THE EFFECTS OF DAILY INTAKE OF THE AQUEOUS FRUIT PULP EXTRACT OF *PICRALIMA NITIDA* ON TOTAL ANTIOXIDANT CAPACITY IN EXPERIMENTAL ALBINO WISTAR RATS

BY

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CERTIFICATION

We the undersigned hereby certify that Emmanuel Opeyemi IBHAZE 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.

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DEDICATION

I dedicate this project work first and foremost to God Almighty who has been there and kept me in good health for the duration of my studies and also to my late grandparents I hope I am making you proud.

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This project work is a product of much research, extensive discussion, and analysis. I want to use this medium to acknowledge the input of various persons at the different stages of its development.

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ABSTRACT

The study investigated the impact of daily intake of aqueous fruit pulp extract of Picralima nitida on the total antioxidant capacity in experimental rats. *Picralima nitida*, known for its rich phytochemical composition, was chosen for its potential health benefits. Thirty- five rats were divided into control and experimental groups, with the latter receiving daily doses of the aqueous fruit pulp extract. After a specified duration, blood samples were collected for the assessment of total antioxidant capacity. The results revealed a significant increase in the total antioxidant capacity in the experimental group B and compared to the control, suggesting a positive influence of *Picralima nitida* extract on the antioxidant defense system. This enhancement in antioxidant capacity may be attributed to the diverse bioactive compounds present in the fruit pulp extract, such as alkaloids, flavonoids, phenolics, and zinc known for their antioxidant properties

CHAPTER ONE

INTRODUCTION

1.0 Background of Study

The most important concern of mankind has been to maintain health and restore well-being. Over the years, different cultures and societies have developed diverse modes of traditional medical practices to maintain and restore health, contributing to improved well-being for a large part of the population (Ortega, 2009). In many cases, natural products and remedies contribute to a greater part of traditional medicine. For thousands of years, natural products have played a very important role in healthcare and prevention of diseases. Moreover, Herbs are one of those natural product sources that have made cultural health maintenance and treatment possible. Human beings have used these herbs for the treatment of diverse ailments for thousands of years (Sofowara, 1982; Hill, 1989; Phillipson, 2001). The information about the medicinal value of plants is commonly based on the empirical knowledge of ancient people, which was passed over several generations. Nowadays, the use of plants as alternative medicine is increasingly more popular among developed societies.

Medicinal plants are a subgroup of plants that are high in phytochemicals, have therapeutic properties, and exert a desired pharmacological effect on a human or animal body. (Natural Products and Drug Discovery, 2018). Only after a plant's biological activity has been properly studied and documented by scientists or ethnobotanists, then it become a recognized therapeutic plant. (Elujoba,1997). *Picralima nitida*, commonly referred to as the Akuamma tree or Akuamma seeds, is a plant species indigenous to certain West African countries, including Ghana, Ivory Coast, and Nigeria (Kapadia *et al.,.,.,* 1993). This plant belongs to the *Apocynaceae* family. Akuamma seeds, extracted

from *Picralima nitida*, have a longstanding history in African traditional medicine due to their therapeutic properties. These seeds contain various alkaloids, which are believed to possess analgesic and anti-inflammatory properties (Erhauyi *et al.,.,.,* 2014). These alkaloids interact with the brain's opioid receptors, similar to the mechanism of action seen with opioids like morphine.

The idea that performing major biological functions necessary for the sustenance of life generates entities, systems that work to take life in itself has perplexed the entire health community, death is a byproduct of life, and life a byproduct of death is a paradox that biochemists is forced to unravel daily (Ibhaze, 2023). This death (free radicals) such as the reactive oxygen species and reactive nitrogen species are generated through endogenous processes such as metabolism, respiration, and phagocytosis. They are also generated by exogenous systems such as pesticides, some pollutants, organic solvents, and radiation (Davies, 1995). However, the generation of these free radicals is normally balanced by an equivalent production of antioxidants through our natural antioxidant defense mechanism, which is the enzymatic antioxidants (superoxide dismutase, glutathione peroxidase, quinone reductase, and catalases) and the non-enzymatic antioxidant (ascorbic acid, α-tocopherol, melatonin, β-carotene) obtained from the diet (Halliwell, 1996; Davies, 2000; Chun-Weng, 2011).

However, when the generation of free radicals overwhelms the antioxidant capacity of the biological defense system; it gives rise to oxidative stress (Zima, 2001). When oxidative stress occurs, it eventually leads to several deteriorating effects on our cellular bio-molecules such as DNA damage, lipid peroxidation, tissue injury, and protein degradation (Chun-Weng, 2011).

1.1 Justification of Study

Despite *Picralima nitida* historical use in the treatment of diabetes, little scientific research has been done to examine its antioxidant capacity Therefore, an in-depth research is required to assess the possible advantages of *Picralima nitida*'s aqueous fruit pulp extract in controlling antioxidant capacity.

1.2 Aim of Research

This research aims to assess the effects of daily intake of the aqueous fruit pulp extract of *Picralima nitida* on total antioxidant capacity in an experimental rat model. The study aims to investigate the potential of this plant extract and provide insights into its mechanisms of action.

1.3 Objectives of the study:

The objective of this study is to determine and compare the total antioxidant capacity in albino rats treated with *Picralima nitida* to those of control groups that did not receive the treatment.

CHAPTER 2

LITERATURE REVIEW

2.0 Free Radical

The recent growth in the knowledge of free radicals and reactive oxygen species (ROS) in biology is producing a medical revolution that promises a new age of health and disease management. (Aruoma, 2003). Ironically, oxygen, an element indispensable for life, under certain situations has deleterious effects on the human body.

Most of the potentially harmful effects of oxygen are due to the formation and activity of several chemical compounds known as ROS, which tend to donate oxygen to other substances. Free radicals and antioxidants have become commonly used terms in modern discussions of disease mechanisms. (Mohammed, 2004). A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. The presence of an unpaired electron results in certain common properties that are shared by most radicals. Many radicals are unstable and highly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants. (Cheeseman,1993)

The most important oxygen-containing free radicals in many disease states are hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical, and peroxynitrite radical. These are highly reactive species, capable in the nucleus, and the membranes of cells of damaging biologically relevant molecules such as DNA, proteins, carbohydrates, and lipids. Free radicals attack important macromolecules leading to cell damage and homeostatic disruption. Targets of free radicals include all kinds of molecules in the body. Among them, lipids, nucleic acids, and proteins are the major targets**.**

There are many different types of free radicals, but some of the most common ones include

REACTIVE OXYGEN SPECIES (ROS): These are free radicals that are produced from oxygen. Some examples of ROS include superoxide, hydroxyl radical, and hydrogen peroxide.

REACTIVE NITROGEN SPECIES (RNS): These are free radicals that are produced from nitrogen. One example of an RNS is nitric oxide.

LIPID PEROXYL RADICALS: These are free radicals that are formed when lipids (fats) are damaged.

Free radicals and other ROS are derived either from normal essential metabolic processes in the human body or from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals. (Bagchi, 19980) Free radical formation occurs continuously in the cells as a consequence of both enzymatic and nonenzymatic reactions. Enzymatic reactions, which serve as sources of free radicals, include those involved in the respiratory chain, in phagocytosis, in prostaglandin synthesis, and the cytochrome P-450 system. (Liu.T.*et al.,.,,* 2000) Free radicals can also be formed in nonenzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing reactions.

Some internally generated sources of free radicals are:

Mitochondria

Xanthine oxidase

Peroxisomes

Inflammation

Phagocytosis

Arachidonate pathways

Exercise

Ischemia/reperfusion injury

Some externally generated sources of free radicals are:

Cigarette smoke

Environmental pollutants

Radiation

Certain drugs, pesticides

Industrial solvents

Ozone

2.1.1 Oxidative Stress

Oxidative stress is necessary for aerobic life, but, under certain conditions, can also be toxic and responsible for causing a variety of diseases including Alzheimer's disease, Parkinson's disease, aging, cancer, neuronal disorders, and cardiovascular disease (Ames, 1983; Aniya *et al.,.,.,* 2002) The term is used to describe the condition of oxidative damage that results when the critical balance between free radical generation and antioxidant defenses is unfavorable. (Rock.CL *et al.,.,.,* 1996) Oxidative stress, arising as a result of an imbalance between free radical production and antioxidant defenses, is associated with damage to a wide range of molecular species, including lipids, proteins,

and nucleic acids. (Mc Cord, 2000) Short-term oxidative stress may occur in tissues injured by trauma, infection, heat injury, hyperoxia, toxins, and excessive exercise. These injured tissues produce increased radical-generating enzymes (e.g., xanthine oxidase, lipogelipogenase, and oxygenase) that activate phagocytes, release free iron and copper ions, or disrupt the electron transport chains of oxidative phosphorylation, producing excess ROS. The initiation, promotion, and progression of cancer, as well as the side effects of radiation and chemotherapy, have been linked to the imbalance between ROS and the antioxidant defense system. ROS has been implicated in the induction and complications of diabetes mellitus, age-related eye disease, and neurodegenerative diseases such as Parkinson's disease. (Rao. *et al.,*2006)

2.1.2 Oxidative Stress and Human Disease

The role of oxidative stress has been postulated in many conditions, including atherosclerosis, inflammatory conditions, certain cancers, and the process of aging. Oxidative stress is now thought to make a significant contribution to all inflammatory diseases (arthritis, vasculitis, glomerulonephritis, lupus erythematosus, adult respiratory diseases syndrome), ischemic diseases (heart diseases, stroke, intestinal ischemia), hemochromatosis, acquired immunodeficiency syndrome, emphysema, gastric ulcers, hypertension, and preeclampsia, neurological disorders (Alzheimer's disease, Parkinson's disease, muscular dystrophy), alcoholism, smoking-related diseases, and many others (Stefanis, 1997). An excess of oxidative stress can lead to the oxidation of lipids and proteins, which is associated with changes in their structure and functions.

Cardiovascular disease continues to be the biggest killer, responsible for about half of all deaths. Oxidative events may affect cardiovascular diseases; therefore, they have the potential to provide enormous benefits to health and lifespan. Polyunsaturated fatty acids

occur as a major part of the low-density lipoproteins (LDL) in the blood, and the oxidation of these lipid components in LDL plays a vital role in atherosclerosis. (Esterbauer *et al.,.,*, 2001) The three most important cell types in the vessel wall are endothelial cells, smooth muscle cells, and macrophages, which can release free radicals that affect lipid peroxidation. With a continued high level of oxidized lipids, blood vessel damage to the reaction process continues and can lead to the generation of foam cells and plaque, the symptoms of atherosclerosis. Oxidized LDL is atherogenic and is thought to be important in the formation of atherosclerosis plaques. Furthermore, oxidized LDL is cytotoxic and can directly damage endothelial cells. Antioxidants like B-carotene or vitamin E play a vital role in the prevention of various cardiovascular diseases. (Neuzil, 1999)

2.1.3 Carcinogenesis

Reactive oxygen and nitrogen species, such as superoxide anion, hydrogen peroxide, hydroxyl radical, and nitric oxide, and their biological metabolites also play an important role in carcinogenesis. ROS induces DNA damage, as the reaction of free radicals with DNA includes strand break base modification and DNA protein cross-links. Numerous investigators have proposed the participation of free radicals in carcinogenesis, mutation, and transformation; their presence in the biosystem could lead to mutation, transformation, and ultimately cancer. Induction of mutagenesis, the best-known biological effect of radiation, occurs mainly through damage to DNA by the HO. Radical and other species are produced by radiolysis, as well as by the direct radiation effect on DNA and the reaction effects on DNA. The reaction of HO Radicals is mainly the addition to the double bond of pyrimidine bases and the abstraction of hydrogen from the sugar moiety, resulting in the chain reaction of DNA. These effects cause cell mutagenesis and carcinogenesis. Lipid peroxides are also responsible for the activation of carcinogens.

Antioxidants can decrease oxidative stress-induced carcinogenesis by directly scavenging ROS and/or by inhibiting cell proliferation secondary to protein phosphorylation. B-carotene may be protective against cancer through its antioxidant function because oxidative products can cause genetic damage. Thus, the photoprotective properties of B-carotene may protect against ultraviolet light-induced carcinogenesis. Immunoenhancement of B-carotene may contribute to cancer protection. B-carotene may also have an anticarcinogenic effect by altering the liver metabolism effects of carcinogens. Vitamin C may help prevent cancer. (Glatthaar The possible mechanisms by which vitamin C may affect carcinogenesis include antioxidant effects, blocking the formation of nitrosamines, enhancement of the immune response, and acceleration of detoxification of liver enzymes. Vitamin E, an important antioxidant, plays a role in immunocompetence by increasing humoral antibody protection, resistance to bacterial infections, cell-mediated immunity, the T-lymphocytes tumor necrosis factor production, inhibition of mutagen formation, repair of membranes in DNA, and blocking micro cell line formation. (Sokol,1998) Hence vitamin E may be useful in cancer prevention and inhibit carcinogenesis by the stimulation of the immune system. The administration of a mixture of the above three antioxidants revealed the highest reduction in risk of developing cardiac cancer.

2.1.4 Free Radical and Ageing

The human body is in a constant battle to keep from aging. Research suggests that free radical damage to cells leads to the pathological changes associated with aging. An increasing number of diseases or disorders, as well as the aging process itself, demonstrate a link either directly or indirectly to these reactive and potentially destructive molecules. The major mechanism of aging is attributed to DNA or the accumulation of cellular and

functional damage. (Cantuti, 2000) Reduction of free radicals or decreasing their rate of production may delay aging. Some of the nutritional antioxidants will retard the aging process and prevent disease. Based on these studies, it appears that increased oxidative stress commonly occurs during the aging process, and antioxidant status may significantly influence the effects of oxidative damage associated with advancing age. Research suggests that free radicals have a significant influence on aging, that free radical damage can be controlled with adequate antioxidant defense, and that optimal intake of antioxidant nutrients may contribute to enhanced quality of life. Recent research indicates that antioxidants may even positively influence life span. (Ashok, 1999)

2.1.5 Oxidative Damage to Protein

Oxidative damage to protein Proteins can be oxidatively modified in three ways: oxidative modification of specific amino acid, free radical-mediated peptide cleavage, and formation of protein cross-linkage due to reaction with lipid peroxidation products. Proteins containing amino acids such as methionine, cysteine, arginine, and histidine seem to be the most vulnerable to oxidation. (Freeman, 2004) Free radical-mediated protein modification increases susceptibility to enzyme proteolysis. Oxidative damage to protein products may affect the activity of enzymes, receptors, and membrane transport. Oxidatively damaged protein products may contain very reactive groups that may contribute damage to membranes and many cellular functions. Peroxyl radical is usually considered to be a free radical species for the oxidation of proteins. ROS can damage proteins and produce carbonyls and other amino acid modifications including the formation of methionine sulfoxide and protein carbonyls and other amino acid modifications including the formation of methionine sulfoxide and protein peroxide. Protein oxidation affects the alteration of signal transduction mechanism, enzyme activity, heat stability, and

proteolysis susceptibility, which leads to aging.

2.1.6 Lipid Peroxidation

Oxidative stress and oxidative modification of biomolecules are involved in several physiological and pathophysiological processes such as aging, atherosclerosis, inflammation and carcinogenesis, and drug toxicity. Lipid peroxidation is a free radical process involving a source of secondary free radicals, which can act as a second messenger or can directly react with other biomolecules, enhancing biochemical lesions. Lipid peroxidation occurs on polysaturated fatty acids located on the cell membranes and it further proceeds with a radical chain reaction. Hydroxyl radical is thought to initiate ROS and remove hydrogen atoms, thus producing lipid radicals and further converted into diene conjugate. Further, by addition of oxygen it forms a peroxyl radical; this highly reactive radical attacks another fatty acid forming lipid hydroperoxide (LOOH) and a new radical. Thus, lipid peroxidation is propagated. Due to lipid peroxidation, several compounds are formed, for example, alkanes, malondialdehyde, and isoprostanes. These compounds are used as markers in lipid peroxidation assay and have been verified in many diseases such as neurodegenerative diseases, ischemic reperfusion injury, and diabetes. (Lovell, 1995)

2.1.7 Oxidative DNA Damage

Many experiments provide evidence that DNA and RNA are susceptible to oxidative damage. It has been reported that especially in aging and cancer, DNA is considered a major target. (Woo,1998) Oxidative nucleotides such as glycol, DTG, and 8-hydroxy-2-deoxyguanosine are found to be increased during oxidative damage to DNA under UV radiation or free radical damage. It has been reported that mitochondrial DNA is more

susceptible to oxidative damage that has a role in many diseases including cancer. It has been suggested that 8-hydroxy-2-deoxyguanosine can be used as a biological marker for oxidative stress (Hattori Y *et al.,.,.,* 1997).

2.2 Antioxidants

The term antioxidant originally was used to refer specifically to a chemical species that prevents the consumption of oxygen. In the late 19th and early 20th century, extensive study was devoted to the uses of antioxidants in important industrial processes, such as the prevention of metal corrosion, the vulcanization of rubber, and the polymerization of fuels in the fouling of internal combustion engines. (Matill,1947) Early research on the role of antioxidants in biology focused on their use in preventing the oxidation of unsaturated fats, which is the cause of rancidity. (German J, 1999)

An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage. These antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property. These low-molecular-weight antioxidants can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Some of these antioxidants, including glutathione, ubiquinol, and uric acid, are produced during normal metabolism in the body. Other lighter antioxidants are found in the diet. Although several enzyme systems within the body scavenge free radicals, the principal micronutrient (vitamins) antioxidants are vitamin E (α-tocopherol), vitamin C (ascorbic acid), and B-carotene. (Halliwell,1995)The body cannot manufacture these micronutrients, so they must be supplied in the diet. Food scientists frequently equate antioxidants to inhibitors of lipid peroxidation because they use antioxidants to prevent rancidity Hence a broader definition; an antioxidant is any substance that when present at low concentrations compared to those of an oxidizable

substrate delays or prevents oxidation of that substrate. (ScienceDirect, 2015). Antioxidants act as radical scavengers, hydrogen donors, electron donors, peroxide decomposers, singlet oxygen quenchers, enzyme inhibitors, synergists, and metal-chelating agents. Both enzymatic and nonenzymatic antioxidants exist in the intracellular and extracellular environment to detoxify ROS.

2.2.1 Mechanism of Action of Antioxidant

Mechanisms of antioxidant action can include: the removal of O2, scavenging reactive oxygen/nitrogen species or their inhibiting ROS/RNS formation, binding metal ions needed for catalysis of ROS generation, and upregulation of endogenous antioxidant defenses of these the two principal mechanisms of action for antioxidants are. (Wolf G, 2005) The first is a **chain-breaking mechanism** by which the primary antioxidant donates an electron to the free radical present in the systems. The second mechanism involves **the removal of ROS/reactive nitrogen species** initiators (secondary antioxidants) by quenching chain-initiating catalysts. Antioxidants may exert their effect on biological systems by different mechanisms including electron donation, metal ion chelation, co-antioxidants, or gene expression regulation. (Knight J, 2005)

2.2.2 Antioxidant as Cellular Immune System

The antioxidants acting in the defense systems act at different levels such as preventive, radical scavenging, repair, and de novo, and the fourth line of defense, i.e., the adaptation

The first line of defense is the preventive antioxidants, which suppress the formation of free radicals. Although the precise mechanism and site of radical formation in vivo are not well elucidated yet, the metal-induced decompositions of hydroperoxides and hydrogen peroxide must be one of the important sources. To suppress such reactions, some antioxidants reduce hydroperoxides and hydrogen peroxide beforehand to alcohols and

water, respectively, without the generation of free radicals and some proteins sequester metal ions. Glutathione peroxidase, glutathione-s-transferase, phospholipid hydroperoxide glutathione peroxidase (PHGPX), and peroxidase are known to decompose lipid hydroperoxides to corresponding alcohols. PHGPX is unique in that it can reduce hydroperoxides of phospholipids integrated into biomembranes. Glutathione peroxidase and catalase reduce hydrogen peroxide to water. (Jacob, 1996).

The second line of defense is the antioxidants that scavenge the active radicals to suppress chain initiation and/or break the chain propagation reactions. Various endogenous radical-scavenging antioxidants are known: some are hydrophilic and others are lipophilic. Vitamin C, uric acid, bilirubin, albumin, and thiols are hydrophilic, radical-scavenging antioxidants, while vitamin E and ubiquinol are lipophilic radical-scavenging antioxidants. Vitamin E is accepted as the most potent radical-scavenging lipophilic antioxidant.

The third line of defense is the repair and de novo antioxidants. The proteolytic enzymes, proteinases, proteases, and peptidases, present in the cytosol and the mitochondria of mammalian cells, recognize, degrade, and remove oxidatively modified proteins and prevent the accumulation of oxidized proteins. (Frie, 1998)

The DNA repair systems also play an important role in the total defense system against oxidative damage. Various kinds of enzymes such as glycosylases and nucleases, which repair the damaged DNA, are known.

There is another important function called adaptation where the signal for the production and reactions of free radicals induces the formation and transport of the appropriate antioxidant to the right site (Hattori Y *et al.,,* 1997).

2.2.3 Types of Antioxidants

Cells are constantly being protected against oxidative stress by an interacting network of antioxidants. This detoxification pathway is the result of multiple enzymatic and nonenzymatic processes. Based on their mechanism of action antioxidants are generally grouped into:

- ENZYMATIC ANTIOXIDANT
- NON-ENZYMATIC ANTIOXIDANT

ENZYMATIC ANTIOXIDANT: These enzymes play a crucial role in neutralizing free radicals within the body by transforming them into less harmful substances, like water and oxygen. examples of these enzymes include:

Superoxide dismutase: Superoxide dismutases (SODs) are a class of closely related enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide. SOD enzymes are present in almost all aerobic cells and extracellular fluids. There are three major families of superoxide dismutase, depending on the metal cofactor: Cu/Zn (which binds both copper and zinc), Fe and Mn types (which bind either iron or manganese), and finally the Ni type which binds nickel (Johnson. *et.al, 2005*). In higher plants, SOD isozymes have been localized in different cell compartments. Mn-SOD is present in mitochondria and peroxisomes. Fe-SOD has been found mainly in chloroplasts but has also been detected in peroxisomes, and CuZn-SOD has been localized in cytosol, chloroplasts, peroxisomes, and apoplasts.

In humans (as in all other mammals and most chordates), three forms of superoxide dismutase are present. SOD1 is located in the cytoplasm, SOD2 is in the mitochondria, and SOD3 is extracellular. The first is a dimer (consists of two units), while the others are

tetramers (four subunits). SOD1 and SOD3 contain copper and zinc, while SOD2 has manganese in its reactive center.

Catalase: Catalase is a common enzyme found in nearly all living organisms, that are exposed to oxygen, where it functions to catalyze the decomposition of hydrogen peroxide to water and oxygen. (Cao X, 2008) Hydrogen peroxide is a harmful by-product of many normal metabolic processes: to prevent damage, it must be quickly converted into other, less dangerous substances. To this end, catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules. All known animals use catalase in every organ, with particularly high concentrations occurring in the liver. (Eisner T, 1999)

Glutathione systems: The glutathione system includes glutathione, glutathione reductase, glutathione peroxidases, and glutathione S-transferases. This system is found in animals, plants, and microorganisms. Glutathione peroxidase is an enzyme containing four selenium-cofactors that catalyze the breakdown of hydrogen peroxide and organic hydroperoxides. There are at least four different glutathione peroxidase isozymes in animals. Glutathione peroxidase 1 is the most abundant and is a very efficient scavenger of hydrogen peroxide, while glutathione peroxidase 4 is most active with lipid hydroperoxides. The glutathione S-transferases show high activity with lipid peroxides. These enzymes are at particularly high levels in the liver and also serve in detoxification metabolism (Brigelius-Flohe, 1999).

Sources of enzymatic antioxidants: Most enzymatic antioxidants are produced naturally in the body.

NON-ENZYMATIC ANTIOXIDANT: These are usually non-proteinous species or substances that can neutralize free radicals in the body they are called non-enzymatic

because they do not perform enzymatic activities within the body examples of these include:

Ascorbic acid: Ascorbic acid or “vitamin C” is a monosaccharide antioxidant found in both animals and plants. As it cannot be synthesized in humans and must be obtained from the diet, it is a vitamin. Most other animals can produce this compound in their bodies and do not require it in their diets. In cells, it is maintained in its reduced form by reaction with glutathione, which can be catalyzed by protein disulfide isomerase and glutaredoxins. Ascorbic acid is a reducing agent and can reduce and thereby neutralize ROS such as hydrogen peroxide. (Meister A, 1998) In addition to its direct antioxidant effects, ascorbic acid is also a substrate for the antioxidant enzyme ascorbate peroxidase, a function that is particularly important in stress resistance in plants.

Glutathione: Glutathione is a cysteine-containing peptide found in most forms of aerobic life. It is not required in the diet and is instead synthesized in cells from its constituent amino acids. Glutathione has antioxidant properties since the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. In cells, glutathione is maintained in the reduced form by the enzyme glutathione reductase and in turn reduces other metabolites and enzyme systems as well as reacting directly with oxidants. Due to its high concentration and central role in maintaining the cell's redox state, glutathione is one of the most important cellular antioxidants. In some organisms, glutathione is replaced by other thiols, such as by mycothiol in the actinomycetes, or by trypanothione in the kinetoplastids. (Matill HA, 1947)

Melatonin: Melatonin, also known chemically as N-acetyl-5-methoxytryptamine, is a naturally occurring hormone found in animals and in some other living organisms, including algae. Melatonin is a powerful antioxidant that can easily cross cell membranes

and the blood–brain barrier. Unlike other antioxidants, melatonin does not undergo redox cycling, which is the ability of a molecule to undergo repeated reduction and oxidation. Melatonin, once oxidized, cannot be reduced to its former state because it forms several stable end-products upon reacting with free radicals. Therefore, it has been referred to as a terminal (or suicidal) antioxidant (Nassar E.*et al.,,* 2007).

Tocopherols and tocotrienols (Vitamin E): Vitamin E is the collective name for a set of eight related tocopherols and tocotrienols, which are fat-soluble vitamins with antioxidant properties. Of these, α-tocopherol has been most studied as it has the highest bioavailability, with the body preferentially absorbing and metabolizing this form. It has been claimed that the α-tocopherol form is the most important lipid-soluble antioxidant and that it protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction.[71] This removes the free radical intermediates and prevents the propagation reaction from continuing. This reaction produces oxidized α-tocopherol radicals that can be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol, or ubiquinol. (Wang X,1999).

Uric acid: Uric acid accounts for roughly half the antioxidant ability of plasma. Uric acid may have been substituted for ascorbate in human evolution. However, like ascorbate, uric acid can also mediate the production of active oxygen species.

Other examples of non-enzymatic antioxidants include lycopene, selenium, zinc, polyphenols, and ubiquinone (Coenzyme Q10).

Sources: common sources include fruits and vegetables citrus, tomatoes, carrots, and spinach.

2.3 Total Antioxidant Capacity

This is said to be the measure/concentration of antioxidants in any biological sample. Antioxidant capacity is the primary measurement to evaluate the state and potential of oxidative stress in aging and other age-related diseases. Since the imbalance between antioxidants and oxidants generates the condition of oxidative stress, estimation of the reducing power/antioxidant capacity is the first step in the prediction of oxidative stress in the aging process. There are several methods to measure total antioxidant capacity in vitro. These methods are based on quenching of free radicals such as 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH·), 2,2-azobis-3-ethylbenzthiazoline- 6-sulfonic acid (ABTS) by antioxidants, inhibition of lipid peroxidation, etc.

TAC assay provides a quantitative assessment of the cumulative antioxidant capacity of a sample some of the common methods used to measure TAC include:

Trolox Equivalent Antioxidant Capacity (TEAC) Assay

Oxygen Radical Absorbance Capacity (ORAC) Assay

Ferric Reducing Antioxidant Power (FRAP) Assay

DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay

2.3.1 Trolox Equivalent Antioxidant Capacity (TEAC) Assay:

The Trolox Equivalent Antioxidant Capacity (TEAC) assay is a method for measuring the antioxidant capacity of a substance. It is based on the ability of antioxidants to reduce a colored radical, ABTS+ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate)), to a colorless form, ABTS. The TEAC value of a substance is expressed as the number of micromoles of Trolox (a water-soluble vitamin E analog) equivalent per liter of sample. The higher the

TEAC value, the greater the antioxidant capacity of the substance. TEAC assay is a relatively simple and rapid assay, and it is widely used to measure the antioxidant capacity of foods, beverages, and supplements. It is also used to measure the antioxidant capacity of biological samples, such as blood and plasma. (Fredrick, 2016)

2.3.2 Oxygen Radical Absorbance Capacity (ORAC) Assay: The Oxygen Radical Absorbance Capacity (ORAC) assay is a method for measuring the antioxidant capacity of a substance. It is based on the ability of antioxidants to protect a fluorescent probe from oxidation by free radicals.

The ORAC assay is a widely used method for measuring the antioxidant capacity of foods, beverages, and supplements. It is a relatively quick and easy assay to perform, and it is relatively inexpensive. However, the ORAC assay has some limitations.

One limitation of the ORAC assay is that it is performed in a cell-free system. This means that it may not accurately reflect the antioxidant capacity of a substance in vivo. Another limitation of the ORAC assay is that it does not take into account the bioavailability of antioxidants. Bioavailability is the percentage of an antioxidant that is absorbed and used by the body. (Journal of Laboratory Assay, 2007)

2.3.3 Ferric Reducing Antioxidant Power (FRAP) Assay: The Ferric Reducing Antioxidant Power (FRAP) assay is a colorimetric method for measuring the total antioxidant capacity of a sample. It is based on the reduction of ferric iron (Fe(III)) to ferrous iron (Fe(II)) by antioxidants present in the sample. The ferrous iron then reacts with a colorimetric probe, 2,4,6-tripyridyl-s-triazine (TPTZ), to form a blue complex. The intensity of the blue color is proportional to the concentration of antioxidants in the sample. The FRAP assay is a simple and rapid method for measuring antioxidant capacity, and it is widely used in various research and industrial applications. It is also a relatively

inexpensive method, and it can be performed in a variety of laboratories.

Applications:

Food and beverage analysis: The FRAP assay can be used to measure the antioxidant capacity of foods and beverages, such as fruits, vegetables, juices, and wines. This information can be used to develop new products with enhanced antioxidant content and to provide consumers with information about the nutritional value of foods.

Plant extract analysis The FRAP assay can be used to measure the antioxidant capacity of plant extracts. This information can be used to identify new sources of antioxidants for use in food and dietary supplements.

Biological fluid analysis: The FRAP assay can be used to measure the antioxidant capacity of biological fluids, such as blood and plasma. This information can be used to assess the antioxidant status of individuals and to identify individuals who may be at risk of antioxidant deficiency.

Research: The FRAP assay is widely used in research to study the role of antioxidants in human health. For example, it has been used to investigate the relationship between antioxidant intake and the risk of chronic diseases such as cancer, heart disease, and Alzheimer's disease. (Journal of Laboratory Assay, 2007)

2.3.4 DPPH (2,2-Diphenyl-1-picrylhydrazyl) Assay: The DPPH assay assesses the ability of antioxidants to scavenge the DPPH free radical. The decrease in absorbance is measured, indicating the antioxidant capacity. (Fredrick, 2016)

2.4 Picralima Nitida

Picralima nitida (Stapf) T. Durand & H. Durand, belonging to the *Apocynaceae*family, is a plant native to West Africa and extensively utilized in African traditional medicine

(Durand and Hook, 2011). Across diverse regions, different components of this plant have been traditionally employed to address various health issues such as fever, hypertension, jaundice, dysmenorrhea, gastrointestinal disorders, and malaria. *Picralima nitida stands* as the sole representative of the *Picralima* genus and shares botanical relations with *Hunteria a*nd *Pleiocarpa*. Commonly known as Picralima, Akuamma, or Pile Plant, it falls within the *Hunterieae* tribe of the *Apocynaceae* family. *See Table 2.1*The geographical distribution of *Picralima nitida* spans the high deciduous forests of West-Central Africa, ranging from Ivory Coast to West Cameroons, and extending across the Congo basin and Uganda (Saxton *et al.,.,.,* 1960). Described as an understory tree, *Picralima nitida* attains a height of 4–35 m, featuring a dense crown and a cylindrical trunk with a diameter of 5–60 m. The wood is characterized by its pale-yellow color, hardness, elasticity, fine-grained texture, and capacity to take a high polish. Noteworthy botanical features include white flowers (approximately 3 cm long) and ovoid fruits that mature to a yellowish hue. The leaves are broad (3–10 cm) and oblong (6–20 cm) with robust lateral nerves numbering 14–24 pairs (Burkill, 1985).

TABLE 2.1

KINGDOM	PLANTAE
ORDER	GENTIANALES
FAMILY	APOCYNACEAE
SUB-FAMILY	RAUVOLFIOIDEAE
TRIBE	HUNTERIEAE
GENUS	PICRALIMA
SPECIES	PICRALIMA NITIDA

(Opeyemi.*et.al,* 2020)

2.4.1 Order *Gentianales*

*Gentianales*is an order of flowering plants that encompasses a diverse array of species, known for their distinctive and often showy flowers. This botanical order is part of the subclass *Asteridae* within the class *Magnoliopsida* (dicotyledons).

Taxonomy and Classification: *Gentianales*is a large order that includes about 16 families and over 87 genera. Some well-known families within this order include *Gentianaceae*, *Apocynaceae, Rubiaceae, and Loganiaceae.*

Morphological Characteristics

1. Leaves: The leaves in *Gentianales* are typically opposite or whorled, and they may be simple or compound.

2. Flowers: Flowers in *Gentianales*are often characterized by a bilateral symmetry, and they can be quite showy. The floral parts are usually in whorls or opposite arrangements.

3. Inflorescence: The arrangement of flowers can vary, with inflorescences often forming clusters or cymes.

4. Fruit: The fruit types are diverse and may include capsules, berries, or drupes.

*Gentianales*s species are found worldwide, but they are particularly diverse and abundant in tropical and subtropical regions. They inhabit various ecosystems, from rainforests to alpine meadows.

Economic Importance: Many species within *Gentianales* have economic significance. Some are cultivated for ornamental purposes due to their attractive flowers, while others

have medicinal properties. The order includes several plants used in traditional medicine, such as those from the *Gentianaceae* and *Apocynaceae* families.

Notable Families within the order *GENTIANALES*

***Gentianaceae*:** Known for the beautiful and often intensely colored flowers, this family includes the Gentians (*Gentiana spp.*).

***Apocynaceae*:** Includes plants like periwinkle (*Catharanthus roseus*), which is a source of vincristine and vinblastine used in cancer treatment.

***Rubiaceae*:** This large family encompasses coffee plants (*Coffea spp*.) and quinine-producing

plants (*Cinchona spp*.).

Ecological Roles: Members of *gentianales* play crucial roles in various ecosystems. They serve as nectar sources for pollinators, and some species have adapted to specific ecological niches.

2.4.2 Family *Apocynaceae*

The *Apocynaceae*family is a diverse and economically significant family of flowering plants, commonly known as the dogbane family. This family is part of the larger order Gentianales, which includes many other plant families. *Apocynaceae* is renowned for its wide distribution and the presence of numerous species with varied ecological roles, ranging from ornamental plants to those with medicinal importance.

Taxonomy and Diversity: *Apocynaceae*is a large family comprising about 200 genera and over 2,000 species. It is characterized by the presence of latex (milky sap), opposite or whorled leaves, and often showy flowers. Notable genera within *Apocynaceae*include

***Vinca*:** Known for ornamental plants like *Vinca minor*.

***Nerium*:** includes the popular ornamental plant *Nerium oleander.*

***Carissa*:** Some species are cultivated for their edible fruits.

***Apocynum*:** includes species like *Apocynum cannabinum*, known for its medicinal uses.

Morphology: Members of the *Apocynacea*family can be found in the form of trees, shrubs, herbs, or vines. The leaves are typically simple and arranged oppositely, sometimes whorled. The flowers are often distinctive and are characterized by their radial symmetry. They may be solitary or arranged in inflorescences, and the fruit types vary widely.

Distribution: *Apocynaceae* has a global distribution, with a concentration of species in tropical and subtropical regions. They thrive in a variety of ecosystems, from rainforests to arid deserts.

Economic Importance: Several members of the *Apocynaceae* family have economic significance. Some species are cultivated for ornamental purposes due to their attractive flowers. Others have medicinal properties, producing compounds with potential pharmaceutical applications. For example, vinca alkaloids derived from plants like *Catharanthus roseus* have been used in cancer treatment.

Latex Production: One distinctive feature of *Apocynaceae* is the presence of latex, a milky sap that often contains alkaloids and other secondary metabolites. This latex can be toxic and is thought to serve as a defense mechanism against herbivores.

Ecological Roles: Beyond their economic importance, *Apocynaceae*species play crucial ecological roles. They serve as habitat and food sources for various organisms, including insects and birds. The presence of toxic compounds in some species may deter herbivores.

2.4.3 Sub-Family *Rauvolfioideae*

*Rauvolfioideae*is a subfamily of flowering plants within the larger *Apocynaceae* family. This subfamily includes a diverse group of plants known for their unique characteristics, distribution, and, in some cases, medicinal properties.

Morphology

Habit: Plants in the *Rauvolfioideae* subfamily exhibit a wide range of habits, including trees, shrubs, and some herbaceous plants.

Leaves: Leaves are usually simple, opposite, and exstipulate, although exceptions exist.

Inflorescence: Flowers are commonly arranged in cymes or umbels, and the inflorescence may vary between species.

Flowers:

Corolla: The corolla is often funnel-shaped, tubular, or salverform.

Calyx: The calyx is usually five-lobed.

Stamens: Stamens are typically inserted on the corolla tube and are equal in number to the lobes of the corolla.

Ovary: The ovary is usually superior.

Fruits

Fruit Types: Fruits vary widely and can be follicles, berries, or drupes.

Seed Dispersal: Seed dispersal mechanisms can include wind, water, or animal dispersal.

Chemical Composition:

Alkaloids: Many plants in *Rauvolfioideae* are known for containing alkaloids, some of which have pharmacological properties. For example, some species are a source of alkaloids used in traditional medicine.

Geographical Distribution: The subfamily is distributed globally in tropical and subtropical regions.

Economic Importance: Medicinal Uses: Some plants in *Rauvolfioideae*, such as those from the genus *Rauvolfia*, have been traditionally used in various cultures for their medicinal properties. Alkaloids from these plants have been studied for their effects on the cardiovascular system and as antihypertensive agents.

Notable Genera:

Rauvolfia: is known for its medicinal properties, particularly in traditional medicine.

Alstonia: Some species are sources of medicinal alkaloids.

Tabernaemontana: includes plants with alkaloids and has been used in traditional medicine.

2.4.4 Tribe *Hunterieae*

The *Hyunterieae* are a tribe within the *Apocynaceae* family, a large family of flowering plants. Here's a comprehensive description of the *Hunterieae* tribe:

Taxonomy and Classification: The *Hunterieae* tribe is classified within the larger *Apocynaceae* family, which includes a diverse array of plants. *Apocynaceae* is known for its ornamental, medicinal, and toxic plants. The tribe Hunterieae is specifically situated within the subfamily *Rauvolfioideae*, which is characterized by the presence of alkaloids

and latex in the plant tissues.

Members and Distribution: The tribe *Hunterieae* includes various genera of plants, and one of the notable members is *Picralima nitida*. This tropical tree is indigenous to West Africa and is recognized for its medicinal properties. *Picralima nitida*, also known as akuamma or pigeon wood, belongs to this tribe and has been traditionally used in African folk medicine.

Morphological Characteristics: Plants within the *Hunterieae* tribe often share certain morphological characteristics. These may include the presence of latex, a milky fluid rich in alkaloids, which is a common feature in the *Apocynaceae* family. The leaves of these plants are typically arranged oppositely, and the flowers are often distinctive, with five petals fused into a tube.

Chemical Composition: Plants in the *Hunterieae* tribe, like other members of the *Apocynaceae* family, are known for their chemical diversity. Alkaloids are a significant component, and they often contribute to the medicinal properties of these plants. *Picralima nitida*, for example, contains various alkaloids with potential pharmacological effects.

Traditional Uses: Some members of the *Hunterieae* tribe, including *Picralima nitida*, have a history of traditional use in African medicine. They are employed to treat various ailments, and different parts of the plants, such as the bark, leaves, and seeds, may be utilized for medicinal purposes. The alkaloids present in these plants can exhibit analgesic, anti-inflammatory, and other pharmacological activities.

2.4.5 Genus *Picralima*

The genus *Picralima* comprises tropical trees, and its most notable and only species is

Picralima nitida.

Taxonomy: Scientific Classification: *Picralima* belongs to the *Apocynaceae* family and the *Hunterieae* tribe.

Species: The genus primarily features *Picralima nitida*, commonly known as Akuamma or Akuammine, which holds medicinal significance

Morphology

Habitat: *Picralima* species, particularly *P. nitida*, are native to West Africa and are prevalent in high deciduous forests from the Ivory Coast to West Cameroon. They are also found in the Congo basin and Uganda.

Plant Size: *P. nitida* typically grows as an understory tree, reaching heights of 4–35 meters.

Bark: The bark of *Picralima* trees is characterized by a grayish color with yellowish slashes that yield scanty latex.

Leaves: Leaves are oblong-lanceolate to broadly oblong-elliptic, dark green, leathery, and glossy glabrous with numerous parallel, rather faint nerves.

Flowers: White flowers, up to 5 cm wide, borne in terminal inflorescences.

Fruits: Broadly ovoid fruits, smooth, glabrous, and leaf green turning yellow or orange when ripe.

Ethnomedicinal Uses: *Picralima nitida* has a rich history in West African traditional medicine.

Various parts of the plant, including leaves, seeds, stem bark, and roots, are used for treating ailments such as fever, hypertension, jaundice, gastrointestinal disorders, and malaria.

Among the local people of Nigeria, *P. nitida* is known as Abere/Erin; this name is used by the southwestern (Yoruba) people, while in the historical lands of Edo State, it is known as Osu. In the southeastern parts of Nigeria, indigenes refer to the plant as Osi or Osu-Igwe; other common native names include ekuama, otoshi, and oso.

Picralima nitida holds significant prominence in West African folk medicine, where various parts of the plant, including leaves, seeds, stem bark, and roots, are utilized by herbalists for addressing conditions such as fever, hypertension, jaundice, gastrointestinal disorders, and malaria (Dalziel *et al.,* 1937). The plant's pharmacological activities, corroborating its ethnomedicinal applications, have been explored through extractions from different plant parts. Indole alkaloids identified in the seeds of Picralima nitida, such as akuammine, akuammidine, akuammicine, akuammigine, and pseudoakuammigine, exhibit opioid analgesic properties. However, the pharmacological potential of these alkaloids has only

been partially investigated, necessitating further research for a comprehensive exploration of their pharmacologic and therapeutic capacities.

Figure 2.1 *Picralima nitida*seed

Figure 2.2 *Picralima nitida* tree

2.4.6 Traditional Medical/Ethnomedicinal Uses of Picralima Nitida

Preparations derived from various plant components are utilized as raw drugs or herbal extracts to provide remedies for diverse human ailments.

SEEDS: In West Africa, particularly in Nigeria, Côte d'Ivoire, and Ghana, the seeds find widespread use as antipyretic and aphrodisiac. They are employed for treating malaria, pneumonia, and various chest conditions, with external application for abscesses in Gabon. In Ghana, a seed decoction is administered as an enema, while crushed seeds are ingested to address chest complaints, pneumonia, and gastrointestinal disorders.

FRUITS: The fruit, utilized in West Africa, serves for treating gastrointestinal disorders and dysmenorrhea. In Ghana, the fruit shell, filled with palm wine, is consumed after absorbing bitter principles, acting as a remedy for fever.

LEAVES: The leaves function as a vermifuge, and their sap is applied as ear drops for otitis in West Africa.

BARK: The bark is employed as laxatives, purgatives, anthelmintics, and for treating venereal diseases. It serves as a febrifuge and is used to address hernia. In Ivory Coast, a bark concoction is consumed for jaundice and 'yellow fever.'

ROOT: The root is utilized as a vermifuge and aphrodisiac, as well as for treating fevers, malaria, pneumonia, and gastrointestinal disorders.

2.4.7 Phytochemistry of Picralima Nitida

Analysis of Picralima nitida has identified the existence of alkaloids, tannins, saponins, flavonoids, terpenoids, steroids, and glycosides within the plant (Obasi *et al.,.,.,* 2005). Further exploration into the plant's phytochemical composition has resulted in the

extraction of several alkaloids, prominent among the seed's major compounds. Additionally, polyphenols have been isolated as other significant phytochemicals from Picralima nitida.

SAPONINS Saponins, a class of glycosidic compounds, are widely distributed in various plant species, showcasing diverse biological activities such as antitumor, antimicrobial, and anti-inflammatory effects. Numerous investigations have delved into unraveling the saponin profile of Picralima nitida. In a notable study, the bark of the tree revealed the presence of 15 different saponins, with picralimides A-D emerging as the most abundant. Another exploration focused on the leaves identified a repertoire of 20 saponins in Picralima nitida, with picralimides A-D and quassinoid saponins dominating the composition. (Adebisi, 2018)

Beyond the prominently mentioned picralimides and quassinoid saponins, additional saponins have been discerned in Picralima nitida, constituting a comprehensive profile:

Picralimides E-H, Quassinoid saponins A-E, Picralimic acid saponins A-B, Picralimidine saponins A-B.(Adebayo, 2020)

ALKALOIDS: The primary group of phytochemicals extracted from Picralima nitida is alkaloids. The initial set of alkaloids identified from this plant comprises the indole alkaloids: akuammine, pseudo akuammine, akuammidine, akuammicine, akuammigine, pseudo akuammiline, akuammiline, and akuammenine, named after the indigenous term 'Akuamma' in Ghana (Henry and Sharp, 1972). Subsequently, numerous alkaloids have been isolated and reisolated from the plant, including picraphylline, paracrine, picraline, picralicine, picratidine, picranitine, burnamine, pericalline, and pericine (Menzies *et al.,.,.,* 1998). While predominantly present in the seeds (3.5–4.8% alkaloid content), these compounds are also found in the leaves, bark, and roots of the plant.

The seeds, particularly rich in alkaloids, feature akuammine as the principal alkaloid in mature seeds. Minor alkaloids include pseudo-akuammicine, picranitine, picratidine (N-methylpicraline), eburnamine (desacetylpicraline), and diacetyl akuammiline (rhazimol) (Menzies *et al.,.,.,* 1996). Akuammine demonstrates potent sympathomimetic and local analgesic properties, comparable to cocaine. It induces significant and lasting hypotension in dogs without affecting respiration. In higher doses, it exerts a pronounced inhibitory effect on intestinal peristaltic movements, coupled with hypertensive activity, albeit weaker yet longer-lasting than yohimbine (Corbett *et al.,.,.,* 1996). It counteracts the impact of adrenaline on the heart, blood vessels, and the regulatory center of the circulatory system. Akuammidine exhibits hypotensive, skeletal muscle relaxant, and local analgesic properties, with local analgesic efficacy approximately three times more potent than cocaine. It selectively acts as a sympatholytic without concurrent parasympatholytic effects. Akuammidine inhibits the irritability of the sympathetic nervous system and opposes akuammine. Pseudo-akuammigine acts as an indirect, reversible, and competitive parasympathomimetic. In low doses, it stimulates, while in high doses, it hinders the central nervous system, respiration, skeletal muscle contraction, and smooth muscle contraction. It prolongs hexobarbital-induced sleeping time and demonstrates local analgesic, anti-inflammatory, cholinesterase-inhibiting, and hypotensive activities. Pericine and pericalline, exclusively found in cell suspension cultures of Picralima nitida, exhibit in vitro opium-antagonist activity.

FLAVONOIDS: The flavonoids are a class of polyphenolic compounds that are found in many plants. Flavonoids are known to have a wide range of biological activities, including antioxidant, anti-inflammatory, and anticancer effects. Several studies have investigated the flavonoid composition of Picralima nitida in one of such about 18 different flavonoids were discovered in the bark of the tree. The most abundant flavonoids were quercetin,

kaempferol, and isorhamnetin

Another study identified 25 different flavonoids in the leaves of Picralima nitida. The most abundant flavonoids of them are quercetin, kaempferol, and myricetin. (Afolayan, 2017).

Other flavonoids present in *P. nitida* include:

Flavonols: quercetin, kaempferol, isorhamnetin, and myricetin

Flavones: luteolin and apigenin

Flavanones: naringenin and hesperidin

Flavanols: catechin and epicatechin

The flavonoids in *P. nitida* are thought to be responsible for many of the plant's medicinal properties. For example, quercetin has been shown to have anti-inflammatory, antioxidant, and anticancer effects. Kaempferol has also been shown to have anti-inflammatory and antioxidant effects. The flavonoids in *P. nitida* have a wide range of potential applications, including:

Anti-inflammatory: The flavonoids in *P. nitida* have been shown to have anti-inflammatory effects. This suggests that *P. nitida* could be used to treat inflammatory diseases such as arthritis, asthma, and inflammatory bowel disease. Kaempferol has been shown and known to have this effect, especially on arthritis

Antioxidant: The flavonoids in *P. nitida* have been shown to have antioxidant effects. This suggests that *P. nitid* could be used to protect cells from damage caused by free radicals. This could help to reduce the risk of chronic diseases such as cancer, heart disease, and Alzheimer's disease.

Anticancer: In *P. nitida* flavonoids have been shown to have anticancer effects. This suggests that *P. nitida* could be used to treat cancer or to prevent cancer from developing. quercetin has shown significant anti-carcinogenic activities (Adebayo, 2020)

In addition to the flavonoids mentioned above, other flavonoids identified in *Picralima nitid* include Apigenin, Catechin, Epicatechin, Hesperidin, Luteolin, Naringenin, Rutin

POLYPHENOLS: Polyphenols represent a category of secondary metabolites present in various plants, exhibiting a broad spectrum of biological activities, encompassing antioxidant, anti-inflammatory, and anticancer effects. Several research endeavors have scrutinized the polyphenolic composition of *Picralima nitida*. A study discerned 18 distinct polyphenols in the tree's bark. Notably, the prevailing polyphenols were flavonoids, with quercetin, kaempferol, and isorhamnetin. Another investigation, detailed 25 diverse polyphenols within the leaves of *Picralima nitida.*, flavonoids, quercetin, kaempferol, and myricetin were the most abundant

2.4.8 Pharmacological Properties of *Picralima Nitida*

Picralima nitida, commonly recognized as 'Osu' or 'Akuamma,' displays a spectrum of biological activities that have become the focal point of scientific exploration. The plant encompasses diverse bioactive compounds, including alkaloids, flavonoids, terpenoids, and phenolics, contributing to its pharmacological profile. Here are some prominent biological activities associated with *Picralima nitida:*

1. Analgesic Potential: *Picralima nitida*has a rich history as a herbal analgesic. Numerous studies have illuminated the analgesic properties of the plant's alkaloids, notably akuammidine, akuammine, and akuammicine. These compounds alleviate pain by interacting with opioid receptors in the central nervous system (Corbett *et al.,.,.,* 1988).

2. Anti-inflammatory Attributes: Scientific investigations have unveiled the anti-inflammatory capacities of *Picralima nitida* extract and compounds. Alkaloids like akuammidine and akuammicine exhibit inhibitory effects on inflammatory mediators and cytokines, contributing to a reduction in inflammation (Duwiejua *et al.,.,.,* 2002).

3. Antibacterial Activity: extracts from *Picralima nitida* have antibacterial efficacy against a spectrum of microorganisms, encompassing bacteria and fungi. The constituents of the plant demonstrate the potential to impede the proliferation of pathogenic germs, indicating its role as a natural antimicrobial agent (Fakeye *et al.,.,.,* 2000).

4. Antioxidant Strength: Enriched with phenolic compounds and flavonoids, *Picralima nitida* manifests potent antioxidant properties. These compounds act as scavengers of free radicals, diminishing oxidative stress and shielding cells from damage. The antioxidant prowess of *Picralima nitida* positions it as a contender in combating diseases associated with oxidative stress (Fakeye *et al.,* 2000).

5. Antimalarial Activity: Some studies have elucidated the antimalarial potential of *Picralima nitida* extracts and alkaloids. Compounds like akuammidine and akuammigine showcase inhibitory effects against Plasmodium falciparum, the malaria-causing parasite. This suggests a potential role for *Picralima nitida*in in the development of antimalarial interventions (Iwu and Klayman, 2002).

6. Anti-diabetic Effects: Investigations propose that *Picralima nitida* harbors anti-diabetic properties. Compounds derived from the plant exhibit the ability to enhance glucose absorption, elevate insulin production, and regulate blood sugar levels, suggesting a therapeutic potential in diabetes management (Furman *et al.,* 2010).

CHAPTER 3

MATERIALS AND METHODS

3.0 Materials

The following materials were used during the research study;

1. Experimental rats, Aqueous *p.nitida* fruit pulp extract, Gavage, Hand gloves, Syringes (2ml and 5ml), Marker, Scissors, Dissecting forceps, Laboratory coat, Face mask, Cotton wool, Masking tape, Gentian Violet paint, Cotton board, Sample container (EDTA, lithium heparin, and plain), Cages (5), Wood shavings, Feed (grower mash and pellet), Glucose strip (Accu-chek), Tissue, Feeding plates, Mortar and pestle

3.1 Machines

1. Glucometer (Accu-chek), made in the United king

2. Weighing scale, made in China

3. Storage system (Haier THERMO COOL), made in Nigeria

3.2 Chemicals and Reagents

1. Chloroform

2. Distilled water

3. Methylated spirit

4. Detergents

3.3 Experimental period.

The experimental period involves all the processes from the beginning to the end of the experiment. which ran for 9-11 weeks

3.3.1. Plant material

The unripe fruits of *Picralima nitida* were obtained from a local vendor in New Benin market, New Benin, Benin City, Nigeria. The fruit was identified and authenticated by Dr. Henry Akinnibosun, a Taxonomist at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria. A voucher number UBHP 424 was obtained and deposited in the Herbarium. The *P. nitida* fruits were washed thoroughly with clean water to remove mud and impurities from the surfaces and left to dry overnight in a sieve, after which the green rind was peeled and separated from the pulp. The pulp was also separated from the seeds using a kitchen knife and then chopped finely into small bits of about 2 cm each. The weight of the pulp was 20 kg.

3.3.2. Preparation of the aqueous fruit pulp extract.

The chopped *P. nitida* fruit pulp was placed on a flat surface and air-dried at room temperature (25°C) to constant weight over 56 days. The dried fruit pulp was pulverized to powder using a mechanical grinder [model: SY-18B Industrial Dry Herbs Grinder, China]. The powdered fruit pulp (5.01kg) was soaked in distilled water for 72 hours at a mass-to-volume ratio of 1: 10 (kg/L) with continuous stirring. The solution was filtered through muslin cloth, cotton wool, and filter paper. The filtrate obtained was freeze-dried [Biobase BK-FD10 Freeze Dryer, China] producing a concentrated jelly-like extract. A total yield of 637.6g (12.7%) was obtained. The freeze-dried extract was preserved in an air-tight clean plastic container and stored at 4°C until required.

3.3.3. Apparatus

Weighing balance (Adventurer: OHAUS CORP, China), Measuring cylinder, Beakers, Test tubes, Stirrer, Micropipette, Shaking water bath (Jinotech, SHZ-82, China), Heating drying oven (Model DHG, Mermert Industries, Germany), Spectrophotometer (721S

Visible Spectrophotometer, China), impact drill (13mm, JEMENS, Germany), Bucket Centrifuge (90-2 electric low-speed centrifuge, China), Hot water bath, Freeze Dryer (Search-tech, British), Incubator (Seradon), Knives, Plastic Bowls, Muslin cloth, Cotton wool, Filter paper, Sterilized or oven dried containers, Filter paper, Foil paper, Gloves, Handkerchief, macro or Micro pipette, Racks, Refrigerator, Spatula, Test tube racks, Funnel, Conical flasks, Cuvette, Washing bottles, Washing brush, 100 ml volumetric flask, Bin bags, Tissue paper, Gallon (10 liter), Rubber tray, Ice packs.

3.3.4. Preparation of the experimental rats.

Healthy male Wistar rats possessing the following characteristics; standing ears, clear sclera, white furs, active, etc) were purchased and kept in clean and serene plastic cages and left to acclimatize for four weeks by feeding them with normal poultry feed called growers mash. The Wistar rats were divided into seven groups with five animals in each group and kept in separate cages. The animals were weighed on a weighing balance to determine their various body weight and each rat was stained on various body parts such as Head, Right hand, Left hand, Right leg, left leg, and tail using gentian violet paint for easy identification purposes. Meanwhile, the last number (seven) wasn't stained in all cages. Making it a total of Thirty-five (35) experimental rats. Arrangements were made from the most weighed to the least weighed.

3.3.5 Acclimatization method.

Each batched rat was fed once daily according to their weight on growers mash at a dose of 142 and their blood glucose concentration was taken once per week. The health integrity of the cages was maintained by changing the bedding's wood shavings every (3) three days to prevent infection and keep it conducive for survival. These activities spanned for four weeks, at the end of which administration of six weeks (42) started.

3.3.6. Administration period

The obtained Aqueous *Picralima* extract stock was stored in the refrigeration system for preservation. The experimental rats were grouped by weight accepting the following criteria**:**

Group A: 270 – 250g

Group B: 250 – 220g

Group C: 220 – 200g

Group D: 200 – 180g

Group E: 160 – 120g

The aqueous extract was measured and mixed with distilled water according to the dosage given and the weight of individual rats was calculated once every week. This mixture was done using a mortar and pestle, to ensure uniformity of the solution. Administration was given in dosage ranges of 100, 200, 250, and 400(mg/g), for four groups, then the last group as control. The experimental rats were gavaged orally.

• Group 1: 400 mg/g

• Group 2: 250 mg/g

• Group 3: 200 mg/g

• Group 4: 100 mg/g

• Group 5: Control.

Calculations were made based on the weight of individual rats and each group's dosage range. The experimental rats with greater weights were administered a higher dosage

accordingly. Thus, for the group with:

- For the group with the weight range of 270 – 250 g, 400 mg/kg of the aqueous was administered.

- For the group with the weight range of 250 – 220 g, 250mg/kg of the aqueous extract was administered

- For the group with the weight range of 220 – 200g, 200 mg/kg of the aqueous extract was administered

- For the group with the weight range of 200 – 180g, 100mg/kg of the aqueous extract was administered

-For the group with the weight range of 160 – 120g, 0mg/kg of the aqueous extract was administered, being the control group.

The cages were maintained by changing the beddings daily using wood shavings, and blood glucose was carried out every three days. The administration was done daily for a total of 5 weeks during the course of which the experimental animals were fed in pellets and their beddings were changed daily to ensure maximum sanitation and comfort of the animals. The animals were weighed weekly to determine the weekly dose for each animal according to their weight. The blood glucose levels of the experimental rats were also taken weekly during this period to monitor the blood glucose concentration of the experimental animals during this stage. After the course of these 5 weeks, the animals were sacrificed.

3.3.7 Sacrifice

On day 42 of administration, the experimental rats were sacrificed by first euthanizing using chloroform and then dissected via a midline incision, the required volume of blood

was obtained via cardiac puncture and decanted into each plain sample bottle, using standard technique to prevent hemolysis. The blood sample collected was left to coagulate and blood retraction was allowed to take place. These samples were used to test for the following assays:

- Testosterone

- Insulin

- C – Peptide

- Total Antioxidant Capacity (TAC)

- Cortisol

- Zinc

3.3.8. Assay process

The principle of Total Antioxidant Capacity (TAC) assays is based on the ability of antioxidants to neutralize or quench free radicals and other reactive species. The assays aim to measure the cumulative antioxidant activity of a sample, which could be a biological fluid, tissue, or substance under investigation. Here are some common principles of TAC assays: using TEAC (Trolox equivalent antioxidant capacity) assay method

Principle

It is based on the ability of antioxidants to reduce a colored radical, ABTS+ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate)), to a colorless form, ABTS. The TEAC value of a substance is expressed as the number of micromoles of Trolox (a water-soluble vitamin E analog) equivalent per liter of sample. The higher the TEAC value, the greater the

antioxidant capacity of the substance. TEAC assay is a relatively simple and rapid assay, and it is widely used to measure the antioxidant capacity of foods, beverages, and supplements. It is also used to measure the antioxidant capacity of biological samples, such as blood and plasma. Trolox is an analog of Vitamin E and has a similar antioxidant capacity to that of Vitamin E. Trolox is used as a reference for other antioxidants. For example, if the T-AOC of Trolox is 1, then the antioxidant capacity of the other substance with the same concentration is shown by the ratio of its antioxidant capacity to Trolox's antioxidant capacity (Fredrick, 2016).

Procedure

1. Prepare a solution of ABTS+ and potassium persulfate.

2. Incubate the ABTS+ solution at room temperature for 16-24 hours to allow the radical to form.

3. Prepare a working solution of ABTS+ by diluting the stock solution in water.

4. Prepare a solution for the sample to be tested.

5. Add the sample solution to the working solution of ABTS+ and measure the absorbance of the solution at 734 nm.

6. Calculate the TEAC value of the sample by comparing the absorbance of the sample solution to the absorbance of a standard solution of Trolox

CHAPTER 4

RESULTS

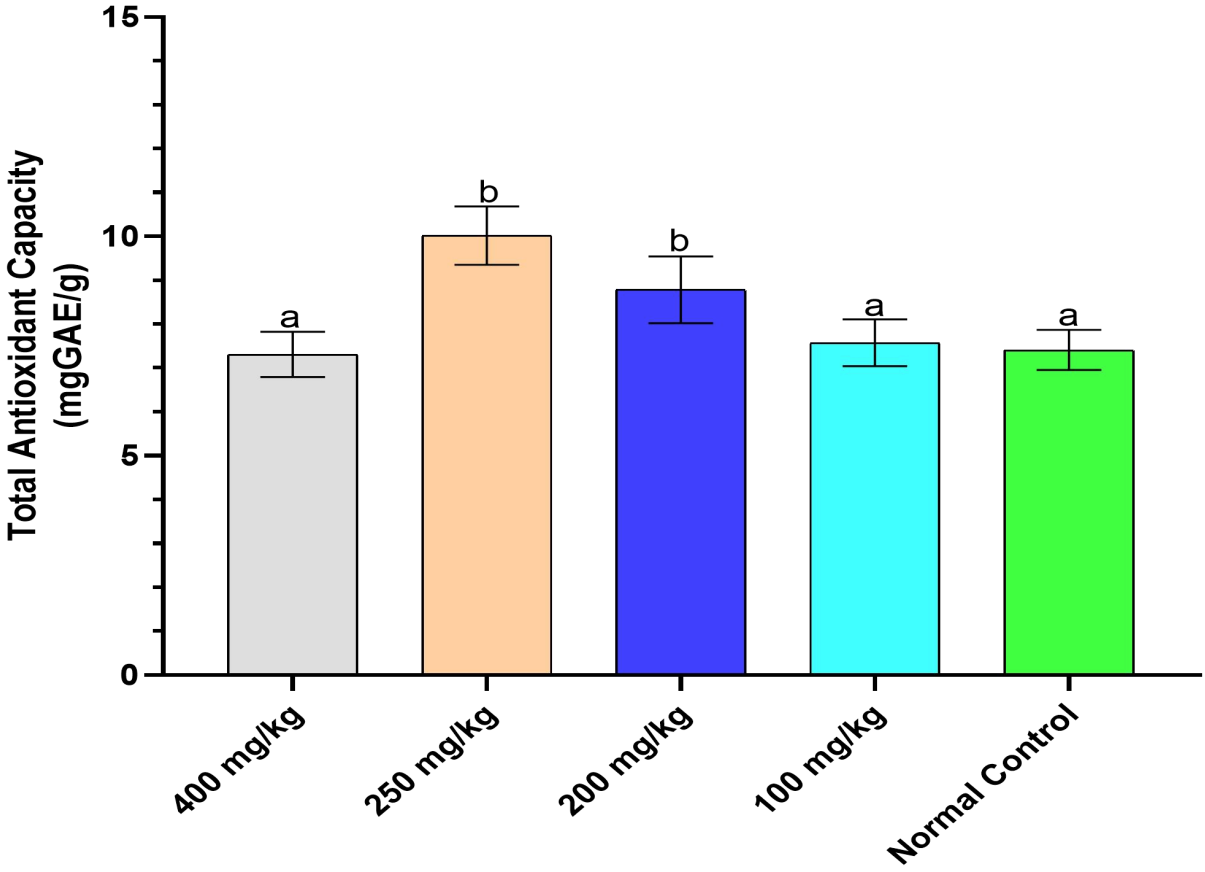

Figure 4.1: Effect of aqueous extract of *Picralima nitida* on total antioxidant capacity (TAC) concentrations in Wistar rats. Values are expressed as mean ± SEM. Means with different superscripts are statistically significant at $P < 0.05$.

From the study above (figure 4.1), there was no significant difference in TAC concentrations in the groups administered 400 and 100 mg/kg aqueous extract of *P. nitida* in comparison with the normal control. However, the groups administered 250 and 200 mg/kg aqueous extract of *P. nitida* showed a significant increase in TAC levels relative to the normal control group.

CHAPTER 5

DISCUSSION

Medicinal plants are a subgroup of plants that are high in phytochemicals, have therapeutic properties, and exert a desired pharmacological effect on a human or animal body. (Natural Products and Drug Discovery, 2018). Medicinal plants are employed globally as an alternative or supplementary form of medicine. Research on these plants encompasses both their pharmacological and toxicological assessments, which is crucial for the advancement of drug development. An example of a medicinal plant is *Picralima nitida*, a plant native to Africa that stands as the sole representative of the *Picralima* genus and shares botanical relations with *Hunteria* and *Pleiocarpa*. Commonly known as Picralima, Akuamma, or Pile Plant, it falls within the *Hunterieae* tribe of the *Apocynaceae* family(Saxton *et al.,* 1960) Across diverse regions, different components of this plant have been traditionally employed to address various health issues such as fever, hypertension, jaundice, dysmenorrhea, gastrointestinal disorders, and malaria known locally within the Benin, Yoruba, and Igbo culture as osu, abeere or osi igwe respectively it has proved to be rich in medicinal properties. (Dalziel *et al.,* 1937). Oxidative stress is the state of unavailability of antioxidants in the presence of free radicals. The term is used to describe the condition of oxidative damage that results when the critical balance between free radical generation and antioxidant defenses is unfavorable. (Rock.CL *et al.,*1996) It was observed that oxidative stress is now thought to make a significant contribution to all inflammatory diseases (arthritis, vasculitis, glomerulonephritis, lupus erythematosus, adult respiratory diseases syndrome). From the study above (figure 4.1), there was no significant difference in TAC concentrations in the groups administered 400 and 100 mg/kg aqueous extract of *P. nitida* in comparison with the normal control. However, the groups administered 250 and 200 mg/kg aqueous extract of *P. nitida* showed a significant increase in TAC levels relative to the normal control group we can thus infer that *P nitida* at different doses kept antioxidant levels at a fairly stable point as against the normal except within the dosage range of 250 and 200mg/kg where *p.nitida* showed increased optimal antioxidant capacity. In a similar study done by (Ikponmwosa-Eweka *et al.,*2023) similar results were obtained on the effect of *Spondias mombin* extracts on antioxidant capacity.

CONCLUSION

Based on this study and results collected during the course of this experiment *Picralima nitida* has been shown to possess medicinal properties, to have potent actions in the regulating of oxidative stress and mopping up of free radicals, and also prevent disease cases related to oxidative stress e.g aging, inflammatory diseases, due to the abundance of phytochemicals e.g flavonoid, phenols, saponins which contain antioxidants.

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APPENDIX

- **How to prepare the Aqueous extract to be administered**

To get the weight of the crude extract to be dissolved per cage, the weight of the animal is considered.

Using the dosage of 400mg/kg as an example, the following formula is used:

$$\frac{\text{Weight of the Animal x Dosage (400mg/kg)}}{1000}$$

This will give the dosage in g/kg.

To get the volume of distilled water used to dissolve the measured extract, the following formula is used.

$$\frac{\text{Dosage of weightiest animal}}{\text{Dosage of specific animal}}$$

This will give the volume of distilled water to dissolve the crude extract.

To get the concentration of the extract, the following formula is used:

$$animal\ volume$$

This will give the concentration in kg/ml