Vegetables are in abundance shortly after the rainy season but become scarce during the dry season during which cultivated types are used. Some eventually find their way to urban markets. One of the most commonly consumed leafy vegetables in Nigeria is Telfairia occidentalis, which is employed both for culinary and medicinal purposes.The main protective action of vegetables has been attributed to the antioxidants present in them. The oxidative stress experienced by a tissue, organelle or organ results from the balance between the production and removal of potentially damaging reactive oxygen species. Antioxidants can prevent the chemical damage caused by reactive oxygen species such as free radicals that are generated by a variety of sources including pesticides, tobacco smoke, exhaust fumes, certain pollutants and organic solvents. Medicinal plants have been used for curing diseases and has been documented in all history of civilisation. Peppers (Capsicum species) are fruits with a high importance in human diet due to their versatility to be consumed as fresh vegetable in salads, cooked meals or dehydrated for spices. This work explores the work on these vegetables in detail.

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Omotosho E. Omolola is a renowned researcher and a university lecturer. She has worked on various international projects in the field of biochemistry and food chemistry. She has authored several articles published in reputable journals and a member of international working groups and professional bodies. Omolola has won various awards.

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PART I

CHAPTER ONE

INTRODUCTION

Leafy vegetables are important items of diet in every home. Apart from the variety which they add to the menu, they are valuable sources of nutrients especially in rural areas where they contribute substantially to protein, mineral, vitamins, fibre and other nutrients which are usually in short supply in daily diets (Mosha and Gaga, 1999). Besides, they add flavour, variety, taste, colour and aesthetic appeal to what would otherwise be a monotonous diet. They are in abundance shortly after the rainy season but become scarce during the dry season during which cultivated types are used. Some eventually find their way to urban markets. One of the most commonly consumed leafy vegetables in Nigeria is *Telfairia occidentalis* which is employed both for culinary and medicinal purposes (Akoroda, 1988).

The main protective action of vegetables has been attributed to the antioxidants present in them. The oxidative stress experienced by a tissue, organelle or organ results from the balance between the production and removal of potentially damaging reactive oxygen species. Antioxidants can prevent the chemical damage caused by reactive oxygen species such as free radicals that are generated by a variety of sources including pesticides, tobacco smoke, exhaust fumes, certain pollutants and organic solvents (Halliwell and Gutteridge, 1989).

Medicinal plants have been used for curing diseases and has been documented in all history of civilisation. 80% of the world population according to the World Health Organisation, uses plant based remedies as their primary form of health care (Evan, 1998). The use of herbal medicines in an evidence-or science-based approach to the treatment and prevention of disease is known as phytotherapy (Joanne *et al.,* 2002).

Selenium is an essential micronutrient of major metabolic significance. The requirement of the trace element selenium for life and its beneficial role in human health has been

known for several decades. This is attributed to low molecular weight selenium compounds, as well as to its presence within at least 25 proteins, named selenoproteins, in the form of the amino acid selenocysteine (Sec) (Laura *et al.,* 2007).

Selenium is an essential component of several major metabolic pathways, including thyroid hormone metabolism, antioxidant defence systems, and immune function. It also has toxic effect as well as beneficial effect to human health (Brown and Arthur, 2007).

Paracetamol is a common analgesic and antipyretic drug that is used for the relief of fever, headaches, and other minor aches and pains. It is also useful in managing more severe pain, allowing lower dosages of additional analgesics to be used, so minimizing overall side-effects. It is a major ingredient in numerous cold and flu medications and many prescription analgesics. It is remarkably safe in recommended doses, but because of its wide availability, deliberate or accidental overdoses are fairly common. Paracetamol is now widely used in a variety of pharmaceutical products.

Paracetamol taken in over doses results hepatotoxicity and nephrotoxicity in men and experimental animals (Vermeulen *et al.,*1992). Hepatotoxicity is a general term for liver damage. The signs and symptoms of hepatotoxicity vary depending on how badly the liver is damaged. Symptoms of liver damage include: nausea vomiting, abdominal pain, Loss of appetite, diarrhea, feeling tired or weak, jaundice (yellowing of the skin and eyes), hepatomegaly (liver enlargement).

There is widespread use of paracetamol may predispose users to liver toxicity. However this effect may be masked due to several factors including nutrition, health, sex etc. The consumption of vegetable based diets such as *Telfairia occidentalis* in Nigeria may be linked to the prevention of liver toxicity in the abuse of paracetamol. This is the basis of this study.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 TELFAIRIA OCCIDENTALIS

Telfaria occidentalis (Fluted pumpkin) is one of the popular and widely grown vegetable crops in Nigeria particularly in the eastern (Anambra, Imo, Abia and Ebonyi States) and mid western areas (Edo, and Delta States) and to an appreciable degree in the south western states (Ondo, Ogun, Ekiti, Oyo and Lagos) (Okoli and Mgbeogu, 1982).

It is a pot-herb cultivated mainly for its succulent young leaves and shoots which are used as vegetables (Akobundu 1987). It is a high-climbing perennial with partial drought tolerance and parenting root system (Tindall, 1968). *Telfairia occidentalis* is a common homestead garden crop in southern Nigeria, mostly cultivated by women (Akoroda, 1988).

The crop is grown close to trees, walls, fences and structures on which the shoots are allowed to climb (Okoli and Mgbeogu, 1982). It could be allowed to creep on the ground or staked (Akoroda, 1988; NIHORT, 1986). Oyenuga (1968) recommended staking as the leaves of Telfairia spp are palatable and nutritious and are very much cherished by goats, while Akoroda *et al* (1990) supported staking because it facilitates harvesting of the leaves and pods.

Telfairia occidentalis belongs to the family Curcubitaceae and has simple, dark green veined leaves that is as wide as 18 cm and long as 35 cm. *T. occidentalis* contains nutrients such as proteins, carbohydrates, vitamins, minerals and fiber .The green leaves of fluted pumpkins are generally referred to as Ugu (Igbo) and these trailing green leaves rich in minerals and vitamins are used in various soup preparations such as Egusi and Edikang Ikong.

The roots and leaves of *Telfairia occidentalis,* have been shown to contain highly toxic alkaloids and saponins, glycosides and triterpenes (Akubue *et al*., 1980; Odoemena and Essien, 1995) The Leaves are also rich in essential and non-essential amino acids, vitamins and minerals (Tindal, 1968; Fasuyi, 2006). Studies have also shown that

Telfairia occidentalis leaf is rich in antioxidant phytochemicals such as vitamin C and phenols (Oboh and Akindahunsi, 2004; Oboh *et al.,* 2006).

2.1.1 MEDICINAL USES OF *TELFAIRIA OCCIDENTALIS*

Plants have been used medicinally in all civilizations. Despite the effectiveness of chemically synthesized medicines, screening for plant drugs will continue for the development of new pharmaceuticals to resolve both old and new health problems.

Aqueous extract of *Telfairia occidentalis* reduces blood sugar and increases haematological indices and reproductive indices in male rats. It also reduces blood glucose levels and therefore could be a hypoglycaemic agent (Salman *et al.,* 2008).

The study of the therapeutic effect of *Telfairia occidentalis* on Protein Energy Malnutrition-Induced Liver Damage shows that it has a restorative ability in the treatment of oxidative stress (Kayode *et al.,* 2009).

Telfairia occidentalis is used for the treatment of convulsion in ethno medicine, where the young leaves of are sliced and stored in a bottle to which coconut water and salt are added. It is also an indigenous plant used in ethnomedicinal treatment of anemia in Nigeria (Ehiagbonare, 2008).

2.2 SELENIUM

Selenium is a chemical element with the atomic number 34, represented by the chemical symbol Se, having an atomic mass of 78.96. It is a non-metal, chemically related to sulfur and tellurium, and rarely occurs in its elemental state in nature. It is a trace mineral that is essential to good health but required in only small amounts. Selenium is a group VI element and has both metallic and non-metallic properties. It can exist in 4 oxidation states (-2, +1, + 2, +6) and forms chemical components analogous to those of sulphur. Selenium is abundant in the earth's crust at concentrations of 50 to 90 $mgkg^{-1}$. High concentrations may be found in volcanic, sedimentary and some carbonate rocks. The concentration of selenium in soil varies from 5 to 1200000 $mgkg^{-1}$. (Francis *et al.,* 2006).

Selenium was discovered by the Swedish chemist Berzelius in 1817, but a biological role for this trace element remained unknown until 1957 when Schwarz and Foltz showed that selenium deficiency could cause necrotic liver degeneration (Schwarz and Foltz, 1957).

However, the first real understanding of the physiological basis for a selenium nutritional requirement did not occur until 1973, when it was shown that selenium was an essential component of mammalian enzymes like glutathione peroxidases (GPx) (Rotruck *et al.,* 1973; Flohe *et al.,* 1973). It is now well established that selenium plays an important biological role in living organisms, mostly through its incorporation in a family of proteins called selenoproteins.

2.2.1 SOURCES OF SELENIUM

Selenium occurs naturally in a number of inorganic forms, including selenide, selenate, and selenite. In soils, selenium most often occurs in soluble forms such as selenate (analogous to sulfate), which are leached into rivers very easily by runoff.

Plant foods are the major dietary sources of selenium in most countries throughout the world. The content of selenium in food depends on the selenium content of the soil where plants are grown or animals are raised. Selenium also can be found in some meats and seafood. Animals that eat grains or plants that were grown in selenium-rich soil have higher levels of selenium in their muscle. In the U.S., meats and bread are common sources of dietary selenium (Pennington and Schoen, 1996).

Specific dietary sources of selenium include brewer's yeast, wheat germ, butter, garlic, grains, sunflower seeds, Brazil nuts, walnuts, raisins, liver, kidney, shellfish (lobster, oyster, shrimp, scallops), and fresh-water and salt-water fish (red snapper, salmon, swordfish, tuna, mackerel, halibut, flounder, herring, smelts). Selenium is also found in alfalfa, burdock root, catnip, fennel seed, ginseng, raspberry leaf, radish, horseradish, onion, chives, medicinal mushrooms (reishi, shiitake), and yarrow.

2.2.2 EFFECT OF SELENIUM ON HEALTH

2.2.2.1 SELENIUM TOXICITY

Although selenium is an essential trace element, it is toxic if taken in excess. Exceeding the Tolerable Upper Intake Level of 400 micrograms per day can lead to selenosis (Zimmerman and Kohrle, 2002). This 400 microgram Tolerable Upper Intake Level is primarily based on a 1986 study of five Chinese patients who exhibited overt signs of selenosis and a follow up study on the same five people in 1992 (Beck *et al.,* 2003). The 1992 study actually found the maximum safe dietary Se intake to be approximately 800 micrograms per day (15 micrograms per kilogram body weight), but suggested 400 micrograms per day to not only avoid toxicity, but also to avoid creating an imbalance of nutrients in the diet and to account for data from other countries (Levander and Beck, 1997).

Symptoms of selenosis include a garlic odour on the breath, gastrointestinal disorders, hair loss, sloughing of nails, fatigue, irritability, and neurological damage. Extreme cases of selenosis can result cirrhosis of the liver, pulmonary edema, and death.

2.2.2.2 SELENIUM DEFICIENCY

Selenium deficiency is relatively rare in healthy, well-nourished individuals. It can occur in patients with severely compromised intestinal function, those undergoing total parenteral nutrition (Gram *et al.,* 1995), and also on advanced-aged people (over 90). People dependent on food grown from selenium-deficient soil are also at risk (Ravaglia, 2000).

Selenium deficiency may contribute to development of a form of heart disease, hypothyroidism, and a weakened immune system. Selenium deficiency is thought to contribute to autoimmune disease by making the body more susceptible to nutritional and biochemical stresses as well as infectious diseases. Three diseases caused directly by selenium deficiency include Keshan Disease, which causes an enlarged heart, Kashin-Beck Disease, which causes osteoarthropathy, and Myxedematous Endemic Cretinism, a form of hypothyroidism which results in mental retardation (Elaine, 2006).

Selenium deficiency has also been seen in people who rely on total parenteral nutrition (TPN) as their sole source of nutrition (Gramm *et al*., 1995). TPN is a method of feeding nutrients through an intravenous (IV) line to people whose digestive systems do not function. Forms of nutrients that do not require digestion are dissolved in liquid and

infused through the IV line. It is important for TPN solutions to provide selenium in order to prevent a deficiency (Abrams *et al.,* 1992.). Physicians can monitor the selenium status of individuals receiving TPN to make sure they are receiving adequate amounts.

2.3 PARACETAMOL

Paracetamol (para-acetyl-amino-phenol) is another name of acetaminophen (N-acetyl-para-aminophenol). It is a painkiller that is popular throughout the world because it is remarkably safe and does not irritate the stomach. It was first discovered to have both analgesic and antipyretic properties in the late nineteenth century (Rocha *et al.,* 2005).

2.3.1 METABOLISM OF PARACETAMOL

Paracetamol is metabolized primarily in the liver, where most of it (60-90% of a therapeutic dose) is converted to inactive compounds by conjugation with sulfate and glucuronide, and then excreted by the kidneys. Only a small portion (5-10% of a therapeutic dose) is metabolized via the hepatic cytochrome P450 enzyme system (specifically CYP2E1).The toxic effects of paracetamol are due to a minor alkylating metabolite , N-acetyl-p-benzo-quinone imine (NAPQI) produced by CYP2E1 (Dahlin *et al*., 1984). The metabolism of paracetamol is an excellent example of toxication, because the metabolite NAPQI is primarily responsible for toxicity rather than paracetamol itself.

At usual doses, the toxic metabolite NAPQI is quickly detoxified by combining irreversibly with the sulfhydryl groups of glutathione to produce a non-toxic conjugate that is eventually excreted by the kidneys.

2.3.2 PARACETAMOL TOXICTY

The toxic dose of paracetamol is highly variable. In adults, single doses above 10 grams or 200 mg/kg of bodyweight, whichever is lower, have a reasonable likelihood of causing toxicity (Dart *et al.,* 2006; Daly *et al,* 2008). Toxicity can also occur when multiple smaller doses within 24 hours exceeds these levels (Daly *et al,* 2008). Following a normal dose of 1 gram of paracetamol four times a day for two weeks,

patients can expect an increase in alanine transaminase in their liver to typically about three times the normal value (Watkins *et al.,*2006). It is unlikely that this dose would lead to liver failure (Dart and Bailey, 2007). Studies have shown significant hepatotoxicity is uncommon in patients who have taken greater than normal doses over 3 to 4 days (Daly *et al.,* 2004). In adults, a dose of 6 grams a day over the preceding 48 hours could potentially lead to toxicity (Daly *et al,* 2008), while in children acute doses above 200 mg/kg could potentially cause toxicity (Tenenbein, 2004). Acute paracetamol overdose in children rarely causes illness or death, and it is very uncommon for children to have levels that require treatment, with chronic larger than normal doses being the major cause of toxicity in children (Daly *et al.,* 2008).

2.4 LIVER

An adult human liver normally weighs between 1.4–1.6 kg (3.1–3.5 lb), and is a soft, pinkish-brown, triangular organ. It is both the largest internal organ (the skin being the largest organ overall) and the largest gland in the human body. The liver is a vital organ present in vertebrates and some other animals.

It also performs and regulates a wide variety of high-volume biochemical reactions requiring highly specialized tissues, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions (Maton *et al.,*1993).

The liver supports almost every organ in the body and is vital for survival. Because of its strategic location and multidimensional functions, the liver is also prone to many diseases. The most common include: Infections such as hepatitis A, B, C, E, alcohol damage, fatty liver, cirrhosis, cancer, drug damage (especially acetaminophen, cancer drugs).

Many diseases of the liver are accompanied by jaundice caused by increased levels of bilirubin in the system. The bilirubin results from the breakup of the haemoglobin of dead red blood cells; normally, the liver removes bilirubin from the blood and excretes it through bile. Diseases that interfere with liver function will lead to derangement of these processes. However, the liver has a great capacity to regenerate and has a large reserve capacity. In most cases, the liver only produces symptoms after extensive damage.

2.5 HEPATOTOXICITY

Hepatotoxicity (from *hepatic toxicity*) implies chemical-driven liver damage. There are several specific conditions that all fall within the general category of hepatotoxicity. These conditions include:

hepatitis -- inflammation of the liver

hepatic necrosis -- death of liver cells

hepatic steatosis -- too much fat in the liver; may be associated with a life-threatening condition called *lactic acidosis.*

Certain medicinal agents when taken in overdoses and sometimes even when introduced within therapeutic ranges may injure the organ. Other chemical agents such as those used in laboratories and industries, natural chemicals (e.g. microcystins) and herbal remedies can also induce hepatotoxicity. Chemicals that cause liver injury are called hepatotoxins. More than 900 drugs have been implicated in causing liver injury (Friedman *et al.,* 2003).

Other risk factors for developing hepatotoxicity include:

infection with hepatitis B or C virus

high levels of certain liver enzymes prior to starting anti-HIV medications

alcohol use

use of other medications that damage the liver

pregnancy

2.6 LIVER FUNCTION TESTS

Liver function tests (LFTs) are a helpful screening tool, which are an effective modality to detect hepatic dysfunction. It could also be defined as groups of clinical biochemistry laboratory blood assays designed to give information about the state of a patient's liver.Since the liver performs a variety of functions so no single test is sufficient to provide complete estimate of function of liver.

Liver function tests have various uses and limitations, and as a result, need for proper interpretations are required. They are helpful in recognizing the pattern of liver disease, such as, being helpful in differentiating between acute viral hepatitis and various cholestatic disorders and chronic liver disease. They are also helpful in assessing the severity and predict the outcome of certain diseases like primary biliary cirrhosis, as well as, being helpful in the follow up of certain liver diseases and evaluating responses to therapy like autoimmune hepatitis.

The liver function tests includes serum albumin, serum bilirubin, serum total protein, liver enzymes (Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphate (ALP), etc.), and prothrombin or clotting time.

The transaminases (aminotransferases) are the most frequently utilized and specific indicators of hepatocellular necrosis. These enzymes- aspartate aminotransferase(AST, formerly serum glutamate oxaloacetic transaminase-SGOT) and alanine amino transferase (ALT, formerly serum glutamic pyruvate transaminase-SGPT) catalyze the transfer of the α amino acids of aspartate and alanine respectively to the α keto group of ketoglutaric acid. ALT is primarily localized to the liver but the AST is present in a wide variety of tissues like the heart, skeletal muscle, kidney, brain and liver. Large increases in mitochondrial AST occur in serum after extensive tissue necrosis. Their activity in serum at any moment reflects the relative rate at which they enter and leave circulation.

2.7 LIPID PEROXIDATION

Lipid peroxidation is a well-established mechanism of cellular injury in both plants and animals, and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides are unstable and decompose to form a complex series of compounds including the reactive carbonyl compounds.

Lipid peroxidation can be defined as the oxidative deterioration of lipids containing any number of carbon-carbon double bonds. It is the process whereby free radicals take electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lie methylene -CH2- groups that possess especially reactive hydrogens. As with

any radical reaction the reaction consists of three major steps: initiation, propagation and termination.

Certain diagnostic tests are available for the quantification of the end products of lipid peroxidation, specifically malondialdehyde (MDA) (Marnett, 1999). The most commonly used test is called a TBARS Assay (thiobarbituric acid reactive substances assay).

Triglycerides are the chemical form in which most fat exists in food as well as in the body. They're also present in blood plasma and, in association with cholesterol, form the plasma lipids. Triglycerides are formed from a single molecule of glycerol, combined with three fatty acids on each of the OH groups, and make up most of fats digested by humans. Ester bonds form between each fatty acid and the glycerol molecule. The triglyceride level is a laboratory test to measure the amount of triglycerides in the blood, and thereby, used to determine one's risk of developing heart disease.

2.8 ANTIOXIDANTS

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves.

Types of antioxidant may be categorized into two; the antioxidant nutrients and the antioxidant enzymes. Antioxidants from our diet appear to be of great importance in controlling damage by free radicals. Each nutrient is unique in terms of its structure and antioxidant function. They include Vitamin E, Vitamin C, beta-carotene and selenium. On the other hand, the antioxidant enzymes includes; superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), which serve as the primary line of defence in destroying free radicals.

Glutathione is a water soluble antioxidant (hydrophilic antioxidant), which basically assist the body in the process of cell cytosol, and help out in the blood plasma. In other

words, they take a hands on approach to ridding the body of harmful free radicals and pollutants.

Glutathione (GSH) is found almost exclusively in its reduced form, since the enzyme that reverts it from its oxidized form (glutathione disulfide) (GSSG) , glutathione reductase, is constitutively active and inducible upon oxidative stress. In fact, the ratio of reduced glutathione to oxidized glutathione within cells is often used scientifically as a measure of cellular toxicity (Pastore *et al.,* 2003).

2.9 BLOOD GLUCOSE

A blood glucose test measures the amount of glucose (type of sugar) in the blood. Glucose comes from carbohydrate foods, and it is the main source of energy used by the body. Normally, blood glucose levels increases slightly after eating and this increase causes the pancreas to release insulin (a hormone) in order to prevent the blood glucose levels from getting too high.

There are several types of blood glucose test that could be used.

Fasting blood sugar (FBS): This measures the blood glucose after one has'nt eaten for at least 8 hours. It is often the first test done to check for prediabetes and diabetes.

2-hour postprandial blood sugar: This measures the blood glucose exactly 2 hours after one starts eating a meal.

Random blood sugar (RBS): This measures blood glucose regardless of when one last ate. Several random measurements may be taken throughout the day. Random testing is useful because glucose levels in healthy people do not vary widely throughout the day. Blood glucose levels that vary widely may mean a problem. This test is also called a casual blood glucose test.

Oral glucose tolerance test: This is a series of blood glucose measurements taken after you drink a sweet liquid that contains glucose. This is used to diagnose prediabetes and diabetes, especially to diagnose diabetes that occurs during pregnancy (gestational diabetes).

2.10 AIMS AND OBJECTIVES

The aims of this research is to:

Study the protective effect of consuming *Telfairia occidentalis* against paracetamol-induced hepatotoxicity.

Study the protective effect of selenium against paracetamol-induced hepatotoxicity.

Study the synergistic protective effect of *Telfairia occidentalis* and selenium against paracetamol-induced hepatotoxicity.

The objectives of this research are to assess the protective effects of either or both *Telfairia occidentalis* and selenium by:

Monitoring liver function by determining serum protein, and liver enzymes such as ALT and AST.

Assessing antioxidant levels by determining lipid peroxidation and reduced glutathione.

Determining haematological indices such as haemoglobin and WBC differentials.

Determining the blood glucose, cholesterol and triglyceride levels.

Checking histological changes on the liver and testes.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 REAGENTS, CHEMICALS AND TEST KITS

The reagents and chemicals used in this research work are shown in the table below:

Table 3.1.10: Reagents and chemicals with manufacturer's name.

	REAGENT NAME	MANUFACTURER
1.	Cholesterol test kit	Linear Chemicals
2.	Triglycerides test kit	Linear Chemicals
3.	GOT (ALT) test kit	Cypress Diagnostics
4.	GPT (ALT) test kit	Cypress Diagnostics
5.	Glucose test kit	Cypress Diagnostics
6.	Thiobarbituric acid	
7.	Hydrochloric acid	Analar
8.	Sodium Chloride	Riedel – de Haen®
9.	Formaldehyde	
10.	Sodium Hydroxide	Riedel – de Haen®
11.	Copper Sulphate	Brekland Scientific
12.	Sodium Potassium Tartarate	T.L.E Scientific
13.	Potassium Iodide	Riedel – de Haen®
14.	Bovine Serum Albumin	Fluka
15.	Tris-chloroacetic acid	Riedel – de Haen®

16.	Dipotassium hydrogen phosphate	Lab Tech Chemicals
17.	Potassium dihydrogen phosphate	
18.	5,5´-dithio(2-nitrobenzoic acid)	
19.	Ethylenediaminetetraacetic acid	Naafco

3.1.2 EQUIPMENTS

The equipments used in this research work are shown in the table below:

Table 3.1.20: Equipments showing their model and manufacturer's names

	EQUIPMENTS	MODEL	MANUFACTURER
1.	Spectrophotometer	Unispec 23D	Uniscope, England
2.	Table Centrifuge Machine	80-2	
3.	Digital top loading weighing balance	Adventurer pro HS 612	Adventurer ®Chaus, Switzerland
4.	Mortar and Pestle		
5.	Measuring cylinder (5ml, 10ml, 250ml, 500ml,1L)		
6.	Biofreezer	DW-40W	Haier thermocool, Nigeria
7.	Microcentrifuge	Mikro 120	Hettich Zentrifugen Germany
8.	Dissecting kit		
9.	Cannula		
10.	Disposable sterile syringes		Axiom drugs limited, Lagos, Nigeria.

11.	Lithium heparin and EDTA bottles		
12.	Oven		
13.	Thermometer		
14.	Magnetic Stirrer	Jenway 1000	Barloworld Scientific, England
15.	Shaking Water bath	SBS 40	Stuart Baloworld, England
16.	Micropipettes	CT 47805	Hirschmann®Laborgerate

3.1.3 PLANT MATERIAL

The plant material used was *Telfairia occidentalis* bought from Sango market in Ota, Ogun state, Nigeria. The plant was identified by Dr. Conrad Omohinmin, a plant scientist and taxonomist in the department of Biological Sciences, Covenant University, Ota.

3.1.4 ANIMALS

The animals used were male albino rats, obtained from University of Agriculture, Abeokuta, Ogun State, Nigeria.

3.2 METHODS

3.2.1 PREPARATION OF REAGENTS

3.2.1.1 PREPARATION OF REAGENTS FOR LIPID PEROXIDATION

HCl (4ml) was added to 250ml of distilled water. 2.96g of thiobarbituric acid (TBA) was added into the HCL - Distilled water mixture and mixed. 75g of Trichloroacetic acid (TCA) was then added to the TCA – HCL – Distilled water mixture and mixed.

200ml of distilled water was then added to make up to 500ml. It was then heated until it dissolved.

3.2.1.2 PREPARATION OF REAGENTS FOR REDUCED GLUTATHIONE

a) 0.1M of PO_4 buffer (pH 8.0) : Dipotassium hydrogen phosphate (K_2HPO_4) (0.373g) and 3.0g of Potassium dihydrogen phosphate (KH_2PO_4) was added to 200ml of distilled water.

b) 0.01% DTNB: DTNB [5, 5′-dithio (2-nitrobenzoic acid)] (0.002g) was weighed and added to 20ml of phosphate buffer.

c) Tris-chloroacetic acid (TCA): TCA (5g) was weighed and added to 100ml of distilled water.

3.2.1.3 PREPARATION OF REAGENTS FOR SERUM PROTEIN BY BIURET

a) 0.2M NaOH : NaOH (8g) pellets were dissolved in 1000ml of distilled water.

b) Biuret reagent: Hydrated $Cu.SO_4.5H_20$ (3g) and 9g of sodium potassium tartarate were dissolved in 500ml of 0.2M NaOH (8g/l). 15g of KI was then added and made up to 1L with 0.2M NaOH.

c) 2% Sodium potassium Tartarate: $KNaC_4O_6.H_2O$ (2g) was dissolved in distilled water and made up to volume of 100ml.

d) Potassium Iodide: KI (5g) was added and made up to 1L with 0.2M NaOH.

e) Bovine Serum Albumin: BSA (30mg) was dissolved in 3ml of normal saline.

3.2.2 PREPARATION OF PLANT MATERIAL

About 20kg of *Telfairia occidentalis* was picked, air-dried and ground to a powdered form.

3.2.3 FEED FORMULATION

Two diets namely control and test were formulated using feedstuffs as shown in table 3.3.30 shown below. The difference between the control and the test diet was the inclusion of 10% *Telfairia occidentalis* powder in the test diet.

Table 3.2.30: Feed formulation for Control and Test diet

Ingredients	Control diet(g)	Test diet(g)
White Maize	8910	7920
Flour Binder	4320	3840
Fish meal	135	120
Soya meal	1890	1680
Wheat Offals	4050	1200
P.K.C	2835	2520
Bone Meal	405	360
Grower Premix	54	48
Salt	54	48
lysine	13.5	12
Methionine	13.5	12
Groundnut cake	4320	3840
Telfairia occidentalis	_	2400

3.2.4 ANIMAL FEEDING AND DOSING

Thirty-five albino male rats obtained were randomly distributed into seven groups namely 1, 2, 3,4,5,6 and 7 consisting of five animals each. The rats were housed in cages and acclimatized for 4 weeks and weighed prior to commencement of feeding. Group 1 was fed control diet and not administered paracetamol or selenium. Group 2 and 6 were also fed control diet and administered paracetamol and selenium respectively. Groups 3, 4, 5 and 7 were the test groups fed with test diets. Group 3 did not receive paracetamol while group 4 received paracetamol. Group 5 were administered both paracetamol and selenium while group 7 received only selenium.

The test feeds were given throughout the experiment, while the paracetamol and selenium administration were given for five days in the last week of the experiment.

3.2.5 WEIGHT OF ANIMALS

The weights of animals were measured at the commencement of the experiment and subsequently every two weeks throughout the duration of the experiment.

3.2.6 COLLECTION OF BLOOD SAMPLES AND ORGANS

Blood samples were collected from the rats through cardiac puncture under mild anaesthesia using diethyl ether. The blood samples of each rat were then put into both heparin and EDTA bottles and a portion centrifuged at 3000g for 15 minutes to obtain plasma. Following the sacrifice of the rats, the liver and testes were excised and preserved in 10% formyl saline for histological studies.

3.2.7 DETERMINATION OF REDUCED GLUTATHIONE (Ellman, 1959)

Sample (0.5ml) was mixed with 0.5ml of 5% TCA in 1mM Ethylenediaminetetraacetic acid (EDTA) and centrifuged at 2000rpm for 10 minutes. 0.5ml of the supernatant was treated with 2.5ml of phosphate buffer (pH 8.0) and 0.2ml of 5,5´-dithio(2-nitrobenzoic acid) (DTNB) in phosphate buffer. Absorbance was read at 412nm against a blank.

3.2.8 DETERMINATION OF LIPID PEROXIDATION

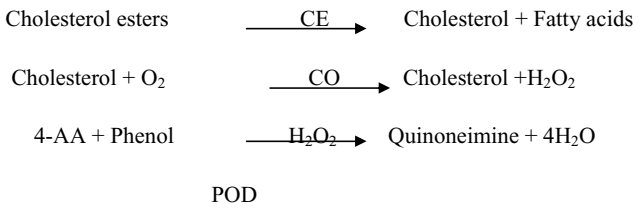
Lipid peroxidation in the liver was estimated spectrophotometrically by thiobarbituric acid reactive substances (TBARS) method of Buege and Aust (1978).

Plasma (0.1 ml) in 0.04M tri- HCl buffer was treated with 1.0ml of TBA – TCA – HCl reagent (thiobarbituric acid 0.37%, 0.25N HCl, 15% TCA) and incubated in water bath for 95°C for 15 minutes. The tube was then placed on ice, centrifuged and the absorbance of clear supernatant was measured against blank at 535nm.

3.2.9 DETERMINATION OF TOTAL CHOLESTEROL (Cypress test kit)

Principle :

This method for the measurement of total cholesterol in serum involves the use of three enzymes: cholesterol esterase (CE), cholesterol oxidase (CO) and peroxidase (POD). In the presence of the former the mixture of phenol and 4-aminoantipyrine (4-AA) are condensed by hydrogen peroxide to form a quinonemine dye proportional to the concentration of cholesterol in the sample.



Procedure:

The reagents and samples were brought to room temperature

The following volumes were then pipette into labeled tubes as shown in the table below:

TUBES	Blank	Sample	CAL. Standard
R1.Monoreagent	1.0ml	1.0ml	1.0ml
Sample	_	10µl	_
CAL.Standard	_	_	10µl

They were then mixed and the tubes incubated for 10minutes at room temperature. The absorbance (A) of the samples and standard were read at 500nm against the reagent blank.

CALCULATIONS:

$$\frac{A \text{ Sample}}{A \text{ Standard}} \times C \text{ Standard} = \text{mg/dL} \quad \text{total cholesterol}$$

3.2.10 DETERMINATION OF TRIGLYCERIDE (Cypress test kit)

Principle:

The method is based on the enzymatic hydrolysis of serum or plasma triglyceride to glycerol and free fatty acids (FFA) by lipoprotein lipase (LPL). The glycerol is phosphorylated by adenosine triphosphate (ATP). In the presence of glycerolkinase (GK) to form glycerol-3-phosphate (G-3-P) and adenosine diphosphate (ADP). G-3-P is oxidized by glycerophosphate oxidase (GPO) to form dihydroxyacetone phosphate (DHAP) and hydrogen peroxide.

A red chromagen is produced by the perioxidase (POD) catalyzed coupling of 4-aminoantiyrine (4-AA) and phenol with hydrogen peroxide (H_2O_2) proportional to the concentration of triglyceride in the sample.

Triglycerides + $3H_2O$ $\xrightarrow{LPL}$ Glycerol + 3FFA

Glycerol + ATP $\xrightarrow{GK}$ Glycerol-3-P + ADP

Glycerol-3-P + O_2 $\xrightarrow{GPO}$ DHA + H_2O_2

4-AA + 4 Phenol $\xrightarrow[POD]{H_2O_2}$ Quinoneimine + H_2O

Procedure:

The reagents and samples were brought to room temperature

The following in the table below were then pipette into labelled tubes:

TUBES	Blank	Sample	CAL. Standard
R1.Monoreagent	1.0ml	1.0ml	1.0ml
Sample	_	10µl	_
CAL.Standard	_	_	10µl

They were then mixed and the tubes allowed to stand for 15 minutes at room temperature. The absorbance (A) of the samples and standard were read at 500nm against the reagent blank.

CALCULATIONS:

$$\frac{A\ \text{Sample}}{A\ \text{Standard}} \times C\ \text{Standard} = \text{mg/dL}\ \ \text{Triglycerides}$$

3.2.11 DETERMINATION OF SERUM PROTEIN (BIURET METHOD)

Principle:

Compounds containing two or more peptide bonds will form violet coloured complexes with copper salts in alkaline solutions. This is due to a coordination of the cupric ions with the unshared electron pairs of the nitrogen of the peptide and the oxygen of water. The intensity of the coloured complex is directly proportional to concentration of the polypeptide present in solution.

PREPARATION OF CALIBRATION CURVE:

Six test tubes were labelled 1 – 6. Different volumes; 0, 0.2, 0.4, 0.6, 0.8, and 1.0ml, of Bovine Serum Albumin (BSA) solution were added to six test tubes. Distilled water was added to the test tubes. 4ml of Biuret's reagent was then added to each of the test tubes. The test tubes were allowed to stand for 30minutes and the absorbance was read at 520nm.

Procedure:

Sample (0.1ml) was added to a clean test tube, followed by 0.9ml of distilled water, then 4ml of Biuret's reagent. The test tube was then allowed to stand for 30minutes and the absorbance was read at 520nm. The calibration curve for the experiment was obtained by plotting absorbance against concentration.

3.2.12 DETERMINATION OF GLUCOSE

This test was carried out using a Glucose Enzymatic-colorimetric test kit. GOD-POD. Produced by CYPRESS DIAGNOSTICS.

Principle:

It is based on the oxidation of glucose by glucose-oxidation (GOD) to gluconic acid and hydroxide peroxide. The formed hydrogen peroxide (H_2O_2) is detected by a chromogenic oxygen acceptor, phenolaminophenazone in the presence of peroxidase (POD).

Glucose + O_2 + H_2O $\longrightarrow$ GOD H_2O_2 + fgvguuluconic acid

H_2O_2 + phenol 4-AP $\xrightarrow{\text{POD}}$ Quinone + $4H_2O$

The intensity of the color formed is proportional to the glucose concentration in the sample.

Procedure:

Wavelength of 505nm (490-550), temperature of 37^0 c($15\text{-}25^0$c) and a cuvette of 1cm light path was used. The instrument was adjusted by zeroing with distilled water. The following below was then pipette into a cuvette.

	Blank	Standard	Sample
Standard	___	10µl	___
Sample	___	___	10µl
Working Reagent	1.00ml	1.00ml	1.00ml

They were then mixed, and incubated for 15minutes at room temperature. The absorbance (Abs) of the sample was read against a standard blank.

3.2.13 DETERMINATION OF ALANINE TRANSAMINASE

This test was carried out using GPT (alt) U.V. Kinetic test kit.According to IFCC, produced by CYPRESS DIAGNOSTICS.

Principle :

Kinetic determination of GPT (ALT) activity according to the following reactions:

α –ketoglutarate + alanine $\xrightarrow{ALT}$ Glutamate + pyruvate

Pyruvate + NADH + H^+ $\xrightarrow{LDH}$ Lactate + NAD^+

The rate of NADH consumption is determined photometrically and is directly proportional to the GPT activity in the sample.

The test is carried out at a wavelength of 340nm and a temperature of 25, 30 or 37°C. The spectrophotometer was adjusted to zero with distilled water or air and 1 ml of the working sample reagent and 0.1 ml of test sample was then pipetted into a cuvette. It was then mixed and left to stand for 1 minute. The initial absorbance was read and the stopwatch was started and absorbances were read every minute for 3 minutes . The differences between the absorbances and the average absorbance differences per minute were calculated.

CALCULATION:

ALT (U/I) = Δ Abs. /min x 1750

3.2.14 DETERMINATION OF ASPARTATE TRANSAMINASE

Aspartate transaminase (AST) test was carried out using a Aspartate transaminase (AST) U.V kinetic test kit produced by CYPRESS DIAGNOSTICS.

Principle:

The kinetic determination of AST activity according to the following reaction;

α-ketoglutarate + Aspartate $\xrightarrow{\text{GOT}}$ Glutamate + Oxalacetate

Oxalacetate + NADH $\xrightarrow{\text{MDH}}$ Malate + NAD^+

The rate of NADH consumption was determined photometrically and is directly proportionally to the AST activity in the sample.

Procedure:

The test is carried out at a wavelength of 340nm and a temperature of 25, 30 or 37°C. The spectrophotometer was adjusted to zero with distilled water or air and 1 ml of the working sample reagent and 0.1 ml of test sample was then pipetted into a cuvette. It was then mixed and left to stand for 1 minute. The initial absorbance was read and the stopwatch was started and absorbances were read every minute for 3 minutes. The differences between the absorbances and the average absorbance differences per minute were calculated.

CALCULATION:

$$\text{AST (U/I)} = \frac{\text{Abs}}{\text{min}} \times 1750$$

3.2.15 DETERMINATION OF FULL BLOOD COUNT

3.2.15.1 PACKED CELL VOLUME (PCV)

The capillary tube was filled two-thirds to three-quarters full with well-mixed, One end of the tube was sealed with the sealant. The filled tube was placed in the microhematocrit centrifuge preset at 3000 rpm for 10 minutes. The tube was placed in

the microhematocrit reader and the hematocrit was read following the manufacturer's instructions on the microhematocrit reading device.

3.2.15.2 TOTAL WHITE BLOOD CELLS COUNT

Tox solution (0.38ml) was pipette and added to 0.02ml of whole blood sample which has been diluted i.e. 50: 1. The counting chamber was charged and the slide is dropped on it. It was placed under the microscope and the number of cells seen was multiplied by 50.

3.2.15.3 DIFFERENTIAL WHITE BLOOD CELLS COUNT

A drop of anticoagulated blood was used to make a thin and even smear or film, air dried for a few minutes and stained using the Lieshman's stain for about 15 to 30 seconds. The slide was removed and excess stain allowed to drain from the edge of the slide. The slide was immersed in distilled water for 5 to 15 seconds till when it became dark blue or when film forms on the surface. Excess water was drained and the back of the slide wiped to reduce background colour. The slide was placed in horizontal position on table and allowed to air dry. Counting the cells commenced when the slide was dry.

The slide was place under the microscope and viewed using the oil immersion objective (red) (100X) eye piece.

100 consecutive white cells were counted and each type of white cell were summed up in percentages until the count totaled 100% using the relative count.

3.3 STATISTICAL ANALYSIS

Results were expressed as mean ± SEM and analysed by one-way ANOVA using SPSS. Significance was checked at $P \leq 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 REDUCED GLUTATHIONE

Table 4.10: Reduced Glutathione levels in control and test groups

TREATMENT GROUPS	REDUCED GLUTATHIONE (mmol/ml)
Group 1(Control)	0.01 ± 0.001
Group 2	0.01 ± 0.001
Group 3	0.01 ± 0.001
Group 4	0.01 ± 0.003
Group 5	0.01 ± 0.000
Group 6	0.01 ± 0.001
Group 7	0.02 ± 0.003^{b}

Each data is expressed as mean ± standard error of mean.

Group 1 = Negative control (No dose of paracetamol and no treatment), and Group 2 = Positive control (dose of paracetamol and no treatment).

b = $P < 0.05$ versus Positive control.

The table above shows a significant increase ($P < 0.05$) in glutathione level of group 7 when compared with the positive control. There were no major changes between the negative and positive control.

4.2 LIPID PEROXIDATION

Table 4.20: Lipid peroxide ation levels in control and test groups

TREATMENT GROUPS	LIPID PEROXIDATION (nmol/ml)
Group 1(Control)	23.29 ± 0.47^{b}
Group 2	17.31 ± 2.83^{a}
Group 3	15.01 ± 0.92^{a}
Group 4	15.35 ± 0.97^{a}
Group 5	17.16 ± 0.67^{a}
Group 6	13.66 ± 0.07^{a}
Group 7	16.02 ± 0.54^{a}

Each data is expressed as mean ± standard error of mean.

Group 1 = Negative control (No dose of paracetamol and no treatment), and Group 2 = Positive control (dose of paracetamol and no treatment).

a = $P < 0.05$ versus Negative control, and b = $P < 0.05$ versus Positive control.

The table above shows the significant changes ($P < 0.05$) in levels of lipid peroxidation between the control and test groups. Group 1 shows the highest value of lipid peroxidation, while group 6 showed the lowest value. Group 2, 3, 4, 5, 6, and 7, showed significant decreases when compared to the negative control. Group 1 showed a significant increase when compared to the positive control.

4.3 TOTAL CHOLESTEROL

Table 4.30: Total cholesterol levels in control and test groups

TREATMENT GROUPS	TOTAL CHOLESTEROL (mg/dL)
Group 1(Control)	252.25 ± 11.41
Group 2	268.11 ± 6.53
Group 3	252.42 ± 3.40
Group 4	248.68 ± 3.16
Group 5	256.28 ± 11.36
Group 6	275.81 ± 4.19[a]
Group 7	243.41 ± 6.41[b,c]

Each data is expressed as mean ± standard error of mean.

Group 1 = Negative control (No dose of paracetamol and no treatment), Group 2 = Positive control (dose of paracetamol and no treatment) and Group 6 = Positive control (dose of selenium and no treatment).

a = $P < 0.05$ versus Negative control, b = $P < 0.05$ versus Positive control (paracetamol), and c = $P < 0.05$ versus Positive control (selenium).

The table above shows significant changes ($P < 0.05$) in total cholesterol levels between the control and test groups. Group 6 shows the highest value of cholesterol, while group 7 showed the lowest value. Group 6 showed significant increase when compared to the negative control. Group 7 showed significant decreases ($P > 0.05$) when compared to both positive control groups having paracetamol and selenium respectively.

4.4 TRIGLYCERIDE

Table 4.40: Triglyceride levels in control and test groups

TREATMENT GROUPS	TRIGLYCERIDE (mg/dl)
Group 1(Control)	229.56 ± 3.55
Group 2	224.83 ± 3.30
Group 3	216.22 ± 1.20
Group 4	214.25 ± 4.38[a]
Group 5	219.37 ± 5.04
Group 6	221.79 ± 6.51
Group 7	215.01 ± 7.27

Each data is expressed as mean ± standard error of mean.

Group 1 = Negative control (No dose of paracetamol and no treatment), and Group 2 = Positive control (dose of paracetamol and no treatment).

a = $P < 0.05$ versus Negative control.

The table above shows a significant decrease ($P < 0.05$) in triglyceride level of group 4 when compared with the negative control. Group 1 shows the highest value of triglyceride, while group 4 showed the lowest value.

4.5 SERUM PROTEIN

Table 4.50: Serum protein levels in control and test groups

TREATMENT GROUPS	SERUM PROTEIN (mg/ml)
Group 1(Control)	4.88 ± 1.32
Group 2	6.34 ± 0.25
Group 3	5.39 ± 0.87
Group 4	6.50 ± 0.66
Group 5	5.80 ± 0.95
Group 6	7.00 ± 0.40
Group 7	6.75 ± 0.85

Each data is expressed as mean ± standard error of mean.

Group 1 = Negative control (No dose of paracetamol and no treatment), and Group 2 = Positive control (dose of paracetamol and no treatment).

The table above indicates that there were no significant differences in the levels of serum protein at $P < 0.05$ in the control and test groups. Group 6 shows the highest value of triglyceride, while group 1 showed the lowest value. The positive control group had an increase in the serum protein level when compared to the negative control at a non significant level ($P > 0.05$).

4.6 GLUCOSE LEVELS

Table 4.60: Glucose levels in control and test groups

TREATMENT GROUPS	GLUCOSE (mg/dl)
Group 1(Control)	188.59 ± 45.48
Group 2	197.60 ± 16.84
Group 3	146.36 ± 23.16
Group 4	180.06 ± 9.03
Group 5	136.83 ± 5.70[b]
Group 6	197.61 ± 9.06
Group 7	166.22 ± 9.35

Each data is expressed as mean ± standard error of mean.

Group 1 = Negative control (No dose of paracetamol and no treatment), and Group 2 = Positive control (dose of paracetamol and no treatment).

b = $P < 0.05$ versus Positive control.

The table above shows a significant decrease in glucose level of group 5 when compared with the positive control. Group 2 shows the highest value of glucose, while group 5 showed the lowest value. The positive control had an increase in glucose level when compared to the negative control, although it was not significant ($P > 0.05$).

4.7 ALANINE TRANSAMINASE (ALT)

Table 4.70: Alanine transaminase levels in control and test groups

TREATMENT GROUPS	ALANINE TRANSAMINASE (IU L^{-1})
Group 1(Control)	18.86 ± 6.69
Group 2	6.13 ± 0.38
Group 3	6.42 ± 0.98
Group 4	28.15 ± 6.49
Group 5	33.98 ± 13.14[b]
Group 6	11.23 ± 4.05
Group 7	13.81 ± 5.64

Each data is expressed as mean ± standard error of mean.

Group 1 = Negative control (No dose of paracetamol and no treatment), and Group 2 = Positive control (dose of paracetamol and no treatment).

b = $P < 0.05$ versus Positive control.

The table above shows a significant increase ($p < 0.05$) in the ALT level of group 5 when compared with the positive control. Group 5 shows the highest value of ALT, while group 2 showed the lowest value. The positive control had a decrease in ALT level when compared to the negative control, although it was not significant ($P > 0.05$).

4.8 ASPARTATE TRANSAMINASE (AST)

Table 4.80: Aspartate transaminase levels in control and test groups

TREATMENT GROUPS	ASPARTATE TRANSAMINASE (IU L^{-1})
Group 1(Control)	20.80 ± 2.53
Group 2	24.27 ± 3.50
Group 3	18.67 ± 3.46
Group 4	26.69 ± 5.31
Group 5	29.17 ± 8.31
Group 6	22.90 ± 6.39
Group 7	33.64 ± 4.02

Each data is expressed as mean ± standard error of mean.

Group 1 = Negative control (No dose of paracetamol and no treatment), and Group 2 = Positive control (dose of paracetamol and no treatment).

The table above indicates that there were no significant differences ($P > 0.05$) in the levels of AST in the control and test groups. Group 7 shows the highest value of ALT, while group 3 showed the lowest value. The positive control had a non-significant increase ($P > 0.05$) in the serum protein level when compared to the negative control.

4.9 HAEMOGLOBIN

Table 4.90: Haemoglobin levels in control and test groups

TREATMENT GROUPS	HAEMOGLOBIN (g dL^{-1})
Group 1(Control)	13.33 ± 1.35
Group 2	11.27 ± 0.13
Group 3	13.92 ± 0.98^{b}
Group 4	12.25 ± 0.77
Group 5	12.92 ± 0.95
Group 6	13.67 ± 1.26
Group 7	15.00 ± 0.33^{b}

Each data is expressed as mean ± standard error of mean.

Group 1 = Negative control (No dose of paracetamol and no treatment), and Group 2 = Positive control (dose of paracetamol and no treatment).

b = $P < 0.05$ versus Positive control.

The table above shows significant differences in haemoglobin levels between the control and test groups. Group 3 and 7 showed significant increases of haemoglobin at $P < 0.05$, when compared with the positive control. Group 7 shows the highest value of haemoglobin, while group 2 showed the lowest value. The positive control had a decrease in haemoglobin value when compared to the negative control, although it was not significant at $P < 0.05$.

4.10 WHITE BLOOD CELLS (WBC)

Table 4.100: white blood cells in control and test groups

TREATMENT GROUPS	WHITE BLOOD CELLS (10^3 mm^{-3})
Group 1(Control)	8666.67 ± 333.33
Group 2	8300.00 ± 200.00
Group 3	8750.00 ± 144.34
Group 4	8375.00 ± 125.00
Group 5	8375.00 ± 375.00
Group 6	8750.00 ± 433.01
Group 7	9500.00 ± 500.00[b]

Each data is expressed as mean ± standard error of mean.

Group 1 = Negative control (No dose of paracetamol and no treatment), and Group 2 = Positive control (dose of paracetamol and no treatment).

b = $P < 0.05$ versus Positive control.

The table above shows a significant increase in WBC of group 7 when compared with the positive control. Group 7 shows the highest value of WBC, while group 2 showed the lowest value. The positive control had a decrease in WBC when compared to the negative control, although it was not significant at $P < 0.05$.

4.11 NEUTROPHILS

Table 4.110: Neutrophils in control and test groups

TREATMENT GROUPS	NEUTROPHILS (%)
Group 1(Control)	79.67 ± 1.76
Group 2	78.60 ± 0.24
Group 3	79.75 ± 1.55
Group 4	78.00 ± 0.41
Group 5	79.50 ± 1.85
Group 6	79.50 ± 1.55
Group 7	80.00 ± 1.00

Each data is expressed as mean ± standard error of mean.

Group 1 = Negative control (No dose of paracetamol and no treatment), and Group 2 = Positive control (dose of paracetamol and no treatment).

The table above indicates that there were no significant differences at $P < 0.05$ in the levels of neutrophils in the control and test groups. Group 7 shows the highest value of neutrophils, while group 4 showed the lowest value, and the positive control had a decrease in the neutrophils level when compared to the negative control at a non-significance of $P < 0.05$.

4.12 LYMPHOCYTES

Table 4.120: Lymphocytes in control and test groups

TREATMENT GROUPS	LYMPHOCYTES (%)
Group 1(Control)	20.33 ± 1.76
Group 2	21.40 ± 0.24
Group 3	20.25 ± 1.55
Group 4	21.75 ± 0.25
Group 5	20.25 ± 1.70
Group 6	20.50 ± 1.55
Group 7	20.00 ± 1.00

Each data is expressed as mean ± standard error of mean.

Group 1 = Negative control (No dose of paracetamol and no treatment), and Group 2 = Positive control (dose of paracetamol and no treatment).

The table above indicates that there were no significant differences at $P < 0.05$ in the level of lymphocytes in the control and test groups. Group 4 shows the highest value of lymphocytes while group 7 showed the lowest value, and the positive control had a decrease in the lymphocyte level when compared to the negative control at a non-significance of $P < 0.05$.

4.13 PACKED CELL VOLUME

Table 4.130: Packed Cell Volume in control and test groups

TREATMENT GROUPS	PACKED CELL VOLUME (%)
Group 1(Control)	40.00 ± 4.04
Group 2	33.80 ± 0.37
Group 3	41.75 ± 2.93[b]
Group 4	36.75 ± 2.32
Group 5	38.75 ± 2.84
Group 6	41.00 ± 3.76
Group 7	45.00 ± 1.00[b]

Each data is expressed as mean ± standard error of mean.

Group 1 = Negative control (No dose of paracetamol and no treatment), and Group 2 = Positive control (dose of paracetamol and no treatment).

b = $P < 0.05$ versus Positive control.

The table above shows significant differences in PCV levels between the control and test groups. Group 3 and 7 showed significant increases of PCV at $P < 0.05$ when compared with the positive control. Group 7 shows the highest value of PCV, while group 2 showed the lowest value. The positive control had a decrease in the PCV value when compared to the negative control, although it was not significant at $P < 0.05$.

4.14 WEIGHT

The weights of the rats were taken before commencement of the experiment, at two weeks interval for eight weeks, and the end of experiment. General increases in the weights of the rats were observed during the period of experiment, but final weight showed in general showed decreases.

4.14.1 WEIGHT OF RATS BEFORE EXPERIMENT

Table 4.14.10: weight of the rats before experiment of the control and test groups

TREATMENT GROUPS	WEIGHT (g)
Group 1 (negative control)	208.67 ± 6.57
Group 2 (positive control)	196.00 ± 8.51
Group 3	205.00 ± 10.08
Group 4	192.50 ± 17.04
Group 5	187.50 ± 6.24
Group 6	190.00 ± 5.94
Group 7	189.33 ± 5.81

Each data is expressed as mean ± standard error of mean.

The table above shows that there were no significant changes at $P < 0.05$ observed between the control and test groups. Differences in values between the groups were also observed. The positive control had a decrease in weight of value, when compared to the negative control.

4.14.2 WEIGHT OF RATS DURING EXPERIMENT

Group 1 = Negative control (No dose of paracetamol and no treatment), and Group 2 = Positive control (dose of paracetamol and no treatment).

a = $P < 0.05$ versus Negative control, and b = $P < 0.05$ versus Positive control.

Table 4.14.20: 1st and 2nd weights of the rats in the control and test groups during experiment

TREATMENT GROUPS	1st WEIGHT (g)	2ndWEIGHT (g)
Group 1 (negative control)	212.00 ± 12.49	234.33 ± 13.86
Group 2 (positive control)	194.40 ± 7.11	208.40 ± 7.28
Group 3	210.50 ± 17.04	242.50 ± 19.62[b]
Group 4	198.25 ± 12.55	240.00 ± 10.80
Group 5	184.50 ± 10.14	219.50 ± 6.08
Group 6	209.00 ± 12.12	229.50 ± 10.21
Group 7	207.33 ± 9.33	204.00 ± 17.44

Each data is expressed as mean ± standard error of mean.

The table indicates that there were no significant changes at $P < 0.05$ observed in the first two weeks (1st weight), although, there were differences in values of the weight between the groups, at non significant level.

There was a significant change observed at the 4th week (2nd weight). There was a significant increase of group 3, when compared to the positive control.

There was a general increase from the first weight and the second weight. Group 7 had a decrease in weight from the first weight to the second weight.

Table 4.14.30: 3rd and 4th weights of the rats in the control and test groups during experiment

TREATMENT GROUPS	3rd WEIGHT (g)	4th WEIGHT (g)
Group 1 (negative control)	234.00 ± 17.78	240.00 ± 18.52
Group 2 (positive control)	212.40 ± 6.65	210.80 ± 7.28
Group 3	243.50 ± 19.09	248.00 ± 19.49
Group 4	244.00 ± 9.56	243.50 ± 12.37
Group 5	223.25 ± 7.43	228.50 ± 7.27
Group 6	239.50 ± 10.21	244.00 ± 10.80
Group 7	200.67 ± 21.67	191.33 ± 24.06[a]

Each data is expressed as mean ± standard error of mean.

There were no significant changes at $P < 0.05$ observed at the end of the sixth week (3rd weight) between the control and test groups, although general increase in weights from the fourth week to the sixth week was observed.

A significant decrease at $P < 0.05$ was observed in group 7 when compared with the negative control. There were changes in weights of all groups from the sixth week to the eighth week. There was an increase in weight from the 3rd weight to the 4th weight of the negative control group, whereas, there was a decrease in weight from the 3rd to 4th weight of the positive control group. This could be exemplified in group 3 which received the test feed and had no administration of paracetamol, having an increase in weight from the 6th to the 8th week, whereas, group 4 which received test feed and administration of paracetamol, having a decrease in weight.

4.14.3 FINAL WEIGHT OF RATS AFTER EXPERIMENT

Table 4.14.40: final weights of the rats after the experiment for the control and test groups

TREATMENT GROUPS	WEIGHT (g)
Group 1 (negative control)	160.33 ± 40.83
Group 2 (positive control)	197.60 ± 11.79
Group 3	244.00 ± 20.77[a]
Group 4	225.50 ± 8.85[a]
Group 5	201.00 ± 13.92
Group 6	216.75 ± 7.78
Group 7	170.00 ± 32.15

The table above shows that there were significant changes at $P < 0.05$ observed between group 3 and group 4, when compared to the negative control. Group 3 had a significant increase, as well as group 4. It was also observed that group 3 had higher weight than group 4, and also, had a smaller decrease in weight from the 4^{th} weight to the final weight, than that of group 4, which had a higher decrease

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The status of *Telfairia occidentalis* and selenium in paracetamol-induced hepatotoxic male albino rats are shown in tables 4.10 – 4.14.40 (chapter four).

Edible vegetable is a vital component of human diet that should be eaten all year round (Aliyu, 2006). Leaves of *Telfairia occidentalis* are highly palatable and consumed largely in a variety of diets in Nigeria especially the South-Eastern region. This study showed increases in weights of the rats as the research progressed, as well as significant changes in weight between groups. This may be as a result of the rich nutritional contents of *Telfairia occidentalis*-supplemented diet.

In a study of *Telfairia occidentalis,* it has been revealed that both the ethanolic and aqueous extracts of *Telfairia occidentalis* leave contain saponin, alkaloid and tannins (oboh *et al.,* 2006). Saponins are known to form large mixed micelles with steroids (Oakenfall *et al.*, 1984). Ingested saponins remain in the gastrointestinal tract and interact with cholesterol producing complexes, which prevent their absorption. The complexes with other materials (bile acid-saponins complexes) are then excreted via faeces and replenished by the metabolism of cholesterol in the liver. Thus, the elimination of bile acid-saponins complexes may result in a decrease in the cholesterol level. This is in line with this study, which showed that *Telfairia occidentalis* was significantly reduced when compared with the control.

Glutathione is one of the most abundant tripeptide, nonenzymatic biological antioxidant present in the liver. It removes free radical species such as hydrogen peroxide, superoxide radicals and maintains membrane protein thiols. Also it is substrate for glutathione peroxidase (GPx) (Prakash *et al.*, 2001). Selenium plays an important role in living organisms, mostly through its incorporation in a family of proteins called selenoproteins, which are important antioxidant enzymes. This selenoproteins includes various types, of which glutathione peroxidase is included (Laura *et al.,* 2007). This study showed a significant increase in glutathione level, for the group containing both selenium and *Telfairia occidentalis.* This is indicative of the fact that selenium has

protection against hepatotoxic effects of acetaminophen (Schnell *et al., 1988*). This was accompanied by an increase in the glutathione levels in selenium-treated animals and an inhibition in the decrease in glutathione content observed in animals receiving hepatotoxic doses of acetaminophen.

Study on the aqueous extract of *Telfairia occidentalis* has been found to be significantly reduce blood glucose (Salmon *et al.,* 2008), and also, selenium has been seen to reduce the high level of serum glucose in diabetic rats (Iizuka *et al.,* 1992). The significant reduction in glucose level for this present study in group 5, suggests a positive synergetic effect between *Telfairia occidentalis* and Selenium in the reduction of blood glucose.

There has been known to be an increase in haematological indices in rats when fed with diet prepared with air-dried leaves of *T. occidentalis* for four weeks (Alada, 2000). This is also in consistent with the observations in the remarkable increase of hemoglobin in animals fed with *T. occidentalis*-supplemented diet (Iweala and Obidoa, 2009). The aqueous extracts of *T. occidentalis* has also been seen to have significant increases in packed cell volume, haemoglobin concentration, red blood cell count and white blood cell count (Salmoan *et al.,* 2008). This can also be accompanied in this present study which showed that long term comsumption of *Telfairia occidentalis*-supplemented diet had significant increases of the haematological indices (packed cell volume, hemoglobin, and white blood cells) at $P < 0.05$. This finding could be related to the presence of some haematological factors including proteins, iron, thiamine, riboflavin and nicotinamide in the leaves of *T.occidentalis* (Tindal, 1968; Fasuyi, 2006). For instance, iron is a well known haemopoietic factor that has direct influence on the production of blood in the bone marrow (Ganong, 2005). It has also been known that selenium may be beneficial for hemoglobin synthesis and erythropoesis (Oster *et al.,* 1988). This study also suggests a positive synergetic effect of *T.occidentalis* and selenium for the higher significant increases in haematological indices.

The changes in the total protein in this study were not statistically significant. This is accompanied by the study of the long term comsumption of *Telfairia occidentalis*-supplemented diet by Iweala and Obidoa (2009), which also showed a non significance of serum protein. This could be associated with the fact that serum proteins are largely

produced in the liver and Oboh (2005) suggested that there could be some level of hepatoprotective effects of *Telfairia occidentalis*-supplemented diet on rats.

The concentration of circulating triglycerides may be an early and reliable indicator of hepatotoxicity in rat and so, its assessment has been recommend as a part of the core list of liver parameters in preclinical studies. This study shows significant decrease in triglyceride level in the group that received *Telfairia occidentalis* and paracetamol. This could be explained by *Telafairia occidentalis* having hepatoprotective properties (Oboh, 2005), as well as the fact that, decreases in triglycerides are metabolic counterparts of an intrinsic toxicity most probably involving an increase in the catabolism of lipids, an impairment of their synthesis, or transportation associated with effects on VLDL particles (Provost *et al.,* 2003).

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PART II

CHAPTER 1

INTRODUCTION

In addition to vitamins and provitamins in fruits and vegetables, the presence of bioactive plant components, often called phytochemicals, has been considered of crucial nutritional importance in the prevention of chronic diseases, such as cancer, cardiovascular disease and diabetes (Aruoma, 2003). Because prevention is a more effective strategy than treatment for chronic diseases, a constant supply of phytochemical-containing plants with desirable health benefits beyond basic nutrition is essential to furnish the defensive mechanism to reduce the risk of chronic diseases in humans.

Peppers are fruits with a high importance in human diet due to their versatility to be consumed as fresh vegetable in salads, cooked meals or dehydrated for spices. Mature red peppers are considered one of the richest sources of natural pigments (carotenoids), and are thus used as a food colourants in the form of ground powder (Hornero-Méndez *et al.*, 2002). Apart from carotenoids, pepper fruit at the red stage is rich in vitamin C and phenolic compounds such as phenolic acids, flavonoids, hydroxycinnamates and flavones (Marín *et al.*, 2004). Moreover, fresh pepper is considered to be one of the vegetables with the highest content of vitamin C within the plant kingdom. Additionally, it is well known that phenolic compounds contribute to fruit sensory and nutritive quality in terms of modifying colour, taste, aroma and flavour, and also providing health-beneficial effects (Tomás-Barberán and Espín, 2001).

Plant-derived antioxidants exert their effects by enhancing the levels of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase or by lowering the levels of lipid peroxides in the blood or liver (Usoh *et al*., 2005; Tseng *et al.*, 1997). It is recognized that antioxidant (mainly polyphenolic) compounds from plant extracts can act by either free radical scavenging (Lodovici *et al*., 2001; Re *et al*., 1999), singlet oxygen quenching (Foley *et al*., 1999), chelating of transitional metal such as iron (Brown *et al*., 1998), as well as a reducing agents and activators of antioxidative defense enzyme systems to suppress radical damage in biological system (Zielinski and

Kozlowska, 2000) Also, when added to foods, antioxidants minimise rancidity, retard the formation of toxic oxidation products, maintain nutritional quality, and increase shelf life (Jadhav *et al.,* 1996).

In general, it seems that the majority of malignant disturbances are associated with an increase in free radical concentration (McBrien and Slater, 1982). Free radicals are undoubtedly involved in many degenerative processes including, cancer, cardiovascular diseases, arteriosclerosis, neural disorders, skin irritations and inflammations. Reperfusion injuries, e.g., in the aftermath of surgery, are also typically characterized by an enhanced formation of free radicals (Bulkley, 1987). Any metabolism involving redox active centers is a possible source of continuous free radical production. This certainly allows the extrapolative conclusion that any stress situation, carries the potential of excess free radical production associated with all the chemical consequences arising thereof. There is increasing evidence from a great variety of studies that even aging is the result of the limited ability of biological systems to avoid stress, especially oxidative stress, and to cope with the resulting molecular damage (Martin *et al.,*1996; Melov *et al.,* 2000; Kirkwood, *et al.,* 2000).

Foods from plant origin usually contain natural antioxidants that can scavenge free radicals. The human body is equipped with an antioxidant defense system that deactivates these highly reactive free radicals (Bulkley, 1987). This includes antioxidants enzymes (made in the body) and antioxidant phytochemicals that counterbalance the excess reactivity that these free radicals have, turning them to harmless particles or waste products that can be gotten rid of (Doblado *et al*., 2005; Oboh and Akindahunsi, 2004; Oboh, 2006). The most likely and practical way to fight against most of these degenerative diseases is to improve body antioxidant status, which could be achieved by higher consumption of food rich in antioxidant phytochemicals. Peppers are very good example of antioxidant rich foods.

This study therefore focuses on *Capsicum species* and their potential to improve the antioxidant status in living systems. It goes further to separate the different chemical components of 5 pepper cultivars into sub-fractions on the basis of polarity. These subfractions are then tested for their ability to scavenge free radicals.

CHAPTER 2

LITERATURE REVIEW

2.1 EXTENSIVE NOTE ON THE GENUS

Capsicum has been known since the beginning of civilization in the Western Hemisphere. It has been a part of the human diet since about 7500 BC (MacNeish, 1964). It was the ancient ancestors of the native peoples who took the wild chili piquin and selected for the many various types known today. Heiser (1976) states that the native Americans had begun to grow chilli plants between 5200 and 3400 BC. Chilies are therefore among the oldest cultivated crops of the America's.

The words pepper, chili, chile, chilli, aji, paprika, and Capsicum are used interchangeably for plants in the genus Capsicum. Capsicum investigators use chili, pepper, or aji, as vernacular terms. *Capsicum* is reserved for taxonomic discussion. (Domenici, 1983). Confusion may occasionally arise within species designation, because *C. annum* was sometimes called *C. frutescens* in the scientific literature (Heiser and Smith, 1953).

The genus Capsicum is a member of the Solanaceae family that includes tomato, potato, tobacco, and petunia. The genus Capsicum consists of approximately 22 wild species and five domesticated species (Bosland, 1994): C. annum, C. baccatum, C. chinense, C. frutescens, and C. pubescens. Capsicum is a perennial small shrub in suitable climatic conditions, living for a decade or more in tropical South and Central America. Capsicum probably evolved from an ancestral form in the Bolivia/Peru area (Heiser, 1976). Chile fruits are considered vegetables, but are berries botanically. Chile types usually are classified by fruit characteristics, i.e. pungency, color, shape, flavor, size, and their use (Smith *et al*., 1987; Bosland, 1992). Despite their vast trait differences most chile cultivars commercially cultivated in the world belong to the species, *C. annum.* The tabasco (*C. frutescens*) and habañero (*C. chinense*), are the best-known exceptions (Smith *et al*., 1987; Bosland, 1992).

The reported life zone for capsicum peppers is 7 to 29 degrees centigrade with an annual precipitation of 0.3 to 4.6 meters and a soil pH of 4.3 to 8.7 . Capsicum species are cold sensitive and generally grow best in well-drained, sandy or silt-loam soil.

Plantings are established by seeding or transplanting. Flowering usually occurs three months after planting. Hot and dry weather is desirable for fruit ripening. Fruit is generally handpicked as it ripens, and then allowed to dry in the sun for spice production in Nigeria. However most Nigerians prefer fresh pepper for culinary purposes. The fruit may be ground intact or after the removal of seeds, placenta parts, and stalks, increasing the fruit color and lowering the pungency. The level of pungency of the Capsicum species depends upon the concentration of capsaicinoids, primarily of capsaicin, in the fruit. Capsicum peppers are classified commercially by the concentration of capsaicinoids, since confusion about the biological identities of some varieties has made other methods unreliable. Paprika comes from plants with 10 to 30 parts per million capsaicinoids, chili peppers from plants with 30 to 600 parts per million, and red peppers from plants with 600 to 13,000 parts per million. The chemical composition of the Capsicum species includes a fixed oil, pungent principles, volatile oil, and carotenoid, mostly capsanthin, pigments. An oleoresin is obtained by solvent extraction. Capsicum frutescens L. is much more pungent than Capsicum annum L(Smith *et al*., 1987; Bosland, 1992).

2.2 BREEDING

In breeding chilies, the choice of breeding method depends on the breeding objective and the plant material being cross-bred (Greenleaf, 1986). The strategy of the chile breeder is to assemble into a cultivar the superior genetic potential for yield, protection against production hazards, and improved quality. Chile cultivars have been developed using selection within introductions and hybridization followed by selection. Hybridization is usually always within a species, but interspecific crosses, especially C. annum by C. chinense have been accomplished successfully. Selection methods have included mass, single plant, backcross, and pedigree. Single seed descent and haploid breeding have also been applied to chiles (Allard, 1960). The only breeding technique so far not applicable to chilies is genetic transformation. This is because the technique to regenerate whole chili plants from single cells has not been ascertained. With the success of other solanaceous crops, e.g. tomato and petunia, the parameters necessary for successful regeneration of chile will be forth coming. Chile plants are considered a self-pollinating crop (Allard, 1960).

2.3 DOMESTIC AND TRADITIONAL USES OF PEPPER

In Nigeria, *Capsicum* species are used fresh or dried, whole or ground, and alone or in combination with other flavoring agents. Capsicum annum L. is used in Tomapep and other red pepper products. The *C. chinens*e popularly called *ata-rodo* in Yoruba is used with the red bell pepper and tomato to make various sauces to eat a large variety of foods (a good example is the popular *ofada* rice). The hausa people of Nigeria use C. annum along with other spices to season foods such as *kilishi and suya*. Fruits of Capsicum annum L., are also widely used as coloring agents. The extracts of Capsicum species have been reported to have antioxidant properties. Chilies and chili pepper from cultivars of Capsicum annum L. and Capsicum frutescens L. are employed as a flavoring in many foods, such as curry powder and Tabasco sauce. Chili powder is a blend of spices that includes ground chilies. Red or hot peppers from Capsicum annum L. and Capsicum frutescens L. are the most pungent peppers and are used extensively in Mexican and Italian foods. Cayenne pepper is the ground product derived from the smaller, most pungent Capsicum species (Carmichael, 1991).

As a medicinal plant, the Capsicum species has been used as a carminative, digestive irritant, stomachic, stimulant, rubefacient, and tonic. The plants have also been used as folk remedies for dropsy, colic, diarrhea, asthma, arthritis, muscle cramps, and toothache (Carmichael, 1991). Capsicum frutescens L. has been reported to have hypoglycemic properties. Prolonged contact with the skin may cause dermatitis and blisters, while excessive consumption can cause gastroenteritis and kidney damage. Paprika and cayenne pepper may be cytotoxic to mammalian cells in vitro. Consumption of red pepper may aggravate symptoms of duodenal ulcers. High levels of ground hot pepper have induced stomach ulcers and cirrhosis of the liver in laboratory animals. Body temperature, flow of saliva, and gastric juices may be stimulated by capsicum peppers (Carmichael, 1991).

The medicinal applications of capsaicinoids have brought innovative ideas for their use. Medicinal use of Capsicums has a long history, dating back to the Mayas who used them to treat asthma, coughs, and sore throats. The Aztecs used chile pungency to relieve toothaches. The pharmaceutical industry uses capsaicin as a counter-irritant balm for external application (Carmichael, 1991). It is the active ingredient in Heet and Sloan's Liniment, two rubdown liniments used for sore muscles. The capsaicin is being

used to alleviate pain. Its mode of action is thought to be from nerve endings releasing a neurotransmitter called substance P. Substance P informs the brain that something painful is occurring. Capsaicin causes an increase in the amount of substance P released (Tewksbury and Nabhan, 2001). Eventually, the substance P is depleted and further releases from the nerve ending are reduced. Creams containing capsaicin have reduced pain associated with post-operative pain for mastectomy patients and for amputees suffering from phantom limb pain (Tewksbury and Nabhan, 2001). Prolonged use of the cream has also been found to help reduce the itching of dialysis patients, the pain from shingles (Herpes zoster), and cluster headaches. Further research has indicated that capsaicin cream reduces pain associated with arthritis. The repeated use of the cream apparently counters the production of substance P in the joint, hence less pain. Reducing substance P also helps by reducing long-term inflammation, which can cause cartilage break down (Tewksbury and Nabhan, 2001).

Birds do not have the same sensitivity to capsaicin, because it targets a specific pain receptor in mammals. Chili peppers are eaten by birds living in the chili peppers' natural range. The seeds of the peppers are distributed by the birds that drop the seeds while eating the pods, and the seeds pass through the digestive tract unharmed. This relationship may have promoted the evolution of the protective capsaicin (Tewksbury and Nabhan , 2001).

Table 2.1:SCIENTIFIC CLASSIFICATION OF PEPPER

Kingdom:	Plantae
(phylum):	Angiosperms
(class):	Eudicots
(sub class):	Asterids
Order:	Solanales
Family:	Solanaceae
Genus:	Capsicum

(Walsh and Hoot, 2001)

BotanicalName:*Capisicum* annum

Common Names: bell pepper(RED).

(green)

traditional name: *Tatase*

BotanicalName: *Capisicum* annum

Common Names: bell pepper

Traditional name: *Tatase dudu*

Botanical Name: Capisicum annum

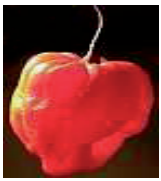
Common Names: Thai peppers.

Traditional Name:*Sombo (Shomboh)*

BotanicalName: Capisicum frutescens

Common Names: cayenne

Traditional name: *shombo (shomboh)*



BotanicalName: *Capisicum chinense*

Common Names: Scotch bonnet

Traditional name: *Ata rodo*

Fig.2.2 *Picture of two different cultivars of capsicum annum, one cultivar of Capsicum frutescence and one cultivar of capsicum chinense.*

2.4 PHYTOCHEMICALS/BIOCHEMICAL COMPOUNDS IN

PEPPER

Peppers are fruits with a high importance in human diet due to their versatility to be consumed as fresh vegetable in salads, cooked meals or dehydrated for spices. Traditionally, commercial growers have harvested peppers at the mature green stage when pericarp becomes thick and the fruit has reached its maximum size. However, in recent years, there has been an increasing interest in picking peppers at the red colour with improved flavour, nutritional aspects and high acceptation by consumers (Frank *et al.*, 2005). In fact, mature red peppers are considered one of the richest sources of natural pigments (carotenoids), and is thus used as a food colourant in the form of ground powder (Hornero-Méndez *et al.*, 2002). Apart from carotenoids, pepper fruit at the red stage is rich in vitamin C and phenolic compounds such as phenolics acids, flavonoids, hydroxycinnamates and flavones (Marín *et al.*, 2004). Moreover, fresh pepper is considered to be one of the vegetables with the highest content of vitamin C within the plant kingdom. Additionally, it is well known that phenolic compounds contribute to fruit sensory and nutritive quality in terms of modifying colour, taste, aroma, flavour and also providing health-beneficial effects (Tomás-Barberán and Espín, 2001).

2.4.1 CAPSAICIN

The chemical capsaicin is the source of the chili pepper's spicy sensation. Pepper plants in the genus *Capsicum* produce capsaicin in glands located inside the pepper at the meeting point of the placenta and the pod. Surprisingly, the seeds do not contain any

capsaicin; rather the white connective pith in the fruit's center contains the highest concentration of capsaicin (Mortensen and Mortensen, 2008).

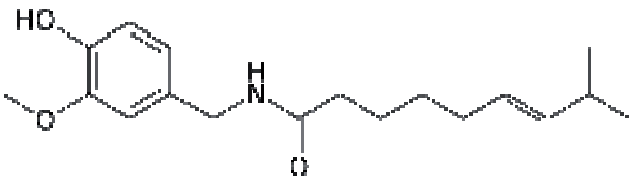


Fig. 2.1 structure of capsaicin (Formula: $C_{18}H_{27}NO_3$)

2.4.1.1 Mechanism of Action

A complex series of reactions causes a person to sense heat when eating capsaicin-containing foods. The specialized capsaicin receptors are located on the taste buds within the papillae of the tongue. The receptor responsible for detecting capsaicin is called transient receptor potential vanilloid-1 (TRPV1). Capsaicin's chemical structure allows it to bind to TRPV1. In the presence of capsaicin, a lipid portion of the receptor called PIP2 separates and allows calcium ions to enter the receptor cell (Mortensen and Mortensen, 2008). A pain message is then carried to the brain by substance P, a neurotransmitter. The bond between capsaicin and TRPV1 is temporary, so feeling of pain subsides when the bond is broken. Accordingly, people with a greater number of taste buds are often more sensitive to foods containing capsaicin. Chili peppers containing capsaicin not only cause the fire in a person's mouth, but they also affect the body in other ways. Peppers increase the production of stomach acids that stimulate the digestive tract to start a cleansing process. Peppers also increases metabolic rates and play a role in the body's fat molecules metabolism (Mortensen and Mortensen, 2008).

Capsaicin causes the brain to release endorphins that cause a sense of well-being or euphoria that can last for several hours (Mortensen and Mortensen, 2008). Capsaicin can also alleviate pain by over-stimulating the release of substance P, which functions as a link between primary receptors and the brain. The over-simulation causes substance P levels to drop and, thus, eliminates the sense of pain (Mortensen and Mortensen, 2008). It has recently been reported that tarantula venom activates the same neurological pathway as capsaicin. It is not clear what the significance of the relationship might be (Mortensen and Mortensen, 2008).

2.5 APPLIED ANTIOXIDANT ACTIVITY

Ai Mey *et al.,* (2008) studied the Effect of cooking on the antioxidant properties of coloured peppers and discovered that Loss of antioxidant activity was greater in the cooked tissues with prolonged boiling time.

Elizabeth (2002) evaluated capsicum as a source of natural antioxidants in preventing rancidity in sunflower oil.

2.6 ANTIMICROBIAL/ BIOCHEMICAL ACTIVITIES OF PEPPER

Ethanol extracts of the fruits of three kinds of Capsicum showed similar potencies in their antimicrobial activities against Gram (+) and Gram (-) bacterias, and fungi, although they contained different levels of capsaicin. Results from Bioautographic tests demonstrated that capsaicin was the main antimicrobial component. At least two other non-polar components of ethanol extract also contributed in the antimicrobial activity and very likely that these compounds were responsible for the activity toward Pseudomonas aeruginosa. (Soetarno *et al*., 1997)

In the sweet *Capsicum annum* L. fruits several new natural compounds have been isolated and identified. The known capsidiol showed batteriostatic properties in vitro against *Helicobacter pylori* with a minimum inhibitory concentration (MIC) of 200 mg/mL when compared with the commercial drug metronidazole (MIC, 250 mg/mL). *H. pylori* is strongly associated with the ulcer disease, and has recently been recognized as a probable cofactor in the development of gastric cancer (Ando, *et al.,* 2006). Thus treatment with capsidiol may be a useful treatment for antibiotic-resistance strains and for patients who do not wish to take synthetic antibiotics (De Marino *et al.,* 2006).

While carrying out some Pharmacokinetic studies, Kamon et al., (2009) postulated that Capsaicin in *Capsicum frutescens* was able to Decrease Plasma Glucose Level. The results of the present study are consistent with a previous report by Tolan (2004). They performed the test on canines under OGTT method and found that the fresh capsicum had lower plasma glucose levels and higher insulin levels than those in the placebo group. They suggested that plasma glucose decrease could be caused by capsicum inducing insulin-secretion (Tolan 2004; Tolan ,2001)

2.7 ANTIOXIDANTS AND FREE RADICALS

When there are more free radicals (reactive oxygen species) in the human body than antioxidants, the condition is called oxidative stress. (Morgan , 1997). Oxidative stress induced diseases encompass cardiovascular diseases, hypertension, chronic inflammatory diseases and diabetes. (Nawar, 1996; Iverson, 1995; Huang and Ferraro 1992; Aruoma *et al.,* 1992). The antioxidant activity of foods has been tested using a wide variety of methods. Antioxidant activity is the capacity to prevent auto-oxidation of free-radical mediated oxidation of the substrate when present in low concentration (Halliwell, 1992).

2.8 FREE RADICALS-MECHANISM OF ACTION

Most of the radicals relevant in biological systems are either derived from or associated with the presence of molecular oxygen, in particular, the superoxide anion (O_2^-) and peroxyl radicals (ROO^-). Other oxygen centered and highly reactive radical species are hydroxyl (^-OH) and oxyl radicals (RO^-) (Chatgilialoglu *et al.,* 1990). Their biological significance is not in doubt but somewhat more in the debate than that of O_2^- and ROO^-. It is not only oxygen-centered free radicals which are of importance and interest. Many others generated both from endogenous as well as exogenous substrates are also of significance. A very relevant example is the thiyl radical, RS^-, which is the one-electron redox intermediate between thiols and disulfides. Thiols and disulfides are two vital classes among the biologically abundant substrates (Chatgilialoglu *et al.,* 1990). The

action of free radicals is generally determined by their chemical reactivity and the availability of a suitable reaction partner in the vicinity of their production site. In some cases, a free radical molecule interaction may directly lead to a biological damage in a few or even just one reaction step (von Sonntag, 1985). Thus, an •OH reaction with the sugar moiety of DNA can result in a strand break due to a radical specific phosphate cleavage reaction (von Sonntag, 1985).

However, such a singular event does, not necessarily imply cell death as there are enzymatic and chemical, possibly even radical associated repair mechanisms. Nature and modern medicine related science have provided us with mechanisms and substrates to cope with free radicals by "deactivation". Enzymes such as the SOD serve this function.

Another important group of compounds in this respect are the antioxidants (Packer and Glazer, 1990; Emerit *et al.,* 1996). The most prominent representatives of antioxidants are vitamin E (a-tocopherol) and vitamin C (ascorbate) which are most effective free radical scavengers in the lipid cell membrane and adjacent, more aqueous compartments, respectively. The same function is observed in many other redox-active compounds such as thiols, carotenoids, quinones, *etc.* Whenever a free radical reacts with a molecular compound it loses its identity and at the same time it transfers its general radical properties to a new radical formed in the reaction. It is therefore necessary to know both the properties of the initiating and the subsequently generated species in order to assess the action of free radicals (Packer and Glazer, 1990; Emerit *et al.,* 1996).

2.9 FREE RADICAL ACTION IN VARIOUS PATHOLOGICAL CONDITIONS.

The involvement of free radicals and oxidative stress has been documented in a number of malignancies. The proposed mechanisms have been postulated to range from free radicals causing DNA double-strand breaks to the possibility of free-radical activation of oncogenes (Reizenstein, 1991).

Harman (1956) was first to suggest that free radicals produced during respiration and cellular metabolism might cause oxidative damage to biological macromolecules. In addition, external factors such as toxic agents, irradiation, and excessive exercise may also raise the level of oxidative stress, and its cumulative effects over the years will lead to cellular damage, tissue and organ malfunctioning, and subsequently aging and death. The discovery of the enzyme superoxide dismutase (SOD) (McCord and Fridovich, 1969) showed that evolution has provided the living organism with antioxidant capacity to neutralize oxygen free radicals such as superoxides. Cutler (1976) observed that the metabolic rate of organisms is inversely related to the life span and aging rate in many animals. The higher the metabolic rate the greater the increase in mitochondrial respiration and production of oxygen free radicals. This reduces the life span observed in aging mammals with some number of exceptions. Very compelling evidence was presented several years ago which demonstrated that the increase of expression of the two antioxidant enzymes catalase and SOD retarded the age-related oxidative damage of *Drosophila melanogaster* (Sohal *et al*., 1993) and increased the life span of these flies (Orr and Sohal, 1994). The free radical theory of aging predicts that one should be able to observe elevated oxidative damage to proteins and enzymes with advanced age (Sohal *et al*., 1993).

In diabetes, long-term elevation of glucose has been shown to cause the phenomenon of non-enzymatic glycosylation or "glycation" of proteins. An increased level of glycosylated hemoglobin has been observed in vivo in diabetic patients (Trivelli *et al.*, 1971), and the extent of hemoglobin glycation has been used in the clinic as an index of progression of glycemia (Kennedy and Baynes., 1984). The molecular mechanism of protein modification due to reaction of glucose with proteins leads to the formation of Schiff base reaction between the protein amines and the aldehydic moiety of sugars. The physiological consequences of this phenomenon has been the modification and alteration of many proteins like collagen (Bailey and Kent, 1989) and serum albumin (Shaklai *et al.,* 1984) and the aggregation of lens proteins in glycosilation associated cataracts (Monnier *et al.,* 1979). Indeed, the stiffness of joints and arteries of diabetic patients has been attributed to increased cross-linkage of collagen, possibly due to non-enzymatic glycosylation of collagen (Bailey and Kent., 1989).

In the process of formation of atherosclerotic plaques, oxidized low-density lipoprotein (LDL) plays a major role. In a review article, the major oxidative changes to the protein component of LDL were summarized (Heinecke, 1997). These include the appearance of tyrosyl radical reactive nitrogen intermediates and the formation of hypochlorous acid (Heinecke, 1997). Careful work has validated that hypochlorite-oxidized proteins including oxidized apoHpoprotein B, are present in atherosclerotic plaques (Hazell *et al*., 1996) This finding supported the notion that tyrosyl radical is involved in the oxidative damage occurring in human atherosclerosis (Leeuwenburgh *et al., 1*997)

In vitro studies of red blood cells from cancer patients that were exposed to lipid or protein oxidation showed that methemoglobin formation was significantly higher in cancer patients compared to controls (Delia *et al*., 1995). Free radicals can cause oxyhemoglobin oxidative stress, which will result in an increase of methemoglobin (Delia, 1995)

An interesting hypothesis was put forward by Beal (1995). In his hypothesis, a defect in energy metabolism lead to neuronal depolarization and an increase in intracellular calcium. This resulted to increased free radical generation. A similar hypothesis was recently advanced by Markesbery (1997) in which the etiology of Alzheimer disease (AD) was attributed to increased oxidative stress observed in the brain of AD patients. These studies and many more, have demonstrated an increase in oxidized proteins in the brains of patients with different neurological diseases (Alzheimer, Parkinson, and Huntington disease) (Carney and Carney, 1994).

Protein oxidation as measured by protein thiol loss and protein carbonyls has been detected in chronic ethanol-induced liver pathology (Rouach *et al*., 1997), in inflammation (Krsek-Staples and Webster , 1993), and in synovial fluid of patients with rheumatoid arthritis (Chapman *et al.,* 1989). Elevated protein carbonyls and oxidation of protein thiols has also been observed in muscles of chickens with inherited muscular dystrophy (Murphy and Kehrer, 1989)

2.10 CLASSIFICATION OF ANTIOXIDANTS

Table 2.1 Classification of major antioxidants

Enzymes	Antioxidant	Role	Remarks
	Superoxide dismutase (SOD) Mitochondrial Cytoplasmic Extracellular	Dismutates $O_2^{\cdot -}$ to H_2O_2	Contains Manganese (Mn.SOD) Contains Copper & Zinc (CuZnSOD) Contains Copper (CuSOD)
	Catalase	Dismutates H_2O_2 to H_2O	Tetrameric hemoprotein present in peroxisomes
	Glutathione peroxidase (GSH.Px)	Removes H_2O_2 and lipid peroxides	Selenoproteins (contains Se^{2+}) Primarily in the cytosol also mitochondria Uses GSH
Vitamins	Alpha tocopherol	Breaks lipid peroxidation Lipid peroxide and $O_2^{\cdot -}$ and $\cdot OH$ scavenger	Fat soluble vitamin
	Beta carotene	Scavenges $\cdot OH$, $O_2^{\cdot -}$ and peroxy radicals Prevents oxidation of vitamin A Binds to transition metals	Fat soluble vitamin
	Ascorbic acid	Directly scavenges $O_2^{\cdot -}$,	Water soluble vitamin

		·OH, and H_2O_2 Neutralizes oxidants from stimulated neutrophils Contributes to regeneration of vitamin E	

-Modified from Fisher, 1988.

2.11 ANTIOXIDANT ENZYMES

Natural antioxidant enzymes manufactured in the body provide an important defense against free radicals. Glutathione peroxidase, glutathione reductase, catalase, thioredoxin reductase, superoxide dismutase, heme oxygenase and biliverdin reductase, are the most important antioxidant enzymes. (Marklund, 1982)

The enzyme superoxide dismutase converts two superoxide radicals into one hydrogen peroxide and one oxygen. To eliminate hydrogen peroxide before the Fenton Reaction can create a hydroxyl radical, organisms use catalase and/or glutathione peroxidase (Marklund, 1982). The brain, which is very vulnerable to free radical damage (due to high metabolic rate, high unsaturated fat in neurons, and the fact that neurons are post-mitotic) has seven times more glutathione peroxidase activity than catalase activity (Marklund, 1982). Moreover, glutathione peroxidase is found throughout the cell, whereas catalase is often restricted to peroxisomes. Nonetheless, the lifespan of transgenic mice has been extended about 20% by overexpression of human catalase targeted to mitochondria (Schriner, 2005).

2.11.1 SUPEROXIDE DISMUTASE (SOD)

The Superoxide dismutase (SOD) molecule in the cytoplasm contains copper and zinc atoms (Cu/Zn−SOD), whereas the SOD in mitochondria contains manganese (Mn−SOD). Unlike exogenous antioxidants, which are generally depleted by antioxidant action, antioxidant enzymes are not depleted because they act catalytically. An exogenous orally effective SOD mixture has been shown to protect against

hyperbaric oxidation damage to DNA that cannot be prevented with Vitamin E or N-Acetyl Cysteine (Muth, 2004).

Superoxide dismutase without glutathione peroxidase or catalase (CAT) to remove hydrogen peroxide is of little value. Insects lack glutathione peroxidase, but experiments have been performed on fruit flies made transgenic by having extra genes for SOD, CAT or both. The flies that were given extra genes for SOD or CAT (but not both) had no more than a 10% increase in mean lifespan, with no increase in maximum lifespan. But flies that had extra genes for both SOD *and* CAT showed maximum lifespan increase by as much as a third, while showing less protein oxidative damage and better physical performance (Orr and Sohal, 1994). A similar experiment using SOD/CAT mimetics in nematode worms increased mean lifespan 44% (Melov *et al.*, 2000). And selective inbreeding of bread-mold fungus resulted in strains with lifespans more than 6 times longer than wild-type — a change that was shown to be due to increased expression of antioxidant enzymes (Vina, 2005). Females express both more Mn−SOD and more glutathione peroxidase than males, and this has been suggested to be the reason females live longer than males in mammalian species (Vina, 2005). The lifespan of transgenic mice has been extended about 20% by overexpression of human catalase targeted to mitochondria (Schriner, 2005).

2.11.2 GLUTATHIONE PEROXIDASE ENZYME

The glutathione redox cycle is a central mechanism for reduction of intracellular hydroperoxides. It is a tetrameric protein 85,000-D. it has 4 atoms of selenium (Se) bound as seleno-cysteine moieties that confers the catalytic activity. One of the essential requirements is glutathione as a cosubstrate (*Halliwell, 1995*).

Glutathione peroxidase reduces H_2O_2 to H_2O by oxidizing glutathione (GSH) (Equation A). Rereduction of the oxidized form of glutathione (GSSG) is then catalysed by glutathione reductase (Equation B). These enzymes also require trace metal cofactors for maximal efficiency, including selenium for glutathione peroxidase; copper, zinc, or manganese for SOD; and iron for catalase (*Halliwell, 1995*).

$H_2O_2 + 2\ GSH \rightarrow GSSG + 2\ H_2O$ (equation A)

$GSSG + NADPH + H^+ \rightarrow 2\ GSH + NADP^+$ (equation B)

2.12 ANTIOXIDANT MOLECULES

Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules in the body are damaged. Although there are several enzyme systems within the body that scavenge free radicals, the principle micronutrient (vitamin) antioxidants are vitamin E, beta-carotene, and vitamin C. Additionally, selenium, a trace metal that is required for proper function of one of the body's antioxidant enzyme systems, is sometimes included in this category. The body cannot manufacture these micronutrients so they must be supplied in the diet

2.12.1 VITAMIN E

Vitamin E is a chain-breaking antioxidant, which means that once lipid peroxidation begins, vitamin E stops it from progressing further. This antioxidant function is quite important with relationship to exercise because exercise increases the throughput of energy in the mitochondria and thereby increases the number of radicals escaping the electron transport chain (Packer, 1994). Vitamin E requirements are dependent upon the degree of oxidative stress, the dietary polyunsaturated fatty acid (PUFA) intake and the intake of other antioxidants. The activity vitamin E is dependent upon an "antioxidant network" involving a wide variety of antioxidants and antioxidant enzymes, which functions to maintain vitamin E in its unoxidized state, ready to intercept and scavenge radicals (Packer, 1994).

2.12.1.1 Antioxidant activity of Vitamin E

Vitamin E functions in vivo as a chain-breaking antioxidant that prevents the propagation of free radical damage in biological membranes (Tappel and Vitam, 1962; Burton and Ingold, 1986; Burton *et al*., 1983; Ingold *et al*., 1987). When lipid hydroperoxides are oxidized to peroxyl radicals (ROO), vitamin E reacts 1000 times faster with these than do polyunsaturated fatty acids in membrane or lipoprotein lipids (Packer L, 1994). The vitamin E radical formed during lipid peroxidation readily reacts with other antioxidants and is reduced itself to the unoxidized form. Vitamin E reductants, which have been demonstrated in vitro to regenerate tocopherol from the

tocopheroxyl radical, include ubiquinol (Stoyanovsky *et al.,* 1995), ascorbate (vitamin C), and thiols (Wefers and Sies, 1988),especially glutathione (McCay, 1985; Niki, 1987; Sies and Murphy, 1991; Sies *et al.,* 1992). Subsequently, ubiquinone, vitamin C, and thiyl radicals can be reduced by various metabolic processes. This process has been termed "vitamin E recycling", where the antioxidant function of the one electron oxidized form of vitamin E is continuously restored by other antioxidants (Packer L., 1994). This antioxidant network depends upon the continuous supply of aqueous antioxidants and the metabolic activity of cells.

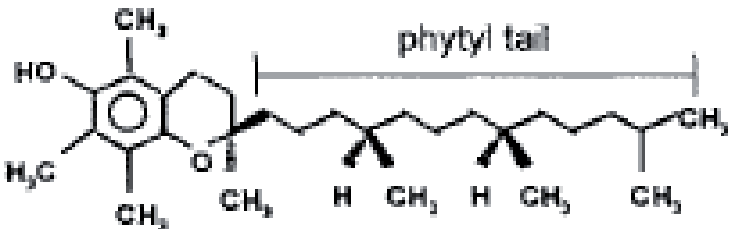


Fig: 2.3 Structure of vitamin E. (α-tocopherol)

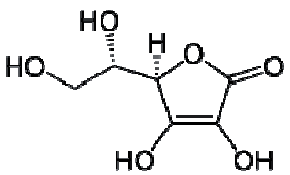


Fig 2.4 structure of L-ascorbic Acid

2.12.2 VITAMIN C/ L-ASCORBIC ACID

Ascorbic acid is well known for its antioxidant activity. Ascorbate acts as a reducing agent to reverse oxidation in aqueous solution. The plasma ascorbate concentration in a patient with oxidative stress (measured as less than 45 µmol/L) is lower than that of a

healthy individual (61.4-80 µmol/L) (Schorah et al., 1996). According to McGregor and Biesalski (2006), increasing the individual's plasma ascorbate level may have therapeutic effects in cases of oxidative stress. Individuals with oxidative stress and healthy individuals have different pharmacokinetics of ascorbate.

Ascorbate usually acts as an antioxidant by being available for energetically favourable oxidation. Many oxidants (typically, reactive oxygen species) such as the hydroxyl radical (formed from hydrogen peroxide), contain an unpaired electron, and, thus, are highly reactive. This can be highly damaging to humans and plants at the molecular level due to their possible interaction with nucleic acids, proteins, and lipids (Schorah et al., 1996). These free radical interactions have damaging effects since they result in a whole chain of free redical reactions. More specifically, the interaction of an initial free radical (often reactive oxygen species) with another molecule changes that molecule itself into a free radical, which than reacts with other molecules and also turning them into free radicals. Ascorbate can terminate these chained radical reactions by being a stable electron donor in interactions with free radicals, being first converted into an ascrobate radical then monodehydroascorbate and then dehydroascorbate. The oxidized forms of ascorbate are relatively stable and un-reactive, and do not cause cellular damage and can be reversed back to ascorbate by cellular enzymes (Schorah *et al*., 1996).

Robert F.C (1985) hypothesizes that the pathogens of most acute infectious diseases depend upon free radical toxicity to defend themselves against immediate destruction by the immune system. If a pathogen produces free radicals at a rate sufficient to exceed the rate at which the host can produce free radical scavengers to protect the immune system, the pathogen will be free to invade and multiply. His postulate is that free radical suppression of the immune system, common to most infectious diseases can be neutralized by ascorbate.

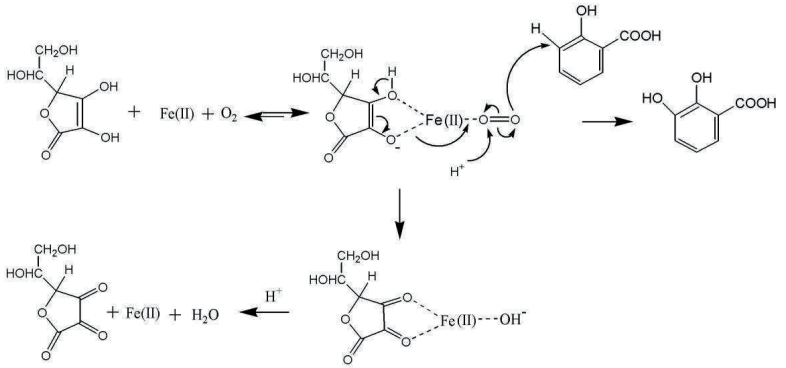


Fig 2.5:Oxidation mechanism of ascorbic acid

2.12.3 SYNTHETIC AND PLANT PHENOLICS

Phenolics represent a vast array of both naturally occurring and synthetic compounds. These compounds inhibit lipid oxidation through their ability to scavenge free radicals, chelate prooxidative metals and inhibit lipoxygenases (Shahidi and Wanasundara, 1992; Laughton *et al.,* 1991; Morgan *et al.*, 1997). Synthetic phenolics such as butylated hydroxyanisole, butylated hydroxytoluene (Fig. 2.6 below), propyl gallate and tertiary butylated hydroxyquinone are commonly used in food products to inhibit oxidative rancidity. Addition of synthetic phenolic antioxidants to foods is strictly regulated with legal concentration limits 338 *Part V: Nutrition* of 200 ppm or less (Nawar, 1996). Numerous studies have evaluated the safety of synthetic phenolics. Consumption of extremely high concentrations (>2 g/day) of synthetic phenolics for several months can produce carcinoma in the rat forestomach (Iverson, 1995; Huang, 1992). These high levels of synthetic antioxidants can inhibit archadonic acid metabolism and can be prooxidative leading to the depletion of glutathione (Iverson, 1995; Huang, 1992). However, lower levels of synthetic antioxidants are capable of altering phase II enzymes in the liver and thus, can reduce the carcinogenic activity of several known carcinogens. These lower levels of synthetic phenolic, which reflect typical human

exposure, are not believed to be harmful to health and may actually be beneficial through their ability to inactivate free radicals in vivo (Iverson, 1995; Huang, 1992). Naturally occurring plant phenolics are much more prevalent in the diet than synthetic phenolics since they are found in a wide range of plant foods. The plant phenolics can be classified as simple phenolics and phenolic acids, hydroxycinnamic acid derivatives, and flavonoids. In addition to the basic hydroxylated benzene ring structure of these compounds, plant phenolics are often associated with sugars and organic acids. Many natural phenolics are capable of inhibiting oxidative reactions (Morgan, *et al*., 1997; Aruoma, *et al., 1992*). However, due to the chemical diversity of these compounds, it is not surprising that antioxidant activities vary greatly. Under certain conditions, some plant phenolics accelerate oxidative reactions, this can be seen with phenolics from soybeans (chlorogenic acid, gallic acid and caflfeic acid) and rosemary (carnosol and carnosic acid) which promote iron-catalyzed oxidation of DNA (Morgan, *et al*., 1997; Aruoma, *et al., 1992*). Despite the fact that phenolics are sometimes prooxidative, their presence in the diet has been positively associated with prevention of diseases such as cancer and atherosclerosis (Huang and Ferraro 1992; Kinsella, *et al.,* 1993).

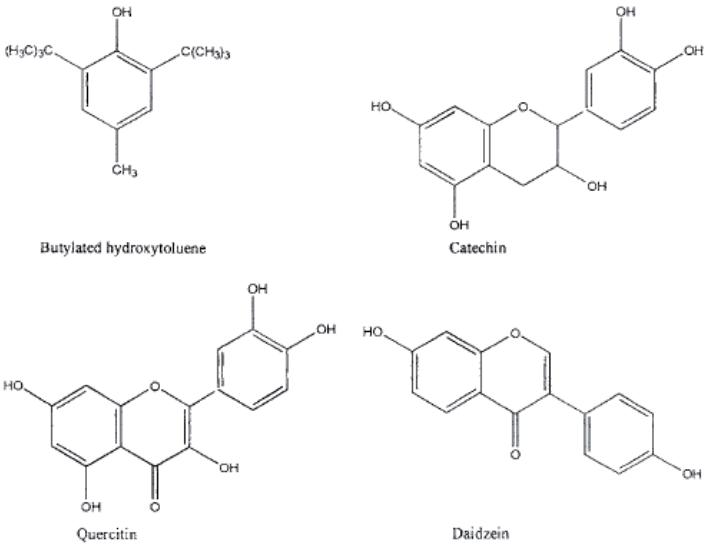


Fig. 2.6: Structures of phenolics commonly found in foods.

2.13 JUSTIFICATION FOR THE RESEARCH

Various species and cultivars of *Capsicum annum* L. have been used by Nigerians for so many purposes, ranging from culinary to medicinal uses. It has also been found to posses some antimicrobial uses, hence its use as a preservative. Peppers are also broadly known to be rich in antioxidants such as vitamin E, vitamin C and carotinoids. These, and many more findings, have stirred up the interest of the scientific community in the antioxidant abilities of peppers.

2.14 AIMS AND OBJECTIVES

The present research work has therefore selected various commercially available pepper cultivars with the following aims:

To separate the different components of the pepper cultivars into sub-fractions. (this separation is based on solvent polarity)

To study the ability of the sub-fractions of different pepper cultivars to scavenge free radicals, in order to find the most potent fraction.

To calculate to total phenols in each sub-fraction.

CHAPTER 3

MATERIALS AND METHODS

3.1 MATERIALS

INSTRUMENTS

U.V. Spectrophotometer, Digital Water bath, Weighing balance, Cuvettes, Refrigerator, Test tubes. Test tube rack, Test tube holder, Micropipette, Dropping Pipette, pH meter, Blender, Stirring rods, Foil paper, Rubber bands, Masking tape, Beakers, Conical Flasks, Measuring cylinders, High quality Camera, Spatula, Reagent bottles, Distilled water.

REAGENTS

1,1-diphenyl-2-picrylhydrazyl,2-deoxy-2-ribose 2.8mM, Acetic acid ($C_2H_4O_2$), Ascorbic Acid (1mM), EDTA 1.04mM, $FeCl_3$ 200µL, Folin-ciocalteau reagent, Gallic acid, Na_2CO_3, Sodium Chloride (NaCl), Sodium hydroxide pellets (NaOH), Trichloroacetic acid, Tris-HCl buffer(50mM, P^H7.4),Vitamin E.

Commercially available samples of *Capsicum annum L.* were obtained from Ota market, Ogun state, Nigeria. They were properly washed in preparation for the experiment. Distilled water was prepared in the Covenant university biochemistry laboratory . All reagents used in this study were of the highest purity commercially available and were obtained from sources such as Sigma and Fisher.

3.1.3 Experimental design

Each pepper cultivar was macerated in acidified methanol-H_2O solvent system to derive five different crude extracts. Each crude extract was sub-fractionated by liquid–liquid partitioning against water with hexane, EtOAc and n-BuOH to derive 5 sub-fractions for each cultivar. There were therefore 25 sub-fractions/samples in all. These samples were screened to characterize their antioxidant properties, using DPPH (1,1-diphenyl- 2-picrylhydrazyl) and hydroxyl free radical-scavenging assays. Also, their total phenol contents were determined. For the purpose of clarity and distinction, their common names and colours were used to code the different samples

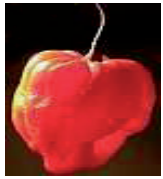
The pepper cultivars used were coded for the experiment as indicated in the figures below:

Code: bell pepper(RED).

Code: bell pepper (green)

Code: Thai peppers.

Code: cayenne peppers.



Code: Scotch bonnet

3.2. Extraction and fractionation

Plant material (100 g) was macerated with 500 ml (60:20:1, v/v/v) methanol: water: acetic acid at room temperature for 12 h with constant stirring. The resulting extract was combined, filtered, concentrated in vacuo and lyophilized. The dried crude extract was suspended in 500 ml ultra-pure water and sequentially extracted with 250 ml hexane, 300 ml ethyl acetate (EtOAc) and 350 ml n-butanol (n-BuOH). The remaining aqueous phase was filtered, concentrated in vacuo and lyophilized. The organic phases were filtered through anhydrous magnesium sulphate and the solvent was removed in vacuo. A schematic of the extraction and fractionation of the plant material is provided below.

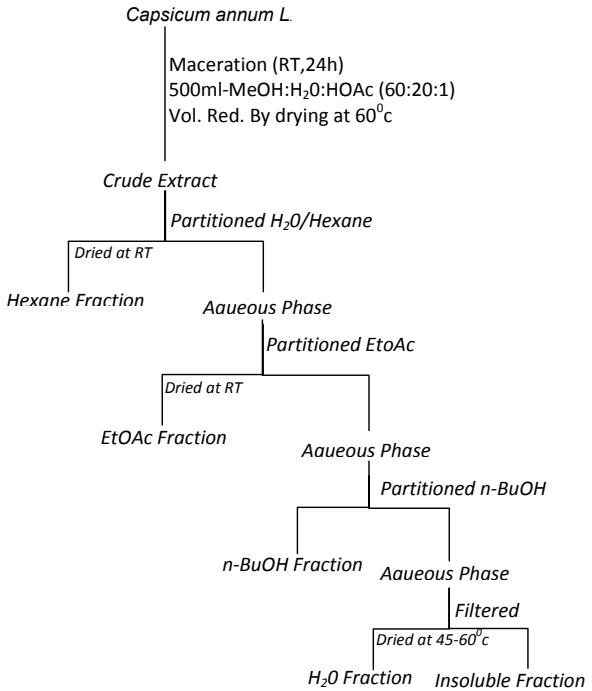


Fig. 3. 1: Fractionation scheme for the production of the *Capsicum annum L.* crude extract and subfractions. Scheme key: RT, room temperature; vol., volume; red., reduced; sol., solvent.

Fig 3.2: Picture of the fractionation process

3.3 Total phenol content

Total phenols were estimated as gallic acid equivalents (Singleton, *et al.,* 1999). To 6.0 ml distilled water, a 100 µl sample, (taken from a 0.1mg/ml solution) was transferred to a 10.0 ml volumetric flask, to which was subsequently added 500 µl undiluted Folin-Ciocalteu reagent. After 1 min, 1.5 ml 20% (w/v) Na_2CO_3 was added and the volume was made up to 10.0 ml with ultra-pure water. After a 30 min incubation at 25^0C, the absorbance was measured at 760 nm and compared to a gallic acid calibration curve.

3.3.1 Diphenyl-2-picrylhydrazyl free radical-scavenging activity

The ability of the extracts to scavenge 1,1-diphenyl- 2-picrylhydrazyl radicals was assessed spectrophotometrically (Gyamfi *et al.,* 1999). A 50 µl aliquot of each extract

(taken from its respective solvent systems) was mixed with 450 µl Tris–HCl buffer (50 mM, pH 7.4) and 1.0 ml 1,1-diphenyl-2-picrylhydrazyl (0.1 mM, in methanol). After a 30 min reaction period, the resultant absorbance was recorded at 517 nm. The percentage inhibition was calculated using equation 1 and IC_{50}s were estimated using a linear regression algorithm.

$$\text{Percentage inhibition} = \left[\frac{(Abs_{control} - Abs_{sample})}{Abs_{control}}\right] \times 100. \quad \text{……equation 1}$$

3.4 Hydroxyl radical scavenging assay

Scavenging of the hydroxyl free radical was measured by the method of (Halliwell *et al.*, 1989) with minor changes. All solutions were prepared freshly. 200µL of 2.8mM 2-deoxy-2-ribose, 5µL methanol extracts of *Capsicum annum L.*, 400µL of 200µM $FeCl_3$, 1.04mM EDTA (1:1 V/V), 200µL of H_2O_2 (1.0mM) and 200µL ascorbic acid (1mM) was mixed to form a reaction mixture. After an incubation period of one hour at 37^{o}C the extent of deoxyribose degradation was measured by the TBA reaction. 1.5ml of 2.8% TCA was added in the reaction mixture and kept for 20 min. at 100^{o}C taking Vitamin E as positive control. Percentage inhibition was calculated (Eq. I).

CHAPTER FOUR

5.1 DISCUSSION

According to the results in chapter four above, there was a very strong association between the Folin-Ciocalteu-reactive (total phenol) content and free radical scavenging capacity for both the DPPH and the hydroxyl free radicals . It is known that chemicals which readily undergo redox reactions are capable of producing a high level of activity in the Folin- Ciocalteu method (Singleton *et al*., 1999), which may explain the high correlation between these two indices. The cardinal mode of action of natural antioxidants is their ability to scavenge free radicals before they can initiate free radical chain reactions in cellular membranes or lipid-rich matrices in foodstuffs, cosmetics or pharmaceutical preparations. In this study, two different free radicals, were used to assess the potential free radical-scavenging activities of the extracts and sub-fractions of the Capsicum species, namely the DPPH and the hydroxyl radical. DPPH is frequently used for the estimation of free radical-scavenging ability (Dorman *et al*., 2003a; Re *et al*., 1998). However, it has been argued that these techniques are only capable of indicating potential antioxidant activity as they do not utilize a food/biologically relevant oxidizable substrate nor a relevant reactive species and, therefore, no direct information on protective performance can be determined (Dorman, *et al.*, 2003b).

The free radical scavenging data presented in chapter four suggests that the crude extracts and all the subfractions are capable of scavenging reactive free radical species in a dose dependent manner.

5.2 1,1-Diphenyl-2-picrylhydrazyl free radical-scavenging activity(DPPH)

IC_{50} value is the effective concentration at which the antioxidant activity is 50%. DPPH is usually used as a substrate to evaluate anti-oxidative activity of antioxidants. The method is based on the reduction of methanol DPPH solution in the presence of a hydrogen donating antioxidant due to formation of the non radical form DPPH-H by the reaction. The extract was able to reduce the stable radical DPPH to the yellow colored diphenyl picrylhydrazine. It has been found that cysteine, glutathione, ascorbic acid, tocopherol, poly-hydroxy aromatic compounds (hydroquinone, pyrogallol, gallic

acid, etc.) reduce and decolorize DPPH by their hydrogen donating ability (Blois, 1958).

Big-red peppers(*Capsicum annum L*)

The ethyl acetate fraction of *Capsicum annum L(* Bell pepper(red)) has the highest antiradical activity by inhibiting DPPH radical with the IC_{50}value of (0.045mg/ml). It appears that the fractions of *Capsicum annum L(* Bell pepper(red)) possess hydrogen donating abilities and acts as an antioxidant in the following order : EtOAc>Hex>BuOH>H_20.

Cayenne peppers (*Capsicum frutescens*)

The hexane fraction of Cayenne peppers has the highest antiradical activity by inhibiting DPPH radical with the IC_{50} value of (0.050mg/ml). It appears that the fractions of *these* Cayenne peppers possess hydrogen donating abilities and acts as an antioxidant in the following order :Hex > BuOH> EtOAc >crude >H_20.

Scotch bonnets(*Capisicum chinense*)

The hexane fraction of *the* scotch bonnet has the highest antiradical activity by inhibiting DPPH radical with the IC_{50} value of (0.018mg/ml). It appears that the fractions of *Capsicum annum L (* Scotch bonnet) possesses hydrogen donating abilities and acts as an antioxidant in the following order :Hex > BuOH> EtOAc >H_20.

Bell peper(green)s(*Capsicum annum L*)

The hexane fraction of *the* Bell peppers(green) has the highest antiradical activity by inhibiting DPPH radical with the IC_{50} value of (0.0195mg/ml). It appears that the fractions of *Capsicum annum L (* Bell peper(green)) possesses hydrogen donating abilities and acts as an antioxidant in the following order : EtOAc > BuOH >Hex >crude >H_20.

Thai pepper (green) (*Capsicum annum L*)

The hexane fraction of *Capsicum annum L(* Thai pepper) has the highest antiradical activity by inhibiting DPPH radical with the IC_{50} value of (0.050mg/ml). It appears that

the fractions of *Capsicum annum L (*Thai pepper) possess hydrogen donating abilities and acts as an antioxidant in the following order :Hex > BuOH> EtOAc >crude >H_20.

5.3 Hydroxyl radical scavenging ability

The extract was examined for its ability to act as $OH^{\cdot}$ radical scavenging agent. Ferric EDTA was incubated with H_2O_2 and ascorbic acid at pH -7.4; hydroxyl radicals were formed in free solution and were detected by their ability to degrade 2-deoxy-2-ribose into fragments that on heating with TBA and low pH forms a pink chromogen (Halliwell *et al*., 1987; Aruoma *et al.*, 1989). The fractions (especially ethyl acetate, hexane and butanol) display a scavenging effect of hydroxyl radical. When the extracts and were added to the reaction mixture they removed hydroxyl radical and prevented the degradation of 2-deoxy-2-ribose. The maximum inhibition (IC_{50} value) and the hydroxyl radical scavenging ability are stated below, beginning from the most potent to the least potent.

Bell pepper(red)s: EtOAc(0.01395 mg/ml) > BuOH (0.11987 mg /ml) >Hex(0.1237mg/ml) >Vit. E(0.155mg/ml)>H_20(0.27mg/ml).

Cayenne peppers: Hex(0.0879 mg/ml) > EtOAc(0.11398 mg/ml) > BuOH0.11796 mg/ml>Vit. E(0.155mg/ml) >crude(0.3674mg/ml) >H_20(27.10 mg/ml).

Scotch bonnets: Hex (0.0333 mg/ml)> EtOAc>Vit. E(0.155mg/ml) (0.161 mg/ml) >BuOH(0.6167 mg/ml) >H_20(2.47 mg/ml).

Big green pappers EtOAc(0.0467 mg/ml) > BuOH(0.145mg/ml)>Vit. E(0.155mg/ml) >Hex(1.177 mg/ml) >crude(4.3 mg/ml) >H_20(4.7348 mg/ml).

Thai pepper(green)s :Hex (0.0334 mg/ml) > BuOH(0.1492mg/ml) >Vit. E(0.155mg/ml)> EtOAc(0.395mg/ml) >crude(1.408mg/ml) >H_20(93.61mg/ml).

5.4 CONCLUSION AND RECOMENDATION

Overall, the most potent fractions were the ethyl acetate and the hexane fractions of all the sub-fractions. The activity of the hexane and EtOAc-soluble components of the crude extract showed particularly strong properties and may find potential application as preservatives to retard free radical-mediated degradation of susceptible components. Furthermore, due to the role free radicals play in the deterioration of human health, the EtOAc subfraction may potentially have beneficial effects upon human biology if incorporated as neutraceutical in appropriate quantities. However, before such claims can be made with confidence, further research for standardization of the qualitative and quantitative chemical composition and in vivo efficacy studies, including absorption and metabolism studies, should be carried out for better utilization of this fruit by interested industries.

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