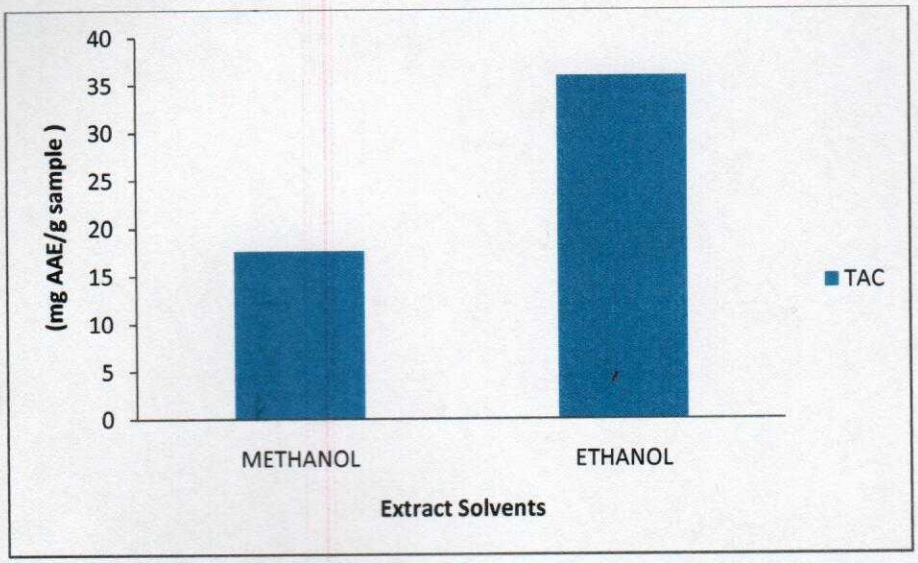


Figure 3: Total antioxidant capacity of Finger millet

The table above showed the result of the total antioxidant capacity of the finger millet in which the ethanol extracted more TAC than the methanol. The methanol extracted 17.612g of the AAE standard while ethanol extracted 35.898g of the sample which implies that ethanol extracted about 53% more than the methanol extract.

Generally, for the total antioxidant activities of the finger millet grain, we are made that understand that the ethanol extract effectively than the methanol which signifies that the best solvent that can be used in extracting antioxidant in plant is the ethanol.

3.2 FREE RADICAL SCAVENGING ACTIVITIES OF FINGER MILLET

3.2.1 Nitric Oxide Radicals:

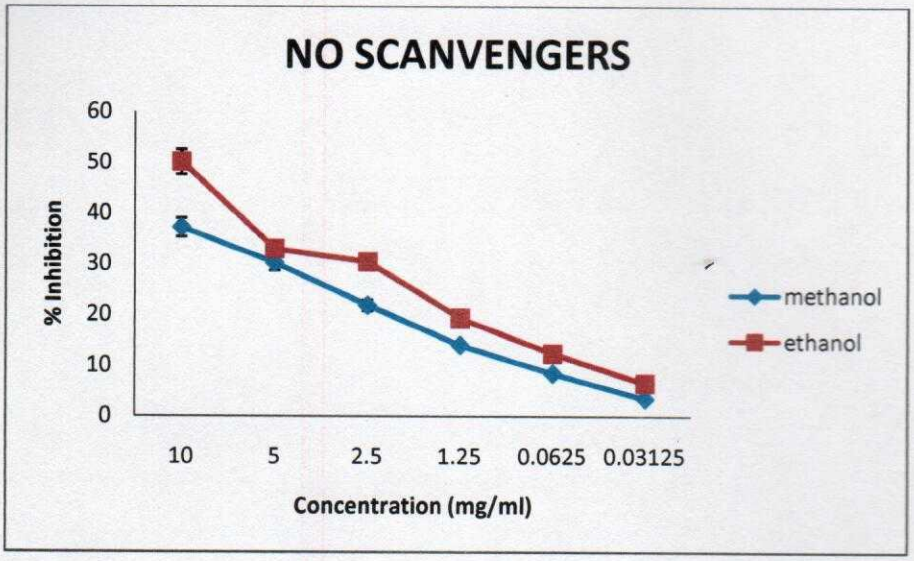


Figure 4: **Nitric oxide radicals**

The figure 4 above showed the result of the scavenging activities of the finger millet grain extracted both in the methanol and ethanol extracting solvent. It showed that as the % inhibition decreases the concentration of the solvent decreases in the methanol extract and also for the ethanol extract as the % inhibition decreases the concentration of the solvent also decreases. The result also pointed out that ethanol extract more nitric oxide radicals more than the methanol. For example, at 10mg/ml concentration the % inhibition of the finger millet by methanol was 37.166 while ethanol was 50.099 which shows a very significant difference.

3.2.2 DPPH Radicals:

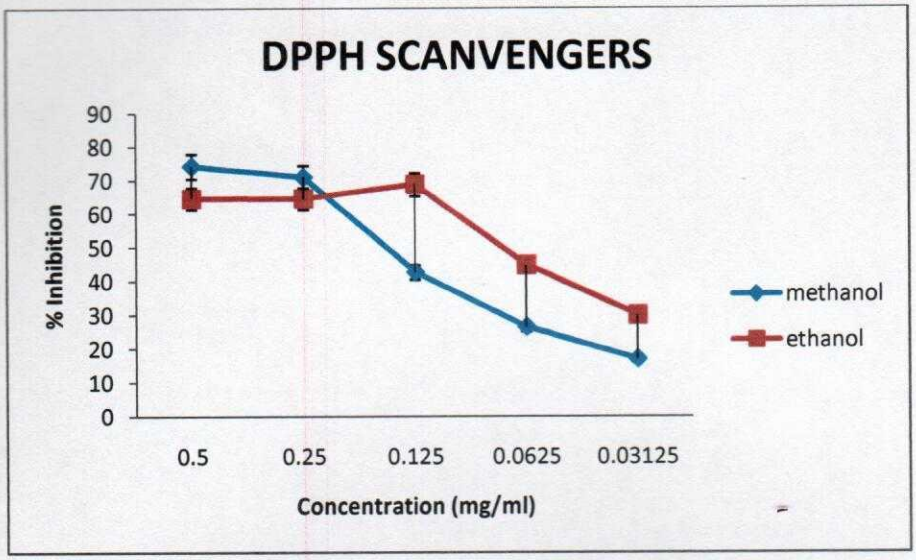


Figure 5: **DPPH Scavengers**

The figure 5 above showed the DPPH scavenging activities of the finger millet grain extracted in both the methanol and the ethanol solvent. The % inhibition decreases as the concentration decreases for the methanol extract as well as the ethanol extract in which the % inhibition decreases as the concentration decreases. For example, 0.5mg/ml concentration inhibited 74.115 for the methanol while it inhibited 64.454 for the ethanol. The figure also showed that at 0.125 concentration was the best point at which both solvent extracted well.

3.2.3 Hydrogen Peroxide (H_2O_2) Radicals:

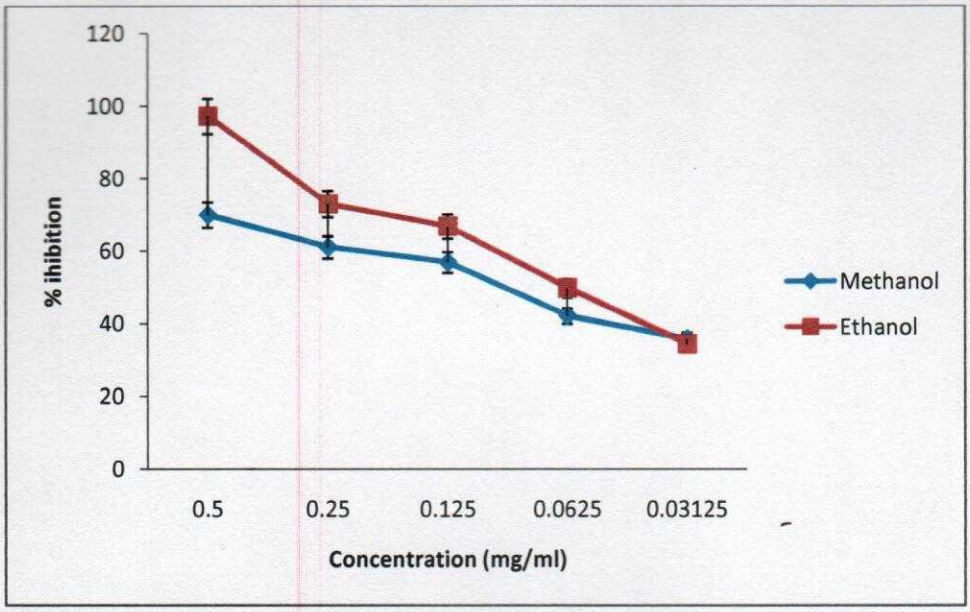


Figure 6: **Hydrogen peroxide (H_2O_2) radicals**

The figure above showed the hydrogen peroxide radicals inhibited by different concentration of the extraction solvent which shows that the ethanol inhibited the radicals higher and more than the methanol solvent. And that the % inhibition decreases as the concentration decreases both in the methanol and the ethanol solvent. For example, 0.5mg/ml concentration inhibited 69.919 for methanol and inhibited 97.154 in ethanol.

For the scavenging radicals in the finger millet it shows that the ethanol inhibited more of the radicals in the ethanol solvent than that of the methanol.

CHAPER FOUR

4.0 DISCUSSION

Free radicals are molecules that contain one or more unpaired electron in its outer orbital and its presence in the body of plant can lead to oxidative stress which can inhibit the growth of the plant. Antioxidants are substances that have the ability of removing these free radicals or neutralizing their effect in the body of organism. Finger millet (*Eleusine coracana)* has been experimented by carrying out different in vitro antioxidant and free radical scavenging assays has been found to have high antioxidant properties using the ethanol as the extraction solvent rather than methanol. Given that the sample has a very high inhibitory effect on the DPPH, Nitric oxide and the Hydrogen peroxide, It obviously has a high antioxidant activity. This was confirmed by the result obtained from both the total antioxidant activities of the finger millet and the scavenging radicals of the finger millet.

Presence of high quality of flavonoid and phenolics according to the results obtained also account for its high antioxidant activity. The medicinal actions of phenolics is mostly ascribed to their antioxidant capacity, free radical scavenging, modulation of gene expression and interaction with the cell signaling pathways. (Soobrattee *et al.*,2005)

4.1 CONCLUSION AND RECOMMENDATION

The study revealed that finger millet has a high antioxidant property such as total flavonoids ranged from14.65-38.19mgQUE/g of sample, total phenolics ranged from 36.46-67.10mgGAE/g of sample and total antioxidant capacity ranged from17.61-35.90 mgAAE/g of sample. The whole grain finger millet posed ability to scavenged free radical nitric oxide and hydrogen peroxide above 50%. Ethanol solvent was effective in extraction of antioxidant in finger millet than methanol. Therefore, whole grain finger millet could be a good and effective antioxidant agent in treatment of human diseases under oxidation stress.

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APPENDICES'

Appendices for the total antioxidant capacity of finger millet grain

Appendix 1: Total flavonoid content of standard quercetin

CONC	Absorbance1	Absorbance2	AVERAGE
0.5	0.182	0.206	0.194
0.4	0.16	0.169	0.1645
0.3	0.14	0.135	0.1375
0.2	0.105	0.106	0.1055
0.1	0.074	0.08	0.077

Appendix 2: Total phenolics content of standard gallic acid

CONC	Absorbance1	Absorbance2	Average
0.1	0.205	0.258	0.2315
0.08	0.178	0.172	0.175
0.06	0.136	0.142	0.139
0.04	0.067	0.083	0.075

0.02	0.04	0.05	0.045

Appendix 3: Total antioxidant capacity of AAE

CONC	Absorbance1	Absorbance2	AVERAGE
0.1	0.307	0.278	0.2925
0.08	0.241	0.219	0.23
0.06	0.181	0.179	0.18
0.04	0.099	0.109	0.104
0.02	0.022	0.024	0.023

Appendix 4: DPPH radical scavenging activity of methanolic extract of finger millet

CONC(mg/ml)	R1	R2	R3	%inhibition	%inhibition	%inhibition	Average inhibition

0.5	0.117	0.119	0.115	74.11504	73.67257	74.55752	74.11504
0.25	0.126	0.143	0.127	72.12389	68.36283	71.90265	70.79646
0.125	0.269	0.253	0.258	40.48673	44.02655	42.92035	42.47788
0.0625	0.324	0.338	0.338	28.31858	25.22124	25.22124	26.25369
0.03125	0.377	0.37	0.382	16.59292	18.14159	15.48673	16.74041
0.015625	0.396	0.427	0.423	12.38938	5.530973	6.415929	8.112094

Appendix 6: DPPH radical scavenging activity of ethanolic extract of finger millet

CONC(mg/ml)	R1	R2	R3	%inhibition	%inhibition	%inhibition	Average inhibition
0.5	0.149	0.168	0.165	67.0354	62.83186	63.49558	64.45428
0.25	0.151	0.135	0.197	66.59292	70.13274	56.41593	64.38053
0.125	0.14	0.128	0.158	69.02655	71.68142	65.04425	68.58407
0.0625	0.249	0.295	0.208	44.9115	34.73451	53.9823	44.54277
0.03125	0.355	0.295	0.302	21.46018	34.73451	33.18584	29.79351
0.015625	0.392	0.367	0.372	13.27434	18.80531	17.69912	16.59292

Appendix 7:DPPH radical scavenging activity of standard vitamin C

CONC(µg/ml)	ABS 1	ABS 2	ABS 3	%inhibition	%inhibition	%inhibition	average inhibition

							n
10	0.477	0.473	0.327	55.127	55.50329	69.23801	59.9561
							35.4343
5	0.676	0.693	0.69	36.4064	34.80715	35.08937	1
2.5	0.846	0.865	0.855	20.41392	18.62653	19.56726	19.5359
							4.95453
1.25	1.02	1.004	1.007	4.045155	5.550329	5.268109	1
							3.16713
0.0625	1.049	1.017	1.022	1.317027	4.327375	3.857008	7
							1.06616
0.03125	1.056	1.053	1.046	0.658514	0.940734	1.599247	5

Appendix 8: Nitric oxide radical scavenging activity of methanolic finger millet

CONC	Absorbance1	Absorbance2	~~%inhibition~~	%inhibition	Average inhibition
10	0.65	0.618	35.57978	38.75124	37.16551
5	0.725	0.684	28.14668	32.21011	30.17839
2.5	0.796	0.783	21.11001	22.39841	21.75421
1.25	0.855	0.88	15.26264	12.78494	14.02379

0.0625	0.896	0.952	11.19921	5.649158	8.424182
0.03125	0.971	0.977	3.766105	3.171457	3.468781

Appendix 9: Nitric oxide radical scavenging activity of ethanolic extract of finger millet

CONC	Absorbance1	Absorbance2	~~%inhibition~~	%inhibition	Average inhibition
10	0.527	0.48	47.77007	52.42815	50.09911
5	0.681	0.674	32.50743	33.20119	32.85431
2.5	0.714	0.692	29.23687	31.41724	30.32706
1.25	0.833	0.797	17.44301	21.0109	19.22696
0.0625	0.879	0.891	12.88404	11.69475	12.2894
0.03125	0.939	0.949	6.937562	5.946482	6.442022

Appendix 10: Nitric oxide radical scavenging activity against the standard

CONC	Absorbance1	Absorbance2	%inhibition	%inhibition
0.1	0.274	0.255	72.8444	74.72745
0.08	0.59	0.455	41.52626	54.90585

0.06	0.793	0.733	21.40733	27.35382
0.04	0.874	0.889	13.37958	11.89296
0.02	0.965	0.941	4.360753	6.739346

Appendix 11: Hydrogen peroxide radical scavenging activity of methanolic extract of finger millet

CONC(mg/ml)	Absorbance1	Absorbance2	%inhibition	%inhibition
0.5	0.113	0.109	69.37669	70.4607
0.25	0.145	0.143	60.70461	61.24661
0.125	0.163	0.156	55.82656	57.72358
0.0625	0.214	0.214	42.00542	42.00542
0.03125	0.24	0.235	34.95935	36.31436
0.015625	0.301	0.31	18.42818	15.98916

Appendix 12: Hydrogen peroxide radical scavenging activity of methanolic extract of finger millet

CONC(mg/ml)	Absorbance1	Absorbance2	%inhibition	%inhibition
0.5	0.113	0.109	69.37669	70.4607

0.25	0.145	0.143	60.70461	61.24661
0.125	0.163	0.156	55.82656	57.72358
0.0625	0.214	0.214	42.00542	42.00542
0.03125	0.24	0.235	34.95935	36.31436
0.015625	0.301	0.31	18.42818	15.98916

Appendix 13: Hydrogen peroxide radical scavenging activity of ethanolic extract of finger millet

CONC(mg/ml)	Absorbance1	Absorbance2	%inhibition	%inhibition
0.5	0.012	0.009	96.74797	97.56098
0.25	0.099	0.101	73.17073	72.62873
0.125	0.121	0.125	67.20867	66.12466
0.0625	0.18	0.192	51.21951	47.96748
0.03125	0.253	0.232	31.43631	37.12737
0.015625	0.298	0.3	19.24119	18.69919

Appendix 14:Graph of absorbance against concentration(total flavonoid content)