CHAPTER ONE

**INTRODUCTION**

**1.1 Background of Study**

The products of biological compounds oxidation, by interaction with important biomolecules, can upset cell homeostasis and act cytotoxically, resulting in different diseases like tumors, heart failure, and cataract, brain dysfunction (Lobo et al., 2010). Antioxidants are the substances able to prevent or inhibit oxidative processes in human body as well as in plant products (Aguilar *et al.,* 2011). The natural antioxidants are a stable part of nutrition as they occur in almost all edible plant products. Polyphenols are the most numerous group of antioxidant components and they are present in fruits and vegetables, their products, leguminous plants, grains, teas, herbs, spices and wines (Barros *et al.,* 2011). Consumption of food containing a lot of polyunsaturated fatty acids raised the significance and usage of substances that protect them against oxidation. The antioxidant supplementation is a generally accepted method of prolonging the stability and storage life of food products, in particular products that contain fat (Aguilar *et al.,* 2012). However, the artificial compounds with antioxidant properties, like butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT), have a limited allowance for food due to their potential carcinogenicity (Berker *et al.,* 2007). The growing demand for natural antioxidants observed in food and cosmetic industries forces the search for new sources of these compounds. Numerous scientific investigations point at consecutive rich sources of antioxidants, especially among plants.

Antioxidants are compounds that help delay and inhibit lipid oxidation and when added to foods tend to minimize rancidity, retard the formation of toxic oxidation products, help to maintain the nutritional quality and increase their shelf life (Moon and Shibamoto, 2009). Antioxidants can eliminate free radicals and other reactive oxygen and nitrogen species, and these reactive species contribute to most chronic diseases. It is hypothesized that antioxidants originating from plants may work as antioxidants in their own right in vivo, as well as bring about beneficial health effects through other mechanisms, including acting as inducers of mechanisms related to antioxidant defense, longevity, cell maintenance and DNA repair (Baur *et al*., 2006). Research focused on natural foods and medicinal plants has grown since evidence of their potential interference in the production of reactive oxygen species was uncovered. These reactive oxygen species play an important role in the progression of a great number of pathological disturbances such as inflammation, atherosclerosis, stroke, heart disease, diabetes mellitus, multiple sclerosis, cancer, Parkinson’s disease, Alzheimer’s disease, etc. They are also responsible for the nutritional value losses, as well as aroma, taste and texture degradation (Galve *et al.,* 2005). Polyphenols are the most numerous group of antioxidant components, and they are present in plant, fruits and vegetables. Polyphenols are present in a variety of plants utilized as important components of both human and animal diets. Fruit and vegetables provide the best polypharmacy against the development of a chronic disease, considering that they contain a vast array of antioxidant components such as polyphenols. Polyphenols make a major contribution to free radical scavenging capacities (Lin *et al.,* 2008). There is a direct relationship between antioxidant activity and total phenolics content in selected herbs, vegetables and fruits. Polyphenols are a broad family of naturally-occurring physiologically-active nutrients. They can be divided into four subgroups. The first group is called bioflavonoids. The next two groups are related compounds of bioflavonoids and are called anthocyanins and proanthocyanidins. The last group is called xanthones. Phenolic compounds act as antioxidants with mechanisms involving both free radical scavenging and metal chelation. They have ideal structural chemistry for free radical-scavenging activities, and have been shown to be more effective antioxidants in vitro than vitamins E and C on a molar basis (Arts *et al.,* 2005; George *et al.,* 2005).

The interest in natural antioxidants and oxidative stress conditions, especially in countries with limited access to conventional treatment method of diabetes, is inadequate. There is an increased demand for natural products with anti-diabetic activity due to the side effects associated with the use of insulin and oral hypoglycemic agents (Lotito and Fraga, 2000). The World Health Organization has also recommended and encouraged the use of natural products for the management of diabetes. Natural antioxidants such as flavonoids and polyphenols are believed to possess antioxidant properties due to their reducing and chelating capabilities. Flavonoids and polyphenols are secondary plant metabolites that are widely distributed in plants leaves and other parts in plants with free radical scavenging abilities (Surai, 2003).

Phytochemicals are bioactive compounds that have been associated with the protection of human health against chronic degenerative diseases. These bioactive compounds are also known as secondary metabolites (Kirshnaiah *et al.,* 2007). There are two types of metabolites produced in plants; primary metabolites and secondary metabolites. Primary metabolites are important for the plants regular metabolism such as growth and development. Secondary metabolites produced by plants may have little need for them. These are synthesized in almost all parts of the plant like bark, leaves, stem, root, flower, fruits, seeds, etc. During past several years, phytochemicals have been used worldwide as the traditional herbal medicine. Because of this, pharmaceutical industries as well as researchers put a greater emphasis on the phytochemical studies (Rice-Evans C., 2001). Also, these phytochemicals present in the different plant parts are used up by the local people for healing of certain disorders. These are also widely used in the field of agriculture. Secondary metabolites are economically important in the production of drugs, flavor and fragrances, dye and pigments, pesticides and food additives. Many of the drugs that are derived from the secondary metabolites are simple synthetic modifications or copies of these naturally obtained substances (Yogish and Raveesha, 2009).

**1.2**  **Statements of Problem**

During the past decades a lot of research has been carried out around antioxidants and their effects on health. There is a lack of a standard procedure to determine antioxidant activity in Nigeria. The antioxidant limitations and metabolism still pose a challenge to future research in this field, and researchers must try and overcome these drawbacks. The new trends in antioxidant treatments include compounds that behave like the enzyme in order to alleviate acute and chronic pain related to inflammation. Another promising research area are genetics, which aim to breed genetically modified plants that can produce higher quantities of specific compounds, yielding higher quantities of antioxidants (Devasagayam *et al.,* 2004; Suntres, 2011).

**1.3 Aim and Objectives**

Phytochemical screenining and in-vitro antioxidant activity of leaf extract of *Pterocarpus soyauxii* (ORA) and *Pterocarpus santalinoides* (UTURUKPA)

**The objectives of this study are**

1. Quantitative and Qualitative ( phytochemical) analysis on *pterocarpus soyanxii* (ora) and *pterocarpus santalinoides* (uturukpa).
2. To estimate flavonoids and flavonoils content on *pterocarpus soyanxii* (ora) and *pterocarpus santalinoides* (uturukpa).
3. To determine reducing power on *pterocarpus soyanxii* (ora) and *pterocarpus santalinoides* (uturukpa).
4. To determine hydrogen peroxide scavenging activity on *pterocarpus soyanxii* (ora) and *pterocarpus santalinoides* (uturukpa).

**CHAPTER TWO**

**Literature review**

**2.1 Oxidative Stress**

Oxidative stress refers to a serious imbalance between reactive species production and antioxidant defense (halliwell and gutteridge, 2007). It occurs due to an increased generation or reduce elimination of reactive species (RS) by the antioxidant defense system. Oxidative stress is usually associated with oxidative damage, which is defined as “the bimolecular damage caused by attack of reactive species (RS) upon the constituents of living organisms. Oxidative damage to cellular components impairs cellular functions; most of the biologically relevant RS are either reactive oxygen species (ROS) or reactive nitrogen species (RNS). Reactive oxygen species include free radicals such as superoxide (O2−) and hydroxyl (OH), and non-free radicals such as hydrogen peroxide (H2O2). Reactive nitrogen species include free radicals such as nitric oxide (NO) and nitrogen dioxide (NO2−), and non-free radicals such as peroxynitrite (OONO−). Besides their toxicities, reactive oxygen species are also required in certain conditions and for physiological functions. For instance, during inflammation, the phagocytes release reactive oxygen species which kill invading bacteria. Reactive oxygen species generated during mild or moderate exercise constitute part of the mechanism of exercise- or training-induced adaptation. (Sachdev and Davies, 2008). The generation of reactive species by aerobic organisms may occur as by-products of metabolism (e.g. During operation of electron transfer chains), intentionally (e.g. During inflammation), or as a result of accidents of chemistry (such as the auto oxidation of unstable biomolecules, e.g. Dopamine) (Halliwell, 2011). Of all the reactive species, significant roles of nitrogen dioxide, nitric oxide, and peroxynitrite have been implicated in diabetic cardiovascular complications (Johansen *et al.,* 2005).

oxidative stress occurs when reactive oxygen species production exceeds than their removal by the cellular defense mechanism (Halliwell and Gutteridge, 2007). It is an imbalance between the production of reactive oxygen species and ability of the biological system to detoxify the reactive intermediates or to repair the resulting damage. The destructive aspect of oxidative stress is the production of reactive oxygen species (ROS) (Halliwell, 2011), which includes free radicals and peroxides that can damage nucleic acids, proteins and cell membranes. In humans, oxidative stress is involved in many diseases, such as diabetes mellitus (DM), atherosclerosis, myocardial infarction (MI), heart failure, Parkinson's disease, chronic fatigue syndrome, Alzheimer's disease and fragile x syndrome. Diabetes mellitus is a continuous source of oxidative stress to the body and there is increased generation of reactive oxygen species. These reactive oxygen species have a very short half-life and cannot remain as such or decreased efficiency of inhibitory and scavenger systems. The stress then may be amplified and propagated by an autocatalytic cycle of metabolic stress, tissue damage, and cell death, leading to a simultaneous increase in free radical production and compromised inhibitory and scavenger mechanisms (Erejuwa *et al.,* 2010a), which further exacerbate the oxidative stress. For practical reasons, neither the rate of oxidant production nor the steady-state levels of reactive oxygen species are easily measured in biological systems. Thus, oxidative stress must be inferred from measurements of oxidative damage as estimated from the kinetics of formation, the steady-state levels, or the extent of accumulation of oxidation products in tissues, plasma, or urine. However, the detection of increased levels of oxidation products in tissues is not sufficient to implicate oxidative stress in the pathology unless the damage can be logically and quantitatively related to the development of pathology and until it can be shown that inhibition of oxidative damage prevents or retards the disease process (Andreyev *et al.,* 2005). In order to prevent oxidative damage, it is important that excess reactive species is eliminated from the cells. Oxidative stress is a regular characteristic of diabetic complications such as cardiovascular complications which are a major cause of death in diabetes. Many mechanisms can result in excessive oxygen radical production leading to oxidative stress. All forms of diabetes increase the long-term complication. These typically develop after many years, but may be the first symptom in those who have otherwise not received diagnosis before that time. The major long-term complications relate to damage to blood vessels. Other “macro vascular” diseases are stroke and peripheral vascular disease (Ock Kyoung Chun and Dae-Okum, 2004). Oxidative stress causes damage to the eyes. This is caused by damage to the blood vessels in the retina of the eye, and can result in gradual vision loss and potentially blindness (ceriello, 2006). . Oxidative stress damage the kidney and can lead to tissue scarring, urine protein loss and eventually chronic kidney disease. (Ceriello *et al.,* 2006).

Oxidative stress in aging can result from an imbalance of prooxidants and antioxidants with excessive, destructive, free radical chemistry (Jones *et al.*, 2006). Due to the expansion of macro vascular and micro vascular complications (Basu *et al.,* 2005). A variety of pathological conditions are induced by oxidative stress such as rheumatoid arthritis, diabetes mellitus and cancer ([El- Faramawy and Rizk, 2011](file:///C:\Users\CHIDIMMA%20EZE\Documents\DIAMEI.htm#b0060)).

There are convincing experimental and clinical evidences that the generation of reactive oxygen species is increased in both types of diabetes and contributes to the onset of diabetes (Jones, 2006). Oxidative stress may be a common pathway linking diverse mechanisms for the pathogenesis of complications. Mechanisms that contribute to increased oxidative stress that may include not only increased non-enzymatic glycosylation, (glycation) and autoxidative glycosylation but also metabolic stress resulting from changes in energy metabolism, alterations in sorbitol pathway activity, changes in the level of inflammatory mediators and the status of antioxidant defense systems. (johansen *et al*., 2005; figueroa-romero *et al*., 2008; giacco and brownlee, 2010).

**2.2 Damages induced by oxidative stress**

Oxidative stress makes damages in tissue or organ caused by free radicals (Hsieh *et al*., 2005).

Free radicals are formed disproportionately in diabetes by glucose autoxidation, polyol pathway and non-enzymatic glycation of proteins (pham-huy, *et al. 2008*). Abnormally high levels of free radicals and simultaneous decline of antioxidant defense systems can lead to the damage of cellular organelles and enzymes, increased lipid peroxidation and development of complications of diabetes mellitus. (bansal and bilaspuri, 2011). Free radicals may play an important role in the causation and complications of diabetes mellitus. In diabetes mellitus, alterations in the endogenous free radical scavenging defense mechanisms may lead to ineffective scavenging of reactive oxygen species, resulting in oxidative damage and tissue injury (Hsieh *et al*., 2005). Oxidative stress is currently suggested as mechanism underlying diabetes and diabetic complications. Enhanced oxidative stress and changes in antioxidant capacity, observed in both clinical and experimental diabetes mellitus, are thought to be the etiology of chronic diabetic complications in recent years, much attention has been focused on the role of oxidative stress, and it has been reported that oxidative stress may constitute the key and common event in the pathogenesis of secondary diabetic complications. Free radicals are continually produced in the body as a result of normal metabolic processes.

## A variety of physiologic and pathophysiologic procedures are believed that reactive oxygen species play an important part in which the expansion of oxidative stress may have a significant function in disease mechanisms. A common pathogenic method in quite a few complications of diabetes such as nephropathy, retinopathy, and atherosclerosis, is too much oxidative stress, which happens as a consequence of an imbalance at the cellular level involving production and abolition of reactive oxygen species (voziyan and hudson, 2005). It is believed that oxidative stress plays important role in the development of vascular complications in diabetes particularly type2 diabetes ([pham-huy, 2008](file:///C:\Users\CHIDIMMA%20EZE\Documents\DIAMEI.htm#b0190)).according to epidemiological studies, diabetic mortalities can be explained notably by an increase in vascular diseases other than hyperglycemia ([pham-huy, 2008](file:///C:\Users\CHIDIMMA%20EZE\Documents\DIAMEI.htm#b0190)).

## 2.3 Reactions with antioxidants

It is a universal truth that oxygen is the major factor that has made the life finite. It is one of the important components of aerobic life. However in some circumstances, this oxygen may be a killer of cells when it generates reactive species that causes necrosis and ultimately the cell death. Reactive nitrogen species RNS and reactive carbon species RCS also cause oxidation by the generation of certain mechanism that interferes with the normal physiological processes inside the cell ([weseler and bast, 2010](file:///C:\Users\CHIDIMMA%20EZE\Documents\DIAMEI.htm#b0225)). Oxidative stress can be defined as any disturbance in the balance of antioxidants and pro-oxidants in favor of the later due to different factors such as aging, drug actions and toxicity, inflammation and/or addiction. It is in general, excess formation or/and insufficient removal of highly reactive molecules such as reactive nitrogen species and reactive oxygen species ([johansen *et al.,* 2005](file:///C:\Users\CHIDIMMA%20EZE\Documents\DIAMEI.htm#b0275)) oxygen is highly reactive specie that has the ability to become part of potentially harmful and damaging molecules (free radicals). Oxidative stress causes healthy cells of the body to lose their function and structure by attacking them. Up until now, pathogenesis of about more than 50 diseases has been implicated by free radicals it is when the antioxidant level is limited that this damage can become debilitating and cumulative damage to dna, proteins, and other macromolecules due to oxidation has been implicated in the pathogenesis of a wide variety of diseases, most notably cancer and heart disease.

**2.4 Oxidative stress induced organ damage:**

|  |  |
| --- | --- |
| **Lungs:** | Asthma, chronic bronchitis |
| **Kidneys:** | Glomerulonephritis, chronic renal failure |
| **Joints:** | Arthritis, rheumatism. |
| **Brain:** | Alzheimer’s disease, parkinson’s disease, memory loss, depression, stroke |
| **Eyes:** | Cataract, retinal diseases. |
| **Fetus:** | Preeclampsia, iu growth restriction (sachdev and davies, 2008). |
| **Heart vessels:** | Arteriosclerosis, hypertension, ischemia, cardiomyopathy, heart failure. |
| **Multi-organs:** | Cancer, diabetes, inflammation infection, aging. |

The term “antioxidant” can be labeled for any substance whose availability, even in minute concentration inhibits or delays the oxidation of a substrate. There are several species or molecules, endogenous (internally synthesized) or exogenous (consumed), that plays a role in antioxidant defense and may be considered as biomarkers of oxidative stress. Antioxidants can be divided as either chain breaking antioxidants or preventive antioxidants, based on their mechanism of action ([somogyi *et al*., 2007](file:///C:\Users\CHIDIMMA%20EZE\Documents\DIAMEI.htm#b0315)). Different types of biological antioxidants include, for instance, glutathione (oxidized/reduced), vitamin c & e, cystine, etc.). Analysis of various clinical tests examining vitamin e therapy had shown that large amounts of vitamin e (more than 400 iu per day) does damage and it can actually cause death (miller *et al*., 2005).

**2.5 Effects of oxidative stress**

Internally, free radicals are produced as a normal part of metabolism within the mitochondria, through xanthine oxidase, peroxisomes, inflammation processes, phagocytosis, arachidonate pathways, ischemia, and physical exercise. External factors that help to promote the production of free radicals are smoking, environmental pollutants, radiation, drugs, pesticides, industrial solvents and ozone. It is ironic that these elements, essential to life (especially oxygen) have deleterious effects on the human body through these reactive species (lobo *et al.,* 2010). The balance between the production and neutralization of Reactive Oxygen species by antioxidants is very delicate, and if this balance tends to the overproduction of ros, the cells start to suffer the consequences of oxidative stress (wiernsperger, 2003).

It is estimated that every day a human cell is targeted by the hydroxyl radical and other such species and average of 105 times inducing oxidative stress (valko *et al.,* 2004). The main targets of ROS and RNS are proteins, DNA (deoxyribonucleic acid) and RNA (ribonucleic acid) molecules, sugars and lipids (lü *et al.,* 2010; craft *et al.,* 2012). Regarding proteins, there are three distinct ways they can be oxidative modified. Oxidative modification of a specific amino acid, free radical-mediated peptide cleavage and formation of protein cross-linkage due to reaction with lipid peroxidation products (lobo *et al.,* 2010). The damage induced by free radicals to DNA can be described both chemically and structurally having a characteristic pattern of modifications: production of base-free sites, deletions, modification of all bases, frame shifts, strand breaks, dnaprotein cross-links and chromosomal arrangements. An important reaction involved with DNA damage is the production of the hydroxyl radical through the fenton reaction. This radical is known to react with all the components of the dna molecule: the purine and pyrimidine bases as well as the deoxyribose backbone. The peroxyl and oh- radicals also intervene in DNA oxidation (Dizdaroglu *et al.,* 2002; Valko *et al.,* 2004). Regarding sugars, the formation of oxygen free radicals during early glycation could contribute to glycoxidative damage. During the initial stages of non-enzymatic Glycosylation, sugar fragmentation produces short chain species like glycoaldehyde

Whose chain is too short to cyclize and is therefore prone to autoxidation, forming the superoxide radical. The resulting chain reaction propagated by this radical can form α and β-dicarbonyls, which are well known mutagens (Benov and Beema, 2003). Lipid peroxidation is initiated by an attack towards a fatty acids side chain by a radical in order to abstract a hydrogen atom from a methylene carbon. The more double bonds present in the fatty acid the easier it is to remove hydrogen atoms and consequently form a radical, making monounsaturated and saturated fatty acids more resistant to radicals than polyunsaturated fatty acids . After the removal, the carbon centered lipid radical can undergo molecular rearrangement and react with oxygen forming a peroxyl radical. These highly reactive molecules can the abstract hydrogen atoms from surrounding molecules and propagate a chain reaction of lipid peroxidation. The hydroxyl radical is the one of the main radicals in lipid peroxidation, it is formed in biological systems, as stated above, by the fenton reaction as a result of interaction between hydrogen peroxide and metal ions. Finally, another way to generate lipid peroxides is through the attack on pufa’s or their side chain by the singlet oxygen which is a very reactive form of oxygen. This pathway doesn’t probably qualify as initiation because the singlet oxygen reacts with the fatty acid instead of abstracting a hydrogen atom to start a chain reaction, making this a minor pathway when compared to the hydroxyl one (halliwell and chirico, 1993). Free radicals have different types of reaction mechanisms, they can react with surrounding molecules by: electron donation, reducing radicals, and electron acceptance, oxidizing radicals (a), hydrogen abstraction (b), addition reactions (c), self-annihilation reactions (d) and by disproportionation (e) (slater, 1984).

These reactions lead to the production of ros, rns and rss whom have been linked to many severe diseases like cancer, cardiovascular diseases including atherosclerosis and stroke, neurological disorders, renal disorders, liver disorders, hypertension, rheumatoid arthritis, adult respiratory distress syndrome, auto-immune deficiency diseases, inflammation, degenerative disorders associated with aging, diabetes mellitus, diabetic complications, cataracts, obesity, autism, alzheimer’s, parkinson’s and huntington’s diseases, vasculitis, glomerulonephritis, lupus erythematous, gastric ulcers, hemochromatosis and preeclampsia, among others (Rahman, 2007; Lobo *et al.,* 2010; Lü *et al.,* 2010; Singh *et al.,* 2010).

2.6 **How the body defends against oxidative stress**

Oxidative stress occurs when the production of free radicals goes beyond the protective defenses in the body. Oxidative stress and free radical damage to cells may initiate the early stages of cancer and heart disease. Free radicals are also suspect in the development of Alzheimer’s disease, arthritis, cataracts, diabetes, kidney disease, age related blindness.

The human body is not without its own defenses against this damage. It creates many different types of molecules antioxidants to combat this free radicals and protect the cells from attack by oxygen. Antioxidants can safely interact with free radicals and stop the chain of damaging reactions before damages are done to cells. There are several enzymes systems in the body that scavenge free radicals, but we can also gain these helpful molecules from foods that we eat. Some vitamins are antioxidants such as vitamin c and e. Some minerals are antioxidants such as selenium and manganese and there are plant compounds that act as antioxidants such as beta carotene and lycopene (Halliwell, 2007).

Scientists began to theorize that free radical damage was involved in the early stages of atherosclerosis and might play a role in the development of many other chronic medical conditions in the 1990s. Studies at the time suggested that people who ate few antioxidant rich fruits and vegetables had a greater risk of developing these medical conditions. So began several clinical trials in which antioxidant supplements like beta carotene and vitamin e were tested for their protection against heart disease, cancer and other conditions. As a result, antioxidants became a buzzword in the 90s and their benefits were glorified by the media by the food industry that began labeling foods as rich in antioxidants and by the supplement industry as they began hyping the health benefits of antioxidant supplement. They were even promoted as anti-aging ingredients in beauty products (Higdon and Frei, 2003).

Despite numerous studies, no substantial health benefits have been demonstrated for supplemental antioxidants. Antioxidants in food, however are considered safe. Until there is more conclusive research the best source of antioxidants is a diet rich in fruits, vegetable and whole grains. Health organizations such as the American heart association, American cancer society and American institute for cancer research determine whether supplements are safe and provide the same benefits as antioxidants found naturally in food. Researchers suggested that whole grains, fruits and vegetables contains antioxidants and antioxidants are beneficial to health. The best way of eating adequate amounts of protective antioxidants is by eating between five and nine fruits and vegetables that representing all the colors of the rainbow every day. Eating small amounts of nuts and drinking wine in moderation can also contribute to antioxidant consumption (Foyer and Noctor, 2003).

**2.7 Antioxidant**

Antioxidants defined as any substance that when present at low concentrations compared with that of an oxidable substrate (halliwell, 2007). Significantly delays or inhibits oxidation of that substrate but later defined them as any substance that delays, prevents or removes oxidative damage to a target molecule (halliwell, 2007). Antioxidants also defined as any substance that directly scavenges reactive oxygen species or indirectly acts to up-regulate antioxidant defense or inhibit reactive oxygen species production. Another property that a compound should have to be considered an antioxidant is the ability, after scavenging the radical, to form a new radical that is stable through intermolecular hydrogen bonding on further oxidation (Halliwell, 1990). During human evolution, endogenous defences have gradually improved to maintain a balance between free radicals and oxidative stress. The antioxidant activity can be effective through various ways as inhibitors of free radical oxidation reactions (preventive oxidants) by inhibiting formation of free lipid radicals by interrupting the propagation of the autoxidation chain reaction (chain breaking antioxidants) as singlet oxygen quenchers; through synergism with other antioxidants as reducing agents which convert hydroperoxides into stable compounds as metal chelators that convert metal pro-oxidants (iron and copper derivatives) into stable products; and finally as inhibitors of pro-oxidative enzymes (lipooxigenases) (Darmanyan *et al.,* 1998; Heim *et al.,* 2002; Min and Boff, 2002; Pokorný, 2007; Kancheva, 2009).

The human antioxidant system is divided into two major groups, enzymatic antioxidants and non-enzymatic oxidants. Regarding enzymatic antioxidants they are divided into primary and secondary enzymatic defenses. With regard to the primary defense, it is composed of three important enzymes that prevent the formation or neutralize free radicals glutathione peroxidase, which donates two electrons to reduce peroxides by forming selenoles and also eliminates peroxides as potential substrate for the fenton reaction catalase, that converts hydrogen peroxide into water and molecular oxygen and has one of the biggest turnover rates known to man, allowing just one molecule of catalase to convert 6 billion molecules of hydrogen peroxide and finally, superoxide dismutase converts superoxide anions into hydrogen peroxide as a subtract for catalase (Rahman, 2007). The secondary enzymatic defense includes glutathione reductase and glucose-6-phosphate dehydrogenase. Glutathione reductase reduces glutathione (antioxidant) from its oxidized to its reduced form, thus recycling it to continue neutralizing more free radicals. Glucose-6-phosphate regenerates nadph (nicotinamide adenine dinucleotide phosphate - coenzyme used in anabolic reactions) creating a reducing environment (Ramble and Burke, 1984; Ratnam *et al.,* 2006). These two enzymes do not neutralize free radicals directly, but have supporting roles to the other endogenous antioxidants. Considering the non-enzymatic endogenous antioxidants, there are quite a number of them, namely vitamins A, enzyme cofactors q10, nitrogen compounds uric acid and peptides glutathione. Vitamin A or retinol is a carotenoid produced in the liver and results from the breakdown of β-carotene. There are about a dozen forms of vitamin a that can be isolated. It is known to have beneficial impact on the skin, eyes and internal organs. The antioxidant activity is the ability to combine with peroxyl radicals before they propagate peroxidation to lipids (palace *et al.,* 1999; jee *et al.,* 2006). Coenzyme q10 is present in all cells and membranes; it plays an important role in the respiratory chain and in other cellular metabolism. Coenzyme q10 acts by preventing the formation of lipid peroxyl radicals, although it has been reported that this coenzyme can neutralize these radicals even after their formation. Another important function is the ability to regenerate vitamin e; some authors describe this process to be more likely than regeneration of vitamin E through ascorbate vitamin C (Turunen *et al.,* 2004).

Uric acid is the end product of purine nucleotide metabolism in humans and during evolution its concentrations have been raising. On the other hand it also prevents lysis of erythrocytes by peroxidation and is a potent scavenger of singlet oxygen and hydroxyl radicals (Kand’ár *et al.,* 2006). Glutathione is an endogenous tripeptide which protects the cells against free radicals either by donating a hydrogen atom or an electron. It is also very important in the Regeneration of other antioxidants like ascorbate (Steenvoorden and Henegouwen, 1997). Despite its remarkable efficiency, the endogenous antioxidant system does not suffice, and humans depend on various types of antioxidants that are present in the diet to maintain free radical concentrations at low levels (Pietta, 2000).

Vitamins C And E are generic names for ascorbic acid and tocopherols. Ascorbic acid

Includes two compounds with antioxidant activity: l-ascorbic acid and l-dehydroascorbic acid which are both absorbed through the gastrointestinal tract and can be interchanged enzymatically in vivo. Ascorbic acid is effective in scavenging the superoxide radical anion, hydrogen peroxide, hydroxyl radical, singlet oxygen and reactive nitrogen oxide (Barros *et al.,* 2011). Vitamin E is composed of eight isoforms, with four tocopherols (α-tocopherol, β-tocopherol, γ-tocopherol and δ-tocopherol) and four tocotrienols (α-tocotrienol, β-tocotrienol, γ-tocotrienol and δ-tocotrienol), α-tocopherol being the most potent and abundant isoform in biological systems. The chroman head group confers the antioxidant activity to tocopherols, but the phytyl tail has no influence. Vitamin E halts lipid peroxidation by donating its phenolic hydrogen to the peroxyl radicals forming tocopheroxyl radicals that, despite also being radicals, are unreactive and unable to continue the oxidative chain reaction. Vitamin e is the only major lipid-soluble, chain breaking antioxidant found in plasma, red cells and tissues, allowing it to protect the integrity of lipid structures, mainly membranes (Burton and Traber, 1990). These two vitamins also display a synergistic behavior with the regeneration of vitamin e through vitamin c from the tocopheroxyl radical to an intermediate form, therefore reinstating its antioxidant potential (Halpner *et al.,* 1998). Vitamin k is a group of fat-soluble compounds, essential for posttranslational conversion of protein-bound glutamates into γ-carboxyglutamates in various target proteins. The 1,4-naphthoquinone structure of these vitamins confers the antioxidant protective effect. The two natural isoforms of this vitamin are k1 and K2 (Vervoort *et al.,* 1997). Flavonoids are an antioxidant group of compounds composed of flavonols, flavanols, anthocyanins, isoflavonoids, flavanones and flavones. All these sub-groups of compounds share the same diphenylpropane (c6c3c6) skeleton. Flavanones and flavones are usually found in the same fruits and are connected by specific enzymes, while flavones and flavonols do not share this phenomenon and are rarely found together. Anthocyanins are also absent in flavanone-rich plants (Lü et al., 2010).The antioxidant properties are conferred on flavonoids by the phenolic hydroxyl groups attached to ring structures and they can act as reducing agents, hydrogen donators, singlet oxygen quenchers, superoxide radical scavengers and even as metal chelators. They also activate antioxidant enzymes, reduce α-tocopherol radicals (tocopheroxyls), inhibit oxidases, mitigate nitrosative stress, and increase levels of uric acid and low molecular weight molecules. Some of the most important flavonoids are catechin, catechin-gallate, quercetin and kaempferol (Rice-evans et al., 1996; Procházková *et al.,* 2011).

Phenolic acids are composed of hydroxycinnamic and hydroxybenzoic acids. They are ubiquitous to plant material and sometimes present as esters and glycosides. They have antioxidant activity as chelators and free radical scavengers with special impact over hydroxyl and peroxyl radicals, superoxide anions and peroxynitrites. One of the most studied and promising compounds in the hydroxybenzoic group is gallic acid which is also the precursor of many tannins, while cinnamic acid is the precursor of all the hydroxycinnamic acids. (Krimmel *et al.,* 2010; Terpinc *et al.,* 2011).

Carotenoids are a group of natural pigments that are synthesized by plants and microorganisms but not by animals. They can be separated into two vast groups:

The Carotenoid hydrocarbons known as the carotenes which contain specific end groups like lycopene and β-carotene; and the oxygenated carotenoids known as xanthophyls, like zeaxanthin and lutein. The main antioxidant property of carotenoids is due to single oxygen quenching which results in excited carotenoids that dissipate the newly acquired energy through a series of rotational and vibrational interactions with the solvent, thus returning to the unexcited state and allowing them to quench more radical species. This can occur while the carotenoids have conjugated double bonds within. The only free radicals that completely destroy these pigments are peroxyl radicals. Carotenoids are relatively unreactive but may also decay and form non-radical compounds that may terminate free radical attacks by binding to these radicals (Paiva and Russel, 1999).

Minerals are only found in trace quantities in animals and are a small proportion of dietary antioxidants, but play important roles in their metabolism. Regarding antioxidant activity, the most important minerals are selenium and zinc. Selenium can be found in both organic (selenocysteine and selenomethionine) and inorganic (selenite and selenite) forms in the human body. It does not act directly on free radicals but is an indispensable part of most antioxidant enzymes (metalloenzymes, glutathione peroxidase, thioredoxin reductase) that would have no effect without it (Tabassum *et al.,* 2010). Zinc is a mineral that is essential for various pathways in metabolism. Just like selenium, it does not directly attack free radicals but is quite important in the prevention of their formation. Zinc is also an inhibitor of Nicotinamide Adenine Diphosphate NADPH oxidases which catalyze the production of the singlet oxygen radical from oxygen by using NADPH as an electron donor. It is present in superoxide dismutase, an important antioxidant enzyme that converts the singlet oxygen radical into hydrogen peroxide. Zinc induces the production of metallothionein that is a scavenger of the hydroxyl radical. Finally, zinc also competes with copper for binding to the cell wall, thus decreasing once again the production of hydroxyl radicals (Prasad *et al.,* 2004).

2.7.1 **Classification of antioxidants**

Antioxidants are grouped into two namely;

* Primary or natural antioxidants.
* Secondary or synthetic antioxidants.

**2.7.2 Primary or natural antioxidants**

They are the chain breaking antioxidants which react with lipid radicals and convert them into more stable products. Antioxidants of this group are mainly phenolic in structures and include the following (Hurrell, 2003):

* Antioxidants minerals - these are co factor of antioxidants enzymes. Their absence will definitely affect metabolism of many macromolecules such as carbohydrates. Examples include selenium, copper, iron, zinc and manganese.
* Anti oxidants vitamins – it is needed for most body metabolic functions. They include-Vitamin C , Vitamin E, Vitamin B.
* Phytochemicals - these are phenolic compounds that are neither vitamins nor minerals. These include:

Flavonoids: these are phenolic compounds that give vegetables fruits, grains, seeds leaves, flowers and bark their colours. Catechins are the most active antioxidants in green and black tea and sesamol. Carotenoids are fat soluble colour in fruits and vegetables. Beta carotene, which is rich in carrot and converted to vitamin a when the body lacks enough of the vitamin. Lycopene, high in tomatoes and zeaxantin is high in spinach and other dark greens. Herbs and spices-source include diterpene, rosmariquinone, thyme, nutmeg, clove, black pepper, ginger, garlic and curcumin and derivatives (Kand’ár *et al.,* 2006).

**2.7.3 Secondary or synthetic antioxidants**

These are phenolic compounds that perform the function of capturing free radicals and stopping the chain reactions, the compound include (hurrell, 2003):

* Butylated hydroxyl anisole (bha).
* Butylated hydroxyrotoluene (bht).
* Propyl gallate (pg) and metal chelating agent (edta).
* Tertiary butyl hydroquinone (tbhq).
* Nordihydro guaretic acid (ndga).

**2.7.4 Antioxidant cell functions**

There are two major groups of antioxidants in living cells: enzymatic antioxidants and non-enzymatic antioxidants. These groups are divided into several subgroups. The enzymatic antioxidants are divided into primary and secondary enzymatic defenses (Caroch *et al.,* 2013). The primary defense is com- posed of three important enzymes that prevent the formation of and neutralize free radicals: glutathione peroxidase, which donates two electrons to reduce peroxides by forming selenols and also eliminates peroxides as potential substrates for the fenton reaction; catalase, which turns hydrogen peroxide into water and molecular oxygen one of the most important and efficient antioxidants known today, when just one molecule of catalase converts 6 billion molecules of hydrogen peroxide (Ratman *et al.,* 2007). And lastly, superoxide dismutase, which converts superoxide anions into hydrogen peroxide as a substrate for subsequent catalase action. The secondary enzymatic defense includes glutathione reductase and glucose-6-phosphate dehydrogenase. Glutathione reductase reduces glutathione antioxidant from it’s oxidized to its reduced form, and by this recycling, to continue neutralizing more free radicals (Ankola *et al.,* 2006). Glucose-6-phosphate regenerates nadph, which creates a reducing environment. These two enzymes support the primary enzymatic defense antioxidants and do not neutralize free radicals directly. The group of non-enzymatic antioxidants contains several subgroups, the main ones being: vitamins (A, E, C), enzyme cofactors minerals zinc and selenium, peptides glutathione, phenolic acids, and nitrogen compounds uric acid (Caroch *et al.,* 2013). There is great importance in maintaining the fragile balance between these antioxidants and the ros molecules. For instance, in humans, disturbing this balance can cause serious health problems, such as cancer, cardiovascular and neurodegenerative diseases, and premature aging (Valko *et al.,* 2007).

2.7.5 **Synthetic antioxidants in pharmaceutical and food industries**

Nowadays, most food and pharmaceutical products contain synthetic antioxidants. These compounds are added to food in order to prolong product shelf life, mainly by preventing the oxidation of unsaturated double bonds of fatty acids. In pharmaceutical products to antioxidants are added to enhance the stability of therapeutic agents that are susceptible to chemical degradation by oxidation. The two most common synthetic antioxidants used today are butylated hydroxyanisole and butylated hydroxyl- toluene. Propylgallate and tertbutylhydroquinone are other widely used synthetic antioxidants in the processed-food industry. For example, tertbutylhydroquinone is usually added to food products such as beef and chicken. Though no harmful effect of these synthetic antioxidants has been shown in man, in 2012, the European Food Safety Authority (EFSA) evaluated information regarding several of these antioxidants and established revised acceptable daily intakes of antioxidants for human consumption, setting a proper scale for their use by food companies. Ascorbic acid derivatives, such as ascorbic acid and erythorbic acid; thiol derivatives, such as thioglycerol, cysteine, dithiothreitol, and glutathione; sulfurous-acid salts, such as sodium sulfite, sodium formaldehyde sulfoxylate, and tocopherols, are widely used in the pharmaceutical industry. Unfortunately, new data indicating that the synthetic antioxidants used in the industry could have carcinogenic effects on human cells resurface every year, thus fueling an intense search for new, natural and efficient antioxidants.

**2.7.6 Antioxidants in plants relation to photosynthesis**

Photosynthesis is an important source of cellular oxidants, and the importance of antioxidants in maintaining high rates of photosynthesis has been shown in many studies (Foyer and Noctor, 2003). Studies showed that photosynthesis is the source of reactive oxygen species and that the photosynthetic electron transport chain operates as a regulatory system for minimizing reactive oxygen species production in an aerobic environment. In addition, there is a need for a strong and efficient antioxidant network to process reactive oxygen species effectively and to maintain intracellular reactive oxygen species pools at low levels (Foyer and Shigeoka, 2011). Originally, reactive oxygen species were recognized as toxic by-products of aerobic metabolism, molecules that have the potential to cause irreversible damage to photosynthetic components and that are removed by antioxidants and antioxidative enzymes. It has become clear that these molecules play an important signaling role in plants, controlling processes such as growth, development, and even programmed cell death (Bailey and Mittle, 2006). Due to the recent findings, it is of great importance to maintain the fragile balance of the reactive oxygen species molecules and antioxidants. Plants usually contain a wide variety of free-radical scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, and more (Agati *et al.,* 2012). Studies have proven that many of these antioxidant compounds exhibit anti-inflammatory, anti- carcinogenic, antibacterial, antitumor, or antimutagenic effects in cells (Chaghaby *et al.,* 2011). Nowadays, the intake of natural antioxidants is associated with reduced risks of cancer, cardiovascular disease, and other diseases. It has been widely proven that green tea leaves contain a high concentration of polyphenols that act as antioxidants both in vitro and in vivo in animal and human cells, thus reducing and controlling reactive oxygen species molecules (frei and higdon, 2003). Studies conducted on chinese medical and other medical herbs demonstrated that some herbs, such as rosemary, sage, thyme, and bay, have much stronger antioxidant activity and contain significantly more phenolic acids than common vegetables and fruits, which are considered good natural sources of dietary antioxidants (Bozin *et al.,* 2006).

2.7.7 **Source of Antioxidants**

There are several sources of antioxidant: those that we can get from food and food supplements e.g. Vitamin e, d and b carotene; and those that are produced within our own bodies they are less well known but vital. The later type includes molecules such as glutathione and uric acid which scavenge free radicals directly; and enzymes such as superoxide dismutase, catalase and glutathione peroxidase which can break free radical into nontoxic products. There are also melatonin which comes as a new member in antioxidant systems besides macromolecules such as caeruloplasmin and transferrin and an array of small molecules including methionine (Bjelakovic *et al.,* 2007). vitamins e & c. The antioxidant can occur endogenously in body e.g.: enzymes and melatonin, or exogenously as they can be obtained from dietary allowance and natural or synthetic drugs such as vitamins and their precursors (Vitamins E And C and carotenoids), selenium and polyphenols. Plant extracts and their constituents as a natural source of antioxidants have been extensively reviewed. This includes different plant organs such as seeds (soybean, peanut, cottonseed, mustard, rapeseed, rice, sesame seed), fruits (grape, citrus, black, pepper, olive), leaves (tea, rosemary, thyme, oregano) and others sweet potato, onion, oat seedling (Schafer and Karger, 2009). Plant extracts containing low molecular mass compounds have been successively used in phytotherapy since ancient times, as reactive oxygen species are involved in several dideases. It has been demonstrated that many naturally occuaring possess notable activity as radical scevengers and lipid peroxidation inhibitors (Hail *et al.,* 2008). In addtion to plant extracts, numerous naturally occurring compounds are useful as antioxidant, ranging from alpha tocopherol and beta carotene to plant antioxidants such phenolic compounds (tannins, flavonoids, anthrocyanins, chalcones, xanthones, xanthones, liganans, depsides, and depsidones etc, terpenes (sesquterpens and diterpineses), alkaloids, organic sulfur compounds .etc. A large number of experiments have been carried out concerning the antioxidant activity of several plant extracts and powders. The results of these experiments reveal that, the activity is due to several secondary metabolites especially phenolic compound e.g.: flavonoids tannins etc (Warner *et al.,* 2004)

**2.7.8 Flavonoids as antioxidant**

Chalcones are biosynthetic intermediates between cinnamic acids and flavonoids. They also show considerable antioxidant activity e.g. Butein, and interestingly, chalcones with only two adjacent hydroxyl groups are almost fully effevtive. Introduction of additional hydroxyl groups leading to only slight increase in their antioxidant activity. Hydrogenation ofrigaloneb Chalcone double bond increase their antioxidant activity (Wilson and Gelb, 2002). e.g.: pent hydroxyl dihydrochlalcone. Flavonoids exerts their antioxidant effects by neutralizing all types of oxidizing radicals including the superoxide and hydroxyl radicals and by chelation. Flavonoid can also act as powerful chain breaking antioxidant due to the electron-donating capacity of their phenolic groups. The potant antioxidant activity of flavonoids; their ability to scavenge hydroxyl radicals. May be the most important function of flavonoids and underlies many of their actions in the body (Xianquan *et al.,* 2005). Flavonoids by acting as free radical scavengers were shown to exert a protective effect in perfusion ischemic tissue damage, and by acting as antioxidants exhibited several beneficial effects as anti-inflammatory, antiallergic, antiviral as well as anticancer activity. They have also been suggested to play a protective role in liver diseases, cataracts and cardiovascular diseases (Godman *et al.,* 2011). They do not only have direct antioxidant activity but also have sparing effects on other antioxidants as Vitamin C And E (Gomes *et al.,*2003). And their capacity to modify membrane dependent process, such as free radical induced membrane lipid peroxidation, is related not only to their structural characteristics but also to their ability to interact with and penetrate the lipid bilayers (Huang *et al.,* 2005).

In addition to their effects on Reactive Oxygen Species (ROS), they have certain actions on rns, polyphenolic compounds are especially susceptible to peroxynitrite- dependant reactions and they are powerful inhibitors of nitrous acid dependant nitration and DNA deamination in vitro, and the role can be exerted in vivo, thus flavonoids may provide gastro-protective effect when high levels of rns are produced (Kacheva V. D, 2009). Flavnoids have chelating activity which allowed them to chelate or binds to metal ions in our bodies to prevent them being available for oxidation. The aerobic oxidation of ascorbic acid in neutral or alkaline solution is catalyzed by copper (p) ions and thought to proceed through a free radical mechanism. The ability of flavoniods to inhibit aerobic oxidation has been attributed to their ability to act as free radical acceptors and to remove catalytic ions through formation of complexes with the metal. The effectiveness of flavonoids to form complexes with metal is undoubtedly influenced by ph of the system (Joung *et al.,* 2004). Flavonoids and phenolic compounds with hydroxyl (or other electro donating) groups can interact with transition metal ions to form chelates, this chelates might be stable, or redox cycling might take place leading to the reduction of the iron or copper to a more pro-oxidant from and the oxidized flavonoid (Kandar *et al.,* 2006). The polyphenolic compounds generally present a tonifying action because of their natural antioxidant properties. Other action to be stressed are the stimulation of protein synthesis and the promotion of ammonia elimination. More specifically, the compounds cause a stabilizing of cell membrane components in cell organelles such as lysosomes and all above, a stabilizing of the plasmatic membrane of erythrocytes, mastocytes, fibrocytes, hepatocytes and other similar cells (Krimme *et al.,* 2010). Another pharmacological action of flavonoids is the protector effects on carcinogenesis by inhibiting the neoplastic effects of chemical carcinogens; their activity as antioxidants on microsomal mono-oxygenase promotes a detoxifying action with an antineoplastic effect (Lambert *et al.,* 2010). Allium sativam . (garlic), a food throughout the world, has been used as a remedy for various ailments since ancient times. More recently, organosulfur compounds, flavonoids, anthocyanins and other phenolics of garlic have been shown to have oxygen radical scavenging and antioxidant properties by several mechanisms as follows: inhibition of ca2+-induced low density lipoproteins (ldls) oxidation, antioxidant against hepatic microsomes stressed by ascorbic acid/fe3+. Hydroxyl radical (oh) scavenger, prevent carbon tetrachloride (ccl4) induced cytotoxicity in liver cells, inhibition of phorbol ester-induced oxygen radical formation by human granulocytes and increase the tissues concentration of the enzymes glutathione-peroxidase and glutathione-disulfide reductase (Ratnam *et al.,* 2006).

2 8 **Types of antioxidants**

**2.8.1 Ascorbic acid**

Ascorbic acid or "Vitamin C" is a monosaccharide antioxidant found in both animals and plants. As one of the enzymes needed to make ascorbic acid has been lost by mutation during human evolution, it must be obtained from the diet and is a vitamin. Most other animals are able to 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, reactive oxygen species such as hydrogen peroxide (antioxidants and cancer prevention, 2007; Ortega, 2006).

**2.8.2 Glutathione**

The free radical mechanism of lipid peroxidation: 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, such as ascorbate in the glutathione-ascorbate cycle, glutathione peroxidases and glutaredoxins, as well as reacting directly with oxidants (Meister and Anderson, 1983). Due to its high concentration and its 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 mycothiol in the actinomycetes, or by trypanothione in the kinetoplastids (Fahey, 2001; Fairlamb and Cerami, 1992).

**2.8.3 Melatonin**

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. Redox cycling may allow other antioxidants (such as Vitamin C) to act as pro-oxidants and promote free radical formation. 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 (Reiter *et al.,* 1997; Tan *et al.,* 2000).

**2.8.4 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, - tocopherol has been most studied as it has the highest bioavailability, with the body preferentially absorbing and metabolizing this form (Herrera and Barbas, 2001). It has been claimed that the -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. This removes the free radical intermediates and prevents the propagation reaction from continuing. This reaction produces oxidized -tocopheroxyl radicals that can be recycled back to the active reduced form through reduction by other antioxidants, such as ascorbate, retinol. This is in line with findings showing that - tocopherol, but not water-soluble antioxidants, efficiently protects glutathione peroxidase (gpx4)-deficient cells from cell death. Gpx4 is the only known enzyme that efficiently reduces lipid-hydro peroxides within biological membranes (Herrera and Barbas, 2001; Packer *et al.,* 2001).

**2.9 Characteristics of antioxidants**

The major antioxidants currently used in foods are monohydroxy or polyhydroxy phenol compounds with various ring substitutions. These compounds have low activation energy to donate hydrogen. Hence, the resulting antioxidants radical does not initiate another free radical due to the stabilization of the delocalized radical electron. Propagation and initiation of free radicals chain reaction can be delayed or minimized by the donation of hydrogen from the antioxidants and metal chelating agent. The resulting antioxidant free-radical is not subject to rapid oxidation due to its stability. Antioxidants free-radicals can also react with lipid free- radicals to form a stable complex compound thereby preventing some of their damages.

**2.10 Antioxidants system in our body**

The body has developed several endogenous antioxidant systems to deal with the production of roi. These systems can be divided into enzymatic and non- enzymatic groups. The enzymatic antioxidants include superoxide dismutase (sod), which catalyses the conversion of normal molecules in the body have two (a paired group) electrons in their outer shell. A molecule with a single electron (unpaired) in its outer shell is called a free radical. Free radicals occur naturally when oxygen in the bloodstream combine with any of a diverse group of chemicals including those commonly found in polluted air, in primary and/or second hand cigarette smoke, in known and damage is accelerated by the normal radiation found in sunlight and by increasing exercise, especially running and other aerobic activities. This is easy to understand in that aerobic exercise can increase oxygen consumption ten to twenty times (10-20) normal values. With more oxygen available in the bloodstream; free radical production soars. The direct muscle destroying activities of the free radicals continue many hours after exercise stops. The destructive effects of free radicals can be prevented with the addition of anti-oxidants in the diet or by anti-oxidant supplements. A good anti-oxidant complex supplement actually has advantages over diet sources in that the complex has many different specific types of anti-oxidants which seek out and destroy free radicals at many various cellular sites. A single anti- oxidant, for example vitamin e, only protects the outer fatty layers of the cell. It will not stabilize DNA which, for example, is one the main effects of the antioxidant vitamin c. The process by which different anti-oxidants disperse through the bloodstream to protect the cells at different sites is referred to in science as "anti-oxidant synergy." when a specific anti-oxidant meets a free radical in the bloodstream at its appropriate activity site, it naturally combines with it and coverts the free radical to harmless water and oxygen. As a result, as anti-oxidant increases due to the supplementation of higher amounts of a greater variety of anti-oxidants, cellular damage lessens and performance and health improves. In fact, aside from the numerous scientifically compelling studies addressing the varied health benefits of anti-oxidant supplementation, there have been studies completed, demonstrating a dramatic decrease in injuries in athletic training with the simple addition of a good anti-oxidant complex supplement. The brain is uniquely vulnerable to oxidative injury, due to its high metabolic rate and elevated levels of polyunsaturated lipids, the target of lipid peroxidation. Consequently, antioxidants are commonly used as medications to treat various forms of brain injury. Here, superoxide dismutase mimetics, sodium thiopental and propofol are used to treat reperfusion injury and traumatic brain injury, are being applied in the treatment of stroke. These compounds appear to prevent oxidative stress in neurons and prevent apoptosis and neurological damage. Antioxidants are also being investigated as possible treatments for neurodegenerative diseases such as alzheimer's disease, parkinson's disease, and amyotrophic lateral sclerosis and as a way to prevent noise- induced hearing loss (warner *et al.,* 2004). Antioxidants can cancel out the cell damaging effects of free radicals. Furthermore, people who eat fruits and vegetables, which happen to be good sources of antioxidants, have a lower risk of heart disease and some neurological diseases and there is evidence that some types of vegetables and fruits in general, protect against a number of cancers. These observations suggested the idea that antioxidants might help prevent these conditions. However, this hypothesis has now been tested in many clinical trials and does not seem to be true, since antioxidant supplements have no clear effect on the risk of chronic diseases such as cancer and heart disease. This suggests that other substances in fruit and vegetables (possibly flavonoids), or a complex mix of substances, may contribute to the better cardiovascular health of those who consume more fruit and vegetables. It is thought that oxidation of low density lipoprotein in the blood contributes to heart disease and initial observational studies found that people taking vitamin e supplements had a lower risk of developing heart disease. Consequently, at least seven large clinical trials were conducted to test the effects of antioxidant supplement with vitamin E, in doses ranging from 50 to 600 mg per day. However, none of these trials found a statistically significant effect of vitamin e on overall number of deaths or on deaths due to heart disease. Further studies have also been negative. It is not clear if the doses used in these trials or in most dietary supplements are capable of producing any significant decrease in oxidative stress. Despite the clear role of oxidative stress in cardiovascular disease, controlled studies using antioxidant vitamins have observed no reduction in either the risk of developing heart disease, or the rate of progression of existing disease. While several trials have investigated supplements with high doses of antioxidants, the "Supplémentation, Vitamines and Mineraux Antioxydants" study tested the effect of supplementation with doses comparable to those in a healthy diet. Over 12,500 French men and women took either low-dose antioxidants (120 mg of ascorbic acid, 30 mg of vitamin e, 6 mg of -carotene, 100 g of selenium and 20 mg of zinc) or placebo pills for an average of 7.5 years. The researchers found there was no statistically significant effect of the antioxidants on overall survival, cancer, or heart disease. Many nutraceutical and health food companies sell formulations of antioxidants as dietary supplements and these are widely used in industrialized countries. These supplements may include specific antioxidant chemicals, like resveratrol from grape seeds or knotweed roots, combinations of antioxidants, like the "aces" products that contain carotene pro-vitamin A, vitamin C, vitamin E and selenium, or herbs that contain antioxidants - such as green tea and jiaogulan. Although some levels of antioxidant vitamins and minerals in the diet are required for good health, there is considerable doubt as to whether these antioxidant supplements are beneficial or harmful (Warner *et al.,* 2004).

2.11 **Effects of antioxidants**

A healthy cell has a mortal enemy which is called a "free radical." free radicals constantly seek out healthy cells and attack their vulnerable outer membranes eventually causing cellular degeneration and death. Free radicals scientists today, carry out the actual destructive work in disease, in infection, in stress and in aging. Additionally, free radicals can negatively affect athletic performance by slowing or halting muscle growth and by lowering aerobic capacity. Further, free radicals are known to cause defects in normal rna as well as in life perpetuating DNA, the genetic material of the cells (Warner *et al.,* 2004).

**2.12 Phytochemicals**

The importance of plants is known to us well. The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe, and efficient and rarely have side effects. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs and antimicrobial drugs, antihepatotoxic compounds. According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants. However, such plants should be investigated to better understand their properties, safety, and efficiency (Arunkumar and Muthuselvam, 2009). Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Edoga *et al.,* 2005). These compounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas (Vasu et al., 2009). a large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms in vitro (Cowan M.M,1999). Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, seeds (Criaga et al., 2001). Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances (Parekh and Chanda, 2007; Mojab et al,. 2003; Parekh and Chanda, 2008).

**2.13 *Pteracarpus soyauxii* and *Pteracarpus santalinoides***

Vegetables are generally succulent parts of plants grown in gardens and consumed as a side dish with starchy staples (Guarino, 1995). Green leafy vegetables constitute an indispensable constituent of human diet in Africa generally and West Africa in particular (Chima and Igyor, 2007). The genus pterocarpus which is tropically and sub-tropically distributed belongs to the family leguminosae. There are about 60 species of the genus of which 20 of these are found in africa in countries such as Nigeria, Cameroon, Sierra Leone and Equatorial Guinea. Fresh vegetables are highly recommended in any diet virtually without quantitative restriction and the roles of vegetables in maintenance of good health are well known (Osuagwe, 2008).the leaves of *Pterocarpus sp*, *P. soyansii* known as “ora” (igbo) and *P. santalinoides* known as “nturukpa” (igbo) are used for soup making in the south eastern part of nigeria. Some tribes in the eastern and Southern Nigeria use the leaf extracts in the treatment of headaches, pains, fever, convulsions, and respiratory disorders and as antimicrobial agents as similarly reported for *Sansevieria trifasciata* (Ogukwe *et al.,* 2004). In addition, green leafy vegetables are used in the diets of postpartum.

Women during which time it is claimed that they aid the contraction of the uterus. It is therefore the objective of this work to determine and compare the phytochemical and proximate composition of *Pterocarpus soyansii* and *Pterocarpus santalinoides* grown in enugu of nigeria.

**CHAPTER THREE**

**MATERIALS AND METHODS**

**3.1 Sample collection**

The fresh leaves of *P. soyansii* and *P. santalinoides* were collected from Nsude in Udi local government area of Enugu State Nigeria. The species *P. soyansii* is locally called “oha” (Igbo), “asunje “(Yoruba) and “alillibi rafii” (Hausa) while *P. santalinoides* is called “nturukpa” (Igbo), “gbengbe” (Yoruba) and “gunduru” (Hausa). The leaves was washed, cut and dried at room temperature for two weeks. After which it was grinded. These leave were homogenized to fine powder and stored in airtight containers and the sample were taken to Pymotech laboratory Abakpa nike, for phytochemical analysis.

**3.2 Preparation of plant extract**

* Dried finely powdered plant material of 10gm was taken from a beaker and 100ml of distilled water was added.
* The mixture was homogenized for 3hrs at a room temperature and it was allowed to settle over night or 24hrs.
* Then the water extract was filtered through filter paper and the filtrate was used for the phytochemical analysis.

**3.3 Solvent extraction (crude plant extract was prepared by soxhlet extraction method).**

* About 10gm of powdered plant material was uniformly packed into a thimble and extracted with 100ml of different solvents separately, solvents used were methanol, ethanol, distilled water, hexene and petroleum ether.
* The process of extraction continued for 24 hours or till the solvent in siphon tube of an extractor become colorless.
* After that the extract was taken in a beaker and kept on hot plate and heated at 30-40ºc till all the solvent got evaporated.

**3.4** **Qualitative phytochemical analysis**

Phytochemical tests were carried out first to establish the presence or otherwise of some specific phytochemicals. The leaves of *Pterocarpus soyansii* and *Pterocarpus santanaloides* were screened for alkaloids, saponins, tannins, flavonoids, polyphenols, steroids, anthraquinone, reducing sugar and terpernoid . The methods used and there corresponding inferences were standard methods (Parekh and Chanda, 2008).

**3.4.1 Test for saponins**

This is divided into two: frothing and emulsion test

**3.4.1.1 Test for frothing**

Crude extract of 3ml was pipette into a test tube and 5ml of distilled water was added to it, then it was shaken vigorously. A persistent frothing movement was observed.

**3.4.1.2 Test for emulsion**

Crude extract of 3ml was pipette into a test tube and 5 drops of olive oil was also incorporated into it and then it was shaken vigorously. Emulsification was observed.

**3.4.2** **Test for alkaloids**

Crude extract of 2ml was added into a test tube and the mixture was heated for 20mins using water bath. The heated mixture was filtered using filter paper and 1ml of the filtrate was measured into a test tube and 0.5ml of wagner’s reagent was added to it. A reddish brown coloration was observed. These show the presence of alkaloids.

**3.4.3 Test for steroid**

Crude extract of 1ml was treated with 0.5ml of acetic acid, 0.5ml of chloroform and 1ml of conc. Sulphuric acid (H2so4) was also added to it. A reddish brown ring was formed at the separating level of the two liquids indicating the presence of steroids.

**3.4.4 Test for terpenoids**

Crude extract of 5ml was mixed with 2ml of chcl3 (chloroform) in a test tube 3ml of conc. Dissolved in 2ml of h2so4 was carefully added to the mixture to form a layer. An interface with reddish-brown coloration was formed. The terpenoid constituent is present.

**3.4.5** **Test for polyphenol**

Crude extract of 2ml was pipette out and 5ml of distilled water added and heated i n a water bath for 10min. 1ml of ferric chloride was added to the mixture followed by 1ml of 1% potassium ferricyanide. The formation of a green-blue coloration indicated the presence of polyphenol.

**3.4.6 Test for flavonoid**

Crude extract of 3ml was pipetted out and 10ml of distilled water was added to it and it was shaken and 1ml of10% naoh was also added into the mixture. A yellow coloration was observed showing the presence of flavonoid.

**3.4.7 Test for tannin**

About 1ml of the extract was measured into a test tube and it was heated and one drop of 10% feric chloride was added to it. The mixture showed a green coloration.

**3.4.8** **Test for anthraquinones**

About 2ml of alcoholic/aqueous extract was shaked with 5ml of 10% ammonia solution added. The mixture was shaken and the presence of a pink, red to violet color in the ammonical (lower) phase indicated the presence of anthraquinones.

**3.4.9 Test for reducing sugar**

The 2ml of the extract in a test tube was added to 5ml of fehling solutions and heated in a water bath at 80oc for 10min. The formation of a brick-red precipitate or solution was taken as evidence for the presence of reducing compounds.

**3.5 Quantitative determination of the phytochemicals from leaf samples**

The quantify of the identified phytochemicals: alkaloid, saponin and phenols were determined according to the method of (Obadoni and Ochuko, 2001). Flavonoids and flavonol were determined according to the method of (Bohm and Kocipai, 1994). Tannin and steroid determination was (Akindahunsi *et al*., 2005).

**3.5.1 Alkaloid determination**

About 5g of the sample was weighed into a 250ml beaker and 200ml of 20% acetic acid in ethanol was added and covered to stand for 4h. This was filtered and the extract was concentrated using a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract untill the precipitation was complete the whole solution was allowed to settle and the precipitate was collected by filtration and weighed (Harborne, 1973; Obadoni and Ochuko, 2001).

**3.5.2**.**Polyphenols determination**.

The extraction of the phenolic component, the fat free sample was boiled in 50ml flask then 10ml of distilled water was added. 2ml of ammonium hydroride solution and 5ml of concentrated amylachohol were also added. The sample were made up to mark left to react for 30min for color development. The absorbance of the solution was read using a spectrophotometer at 805nm wavelengths (Harborne, 1973; Obadoni and Ochuko, 2001).

**3.5.3 Saponin determination**

The samples were ground. 5g of each leave samples were dispersed in 200ml of 20% ethanol. The suspension was heated over a hot water bath for 4h with continuous stirring at about 55oc. The mixture was filtered and the residue re-extracted with another 200ml of 20% ethanol. The combined extract wass reduced to 40ml over water bath at about 90oc. The concentrate was transferred into a 250ml separating. Funnel and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated, 60ml of n-butanol was added. The combined n- butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath, after evaporation, the samples were dried in the oven to a constant weight. The saponin content was calculated in percentage (Obadoni and Ochuko, 2001).

**3.5.4 Flavonoid determination**

About 5g of the leave sample were extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper no. 42 (125mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed (Boham and Koctpat, 1994).

**3.5.5 Steroid determination**

The leave sample of 5g was weighed into a 250mlconical flask. 100ml of distilled water was dispensed into the sample. The mixture was the agitated in a vibrator/ shaker at relatively low speed for 3hours, then it was allowed to stand over night. The mixture was then filtered into a beaker with the aid of a whatman filter paper. The filtrate was eluted with 10ml nh4oh 2ml of the elute was put into a test tube and mixed with 2ml of chloroform. 3ml of acetic anhydride was added to the mixture followed by 2ml of concentrated sulphuric acid. Then the absorbance was read in a uv visible spectrophotometer at 420nm (Akindahunsi *et al*., 2005).

**3.5.6 Flavonol determination**

The method of Kumaran and Karunakaran was adopted for the determination of total flavonol. An amount of 2.0ml of the sample, 2.0ml of aluminium chloride, ethanol solution and 3.0ml of (50g/l) sodium acetate solution were all added together in a test tube. After incubation at 20oc for 150min, the absorbance was read at 44nm (Bohm and Kocipai, 1994).

**3.5.7 Tannin determination**

Folin-Denis method was used. About 1g of sample was despensed into 50ml distled water and agitated. The mixture was allowed to stand at room temperature for 30minutes. Then, the filterate extract was collected through a whatman filter paper 1. The extract was further centrifuged at 3000rpm for 5mins. 2.5ml of supemetant was dispensed into a 50ml volumetric flask. Similarly 2.5ml of standard tannic acid was dispensed into a 50ml volumetric flask. Exactly 1.0ml folin denis reagent was measured into each flask filtered by 2,5ml of saturated sodium trioxocarbonate solutio (10%). Then the mixture was diluted to mark in the flask and incubated for 90mins at room temperature the absorbance was measured at 250nm using spectrophotometer.

**3.6 DETERMINATION OF REDUCING POWER**

The method of yen and chen was used to determine the reducing power of the extract. 5gm of the sample was weighed; 100ml of distilled water was measured and mixed with the sample inside a conical flask. It was shaken for 2hrs at room temperature, and then the extract was filtered.the crude extract was used to determine the reducing power. The concentration range between 0.125 and 1.0 mglml each for the leaf and ascorbic acid were separately prepared, then 1ml each of the concentrations was mixed with a mixture containing 2.5ml of potassium ferriayanide (1% w/v) and 2.5ml of 0.2 MOI/L phosphate buffer (ph 7.0). the resulting was incubated at 50˚c for 20mins, about 2.5ml of trichloroacetic acid (10% w/v) was further added to the mixture and then centrifudged at 3000 r/min for 10min. the upper layer of the solution (2.5ml) was collected and mixed with 0.5ml of ferrous chloride (0.1% w/v) and 2.5 of distilled water. The absorbance was taken using UV light at 700nm against a blank surface. Higher reducing power of the plant extract was indicated by an increased absorbance of the reaction mixture.

**3.7 DETERMINATION OF HYDROGEN PEROXIDE SCAVENGING ACTIVITY**

The method of Ruch et al was adopted to determine the hydrogen peroxide scavenging activity of the plant extract. About 4ml of leaf extracts and ascorbic acid at various concentrations of 0.125-1.0mg/ml each was mixed with 0.6ml of 4mmoi/l hydrogen peroxide solution prepared in phosphate buffer (0.1moi/l; ph 7.0). The mixture was incubated at room temperature for 10min. the absorbance of the solution mixture was read using UV light at 230nm against a blank solution containing only the plant extract without hydrogen peroxide. The amount of hydrogen peroxide radical inhibited by the extract was calculated using the equation Hydrogen Peroxide Radical Scavenging Activity= [(Absorbance Control-Absorbance sample)/ (Absorbance control)] × 100. Where, Absorbance Control was the Absorbance of Hydrogen Peroxide Radical + Solvent; Absorbance sample was the Absorbance of Hydrogen Peroxide Radical + Sample extract or standard.

**CHAPTER FOUR**

**4.0 RESULTS**

The result of the quantitative phytochemical analysis of Pterocarpus soyansii and *Pterocarpus santalinoides* leaves is summarized in Table 1 and 2. The results revealed the presence of phytochemicals such as alkaloids, saponins, tannins, flavonoids, steroids, phenols and flavonols.

**Table 1: Qualitatitive phytochemical screening of *Pterocarpus soyansii***

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample** | **Parameter** | **Hexane** | **H2O** | **Ethanol** | **Petroleum ether** | **Methanol** |  |  |  |  |  |  |  |
| *P. soyauxii* | Alkaloid | ̶ | ++ | ̶ | ̶ | ̶ |  |  |  |  |  |  |  |
|  | Saponin Frothing Emulsion | ̶  ̶ | ++  ++ | ++  ++ | ̶  ̶ | ̶  ̶ |  |  |  |  |  |  |  |
|  | Steroid | ̶ | ̶ | ++ | ̶ | ̶ |  |  |  |  |  |  |  |
|  | Flavonoid | ̶ | ++ | ̶ | ̶ | ̶ |  |  |  |  |  |  |  |
|  | Tannin | ++ | ̶ | ++ | ++ | ++ |  |  |  |  |  |  |  |
|  | Anthraquinine | ̶ | ̶ | ̶ | ̶ | ̶ |  |  |  |  |  |  |  |
|  | Reducing sugar | ̶ | ̶ | ̶ | ̶ | ̶ |  |  |  |  |  |  |  |
|  | Terpenoid | ̶ | ++ | ̶ | ̶ | ̶ |  |  |  |  |  |  |  |
|  | Polyphenol | ̶ | ++ | ̶ | ̶ | ++ |  |  |  |  |  |  |  |

++ = Present

- = Absent

**Table 2: Qualitatitive phytochemical screening of *Pterocarpus santalinoides***

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample** | **Parameter** | **Hexane** | **H2O** | **Ethanol** | **Petroleum ether** | **Methanol** |  |  |  |  |
| *P. santalinoides* | Alkaloid | *̶* | ̶ | ̶ | ̶ | ̶ |  |  |  |  |
|  | Saponin Frothing Emulsion | ̶  ̶ | ++  ++ | ++  ̶ | ̶  ̶ | ++  ̶ |  |  |  |  |
|  | Steroid | ̶ | ++ | ++ | ̶ | ̶ |  |  |  |  |
|  | Flavonoid | ̶ | ++ | ̶ | ̶ | ̶ |  |  |  |  |
|  | Tannin | ++ |  | ++ | ++ | ++ |  |  |  |  |
|  | Anthraquinine | ̶ | ̶ | ̶ | ̶ | ̶ |  |  |  |  |
|  | Reducing sugar | ̶ | ̶ | ̶ | ̶ | ̶ |  |  |  |  |
|  | Terpenoid | ̶ | ̶ | ̶ | ̶ | ++ |
|  | Polyphenol | ++ | ++ | ̶ | ̶ | ++ |

++ = Present

- = Absent

**4.1 QUANTITATIVE RESULT**

The result of the quantitative phytochemical analysis of Pterocarpus soyauxii and *Pterocarpus santalinoides* leaf extracts is summarized in Table 3. The result of the phytochemicals indicates that the concentration of tannins (0.238) was higher in *P. soyauxii* than in *P. santalinoides* which contained (0.0691). *Pterocarpus soyauxii* contained alkaloids (24.298), saponins (6.988), phenols (0.422), steroids (0.0148), flavonols (46.8) and flavonoids (0.876), while *P. santalinoids* does not contain alkaloids, (6.988) of saponins, (0.352) of phenols and (0.0133) of steroids, flavonoids (1.572) and flavonols (185.6). Quantitative estimates of other phytochemicals showed that P. santalinoides contained significantly higher concentration of flavonoids, (1.572) and flavonols (185.6). as compared to P. soyansii which contained (0.876) of flavonoids, and (46.8) of flavonols. The result of the proximate analysis clearly shows that the leaves of both species of Pterocarpus have high nutritional value.

**Table 3: Quantitative phytochemical screening of *P. soyauxii and P. santalinoides***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Sample** | **Flavonoid(%)** | **Flavonoid (%)** | **Saponin (%)** | **Steroid (%)** | **Phenol (%)** | **Tannin (%)** | **Flavanol (mg/L)** |
| *P.soyanxii* | 0.876 | 24.298 | 6.988 | 0.0148 | 0.422 | 0.238 | 46.8 |
| *P.santalinoides* | 1.572 | *̶* | 6.746 | 0.0133 | 0.352 | 0.0691 | 185.6 |

**4.2** REDUCING POWER RESULTS

Reducing  power  is  associated  with  antioxidant  activity  and  may  serve  as  a  significant  reflection  of  the  antioxidant  activity .  Compounds  with  reducing  power  indicate  that  they  are  electron  donors  and  can  reduce  the  oxidized  intermediates  of  lipid  peroxidation  processes,  so  that  they  can  act  as  primary  and  secondary antioxidants. The higher the absorbance, the higher the reducing power activity.

**Figure 1: Reducing Power of *P. soyauxii***

1000 × Concentration × absorbance

Weight of sample x 1

**Figure 2: Reducing power of *P.santalinoids***

1000 × Concentration × absorbance

Weight of sample x 1

**4.3** DETERMINATION OF HYDROGEN PEROXIDE ACTIVITY RESULTS

**Figure 3: Hydrogen peroxide scavenging activity of *P.soyanxii***

Absorbance control – absorbance sample × 100

Absorbance of control

The higher the absorbance, the higher the hydrogen peroxide scavenging activity.

**Figure 4: Hydrogen peroxide scavenging activity of *P.santalinoids***

Absorbance control – absorbance sample

Absorbance of control x 100

The higher the absorbance, the higher the hydrogen peroxide scavenging activity.

CHAPTER FIVE

**5.1 DISCUSION**

The results indicate that some of the phytochemical and nutritive constituents vary significantly. Various studies have shown that saponins although non toxic can generate adverse physiological responses in animals that consumes them. They exhibited growth inhibition against a variety of cells making them have anti-inflammatory and anti cancer properties. They also show tumor inhibiting activity on animals. Tannins exhibited antimicrobial activities by iron deprivation, hydrogen bonding or specific interactions with vital protein interaction. Leaves that have tannins are used for the treatment of intestinal disorders such as diarrhea and dysentery (Akindahunsi and Salawu, 2005). Tannins have astringent properties, hasten the healing of wounds and inflamed mucous membranes. Phenols protect plants from predators and pathogens. They produce poisons that protect the plants. The presence of phenolic compounds in the plants indicates that these plants may be anti-microbial agents. Phenols are used to eliminate bacteria and also used as poisons to burn up parasites (Sofowora, 1993). Steroidal compounds are of importance and interest in pharmacy due to their relationship with such compounds as sex hormones. Sterols are used to lower cholesterol (Edeoga et al., 2005). Flavonoids have been shown to have antibacterial, anti-inflammatory, anti allergic and anti tumour and protect organisms from free radicals attack. Flavonoids, are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anticancer activity. Flavonoids in intestinal tract lower the risk of heart disease (Okwu, 2004). This may be the reason for the use of the two *Pterocarpus* species in the treatment of intestinal trouble when consumed in soup. Apart from saponins, other secondary metabolite constituents of *P. soyansii and P. santalinoides* detected include the alkaloids and flavonoids. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects (Stray, 1998; Okwu and Okwu, 2004). Alkaloids are mainly blended from amino acids and also protect the plant from herbivorous animals and may also be useful pharamacologically. Natural antioxidant mainly come from plants in the form of phenolic compounds such as flavonoid, flavonols etc (Ali *et al.,* 2008).Tannins bind to proline rich protein and interfere with protein synthesis. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms. and reducing  power  is  associated  with  antioxidant activity  and  may  serve  as  a  significant  reflection  of  the  antioxidant  activity .  Compounds  with  reducing  power  indicate  that  they  are  electron  donors  and  can  reduce  the  oxidized  intermediates  of  lipid  peroxidation  processes,  so  that  they  can  act  as  primary  and  secondary antioxidants. The higher the absorbance, the higher the reducing power activity

**5.2 CONCLUSION**

The results of the quantitative phytochemical study of the leaves of *P. soyauxii* and *P. santalinoides* showed the presence of alkaloids, tannins, steroids, saponins, phenols and flavonoids. The extensive study of these phytochemicals and establishment of good correlation among the plant phytochemicals is essential for ensuring efficiency and quality of the herbal medicine. The result of the proximate analysis clearly shows that the leaves of both species of Pterocarpus have high nutritional value. Their leaves are used for soup making in the south eastern part of Nigeria. Some tribes in the Eastern and Southern Nigeria use the leaf extracts from *P. soyauxii* and *P. santalinoides* in the treatment of headaches, pains, fever, convulsions, and respiratory disorders. However, adequate and proper care of these vegetables during processing and storage will ensure the conservation of their usefulness. The results of this study also showed that leaf extracts of *P. soyauxii* and *P. santalinoides* possessed reducing power ability, as well as the ability to scavenge hydrogen peroxide, which could otherwise result in oxidative stress conditions in vivo. Compounds  with  reducing  power ability indicate  that  they  are  electron  donors  and  can  reduce  the  oxidized  intermediates  of  lipid  peroxidation  processes,  so  that  they  can  act  as  primary  and  secondary antioxidants.

**REFERENCES**

Aguillar F, Crebelli R, Dusemund B, Galtier P, Gilbert J, Gott D.M, Gundert- Remi U, Koenig J, Lambré C, Leblanc J.C, Mortensen A, Mossesso P, Parent-Massin D, Rietjens I.M.C.M, Stankovic I, Tobbcak P, Waalkens- Berendsen I, Woutersen R.A, Wright M (2011). Scientific opinion on the re- evaluation of butylated hydroxyanisole – BHA (E 320) as a fodd additive. *EFSA Journal.* doi*:* 10.2903/j.efsa.2011.2392.

Aguillar F, Crebelli R, Dusemund B, Galtie P., Gilbert J, Gott D.M, Gundert- Remi U, Koenig J, Lambré C, Leblanc J.C, Mortensen A, Mossesso P, Parent-Massin D, Rietjens I.M.C.M, Stankovic I, Tobbcak P, Waalkens- Berendsen I, Woutersen R.A, Wright M (2012). Scientific opinion on the re- evaluation of butylated hydroxytoluene BHT (E321) as a fodd additive. *EFSA Journal*. doi: 10.2903/j.efsa.2012.2588.

Akindahunsi, A.A. and Salawu, S.O. (2005). Phytochemical screening of nutrient and antinutrient composition of selected tropical green leafy vegetables. *Afr. Journal. Biotech.,* 4: 497-501.

Ali S.S., Kasoju N., Luthra A., Singh A., Sharanabasava H, Sahuand A., Bora U. (2008). Indian medicinal herbs as source of antioxidants. *Food Res.* Int., 41: 1-15.

Andreyev A. Y, Kushnareva, Y. E, Starkov A. A. (2005). Mitochondrial metabolism of reactive oxygen species. *Biochemistry (Mosc),* Vol. 70, No. 2, pp. 200-214.

Arslanian S.A, Bacha F, Gungor N, Saad R. (2006). Are obesity-related metabolic risk factors modulated by the degree of insulin resistance in adolescents? *Diabetes Care* 29:1599-1604.

Arunkumar S, Muthuselvam (2009). Analysis of phytochemical constituents and antimicrobial activities of aloevera L. against clinical pathogens. *World Journal Agril. Sc.*, 5(5): 572-576.

Aslan M, Orhan D D, Orhan N, Sezik E and Yesilada E (2007).  *Journal Med Food*., 10, 396-400

Barrou B, Bourny E, Deybach C, Deray G, Launary- Vacher V, Izzedine H (2005). Drug induced diabetes mellitus. Expert opinion on safety, Vol. 6, NO. 4, PP. 1097-109; 10; 1517/14740338.4.6.1097 PMID 16255667.

Barros A.I.R.N.A, Nunes F.M, Gonçalves B, Bennett R.N, Silva A.P (2011). Effect of cooking on total vitamin C contents and antioxidant activity of sweet chestnuts (Castanea sativa Mill.). *Food Chem*. 128, 165-172.

Basu A.K, Banerjee R, Mandal S, Pal S.K. (2005). A study on micro-albuminuria-an independent risk factor for vasculopathy in diabetes mellitus.  *Journal Indian Med Assoc 103*:374-375, 382.

Berker K.I, Güclü K, Tor I, Apak R (2007). Comparative evaluation of Fe(III) reducing power-based antioxidant capacity assays in the presence of phenanthroline, batho-phenanthroline, tripyridyltriazine (FRAP), and ferricyanide reagents. *Talanta* 72, 1157-1165.

Brownlee M, Giacco F. (2010). Oxidative stress and diabetic complications. *Circ Res*, Vol. 107, No. 9, pp. 1058-1070.

Bjelakovic G, Nikolova D, Gluud LL, Simonetti, R.G; Gluud, C. (2007). Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. JAMA 297(8): 842–857.

Cave A.C, Brewer A.C, Narayanapanicker A, Ray R, Grieve D.J, Walker S, Shah A.M. (2006). NADPH oxidases in cardiovascular health and disease. Antioxid Redox Signal 8: 691–728.

Ceriello A. (2006). Oxidative stress and diabetes-associated complications. Endocr Pract 12 Suppl 1:60-62.

Chanda S, Dave R (2009). In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties:  An  overview. *African  Journal  of  Microbiology  Research* 3(13):981‐996.

Cowan M.M. (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev. 564-582.

Criagg G.M, David J.N. (2001). Natural product drug discovery in the next millennium. *Journal Pharm. Biol.*, 39: 8-17.

Edoga, H.O, Okwu, D.E, Mbaebie, B.O. (2005). Phytochemicals constituents of some Nigerian medicinal plants. *African Journal Biotechnol.,* 4(7): 685-688.

Edoga H.O, Okwu D.E, Mbaebie, B.O (2005). Phytochemicals constituents of some Nigerian medicinal plants. *African Journal Biotechnol*., 4(7): 685-688.

Erejuwa O, Gurtu S, Sulaiman S. A, Sirajudeen K. N, Salleh M. S, Wahab A.B. (2010a). Hypoglycemic and antioxidant effects of honey supplementation in streptozotocin-induced diabetic rats. *Int Journal Vitam Nutr Res*, Vol. 80, No. 1, pp. 74-82.

Ergul A.K, Harris A.J, Johansen J.S, Rychly, D. (2005). Oxidative stress and the use of diabetes: linking basic science to clinical practice. Cardiovasc Diabetol, Vol. 4, No. 1, pp.

Figueroa-Romero C, Feldman E. L, Sadidi M. (2008). Mechanisms of disease: the oxidative stress theory of diabetic neuropathy. Rev Endocr Metab Disord, Vol. 9, No. 4, pp. 301-314.

Galvez M, Martin-Cordero C, Houghton P.J, Ayuso M J (2005).  *Journal Agric Food Chem*53, 1927-1933. 3.

Godman, M., Bostick, R.M., Kucuk, O., Jones, D.P (2011). Clinical trials of antioxidants as cancer prevention agents: Pas, present and future. Free Radic. Biol. Med. 51, 1068-1084.

Gomes, C.A., Cruz, T.G., Andrade, J.L., Milhazes, N., Borges, F., Marques, M.P.M (2003). Anticancer activity of phenolic acids of natural or synthetic origin: A structure-activity study. *Journal Med. Chem*. 46, 5395-5401.

Gutteridge J. M. C, Halliwell B (2007). Free Radicals in Biology and Medicine 4th. Edn, Clarendon Press, Oxford. .

Hail N, Cortes M, Drake EN, Spallholz JE (2008). Cancer chemoprevention: a radical perspective. Free Radic. Biol. Med., 45(2): 97-110.

Halliwell B. (2011). Free radicals and antioxidants. Trends Pharmacol Sci, Vol. 32, No. 3, pp. 125 130. Jones D.P (2006). Extracellular redox state: refining the definition of oxidative stress in aging. Rejuvenation Res 9:169-181.

Hou W C, Lin R D and Cheng K T (2003) Phytomedicine, 10, 170-175.

Hsieh R.H, Lien L.M, Lin S.H, Chen C.W, Cheng H.J, Cheng H.H (2005). Alleviation of oxidative damage in multiple tissues in rats with streptozotocin-induced diabetes by rice bran oil supplementation. Ann N Y Acad Sci 1042:365-371.

Huang D, O.U, B, Prior, R.L (2005). The chemistry behind antioxidant capacity assays. *Journal Agric. Food Chem.* 53, 1841-1856.

Johansen JS, Harris AK, Rychly DJ, Ergul A. (2005). Oxidative stress and the use of antioxidants in diabetes: Linking basic science to clinical practice, Cardiovascular Diabetology, , 4: 5–9.

Joung T, Nihei, K, Kubo, I. (2004). Lipoxygenase inhibitory activity of octyl gallate. *Journal Agric. Food Chem*. 52, 3177-3181.

Kancheva, V.D. (2009). Phenolic antioxidants – radical-scavenging and chain-breaking activity: A comparative study. Eur*. Journal Lipid. Sci. Technol.* 111, 1072-1089.

Kand’ár, R., Žáková, P., Mužáková, V. (2006). Monitoring of antioxidant properties of uric acid in humans for a consideration measuring of levels of allantoin in plasma by liquid chromatography. Clinica Chimica Acta 365, 249-256.

Kim Y. M, Jeong Y K, Wang M. H, Lee W .Y, Rhee H. I (2005). *Nutr.* 21, 756-761.

Krimmel B, Swoboda F, Solar S, Reznicek G. (2010). OH-radical induced degradation of hydroxybenzoic- and hydroxycinnamic acids and formation of aromatic products – A gamma radiolysis study. *Radiat. Phys. Chem*. 79, 1247- 1254.

Kukic J, Petrovic S, Niketic M (2006) *Biol Pharm Bull*. 29, 725-729. 2.

Lambert J.D, Elias R.J (2010). The antioxidant and pro-oxidant activities of green tea polyphenols: A role in cancer prevention. Arch. Biochem. Biophys. 501, 65-72.

Lin, Yong, Shi, Ranxin, Wang, Xia, Shen, Han-Ming (2008). Luteolin, a Flavonoid with Potential for Cancer Prevention and Therapy. *Current Cancer Drug Targets*, Volume 8, 634-646. 67.

Lobo V, Phatak A, Chandra N (2010). Free radicals and functional foods: Impact on human health. *Pharmacogn*. Rev. 4, 118-126.

Lotito S.B and Fraga C.G (2000). Ascorbate protects (+)-catechin from oxidation both in pure chemical system and human plasma. *Biol*. Res. 33: 151-157.

Mann, J (1978). Secondary Metabolism. Oxford University press, London, pp. 154.

Miller E.R, 3rd, Pastor-Barriuso R, Dalal D, Riemersma R.A, Appel L.J, Guallar E (2005). Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. Ann Intern Med 142:37-46.

Moon J, Shibamoto T (2009). Antioxidant assays for plant and food components. *Journal Agric. Food Chem*. 57, 1655-1666.

Mojab F, Kamalinejad M., Ghaderi N., Vanidipour H.R. (2003). Phytochemicals screening of some species of Iranian plants. *Iran. Journal Pharm. Res.,* 3: 77-82.

Obadoni B.O. and Ochuko P.O (2001). Phytochemical Studies and Comparative Efficacy of the crude extracts of some homeostatic plants in EDO and Delta States of Nigeria. *Global Journal Pure and Applied Sciences* 8:203 -208.

Ock Kyoung Chun and Dae-Ok Kim (2004). Food Research International, Volume 37: 337-342. http://www.ncbi.nlm.nih.gov/pubmed/22455351.

Oktay M, Gulcin I, Kufrevioglu O.I (2003). Determination of invitro antioxidant activity of fennel  (Foeniculum  vulgare)  seed  extracts. Lebensum.­Wiss.U.­Technol ; 36:263‐271.

Okwu D.E (2004). Phytochemicals and vitamin content of indigenous species of southeastern Nigeria. *Journal Sustain. Agric. Environ.,* 6(1): 30-37.

Parek J., Chanda, S.(2007). Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *African Journal Biomed. Res.,* 10: 175-181.

Parekh J, Chanda S. (2008). Phytochemicals screening of some plants from western region of India. Plant Arch., 8: 657- 662.

Pfundstein B, El Desouky S K, Hull W E, Haubner R, Erben G and Owen R W (2010). Phytochem., 71, 1132-1148.

Ratnam D.V, Ankola D.D, Bhardwaj V, Sahana D.K, Kumar N.M.V.R. (2006). Role of antioxidants in prophylaxis and therapy: *A pharmaceutical perspective. Journal SSControl Release*. 113, 189-207.

Rice-Evans, C. 2001. Flavonoid Antioxidants .Current Medicinal Chemistry, Volume 8, 797 807(11).

Schäfer ZT, Karger AG (2009). Nature DOI :10.1038/nature08268 http://www.sciencedaily.com.

Shaw L.J, Berman D.S, Hendel R.C, Alazraki N, Krawczynska E, Borges-Neto S, Maddahi J, Cerqueira M (2006). Cardiovascular disease risk stratification with stress single- photon emission computed tomography technetium-99m tetrofosmin imaging in patients with the metabolic syndrome and diabetes mellitus. *Am Journal Cardiol* 97:1538-1544.

Surai, P.F. (2003). Selenium-vitamin E interactions: Does 1 + 1 equal more than 2? In: Nutritional Biotechnology in the Feed and Food Industries (T.P. Lyons and K.A. Jacques, eds.) Nottingham University Press, Nottingham, UK.

Vasu, K, Goud, J.V, Suryam, A, Singara, Chary, M.A. (2009). Biomolecular and phytochemical analyses of three aquatic angiosperms. *African Journal Microbiol. Res.,* 3(8):418-421.

Voziyan P.A, Hudson B.G (2005) Pyridoxamine as a multifunctional pharmaceutical: targeting pathogenic glycation and oxidative damage. Cell Mol Life Sci 62:1671-1681.

Warner D, Sheng H, Batini-Haberle I (2004). Oxidants, antioxidants and the ischemic brain. *Journal Exp. Biol.,* 207(18): 3221-3231.

Wilson J, Gelb A (2002). Free radicals, antioxidants, and neurologic injury: possible relationship to cerebral protection by anesthetics. *Journal Neurosurgical Anesthesiol.,* 14(1): 66-79.

Xianquan S, Shi J, Kakuda Y, Yueming J (2005). Stability of lycopene during food processing and storage. *Journal Medicinal Food*, 8(4): 413–22.

**APPENDIX**



**Sample in the waterbath shaker**



**Sample in the conical flask**



**The Samples: Pterocarpus soyauxii (Oha) and Pterocarpus santalinoides (Uturukpa)**