Isolation, Characterization and Radical-Scavenging Potentials of Bioactive Components of *Combretum dolichopentalum* Leaves

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CERTIFICATION

This is to certify that this work "**Isolation, Characterization and Radical-Scavenging Potentials of Bioactive Components of *Combretum dolichopentalum* Leaves**" was carried out by Ujowundu, Favour Ntite (20124771738) in partial fulfilment for the award of the Degree of Doctor of Philosophy (Ph.D) in Medical Biochemistry in the Department of Biochemistry Federal University of Technology, Owerri.

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DEDICATION

I dedicate this work,

To God, The father, the giver of academic success,

God, The son, the lover of my soul, and

God, The Holy Spirit, the divine intervener,

For the success of this study.

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ABSTRACT

The present study isolated, identified and characterized the bioactive components of *Combretum dolichopentalum* leaves and determined the antioxidant potentials of the extracts of the leaves on some biochemical and physiological parameters. Biochemical methods such as ion exchange chromatography, column chromatography, thin layer chromatography, gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS), spectrophotometric analyses, atomic absorption spectrometry and Fourier transform infra red (IR) were used. Preliminary phytochemical analyses showed the presence of flavonoids, alkaloids, saponins, tannins, oxalate and phytate. Further screening show the presence of flavonoids: kaempferol, luteolin, isorhamnetin and apigenin; alkaloids: akuamidine, undulantine, powelline and their hydroxylated compounds, voacangine and buphranidine; saponins: hicogenin, sapogenin, tribuloin and tannic acids. The use of GC-MS aided the identification of organic acids: (heptedecanoic acid), organic esters (ethyl octadecanoate), ketones (6,10-dimethyl-2-undecanone), alcohols (3,7,11,15-tetramethyl-2-hexadecen-1-ol) and phenol derivatives (Phenol-2,6-bis (1,1-dimethyl)-4 -methyl, methyl carbamate). Also, the eluates obtained from column and thin layer chromatography were analysed with IR and phenol-2,6-bis (1,1-dimethyl)-4-methyl, methyl carbamate, nitrocyclohexane, fluorodecane and others were identified. Proximate analysis showed the presence of carbohydrates, proteins, lipids, sodium, potassium, phosphorus, vitamins A, E and B-vitamins. Amino acid profile using the technicon sequential multi-sample amino acid analyser (TSM) showed the presence of essential and non essential amino acids. The effects of isolates such as flavonoids, saponins, alkaloids and tannins were determined for *in vitro* hydrogen peroxide scavenging ability. The effects of the extract in comparison with a standard drug (silymarin) on animal organ/tissue functions and on serum concentrations of electrolytes, beneficial transition minerals, lipids, antioxidants and markers of lipid peroxidation were determined. The effects of the crude extract in comparison with standard plant antioxidant (quercetin) and butylated hydroxyl toluene (BHT), for *in vitro* scavenging potentials showed dose dependent scavenging potentials on nitric oxide, hydroxyl radical, H_2O_2 induced lipid peroxidation and reducing power. Intoxication with CCl_4 after pre-treatment with the ethanol extract and crude flavonoid, saponin, alkaloid and tannin fractions indicated hepatoprotective and antinephrotoxic potentials except saponins isolate which showed a significant ($P \square 0.05$) adverse effect as indicated by some biochemical and physiological parameters. Flavonoid and alkaloid extracts are the major fractions that elicited biochemical and physiological benefits in CCl_4 intoxication.The *in vitro* and *in vivo* radical scavenging properties of extracts of *C. dolichopentalum* make a promising drug with strong antioxidant potentials and inhibitory against *Escherichia coli* and *Salmonella typhi*.The present study isolated and characterized the phytochemicals in *C. dolichopentalum* leaves which could be further exploited for the treatment of oxidative stress and microbial induced disorders and diseases especially in light of the need to produce more effective agents.

Keywords: *Combretum dolichopentalum*, Gas chromatography, GC-mass spectrometry, Infra red spectrometry, Atomic absorption spectrometry, Phytochemicals, Radical-scavenging, Antimicrobials, Carbon tetrachloride, Oxidative stress, Antioxidants.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

It is clear that most measures of physiological function decline in oxidatively stressed organisms. How can we decipher the molecular basis of this loss of functional capacity? A fundamental part of oxidative damage is simply reflected by unwanted chemical processes resulting in the spontaneous appearance of side products of normal metabolism; the formation of mutated, less active, and potentially toxic species of DNA, RNA, proteins, lipids and small molecules to the extent that organisms can minimize the accumulation of these altered bio-molecules to the extent they can tolerate.

In this sense therefore, oxidative stress may be seen as a battle between biochemistry and chemistry. Organisms have evolved biochemical systems where the right DNA sequences encode the right sequences of RNA and proteins, which fold in just the right way to make both catalysts and architectural structures. The catalyst combines speed and specificity to ensure that thermodynamically favourable but kinetically unfavourable reactions occur that lead to metabolic pathways for energy generation, biosynthesis, and signal transduction. Since there are a number of possible chemical reactions for each metabolic intermediate, the provision of enzymes that catalyze just one of the possible reactions can lead to a rapid and smooth metabolic conversion of reactants to products with few side products. All these represent the beauty of biochemistry in making life possible. What works against the beauty of biochemistry is chemistry itself. While enzymes can speed up reactions, it is more difficult to slow down reactions. Side reactions still go on, and the more time elapses, the more unwanted side products are formed. Importantly, these side products are not just small molecules, but all types of bio-molecules including nucleic acids and proteins. Almost all the molecules from small metabolites to proteins that make up living systems are not thermodynamically stable (carbon dioxide and water may be the exceptions). Thus, from the

moment that bio-molecules are synthesized, a slow process of non-enzymatic decomposition begins, leading to simple products. These spontaneous chemical reactions (or side reactions) over time result in the modification of the biochemical species required for the orderly processes of life described above into less functional species (Clarke, 2003). Perhaps the clearest example of this is the decomposition of DNA, including phytochemical alterations, the hydrolytic loss of bases and oxidative modifications, all of which lead to altered structures and mutations. Free radical oxidation is one of the biological side reactions that can take place spontaneously and cause damages (Baynes, 2000). Modification of biological molecules caused by oxidative stress is the most popular harmful biological side reaction leading to pathological changes including ageing of organisms (Harman, 1981; Yu, 1996; Sohal, 2002). Free radical oxidations do not belong to normal physiological and biological processes, they are specially termed as "biological side reactions". In fact free radical oxidations have covered most modification possibilities of biological 'fuel' associated damaging process (Boritz, 1986; Yin, 2003).

Reactive oxygen species (ROS) are essential for life because of their role in many vital processes such as signal transduction and the ability of phagocytes to carry out their bactericidal activity. ROS include oxygen-centred free radicals such as hydroxyl and superoxide radicals which are substances with one or more orbital electrons with unpaired spin states and non radicals including hydrogen peroxide and singlet oxygen. Although carefully controlled processes regulate the production of ROS for essential functions, many cellular processes result in the generation of ROS (Figure 2.1). An important site of this non essential generation of ROS which constitutes oxidative stress is the electron transfer chain (ETC) that resides within the inner membrane of mitochondria. Normally, electrons are transferred from complex I (NADH dehydrogenase) and complex II (succinate dehydrogenase) to coenzyme Q10 and then to complex III, cytochrome C, and complex IV. Finally, four electrons are transferred to oxygen with the formation of water (Conklin, 2004). In the

process, coupling of electron transfer to oxidative phosphorylation results in ATP generation. Although, this process is very efficient, about 2 % of the electrons escape the ETC and react with molecular oxygen to form superoxide radicals.

However,the superoxide radical is not highly toxic, mitochondrial superoxide dismutase generates hydrogen peroxide from superoxide radicals, and in the presence of reduced iron or copper, highly toxic hydroxyl radical is formed via Fenton or Haber Weiss reactions. The cytochrome P450 mono-oxygenase system of hepatic endoplasmic reticulum (microsomes) also generates a substantial amount of ROS in the process of metabolizing chemically diverse group of compounds that include most of the drugs that we administer as well as environmental substances. Enzyme systems such as the xanthine oxidase system can also generate ROS.

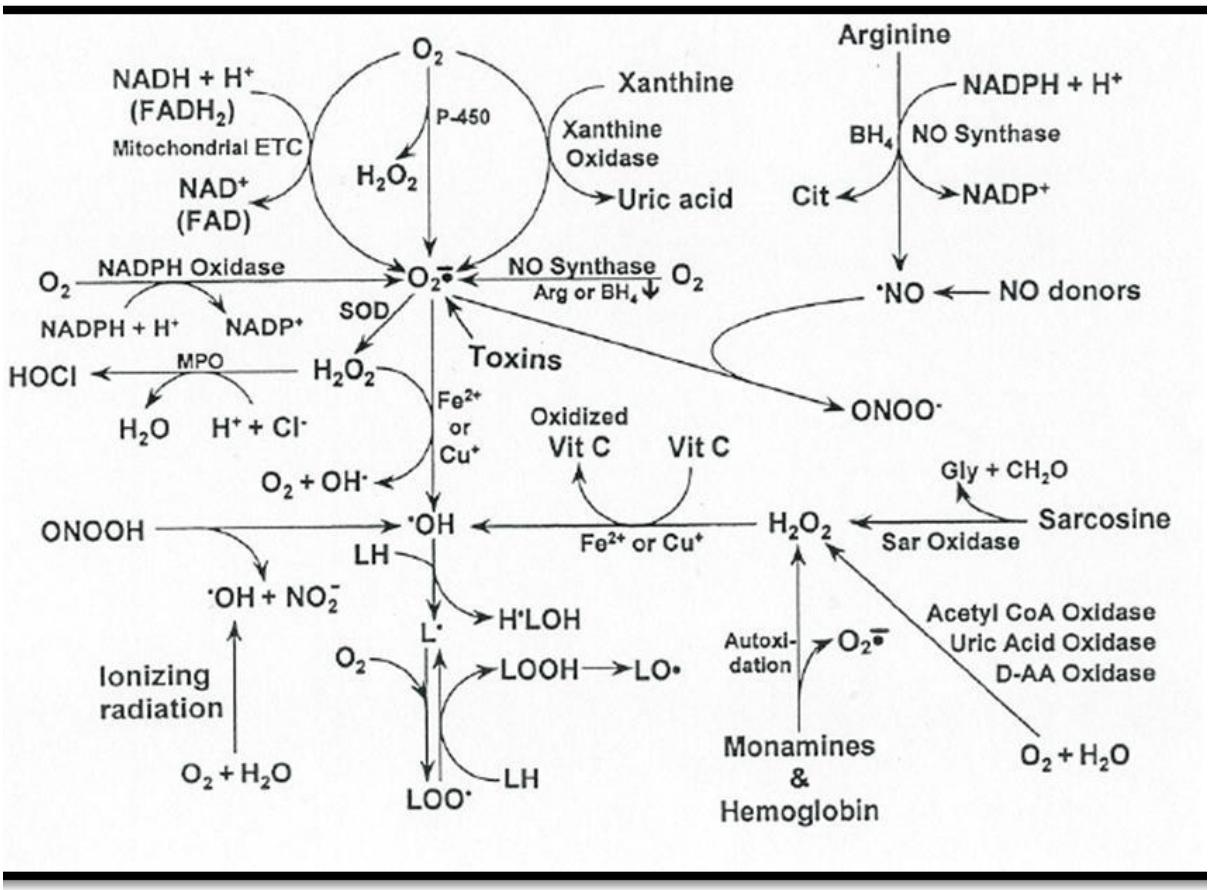


Figure 1.1: Oxygen and nitrogen-based free radicals and associated reactants that are generated in cells by various processes. Source: Reiter *et al*. (2003).

ROS can interact with cellular macromolecules, including DNA, proteins and lipids, and interfere with vital cellular functions. Mutations caused by ROS can result in malignant transformation and the development of cancer. ROS are also implicated in the aetiologic and progression of many other diseases (van Gaal *et al.,* 2006; Rattan, 2006). Under normal conditions, antioxidant mechanisms, including small molecular weight antioxidants and antioxidant enzymes scavenge ROS and protect the organism from the damaging effects of oxidative stress. Tissue antioxidants help to control oxidative stress *in vivo*, but tissue antioxidant interception and scavenging of ROS is not 100 % efficient.

There is always a need to enhance the potentials of endogenous antioxidants by introducing antioxidants of plant origin which possess free radical scavenging bioactive compounds such as flavonoids, tannins, and polyphenols. *Combretum dolichopentalum* is one of such plants. *Combretum* makes up the type genus of the family combretaceae. Several species are used in African and Indian traditional medicines. *Combretum dolichopentalum* commonly known as 'food for the small bird' is one of the medicinal plants popularly used in treating disease conditions of the alimentary tract in Igbo ethnomedicine. *C. dolichopentalum* is used for the treatment of stomach ache, gastro- intestinal disorders, such as dysentery, passage of bloody stool, diarrhoea and stomach ulcer in and around Ogwa of Imo State of Nigeria. Also in Mbaise, Imo State, *C. dolichopentalum* is taken by women after parturition for reconditioning of the uterus after pregnancy. The leaves are cooked until the fluid content turns red, and is prepared as soup for drinking (Asuzu & Onu, 1998). Plant based drugs used in traditional medicine practice have become the focus of current research because they are cheap, very efficacious, possess little side effects and according to WHO, about 80 % of world population still rely on herbal remedies (Dhanmasin *et al.,* 2003).

It is well known that ROS cause damage by the mechanism of covalent bonding, cross-linking, and lipid peroxidation with subsequent tissue injury. Liver damage remains one of the most serious

health problems. The liver being a versatile organ has specialized functions, reflected in its anatomy and metabolic activity. It regulates internal chemical environment (Gole *et al.,* 1977) and plays a central role in metabolic function such as, secretory functions, excretory functions, synthesis of certain blood coagulation factors including factors V, VI, X, and fibrinogen; synthesis of albumin and globulin, detoxification function, and storage function. Antioxidant agents of natural origin have attracted special interest because they can protect humans from free radical damage (Osawa *et al.,* 1990; Babu *et al.,* 2001; Raja *et al.,* 2007) and protect the liver from damage initiated by hyperlipidaemia (Alisi *et al.,* 2008). Numerous medicinal plants and their formulations are used for liver disorders in ethno-medicinal practices as well as in traditional systems of medicine in Africa, India and Asia (Gowchi *et al.,* 2001; Nwaogu *et al.,* 2006; Igwe *et al.,* 2008; Ujowundu *et al.,* 2008; Alisi & Onyeze, 2008; Ojiako & Nwanjo, 2009).

1.2 Justification for the Study

There are very few reported studies on the roots of *C. dolichopentalum* and little/or none on the leaves. This study will elucidate the bioactive components of *C. dolichopentalum* leaves. The knowledge of the possible bioactive components of the leaves may enhance the effective utilisation of the plant.

1.3 Aim of Study

The aim of this study was to isolate and characterize the bioactive components of *C. dolichopentalum* leaves and determine their *in vitro* and *in vivo* radical-scavenging potentials.

1.4 **Objectives of the Study**

The following were the objectives of the study

To estimate, the proximate and phytochemical composition of *C. dolichopentalum* leaves.

To determine the amino acid content of *C. dolichopentalum* leaves.

To isolate and identify the subclasses of flavonoids, alkaloids, saponins, and tannins of the leaves using gas chromatography and infra red spectroscopy.

To elucidate the structures of the bioactive organic compounds using gas chromatography-mass spectral analysis and infra red spectroscopy.

To evaluate the free radical scavenging potentials of *C. dolichopentalum leaves.*

To determine the antimicrobial potentials of the extract

To analyse the hepatoprotective properties of the extract

To determine the antioxidant effects of the extract

To analyse the antinephrotoxic potentials of the extract

To determine the extracts' effect on haematological parameters

To undertake histopathological study on the liver and kidney of wistar albino rats administered the plant extract.

1.5 Hypotheses

The hypotheses shall be in the null and alternate form:

Null hypothesis

H_0: Extract of *C. dolichopentalum* leaves has no free radical scavenging, antioxidant, antimicrobial, hepatoprotective, nephroprotective, and erythopoietic potentials.

Alternate hypothesis

H_1: Extract of *C. dolichopentalum* leaves has free radical scavenging, antioxidant, antimicrobial, hepatoprotective, nephroprotective, and erythopoietic potentials.

CHAPTER TWO

LITERATURE REVIEW

2.1 Free Radical Oxidative Stress

According to the free radical theory of ageing, senescence and a variety of degenerative diseases associated with it are attributed to the deleterious attack of oxygen free radicals on cellular constituents, including connective tissues, chromosomes and mitochondrial DNA (Esterbauer *et al.,* 1991; Halliwell & Gutteridge, 1999). Univalent reduction of oxygen gives rise to damaging oxidative species:

$$O_2 \xrightarrow{e} O_2^{-} \xrightarrow{e} H_2O_2 \xrightarrow{e} HO^{\cdot} \xrightarrow{e} H_2O$$

Mitochondria are responsible for the majority of cellular O_2 consumption and are a major source of ROS in healthy cells. Free radical production ranges from 0.1 to 4 % of the oxygen consumed. It is established that complex I (Kushnareva *et al.,* 2002), complex III (Miwa & Brand, 2005), glycerol-3-phosphate dehydrogenase (Tretter *et al.,* 2007) and α-ketoglutarate dehydrogenase (Tretter & Adam, 2005) produce free radicals in isolated mitochondria.

2.2 Oxidative Damage of Mitochondrial DNA, Lipids and Proteins

The ROS can damage many different kinds of cellular macromolecules including lipids, proteins and DNA (Figure 2.2). Damage to DNA can lead to irreversible loss or alteration of genetic information in post mitotic cells. Mitochondrial DNA (MtDNA) is situated very close to or even in contact with the site of mitochondrial ROS production. Thus, it is known that the most probable reason for the higher level of oxidative damage in MtDNA than nuclear DNA (nDNA) is its (inner mitochondria membrane) closeness to, even its contact with the ROS generation of healthy cells (Barja *et al.,* 1994a, Barja *et al.,* 1994; Barja 2004). ROS can also attack DNA directly at the sugar-phosphate backbone or at the bases. This produces many different oxidatively modified purines and pyrimidines, including the most commonly measured 8-oxo-7,8-dihydro-2'deoxyguanosine (8-

oxodG), as well as single and double strand breaks in both rats and human DNA and chromosomes (Quiles *et al.*, 2004a; Quiles *et al.*, 2004b; Ramirez-Tortosa *et al.*, 2004).

The oxidation of proteins plays an essential role in the pathogenesis of a number of degenerative diseases and in ageing (Stadtman & Berlett, 1998). The major molecular mechanisms leading to structural changes in proteins are metal catalyzed protein oxidation, characterized by carbonyl formation (Stadtman & Levine, 2003), loss of protein sulphydryl groups (Shacter, 2000a), nitrotyrosine (NT) (Stadtman & Levine, 2003) and advanced oxidation products (AOPP) formation (Loeckie *et al.*, 1999). Protein modifications elicited by direct oxidative attack on lysine, arginine, proline or threonine and by secondary reaction of cysteine, histidine or lysine residues with carboxyl compounds can lead to the formation of protein carboxyl derivatives. The SH moiety of

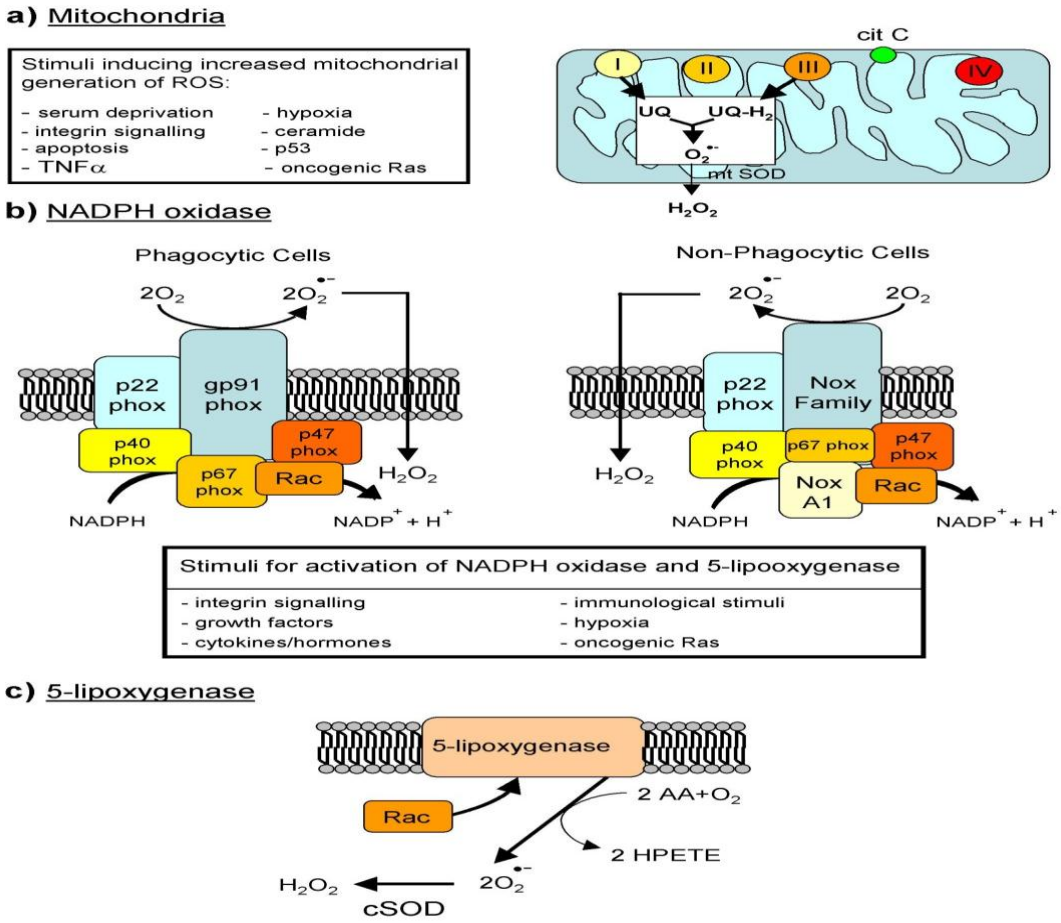


Figure 2.1: Major sources of ROS (Speakman & Selman, 2011).

cysteine is highly prone to oxidative attack by several mechanisms leading to the formation of disulphide bonds and thioyl radicals (Shacter, 2000b).

Oxidation of cysteine–SH can also give rise to intra or inter-protein cross-linked derivatives. Peroxynitrite ($ONOO^-$) is cytotoxic specie generated when superoxide radical combines with nitric oxide ($NO^{\cdot}$); and its formation has been implicated in both the origin and in the progression of protein oxidation (Beal, 2002). Nitration of the ortho position of tyrosine is a major production of $ONOO^-$ attack on proteins, leading to nitrotyrosine (NT), and it has been considered a specific marker for the detection of $ONOO^{—}$ *in vivo* (Beal, 2002; Halliwell & Gutteridge, 2007). $ONOO^{—}$ is very toxic due to its ability to oxidize thiol groups, lipids, proteins, enzymes and DNA.

Unsaturated fatty acids of cellular membranes are biomolecules most susceptible to oxidative damage in cells, and this sensitivity increases as a function of their double bonds. Lipid peroxidation is mainly initiated by hydrogen abstractions from unsaturated fatty acids by oxygen centred radicals followed by the formation of hydroperoxides (Figure 2.2). Degradation of hydroperoxides results in a variety of derivatives including various carbonyl products (Schauenstein & Esterbauer, 1979; Esterbauer *et al.*, 1991). Such unsaturated carbonyls include enals, dienals, trienals, hydroxylenals, 2-ketoaldehydes, deoxyosones and various reductions that are very reactive and toxic to almost all cellular and extracellular biomolecules (Esterbauer *et al.*, 1991; Yin & Brunk, 1995; Baynes, 2000). Many carbonyls react readily even at neutral pH and room temperature, with important biochemical groups such as amino, thiol or hydroxyl. A secondary functional group of the carbonyls increases their reactivity potential and may induce irreversible reaction products, or result in cross-linking reactions (Bjorksten, 1968). Napetschnig (1981) reported that 4-hydroxyl alkenals could react with nearly all amino acids under appropriate conditions. Due to its reactivity, the carbonylic products, particularly α,β-unsaturated carbonyls of lipid peroxidation, are implicated in various types of cell damage, including depletion of glutathione, protein modification, disturbance of calcium homeostasis, cell membrane destruction,

tissue injuries, enzyme inhibition and disruption in DNA, RNA, and protein synthesis (Esterbauer *et al.*, 1991; Schaur, 2003; Eckl, 2003; Zarkovic, 2003).

Compared with vanishing oxygen free radicals, toxic carbonyls can pass a long distance through organ, tissue and cellular membrane and reach almost any part of the body (Esterbauer *et al.*, 1991). Lipid peroxidation generates aldehydic products, like malondialdehyde (MDA) and others that covalently attach to protein lysine residues. Some common enals that cause DNA damage analogously to proteins are MDA, acrolein, and 4-hydroxynonenal, among others (Pamplona & Barja, 2006). Common adducts arising from enals are exocyclic adducts such as ethno adducts, and M1dG. These DNA damage markers are mutagenic, carcinogenic and have powerful effects on signal transduction pathways (Pamplona & Barja, 2006). Thus oxidative damage is not limited to direct attack by ROS, since cross-reactions of side and final products of peroxidation of different kinds of macromolecules also occur. This is the case for glycoxidation and lipid peroxidation – derived reactions, which can finally lead to protein and DNA modification.

2.2.1 Factors Affecting Toxic Responses

The disposition of toxic compounds in biological systems can be divided into four interrelated phases: absorption, distribution, metabolism, and excretion (Rozmann & Klaasen, 2001). Absorption is necessary for the chemical to exert a systematic biological or toxic effect and involves crossing membranes. The following physiochemical characteristics with regard to the passage of foreign potentially toxic molecules through membranes are important. These are size/shape, lipid solubility/hydrophobicity, structural similarity to endogenous molecule, and charge/polarity of the foreign molecule (Florence & Atwood, 2005).

There are five types of transport along with their important features:

1. Filtration: Small (less than 100 molecular weight) water soluble molecules.
2. Passive diffusion: Lipid soluble, non charged molecules, concentration gradient, first order, and pH partition theory.

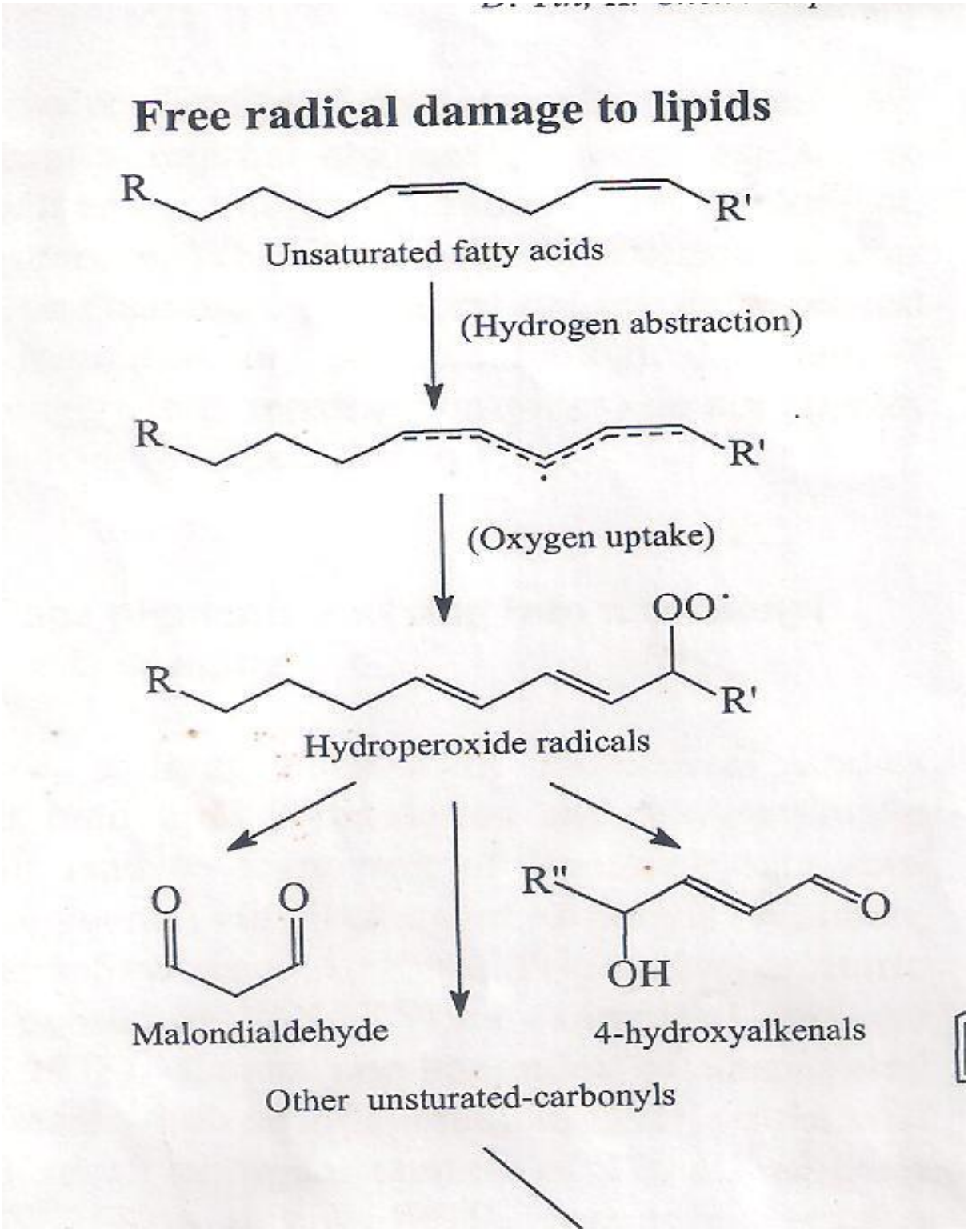


Figure 2.2: Biological side reaction; Lipid peroxidation (Yin & Chen, 2005)

3. Active transport: Specific membrane carrier, energy required, operates against concentration gradient, inhibitable, saturable, possible competition, and zero order.
4. Facilitated diffusion: Specific carrier, saturable, possible competition, operates with concentration gradient, no energy required.
5. Endocytosis: Phagocytosis/pinocytosis requires energy, large, insoluble molecules (Pratt, 1990).

There are three main sites of absorption viz:

Skin: The skin is characterized by a large surface area, poorly vascularised, and not readily permeable.

Gastrointestinal tract: Major site, well vascularised, with variable pH, large surface area, transport processes, food, gut bacteria.

Lungs: The lungs have a very large surface area, well vascularised and readily permeable.

Compounds may be administered by direct injection i.e. intraperitoneal (i.p), intramuscular (i.m), subcutaneous (s.c), and intravenous (i.v). The end result of the absorptive phase is that the compound may pass through tissues and enter the blood.

Distribution is the phase in which the compound is carried to tissues by the blood stream or lymphatic system. Compounds are usually first absorbed into the portal venous system after oral administration, directing them to the liver where they may be removed (extracted/metabolized) (first pass effect). The plasma level reflects the concentration at target/receptor and is governed by distribution. Distribution depends on passage through membranes (passive diffusion, carrier mediated transport, etc.) and may be limited by binding to plasma proteins (e.g albumin). Protein binding may involve ionic, hydrophobic, hydrogen and van der Waal bonding. It may show saturation, displacement, and competitive inhibition; therefore, threshold effects may occur (Ahday *et al.*, 1981). Chemicals may be sequestered and accumulated in tissues (compartments) depending

on certain factors (e.g, lipid solubility, pKa); distribution can change with pH of blood or tissue. Some tissues are poorly accessible (e.g brain) (De Boer *et al.*, 2003).

Blood levels may be used to derive kinetic parameters such as half-life, elimination rate constant and volume distribution. Comparison of blood levels after oral and intravenous administrations may be used to calculate bioavailability. Half-life can be used to predict the effect of repeated dosing (Tune, 1998).

Excretion is the elimination of the molecules from the organism by one of several routes. The urine is the major route, but expired air and bile may also be used. Urinary excretion involves filtration, passive diffusion and active transport. Biliary excretion involves active transport and there is no molecular weight threshold for compounds, above which excretion by this routes become more important. Biliary excretion may lead to enterohepatic recirculation. Excretion by these routes can be saturated leading to accumulation. Volatile chemicals are transported by passive diffusion from the blood into the lungs prior to inhalation. Excretion into milk is important for exposure of the new born animals.

The concentration and physiochemical properties (ionization/charge, lipid solubility, size) of the molecules have the major impact on each of the phases of its disposition.

Membrane transporter proteins (Multi drug resistance (MDR), ATP-binding cassette (ABC) transporter protein) such as P-glycoprotein are crucially important in the process of excretion and also in absorption, distribution and elimination of chemicals from cells. These transport organic anions or cations and neutral compounds across membranes, pump unwanted chemicals out of the cells such as in gut, placenta, and brain, transport chemicals into bile from liver cells, and facilitate excretion from the kidney (Ayrton & Morgan, 2001).

2.2.2 Carbon Tetrachloride

Carbon tetrachloride (CCl_4) is a colourless liquid with a sweetish odour (NLM, 2003; Lewis, 1997). Its synonyms include tetrachloromethane and perchloromethane (NLM, 2003; O'Neil & Smith, 2001).

Absorption: CCl_4 is rapidly absorbed by any route of exposure. CCl_4 is readily absorbed through the GIT in humans and other animals. Administration of CCl_4 in vehicles changes the rate and percentage of gastrointestinal absorption. Pharmacokinetics data suggested that corn oil vehicle resulted in slower absorption from GIT and subsequently lower peak blood concentrations and delayed removal from the blood stream (Kim *et al.,* 1990a)

Distribution: Once absorbed, it is widely distributed in tissues, especially with high lipid contact, reaching peak concentrations in < 1-6 hours, depending on exposure concentration or dose. Animal studies indicate that the largest fraction of an absorbed oral dose of CCl_4 is initially distributed to fat deposit or tissues (Marchand *et al.,* 1970). Other studies supported this distribution to liver, kidney, brain, and lungs (Fowler, 1969).

Metabolism: CCl_4 is metabolized in the body primarily by the liver and also by the kidney, lung and other tissues containing CYP450. The metabolism of CCl_4 has been extensively studied *in vivo* in mammalian systems. Based on the available data, a proposed scheme for CCl_4 is illustrated in Figure 2.3.

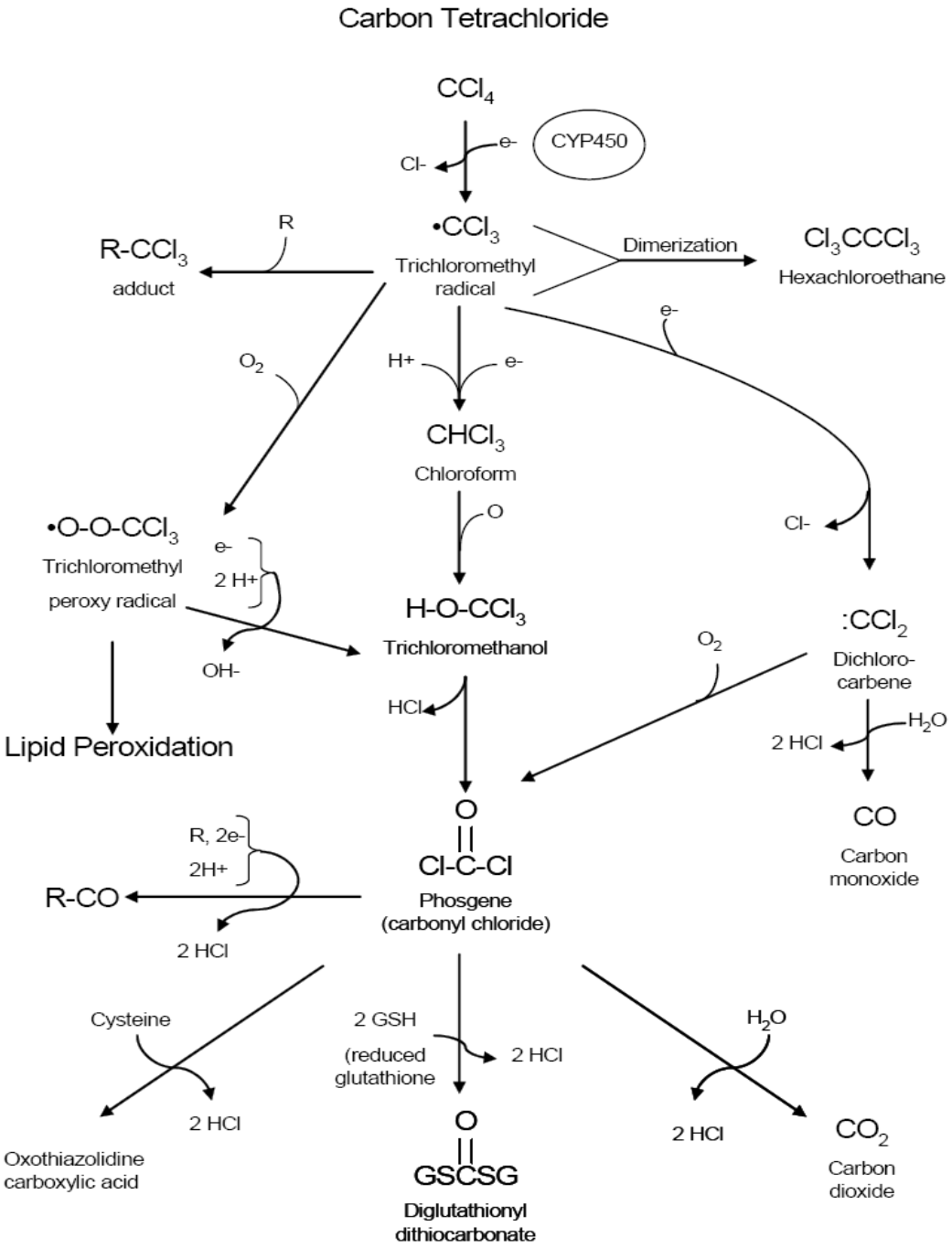
Elimination: In humans and other animals exposed to CCl_4 by any route, the unmetabolized parent compound is excreted in exhaled air. Additionally, animal studies showed that volatile metabolites are released in exhaled air, whereas non-volatile metabolites are excreted in faeces and to a lesser degree in urine (Stewart *et al.,* 1965; Morgan *et al.,* 1970; Sato and Nakajima, 1987; Sanzgiri and Buckner *et al.,* 1997).

Biotransformation of carbon tetrachloride

There is considerable evidence that the initial step in biotransformation of carbon tetrachloride is reductive dehalogenation: reductive cleavage of one carbon-chlorine bond to yield chloride ion and the trichloromethyl radical (Tomasi *et al.,* 1987; Reinke &Janzen, 1991). The initial reaction step is catalyzed by a nicotinamide adenine dinucleotide phosphate (NADPH)-dependent CYP450 that is inducible by phenobarbital or ethanol (Castillo *et al.,* 1992). In humans and animals, CYP2E1 is the primary enzyme involved with carbon tetrachloride bioactivation, while CYP3A may be involved under high exposure conditions (Zangar *et al.,* 2000). As demonstrated in studies with CYP2E1 genetic knockout mice, this enzyme is required for the development of hepatotoxicity (as measured by elevated liver enzymes and liver histopathology) in mice exposed to carbon tetrachloride (Wong *et al.,* 1998).

The fate of the trichloromethyl radical is dependent on the availability of oxygen and includes several alternative pathways for anaerobic or aerobic conditions. Anaerobically, the trichloromethyl radical may dimerize to form hexachloroethane, which has been detected in animal tissues (Uehleke *et al.,* 1973). Addition of a proton and an electron to the radical results in the formation of chloroform ($CHCl_3$), which has been detected in exposed rats and rabbits (Reynolds *et al.,* 1984). The trichloromethyl radical can undergo further reductive dehalogenation catalyzed by CYP450 to form dichlorocarbene ($:CCl_2$), which can bind irreversibly to tissue components or react with water to form formyl chloride (HCOCl), which decomposes to carbon monoxide (Pohl *et al.,* 1984). The trichloromethyl radical can bind directly to microsomal lipids and proteins (Fanelli & Castro, 1995), as well as the heme portion of CYP450.

Aerobically, the trichloromethyl radical can be trapped by oxygen to form the trichloromethyl peroxy radical, which can bind to tissue proteins (Galelli & Castro, 1998) or decompose to form phosgene ($COCl_2$) (Pohl *et al.,* 1984) and an electrophilic chlorine (Pohl *et al.,* 1984).



CYP450, usually CYP2E1, but also CYP3A; R = acceptor molecule, such as protein or lipid.

Figure 2.3: Metabolism of CCl_4 (ACGIH, 2001).

The rate of conversion of the trichloromethyl radical to the trichloromethyl peroxy radical (and to downstream reaction products with amino acids and lipids) has been estimated to be approximately 108–109 mol/l (Slater, 1981; Russell *et al.*, 1990). Rates such as 108–109 mol/l are sufficiently

high to suggest that the rate of production of the trichloromethyl peroxy radical (and, thereby, the rate of elimination of the trichloromethyl radical) may be diffusion limited (1010–1012 L/mols) (Atkins, 1998). Therefore, limiting factors in the oxidative elimination of the trichloromethyl radical are likely to be reactant concentrations at the site of production of the trichloromethyl radical (e.g., O_2) and/or factors that limit diffusion of the trichloromethyl radical (e.g., diffusion coefficient in cytosol). The trichloromethyl peroxy radical is the primary initiator of lipid peroxidation that occurs from exposure to carbon tetrachloride (McCay *et al.,* 1984; Boll *et al.,* 2001). Carbon dioxide is generated by the hydrolytic cleavage of phosgene (Shah *et al.,* 1979). Phosgene may also be conjugated to reduced glutathione (GSH) to form diglutathionyl dithiocarbonate or to cysteine to form oxothiazolidine carboxylic acid (U.S. EPA, 2001), thus reducing the cellular thiol redox status.

2.2.3 The Antioxidant System

An antioxidant is a molecule capable of preventing or inhibiting the oxidation of other molecules. The body has several mechanisms to counteract oxidative compounds or the actions of free radicals by producing antioxidants, either naturally generated i*n vivo* (endogenous antioxidant), or externally supplied through foods (exogenous antioxidant). The roles of antioxidants are to neutralize the excess of free radicals, to protect the cells against their toxic effects and to contribute to disease prevention and defence system (Pham-Hug *et al.,* 2008).

Antioxidants are basically classified into two according to their sources, as endogenous and exogenous antioxidants. Another classification grouped antioxidants into enzymatic or non enzymatic antioxidants. Endogenous antioxidants in cells can be enzymatic or non enzymatic, while exogenous antioxidants are only non enzymatic.

The major antioxidant enzymes (Figure 2.4) directly involved in the neutralization of ROS and RNS are: superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) (Wilcox *et al.,* 2004; Pham-Hug *et al.,* 2008). Superoxide dismutase, the

first line of defence against free radicals, catalyses the dismutation of superoxide anion radicals ($O^{-}{}^{\cdot 2}$) into hydrogen peroxide (H_2O_2) by reduction. The oxidant formed is transformed into water and oxygen (O_2) by catalase or GPx. The selenoprotein, glutathione peroxidase, removes H_2O_2 by using it to oxidize reduced Glutathione (GSH), into oxidized glutathione (GSSG). Glutathione reductase (GR), a flavoprotein, regenerates GSH from GSSG, with NADPH as a source of reducing power. Besides H_2O_2, GPx also reduces lipid or non lipid peroxides while oxidizing GSH (Bahorun *et al.*, 2006; Halliwell, 2007; Pham-Hug *et al.*, 2008). Non enzymatic antioxidants are also divided into nutrient (e.g vitamins A, C, and E, selenium, zinc, flavonoids e.t.c), and metabolic (lipoic acid, GSH, and co-enzyme Q_{10}) (Droge, 2002; Wilcox *et al.*, 2004) antioxidants. Nutrient antioxidants deficiency is one of the causes of numerous chronic and degenerative pathologies. Each nutrient is unique in terms of its structure and antioxidant function (Wilcox *et al.*, 2008).

Mechanism of antioxidant activity

When an antioxidant destroys a free radical, the antioxidant itself becomes oxidized. The antioxidant process can function in one of two ways: (i) Chain-breaking or (ii) prevention of initiation phase. Antioxidant enzymes like SOD, Catalase, GPx can prevent oxidizing species by reducing the rate of chain initiation, for example, by either initiating free radicals scavenging (Figure 2.4) or by stabilizing transition metal radicals such as copper and iron (Young and Woodside, 2001; Pham-Hug *et al.*, 2008). Thus, the major functions of antioxidants are: (i) to provide scavengers for ROS and RNS, (ii) to keep the cellular thiol redox status in the reduced form, (iii) to prevent or repair the oxidation of lipids and (iv) to sequester redox-active metals and finally (v) to prevent Fenton type reactions (Boesterli, 2008).

2.3 The Role of Medicinal Plants in Health Care

The use of medicinal plants as fundamental components of the African traditional health care system is perhaps the oldest and the most assorted of all the therapeutic systems (Mahomodally,

2013). In many parts of Africa, medicinal plants are the most easily accessible and affordable healthcare resources available to the local communities. Medicinal plants are used and marketed worldwide as herbal drugs or as single active ingredients over centuries. Besides their popular consumption to treat and cure human illness, plant derived natural products play important roles as a source of pharmacological tools to enable the understanding of the biochemical pathways and the etiology of diseases (Rishton, 2008).

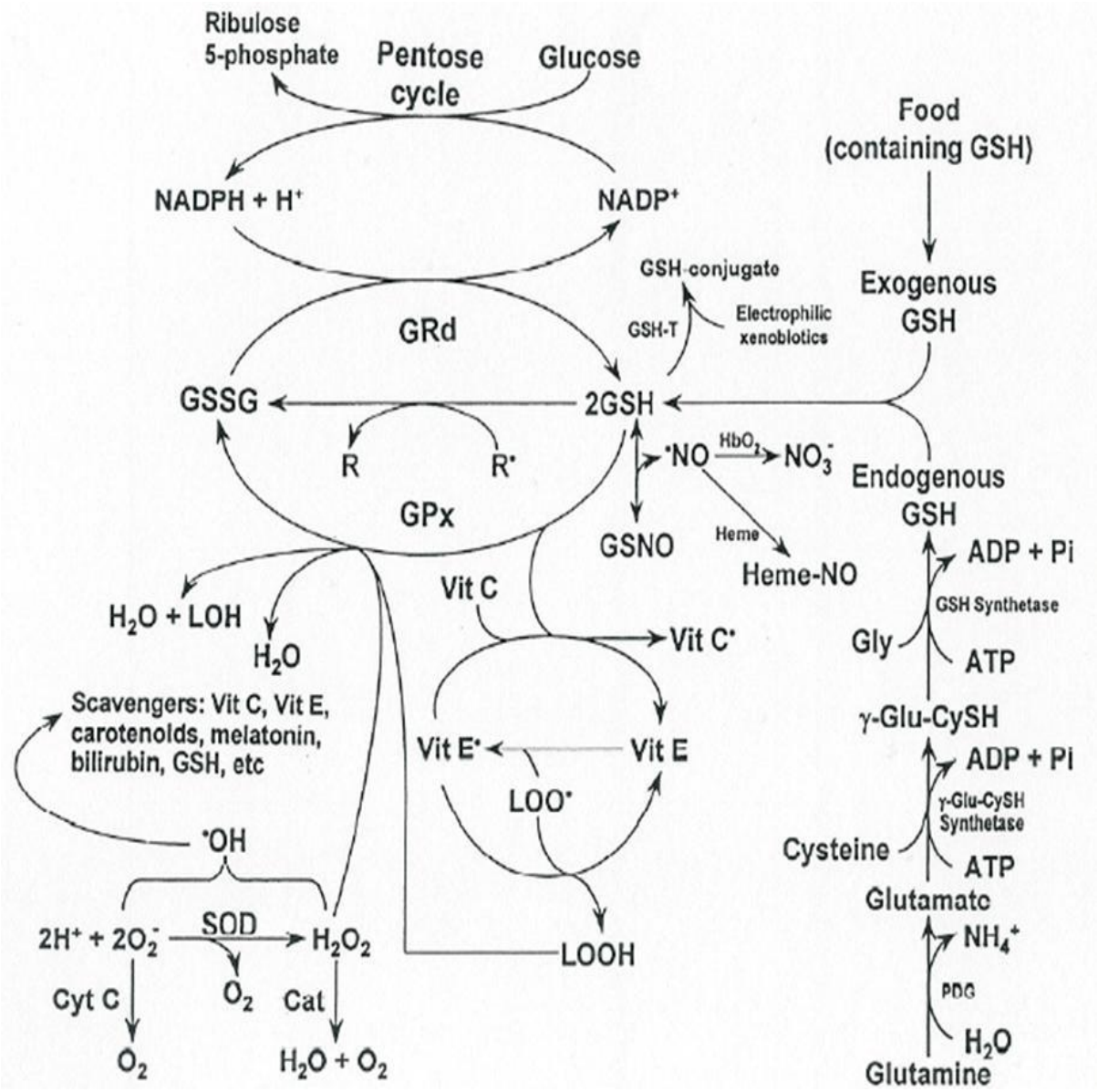


Figure 2.4: Schematic representation of antioxidative enzyme activities on free radicals and other reactants (Reiter *et al*., 2003).

Plants are sources of potential therapeutic agents against various diseases due to the presence of a wide array of bioactive phytochemicals and biodiversity and (Farombi, 2013). The use of medicinal plants in the management of diseases is an important alternative therapy widely employed in developing countries. Several investigations have yielded compounds with properties useful for the development of modern synthetic drugs for the management of several diseases (Iweala *et al.*, 2013).

It is estimated that 80 % of metabolites/plant extracts used as drugs and sold worldwide are derived from natural products and that over 100 new natural product-based lead drugs are in clinical development (Butler, 2008; Bhutani and Gohil, 2010). Aspirin, atropine, artemisinin, colchine, digoxin, ephedrine, reserpine, taxol, tubocurarine, vincristine and vinblastine are few important examples of what medicinal plants have given us in the past (Noveas and Leite, 2011). Due to growing drug discovery from natural products, researchers and pharmaceutical industries have increasing interest in traditional health practices used around the world. This interest has been rekindled for decades due to systemic demonstration that plants are the richest source of drugs for traditional system of medicine, modern medicines, naturaceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer *et al.*, 1999).

2.3.1 Phytochemicals

Phytochemicals are biologically active, naturally occurring secondary compounds found in plants, which provide health benefits for humans more than those attributed to macronutrients and micronutrients (Hasler & Blumberg, 1999).

They contribute to plant colour, aroma, flavour, and protect plants from diseases (Brown & Authur, 2001). Generally, plant chemicals that protect plant cells from environmental hazards such as

pollution, stress, drought, UV- exposure and pathogenic attack are known as phytochemicals (Gibson *et al.,* 1998; Mathai, 2000).

Phytochemicals are classified based on their protective functions, physical and chemical characteristics (Meagher & Thompson, 1999). Phytochemicals accumulate in different plant parts, such as roots, stems, leaves, flowers, fruits, and seeds (Costa *et al.,*1999; Mathai, 2000); some are concentrated in the outer layer of the various plant tissues, especially the pigment molecules with their levels varying from plant to plant depending on the variety, processing method, cooking and growth conditions (King & Young, 1999). Absence or deficiency of phytochemicals in processed foods may contribute to increased risk of disease conditions (Reader's Digest, 1998).

Phytochemicals have biological properties, such as antioxidant activity (Figure 2.5) (Aruoma *et al.,* 2010), antimicrobial effect (Gibson *et al.,* 1998; Alisi *et al.,* 2008), and are associated with a lower incidence of heart disease, ischemic stroke, and other chronic diseases (Verlengieri *et al.,* 1985; Joshipura *et al.,* 1999; Riboli & Norat, 2003; Peterson *et al.,* 2003; Bosetti *et al.,* 2005). Phytochemicals can detoxify substances that cause cancer, by neutralizing free radicals, inhibiting enzymes that activate carcinogens and also activate enzymes that detoxify carcinogens (Meagher & Thompson, 1999; Narasinga, 2003). Examples of phytochemicals found in plants include: flavonoids, alkaloids, saponins, tannins, oxalate and phytate.

2.3.1.1 Flavonoids

A flavonoid is any member of a class of widely distributed biological natural products containing aromatic heterocyclic skeleton of flavan (2- phenylbenzopyran) but no nitrogen atom in the molecule. Flavonoids are derived from the Latin word flavus, meaning yellow. They were referred to as vitamin P from the mid- 1930 to early 1950s, probably due to the effect they had on the permeability of vascular capillaries but the term has since fallen out of use. Flavonoids are the most important plant pigments for fruits, leaves, and flower colouration producing yellow or red/blue

pigmentation in petals designed to attract pollinators. In higher plants, flavonoids are involved in UV-filtration, forming antifungal barriers, antimicrobials, insecticidal and oestrogenic activities and symbiotic nitrogen fixation (Beecher, 2003; Lushsinger & Mayeux, 2004; Morris, 2005; Vita, 2005).

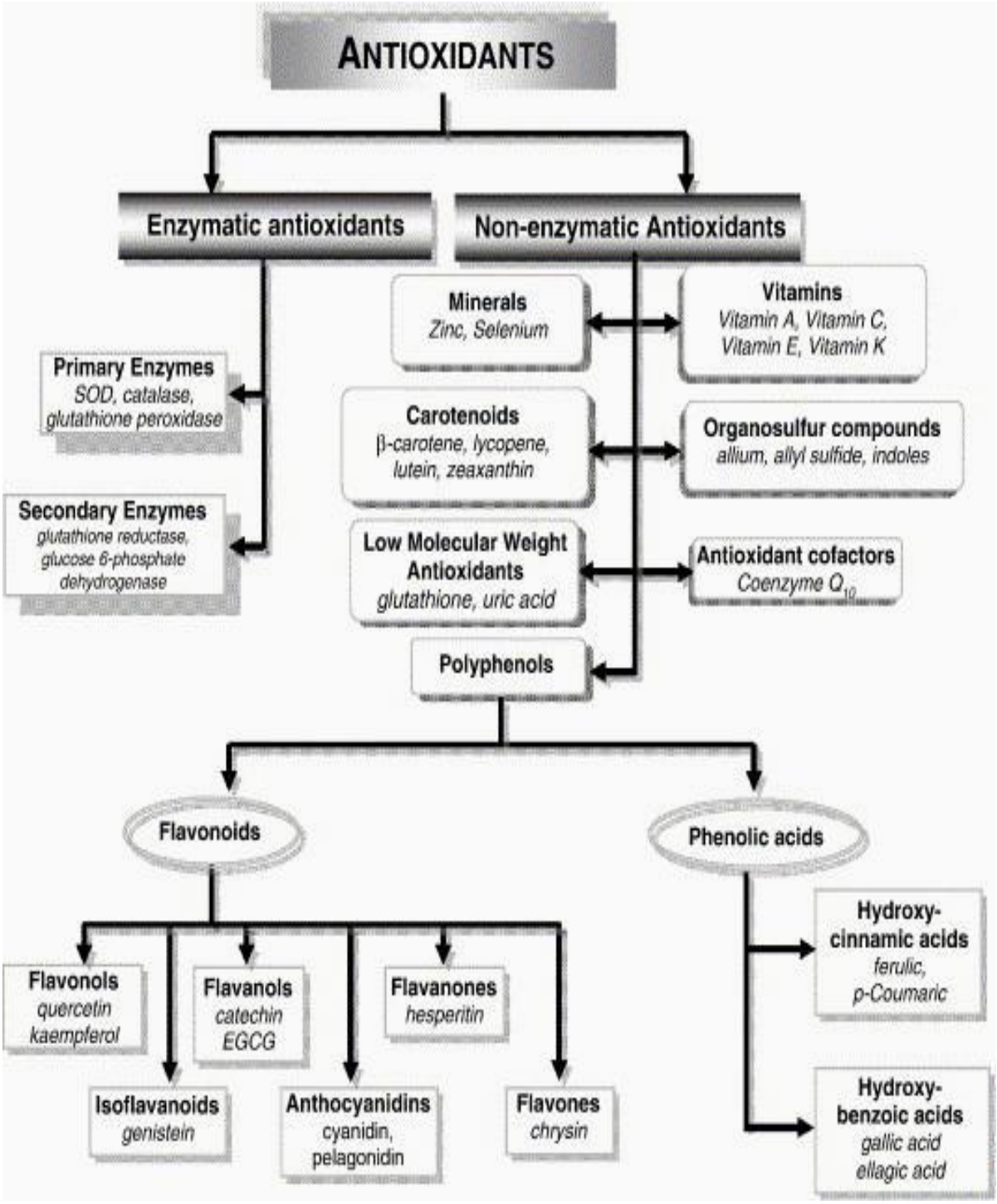


Figure 2.5: Phytochemicals as antioxidants (Reiter *et al*., 2003).

At cellular level, flavonoids have been found to exert a variety of biological effects (Middleton *et al.,* 2000), presumably mediated by specific interactions with molecular targets. Indeed flavonoids have been shown to interact with biological macromolecules such as nucleic acids (Kanakis *et al.,* 2005), polysaccharides (Zheng *et al.,* 2005; Calabro e*t al.,* 2005) and proteins (Jacobs & Lewis, 2002; Williams *et al.,* 2004).

The capacity of flavonoids to act as antioxidants, depend on their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. Flavonoids are usually classified into sub-groups according to their structural pattern (Pekkarinen *et al.,* 1999), i.e **flavonols** (quercertin, rhamnetin, kaempferol e.t.c), **flavones** (apigenin, luteolin, tangeritin e.t.c.), **lsoflavones** or 3-phenylchromen-4-one (genistein, prunetin, diadzin e.t.c), **flavanones** (hesperidin, naringenin, pinocembrin), **flavanols** (catechins, epicatechin, theoflavin, gallocatechin**), anthocyanidins** (malvidin, europinidin, rosinidin e.t.c) .

2.3.1.2 Alkaloids

Although numerous alkaloids exist, they have similar properties, when separated. In general, they are colourless, crystalline solids that are basic with bitter taste. However some exceptions are known, for instance, some alkaloids are not basic and others are brightly coloured or liquid. They naturally occur in seed bearing plants and are found in berries, bark, roots, fruits and leaves of plants (Cordell, 1981).

Use of analytical separation techniques, such as chromatography and mass spectroscopy, led to elucidation of the chemical structure of alkaloids. The term for these compounds is thought to have originated from the fact that the alkaloid morphine had similar properties to basic salts derived from the alkali ashes of plants. Thus, it was called a "vegetable alkali" or "alkaloid" (Raffauf, 1996). Most alkaloids are also chiral molecules, meaning they have non-superimposable mirror images.

This results in isomers that have different chemical properties (Raffauf, 1996).The physiological effects of alkaloids have made them important compounds in medicine. They have been used as painkillers, stimulants, muscle relaxers, tranquilizers and anaesthetics. The four types of alkaloids that have the most important economic impact include opiates, cocaine, caffeine, and nicotine (Rao *et al.,* 1978; Wink *et al.,* 1998; Agunu *et al.,* 2005).

Alkaloids are so numerous, and involve such a variety of molecular structures that their rational classifications are difficult. However, the best approach to the problem is to group them in families, depending on the type of heterocyclic ring present in the molecule (Krishnan *et al.,* 1983). The various classes of alkaloids according to the heterocyclic ring system they contain are:

(a) Pyridine alkaloids: they have piperidine (hexahydropyridine) ring system, examples are coniine, piperine and lsopelletierine.

(b) Pyrrolidine-pyridine alkaloids: they have a pyrrolidinepyridine e.g myosmine, nicotine.

(c) Pyridine-piperidine alkaloids: they contain a pyridine ring joined to piperidine ring system. The simplest member is anabasine isolated from a poisonous Asiatic plant *Anabasis aphyllan*.

(d) Quinoline alkaloids: these have the basic heterocyclic ring system. A typical example is quinoline, an anti-malaria drug which occurs in the bark of Cinchona tree.

(e) Isoquinoline alkaloids: they contain heterocyclic ring system isoquinoline e.g opium alkaloids such as nicotine, papaverine, morphine, codeine, and heroine.

Alkaloids are significant for the protection and survival of plants against microorganisms, insects and herbivores and also against plants by means of allelopathically active chemicals (Molyneux *et al.,* 1996).The position of the nitrogen atom in the carbon ring varies with different alkaloids and with different plant families. In fact, it is the precise position of the nitrogen atom that affects the properties of these alkaloids. Alkaloids can also be classified based on their biosynthetic pathways as many alkaloids are synthesized from ornithine, tyrosine, tryptophan, pyridine or lysine (Raffauf, 1996).

Table 2.1: Some Alkaloids and Their Medicinal Applications (Meyers, 2001)

Alkaloid	**Action**
Ajmaline	Antiarrhythmic
Atropine, opolamine, hyoscyamine	anticholinergic
Caffeine	stimulant, adenosine receptor antagonist
Codeine	cough medicine, analgesic
Colchicine	remedy for gout
Emetine	antiprotozoal agent
Ergot alkaloids	sympathomimetic, vasodilator, antihypertensive
Morphine	analgesic
Nicotine	stimulant, nicotinic acetylcholine receptor agonist
Physostigmine	inhibitor of acetylcholinesterase
Quinidine	antiarrhythmic
Quinine	antipyretics, antimalarial
Reserpine	antihypertensive
Tubocurarine	muscle relaxant
Vinblastine, vincristine	antitumor
Vincamine	vasodilating, antihypertensive
Yohimbine	stimulant, aphrodisiac

Medical uses of alkaloid-containing plants have a long history, and, thus, when the first alkaloids were isolated in the 19th century, they immediately found application in clinical practice (Hesse, 2002a). Many alkaloids are used in medicine (Table 2.1), usually in the form of salts (Hesse, 2002b). Many synthetic and semisynthetic drugs are structural modifications of the alkaloids, which were designed to enhance or change the primary effect of the drug and reduce unwanted side-effects (Hesse, 2002c). For example, naloxone, an opioid receptor antagonist, is a derivative of thebaine, present in opium (Dewick, 2002).

2.3.1.3 Saponin

Saponins belong to the triterpenoid class of terpenes. They consist of a polycyclic aglycone that is either a choline steroid or triterpenoid, attached via C_3 on/or bonded to a sugar side chain. The aglycone is referred to as the sapogenin and the steroid saponins are called saraponins. The number of saccharide chains attached to the sapogenin core can vary in length and types, with the most common components of the attached chains being dietary monosaccharides like D-glucose and D-galactose. According to the structure of the sapogenins, two kinds of saponins are recognized: the steroidal (commonly tetracyclic triterpenoids) and the pentacyclic triterpenoid types. Both have a glycosidic linkage at C_3 and have a common biogenetic origin via mevalonic acid and isoprenoid units (Evans, 2005).

Saponins are a class of natural products which on intravenous injection into animals get complexed with cholesterol to form pores in erythrocytic cell membrane bilayers, which results in cell lysis and thus are highly toxic. However, because of their amphipathic nature, they act as surfactants that can be used to enhance penetration of macromolecules such as proteins through cell membranes. Steroidal saponins are of great pharmaceutical importance because of their relationship with compounds such as sex hormones, cortisone, diuretic steroids, vitamin D, and cardiac glycosides. They also have relationship with the hormone, oxytocin, which is involved in controlling the onset of labour in women and subsequent release of milk (Okwu & Okwu, 2004). Saponins have

detergent properties, and thus are sometimes used as foaming agents in foods and cosmetics. They prevent cancer by protecting DNA from damage, they have been shown to be antiviral from *in vitro* studies, and directly inhibit colon cancer. Saponins can be cardioprotective by their ability to lower cholesterol levels (Oakenfull, 1981). They have a potential role in cancer prevention by acting as antioxidant and anti-mutagenic agents.

2.3.1.4 Tannins

Tannins are naturally occurring astringent, bitter, polyphenolic compounds of plants that bind, and precipitate proteins. Thus they have a large influence on the nutritive value of many foods eaten by humans and animals. However not only tannins bind and precipitate proteins. Other phenolics such as pyrogallol and resorcinol also have this property, but not all polyphenols precipitate proteins or form complexes with polysaccharides (Waterman & Mole, 1994; Onwuliri *et al.,* 2002). The astringency of tannin is what causes the dry and pucker feeling in the mouth following the consumption of unripe fruit or red wine (Kadam & Charan, 2000). Tannins proffer defence mechanisms in plants against pathogens, herbivores and hostile environmental conditions (Hagerman & Klucher, 1986). They significantly destroy microbes through effects on their metabolism involving deprivation of iron and other metal ions and thus restriction of oxidative phosphorylation (Ajao *et al.,* 1985). Tannins hasten the healing of wounds and inflamed mucous membranes. They decrease organic matter and fibre digestion because of their interaction with cellulose enzymes and rumen bacteria. Protein digestibility is also affected, since the complexes formed during its interaction with tannins make it more difficult to digest (Giner-Chavez, 1996; Onwuliri *et al.,* 2004).

2.4 Organ Function

2.4.1 Liver

The liver is a versatile organ involved in many biochemical reactions, secretory functions, metabolic functions, excretory functions, and synthesis of certain blood coagulation factors such as

factors V, VII, X, and fibrinogen, synthesis of albumin and globulin, detoxification function, storage function and others. Tests used in the study of liver and biliary tract diseases can be classified according to the specific function of the liver (Chatterjea & Shinde, 2007), and are generally termed liver function test (LFT).

Abnormalities of bile pigment

The haem group of haemoglobin, from senescent erythrocytes is degraded to yield free iron (ii) and bilirubin. Bilirubin is largely insoluble and is transported in blood bound to albumin (unconjugated bilirubin). In the liver, albumin-bound bilirubin is converted to bilirubin diglucuronide (conjugated bilirubin) catalyzed by the enzyme UDP – glucoronyl transferase. Impaired function of the liver or block in bile secretion causes bilirubin to leak from the liver into the blood resulting in the yellowing of the skin and eyeball, a condition called jaundice (Nelson & Cox, 2005; Raju & Madala, 2005).

Changes in plasma proteins in diseases

Determination of total protein, albumin, globulin and albumin-globulin ratio (A:G) yields useful information in liver diseases. The liver is the site of albumin synthesis and possibly of some α and β – globulins. Plasma proteins contribute amino acids for tissue protein synthesis. Plasma proteins play an important role in fluid exchange, buffering action, blood coagulation and fibrinolysis, immunological function, binding and transport function; they act as enzymes and also account for the viscosity of blood.

Abnormalities of lipids in diseases

Lipids are heterogeneous bio-organic molecules, which are sparingly soluble in water but are freely soluble in organic solvents such as ether, chloroform, and benzene. Lipids are structural component of biological membranes e.g phospholipids and cholesterol. They are the major storage form of energy in the body e.g triglycerides. Some vitamins and hormones are lipid derivatives e.g vitamin D2, D3, steroidal hormones and amphipathic bile acids (catabolic products of cholesterol) aid digestion and absorption of lipids. Chylomicrons synthesized in the intestine act as a carrier for

exogenous triglycerides and cholesterol. Very low density lipoprotein (VLDL) synthesized in the liver acts as carrier of endogenous triacyglycerides (TG) and cholesterol from the liver to extrahepatic tissues for storage or membrane formation.

Low density lipoprotein (LDL) is a degradation product of VLDL. It results from the hydrolytic removal of triacyglycerides from VLDL. It is rich in cholesterol and cholesterol esters; LDL regulates cholesterol synthesis in extrahepatic tissues. High density lipoprotein (HDL) synthesized in the liver and intestine, scavenges body and blood vessel wall cholesterol from peripheral tissues to liver for degradation.

Serum enzymes in liver disease

Transamination is a reversible reaction in which an $\alpha - NH_2$ group of one amino acid is transferred to a keto acid resulting in the formation of a new amino acid and a new keto acid. Two specific transaminases of clinical importance in the body (in that they use specific amino acid and specific keto acids) are alanine aminotransferase (ALT) and aspartate amino transferase (AST). ALT is found mainly in the liver; it is entirely cytoplasmic and most helpful in diagnosis of liver disease. AST is concentrated in the myocardium (heart muscles) and in the liver, and it is also distributed in other tissues such as muscles and kidneys. Increases in activities of both transaminases are found in liver diseases with ALT much higher than AST. Alkaline phosphatase (ALP) is found in a number of organs, with the highest levels in bones and liver, then, in the small intestine, kidney and placenta.

2.4.2 Renal Function Test

The major function of the kidney is to get rid of waste products of metabolism such as urea, creatinine and uric acid. It gets rid of foreign and non-endogenous substances, maintains salt-water balance as well as maintaining acid-base balance. The removal of excess ammonia (NH_3) derived from amino acid catabolism in the tissues or from bacterial action in the gut is accomplished by the

production of urea, which is excreted in the urine. Urea formation takes place in the liver, and enzymes involved are partly mitochondrial and partly cytoplasmic (Nelson & Cox, 2008).

The amount of creatinine formed is proportional to muscle mass and remains remarkably constant from day to day. Creatine is excreted normally as creatinine. Creatinine is formed in muscle by non-enzymatic dehydration of creatine. Creatinine is excreted by the kidneys and the level of excretion (creatinine clearance rate) is a measure of renal function (Raju & Madala, 2005).

Water and electrolyte balance

Electrolytes are substances which dissociate in solution into cations and anions e.g NaCl, KCl, etc. They are important both in the distribution of and retention of body water. The electrolyte composition of tissue fluid is similar to that of plasma except that chloride ion largely replaces proteins as anions. The predominant cation is sodium ion. The law of electrical neutrality states that fluid in any body compartment will contain equal number of cations and anions (Chatterjea & Shinde, 2007).

Acid-base balance

The pH of blood is maintained in a narrow range of 7.35 – 7.45 and it is maintained approximately at 7.4. Maintenance of this constant blood reaction is one of the prime requisites of life and any other material variation on either side adversely disturbs the vital body processes and may lead to death. Relatively small changes in this pH value of blood can lead to severe metabolic consequences. Therefore, blood buffering is extremely important in order to maintain homeostasis. The pH value < 7.3 leads to acidosis and pH value > 7.5 leads to alkalosis. Large hydrogen ion (H^+) are continually contributed to this fluid from intracellular metabolic reactions, hence to maintain constancy, it is necessary and imperative that they are removed from the fluids effectively and promptly. The mechanism of neutrality regulation is concerned, therefore, with maintaining a state of equilibrium between production i.e, introduction of H^+ ions and removal of the same. Although the blood contains numerous cations (e.g Na^+, K^+, Ca^{2+}, and Mg^{2+}) and anions (e.g Cl^-, PO_4^- and

SO_4^{2-}), that can play a role in buffering; the primary buffers in blood are haemoglobin in erythrocytes and bicarbonate ion (HCO_3^-) in the plasma. Buffering by haemoglobin is accomplished by ionization of the imidazole ring of histidines in this protein (Raju & Madala, 2005; Chatterjea & Shinde, 2007).

2.4.3 Metabolically important minerals

Metabolically important minerals are grouped under two classes: Macro-elements and trace elements. Macro elements are those which are required in quantities more than 100 mg/day, of which examples are sodium, potassium, chloride, calcium, phosphorus and magnesium ions. Trace elements are those which are required in less than 100 mg/day; examples are cobalt, copper, iodide, iron, manganese, molybdenum, zinc, selenium, and fluoride ions (Raju & Madala, 2005). Most minerals with the exception of sodium and potassium form salts with other compounds that are relatively insoluble and not readily absorbed. Specific carrier proteins are often required for mineral absorption; the synthesis of these proteins serves as an important mechanism for control of mineral levels in our body. Specific proteins are also required for the transport and storage of many minerals. Most minerals are excreted in urine, and many are also secreted into the digestive juice and bile.

Deficiencies are usually secondary to malabsorption, chronic bleeding, renal disease or other clinical problems. Toxicity is usually noticed when control of absorption fails in some way (Raju & Madala, 2005).

Sodium

The principle cation in the extracellular fluid (ECF) is sodium (Na^+). Sodium in the body is mainly associated with the anions chloride (Cl^-) and bicarbonate (HCO_3^-) as NaCl and $NaHCO_3$. Sodium is absorbed actively by sodium pump situated in basal and lateral plasma membrane of intestinal and renals by the sodium pump called Na^+ - K^+ ATPase. Sodium is concerned with fluid balance (retains

water in ECF), neuromuscular excitability, acid- base balance, maintenance of viscosity of blood. Sodium also plays a role in resting and action potentials of membranes.

Potassium

Potassium is the major intracellular cation. This high intracellular level is maintained by active transport of the sodium potassium pump. Potassium is continually filtered by the glomeruli of the kidney and reabsorbed by the cells of proximal convulated tubules. Many functions of sodium and potassium are carried out in coordination with each other and are common. Potassium influences muscular activity. It is involved in acid base balance, neuromuscular irritability and nerve conduction process. It plays important role in cardiac function and certain enzymes such as pyruvate kinase which requires K^+ as cofactor.

Calcium

Calcium is an important mineral found mainly in bones and teeth in the form of hydroxyapatite (calcium phosphate with some hydroxyl groups) associated with the cartilage (protein) matrix. Calcium is absorbed from the gut incompletely against electrical and concentration gradients by simple diffusion and an active transport process involving energy and Ca^{2+} pump. Both processes require calcitriol (1,25–dihydroxy – D3) which regulates the synthesis of Ca- binding proteins and transport and also, a Ca^{2+} - dependent ATPase. Dietary factors which influence the absorption of calcium include fatty acids, sugars, organic acids, phytic acid, oxalates, fibres, minerals like magnesium, iron and vitamin D. Calcium plays a role in blood coagulation, calcification of bones and teeth, neuromuscular transmission, excitability of nerves, muscle contraction, normal excitability of heart. Calcium also plays a role as secondary or tertiary messenger in hormone action as well as in permeability of gap junctions.

Iron

Iron is one of the most essential trace elements in the body. Naturally iron can only be excreted by menstruation, haemorrhage or sloughing of cells from the body, but drugs such as deferoxamine can

be used to reduce iron overload. Sixty nine percent (69 %) of the total iron content of an adult (3.7 g of 70 kg) is present in haemoglobin; the rest (almost 29 %) is stored as ferritin, and the remainder is in myoglobin, cytochromes, catalase, peroxidase (e.g glutathione peroxidase) and iron requiring enzymes, such as xanthine oxidase, cytochrome C reductase, acyl CoA dehydrogenase, NADH – reductase (enzymes that use riboflavin as coenzymes). Iron is always found in bound form because free iron is chemically very reactive and toxic. i.e it binds non specifically to many proteins, with deleterious consequences to the structures, acts catalytically in assorted oxidation reactions e.g free iron catalyzes the conversion of superoxide (O_2^-) to hydroxyl (OH^-) radicals, which initiate peroxidation of unsaturated fatty acids in cellular membranes. Since iron is in bound form, it does not get excreted. Iron is lost from the body only by processes such as bleeding, sloughing of cells, menstrual flow and transfer to a developing fetus. The mechanism by which the body's iron content gets regulated is absorption. The absorption of iron from intestinal lumen into mucosal cells takes place as Fe^{2+}. Gastroferrin, a glycoprotein in gastric juice is believed to bind iron and facilitate its uptake in duodenum and jejunum. Oxalates and phytates present in foods chelate iron and renders it non absorbable.

Chlorine

Chlorine is taken in diet as sodium chloride. Many vegetables and meat have small proportions of chloride. It is also available in the chlorinated water normally supplied as a process of purification of water for drinking purpose. Absorption of chloride occurs in the small intestines. The mechanism of chloride uptake is unclear but it appears to depend on an exchange process with the HCO_3^-, whilst the accompanying sodium ion exchanges for a hydrogen ion. Chloride is important in the production of HCl in the gastric juice. It is also important in chloride shift.

Zinc

Zinc is distributed in different parts of the body as follows: skin, prostate (testes), bones, teeth, kidney, muscles, heart, pancreas and spleen and very low in brain and lungs. Only a small

percentage of dietary zinc is absorbed from duodenum and ileum. It has been reported that low molecular weight zinc – binding factor is secreted by the pancreas, which forms complex with zinc and helps in its absorption (Chatterjea & Shinde, 2007). High amounts of dietary calcium, phosphates and phytic acid have been found to interfere with zinc absorption. Zinc is lost in sweat, faeces and urine. The normal serum level of zinc is about100 µg/dl, one third of which is loosely bound with albumin and two thirds is firmly bound to globulins. There are more than 100 zinc metalloenzymes, including large number of nicotinamide adenine dinucleotide (NADH) dehydrogenases, RNA and DNA polymerases, alkaline phosphatase, superoxide dismutase, carbonic anhydrase and retimine reductase. Zinc plays a role in vitamin A metabolism, insulin secretion, growth and reproduction, wound healing and in biosynthesis of mononucleotides (Wardlaw *et al.*, 2004).

2.4.4 Vitamins with Antioxidant Activity

Both fat and water soluble vitamins are essential organic substances needed in small amounts in the diet for normal function, growth and maintenance of body tissues. Their most prominent function is as co-factors in enzymatic reactions. The most distinguishing feature of vitamins in general is that they cannot be synthesized by human cells and, therefore, must be supplied in the diet. If the vitamin content is insufficient to meet needs, a deficiency occurs, accompanied by a measurable decline in health. Vitamins have proved useful as pharmacological agents in treating a limited number of non deficiency diseases (Wardlaw *et al.*, 2004).

Vitamins differ from other organic foodstuffs in that they do not undergo degradation to provide energy, unlike carbohydrates and lipids; several B-complex vitamins play a role as ‘co-enzymes’ in several energy yielding chemical reactions (Wardlaw *et al.*, 2004; Raju & Madala, 2005; Chatterjea & Shinde, 2007). Except for vitamin K, the fat soluble vitamins are not readily excreted from the

system. In contrast, most water soluble vitamins are generally lost from the body via the kidneys. Two exceptions are vitamin B12 and vitamin B6, which are stored much more readily than the other water-soluble vitamins. Because of the limited storage of many vitamins, they should be consumed in the diet daily. Fat malabsorption resulting from various diseases is associated with poor absorption of the fat-soluble vitamins (FNB, 1998; FNB, 2002; Giovannucci, 2002).

Heat, light, exposure to air, cooking in water and alkalinity are all factors that can destroy vitamins. Adequate absorption of fat soluble vitamins depends on efficient fat absorption. This in turn, depends on fat digestion, utilizing bile salts, and the enzyme lipoprotein lipase in the small intestinal wall (Wardlaw *et al.*, 2004).

Vitamin A

Vitamin A consists of three biologically active molecules: retinol, retinal, and retinoic acid. Each of these compounds is synthesized from the plant precursor β-carotene. β-carotene consists of two molecules of the provitamin form of vitamin A. Ingested β-carotene is cleaved in the lumen of the intestine by β-carotene dioxygenase to yield retinal. Retinal is reduced to retinol by retinaldehyde reductase, an NADPH requiring enzyme within the intestines. Retinol is esterified to palmitic acid and delivered to the blood via chylomicrons. Human liver is the only organ where carotenes are converted to vitamin A. The uptake of chylomicron remnants by the liver results in the delivery of retinol to the liver for storage as a lipid ester within the lipocytes. Transport of retinol from the extrahepatic tissues occurs by binding to specific retinol binding protein (RBP). Within extrahepatic tissues, retinol is bound to cellular retinol binding protein (CRBP). Plasma transport of retinoic acid is accomplished by binding to albumin (Raju & Madala, 2005; Chatterjea & Shinde, 2007). Vitamin A acts as a very effective antioxidant and is said to reduce the risk of cancers initiated by production of free radicals. Vitamin A also plays a role in vision, reproduction, epithelialisation, bone and teeth formation, growth, and may be involved in protein synthesis and metabolism of DNA.

Vitamin C

Ascorbic acid is more commonly known as vitamin C. Ascorbic acid is derived from glucose via the uronic acid pathway. Primates lack the enzyme L-gluconolactone oxidase responsible for the conversion of gluconolactone to ascorbic acid. Hence primates require ascorbic acid in their diet. The main function of ascorbate is as a reducing agent in a number of reactions. Vitamin C has the potential to reduce cytochromes a and c of respiratory chain as well as molecular oxygen (Raju & Madala, 2005). Its strong reducing property depends on the liberation of H-atoms from the enediol-OH groups, on C_2 and C_3, the ascorbic acid being oxidized to dehydroascorbic acid, e.g by air, H_2O_2, $FeCl_3$, ferricyanide, 2,6-dichlorophenol indophenols, etc. Besides its role as an antioxidant, vitamin C is required in the formation of 5-OH tryptamine in the pathway of biosynthesis of serotonin. Ascorbic acid is necessary for the formation of tissue ferritin. Ascorbic acid in food helps in the absorption of iron by converting the inorganic ferric ion to the ferrous form. Ascorbic acid in combination with folic acid helps in the maturation of red blood cells. Vitamin C is capable of both activating and inhibiting different groups of enzymes. Arginase and papain are activated, whereas, the activity of urease and β amylase from plants is inhibited. Vitamin C is required as a cofactor in the hydroxylation of proline residues in collagen. Vitamin C is therefore required for the maintenance of normal connective tissue as well as for wound healing since synthesis of connective tissues is the first event in wound tissue remodeling. Vitamin C is needed as a cofactor in the synthesis of bile acids and epinephrine from tyrosine. Deficiency of vitamin C leads to the disease scurvy due to the role of the vitamin in the post-translational modifications of collagen. Scurvy is characterized by easily bruised skin, muscle fatigue, soft swollen gums, decreased wound healing, hemorrhage, osteoporosis, and anemia (Raju & Madala, 2005). Vitamin C is readily absorbed and so the primary cause of vitamin C deficiency is poor diet and/or an increased requirement.

The primary physiological state that can lead to an increased requirement for vitamin C is severe stress (or trauma). This is due to a rapid depletion in the adrenal stores of the vitamin.

Vitamin E

Vitamin E is a mixture of several related compounds known as tocopherols. The α-tocopherol is the most potent of the tocopherols. Vitamin E is absorbed from the intestines, packaged in the chylomicrons. It is delivered to the tissues via chylomicron transport and then to the liver through chylomicron remnant uptake. The liver can export vitamin E in VLDLs. Due to its lipophilic nature vitamin E accumulates in cellular membranes, adipose tissue and other circulating lipoproteins. The major site of vitamin E is in the adipose tissue.

Also the major function of vitamin E is to act as an antioxidant by scavenging free radicals and singlet-oxygen. In particular, vitamin E is important for preventing peroxidation of polyunsaturated membrane fatty acids. The vitamins C and E are interrelated in their antioxidant capabilities. Active α-tocopherol can be generated by interaction with vitamin C following scavenging of a peroxy free radical. Alternatively, α-tocopherol can scavenge two peroxy free radicals and then be conjugated to glucuronate for excretion in the bile. The major symptom of vitamin E deficiency in humans is an increase in red blood cell fragility. Since vitamin E is absorbed from the small intestines in chylomicrons, any fat malabsorption disease can lead to deficiencies in vitamin E intake. Neurological disorders have been associated with malabsorptive disorders and vitamin deficiency.

2.4.5 Essential and Non Essential Amino Acids

Proteins can be broken down hydrolytically to form amino acids. The general structure of amino acids includes an α-amino (α-NH_2) group, an α-carboxylic (α-COO^-) group and a variable side chain (R group). The α-amino group has a pKa of 9.8, therefore ionized (+vely charged) at physiological pH. The α-carboxylic group, has a pKa of 2.5, therefore ionized (-vely charged) at physiological pH (Raju & Madala, 2005).

Amino acids can be classified as neutral, basic and acidic depending on their reaction in solution. Nutritionally, amino acids are of two types: (i) essential amino acids; they are not synthesized by the organism and need to be supplied in the diet (e.g methionine, threonine, tryptophan, valine, isoleucine, leucine, phenylalanine, and lysine) and (ii) non essential amino acids; these can be synthesized by the body and may not be requisite component of the diet. There is a third group of semi- essential amino acids that are growth promoting factors, since they are not synthesized in sufficient quantity during growth. They include arginine and histidine. They become essential in growing children, pregnancy, and lactating women.

Amino acids serve variety of functions:

(a) Amino acids are glucogenic; (aspartate, glutamate, alanine e.t.c)

(b) Specific amino acids give rise to specialized products; e.g thyroid hormones; T3 and T4, catecholamines, melanin, niacin, serotonin (from 5-hydroxyltryptamine), creatine (glycine, arginine, and methionine), bile salts (glycine and cysteine), glutathione (glutamate, cysteine, glycine), histamine (histidine), heam (glycine), pyrimidines (aspartate and glutamate) and purines (glycine, aspartate, glutamate, and serine).

(c) Glycine and cysteine are used as detoxicants of specific substances.

(d) Methionine acts as 'active' methionine (S-adenosylmethionine) and transfers methyl group to various substances by transmethylation.

(e) Cysteine and methionine are sources of sulphur.

(f) Precursors of hormones and neurotransmitters.

(g) Buffers

(h) Precursors of proteins

(i) Transmitters.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals, Reagents and Kits.

All chemicals used in this study were of analytical grade and are products of May and Baker, England and Merck, Germany. Quercetindihydrate (Sigma-Aldrich, MO USA), catechin (Sigma-Aldrich, MO USA), Folin-Denis reagent (FlukaChemie, Switzerland), nitrobluetetrazolium (NBT) (FlukaChemie, Switzerland), riboflavin, sodium nitroprusside (SNP) (M&B Ltd England). Napthylethylenediaminedihydrochloride (NED) (Fieser, Germany), Sulfanilamide (Fieser, Germany), 2-Thiobarbituric acid (TBA) (Sigma-Aldrich, MO USA), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (FlukaChemie, Switzerland) , Ascorbic acid (Labosi, Paris, France) sodium dodecyl sulphate (SDS) (FlukaChemie, Switzerland), 5,5-dithiobis -2-nitrobenzoic acid (Sigma-Aldrich, MO USA), Bovine Serum Albumin (BSA) (Sigma-Aldrich, MO USA), Butylated hydroxyl toluene (FlukaChemie, Switzerland), Triphenyl-formazan (TPF) (Sigma-chemicals, MO USA), 2,3,5-triphenyltetrazolium chloride (TTC) (BDH Poles, England).

Others include adrenaline (FlukaChemie, Switzerland), glutathione (GSH) (FlukaChemie, Switzerland), 5,5-dithiobis-2-nitro-5-thiobenzoic acid (FlukaChemie, Switzerland), Folin-ciocaltaues reagent (Sigma-Aldrich, MO USA), Folin-Dennis reagent (Sigma-chemicals, MO USA), Potassium bromated, Nujoe oil, helium, Acetone, 2 M HCl, Vanadate – Molybdate yellow solution. All other chemicals and reagents used were from varied sources and of analytical grade. Reagents and solvents such as ethanol, chloroform, diethyl ether and hexane used for GC- MS were all Analar grade obtained from Merck Germany.

3.1.2 Equipment and Apparatus Used for Analyses.

This study used different equipment and apparati from different manufacturers which include; Buck 530 gas chromatograph, equipped with an on-column automatic injector, flame ionization detector, HP 88 capillary column (100m x 0.25µm film thickness,) CA, USA. Infrared spectrophotometer Model 530 Buck Scientific, CA, USA. Digital pH meter (Labtec lndia), lntubator, UV-visible spectrophotometer model D_{20} (Bausch and Laumb, Germany), Digital spectrophotometer 390 (Tuner, USA), Hot air oven (England), Water bath (Grant, England), Digital weighing balance-Mettler PT 320 (Mettler-Wagen, Switzerland), Rotary shaker (Marrienfeld, Germany), Deep freezer (Freshpoint FDF-196,), Rotary evaporator (Buchi Rotavapour-Switzerland), Bench centrifuge (Clay Adams, USA), Muffle furnace (Cabolit, England), Automatic micropipettes (TECO diagnostics, USA), Tissue homogenizer BLK 397 (Kenwood ltd, Japan), Separating funnel, Heating mantle. GC- analyses were carried out with SHIMAZU Japan Gas Chromatography 5890-11 with a fused GC column OV 101 coated with polymethyl silicon. The Mass Spectrometer was also equipped with a computer fed Mass Spectra data bank, HERMCE Z 233 MZ centrifuge Germany was used. Heating mantle, Crucible, Glass rod, Flame photometer, 100 ml volumetric flask, Whatman No. 1 filter paper, Wash bottle, 10 ml pipette, funnel.

3.1.3 Plant Sample

Fresh leaves *of C. dolichopentalum* were harvested from a farm in Obinze in Owerri West Local Government Area of Imo state. The plant was authenticated by Mr. A. Ozioko, of the Bioresource Development and Conservation Program (BDCP), Research Centre at the University of Nigeria, Nsukka, Enugu State, Nigeria. The fresh leaves were plucked from their stems, washed with distilled water and allowed to dry at room temperature. The dried samples were pulverized (using electric blender) and stored in an airtight container kept in a dessicator for 3 days.

3.2 METHODS

3.2.1 Proximate Analysis

Moisture content

This involves measurement of the mass lost due to evaporation of water at or near the boiling point of water. It was carried out as described by AOAC (1984).

Procedure: The weight of a glass Petri dish (w_1) was taken before and after adding 5.0 g of the sample (w_3). The Petri dish and its content were placed in an air circulating oven set at 105^{o}C. The sample was allowed to dry for 4 hours. The container and content were allowed to cool, and the weight taken (w_2). This process was repeated until two consecutive weighing results gave a constant value.

Calculation:

$$X\ \% = \frac{w_3 - w_2}{w_3 - w_1} \times \frac{100}{1}$$

Where $w_3 - w_2$ = weight of moisture

$w_3 - w_1$ = weight of sample

Ash content

The ash content of a material is the residue remaining after ignition at 500-600^{o}C for 2-4 hours, to burn off all the organic matter, as described by the method of AOAC (1984).

Procedure: The weight of a porcelain dish was taken (w_1). Three grams (3.0 g) of the sample were placed in the platinum dish and the weight recorded (w_2). The Petri dish and its content were placed in a muffle furnace set at 550^{o}C for 3 hours. This was cooled completely and the weight taken (w_3).

Calculation: $$X\ \% = \frac{w_3 - w_1}{w_2 - w_1} \times 100$$

Where $w_3 - w_2$ = weight of ash

$w_2 - w_1$ = weight of sample

Total lipid content

The lipid content was determined by method described by Pearson (1976).The total lipid content may be considered as consisting of the free and bound lipid constituents. Free lipid constituents can be extracted by the less polar solvents such as light petroleum fractions, diethyl ether, petroleum ether, whereas 'bound' lipid constituents require more polar solvents such as alcohols for their extraction.

Procedure: The weight of a thimble plugged with cotton wool was taken (w_1), and 10.0 g of the sample was placed in the thimble and the weight taken (w_2). Using a soxhlet extraction apparatus, the sample was extracted with ethanol at the boiling temperature of ethanol (78^oC) for 9 hours. At the end of the extraction, the thimble and its content were transferred to an oven set at the evaporation temperature of the extraction solvent for 90 minutes. This was cooled and the weight taken (w_3). The percentage lipid content was calculated using the relationship:

$$X\ \% = \frac{w_2 - w_3}{w_2 - w_1} \times 100$$

Where, X % = % lipid content

W_2 = weight of thimble, cotton wool and sample before extraction

W_3 = weight of thimble, cotton wool and sample after extraction

W_1 = weight of thimble and cotton wool alone

Protein content

This was done by the Kjeldahl method of AOAC (1984). It involves the wet combustion of the sample by heating with concentrated H_2SO_4 in the presence of metallic and other catalysts to effect the reduction of organic nitrogen to ammonia, which is retained in solution as ammonium sulphate. The digest, having been made alkaline is distilled or steam distilled to release the ammonia which is trapped and is later titrated.

Procedure: Two grams (2.0 g) of the sample were measured into a Kjeldahl flask containing boiling chips. With a spatula, 1 g of Kjeldahl copper and sodium sulphate catalyst were added to raise the temperature. A quantity, 30.0 ml of concentrated H_2SO_4 was carefully poured into the flask

and 5 glass beads were added to assist the oxidation process and prevent bumping during heating. Digestion took place on an electrical digestion flask. This was cooled and its content poured into 50.0 ml of cold distilled water in an Erlenmeyer flask placed in an ice bath. The content of the flask was transferred to a measuring cylinder and made up to 200.0 ml. A blank was prepared in the same manner without the sample. Soonafter, 50.0 ml of the diluted digest was placed in a clean dry Kjeldahl flask. Furthermore, 50.0 ml of 0.1 M H_2SO_4 acid solution was poured into a beaker with 3 drops of methyl orange indicator. The flask was connected to complete a distillation set up. The beaker was connected in such a way that the tip of the condenser column just dipped into the acid solution. Then 50.0 ml of 40 % NaOH solution was released into the Kjeldahl flask. The distillation process was allowed to run until the total volume in the receiver beaker was 90.0 ml. A burette was filled with 0.1 M H_2SO_4. The same procedure was carried out for the blank experiment.

$$\%\text{Crude protein} = \frac{(T-B) \times M\ H_2SO_4 \times 6.25 \times vol \times 0.014 \times 100}{\text{Aliquot} \times Mass\ of\ Sample\ used}$$

3.2.2 Quantitative Determination of the Mineral Elements of *C. dolichopentalum Leaves*

Determination of Calcium, Potassium and Sodium

The ash of the powdered sample was digested by adding 5 ml of 2 M HCl to the ash in the crucible and heated to dryness on a heating mantle. Exactly 5 ml of 2 M HCl was added again, heated to boiling, and filtered through a Whatman No. 1 filter paper into a 100 ml volumetric flask. The filtrate was made up to mark with distilled water, stoppered and made ready for reading of concentration of calcium, potassium and sodium on the Jenway Digital Flame Photometer (PFP7 Model) using the filter corresponding to each mineral element.

The concentration of each of the element was calculated using the formula:

$$\% \text{ Ca (or \% K or \% Na)} = \frac{\text{Meter Reading (MR) x Slope x Dilution factor}}{1000}$$

NB: MR x slope x dilution factor gives the concentration in part per million (ppm or mg/kg). Concentration in % was achieved by dividing by 1000.

Determination of phosphorus

Phosphorus concentration was determined by the modified Vanado-molybdate spectrophotometric method (APHA, 1985).

Procedure: The ash of the powdered sample was treated with 2 M HCl solution as described for calcium determination above. Exactly 10 ml of the filtrate solution was pipetted into 50 ml standard flask and 10 ml of vanadate yellow solution was added and the flask was made up to mark with distilled water, stoppered and left for 10 minutes for full yellow colour development. The concentration of phosphorus was obtained by taking the optical density (OD) or absorbance of the solution on a Spectronic 20 spectrophotometer at a wavelength of 470 nm.

The percentage of phosphorus was calculated using the formula:

$$\% \text{ Phosphorus} = \frac{\text{Absorbance x Slope x Dilution factor}}{10000}$$

The meter reading for each element was used to calculate the concentration of each element using the formula:

Ppm or mg/kg (any of the elements) = Meter reading x Slope or Gradient x dilution factor. % (any of the elements) = ppm or mg/kg divided by 10000

Determination of the amounts of the minerals Mg, Fe and Zn using Atomic Absorption Spectrophotomer

Mineral analysis was conducted using Varian AA20 Atomic Absorption Spectrophotomeer according to the method of American Public Health Association (APHA), (1995).

Working principle: Atomic absorption spectrometer's working principle is based on the sample being aspirated into the flame and atomized when the AAS's light beam is directed through the flame into the monochromator, and onto the detector that measures the amount of light absorbed by the atomized element in the flame. Since metals have their own characteristic absorption wavelength, a source lamp composed of that element is used, making the method relatively free from spectral or radiational interferences. The amount of energy of the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample.

Sample digestion

Two grams (2 g) of the plant sample was measured into a digestion flask and 200 ml of the acid mixture (650 ml concentrated $HN0_3$; 80 ml perchloric acid; 20 ml concentrated H_2S0_4) added. This was heated until a clear digest was obtained. The digest was diluted with distilled water to 100 ml and appropriate dilutions were then made for each element.

Preparation of reference solution

A series of standard mineral solutions in the optimum concentration range was prepared, the reference solutions were prepared daily by diluting the single stock element solutions with water containing 1.5 ml concentrated nitric acid/litre. A calibration blank was prepared using all the reagents except for the mineral stock solutions.

Calibration curve for each mineral was prepared by plotting the absorbance of standards versus their concentrations

3.2.3 Quantitative Determination of Vitamin Contents of *C. dolichopentalum* Leaves

(Eitenmiller and Landen, 1999; A.O.A.C, 2005).

Vitamin B2

Procedure: One gram (1 g) of the powdered sample was weighed into a 250 ml volumetric flask and 5 ml of 5 M HCl was added, followed by the addition of 5 ml of dichloroethene. The mixture was shaken and 90 ml of deionized water was added. The mixture was thoroughly shaken and heated on a steam bath for 30 minutes to extract the riboflavin. The mixture was cooled and made up to volume with deionized water. It was then filtered, discarding the first 20 ml of the aliquot. Then 2 ml of the filtrate obtained was pipetted into another 250 ml volumetric flask and made up to mark with deionized water.

A standard solution was prepared by dissolving 0.05 mg riboflavin into 100 ml of distilled water. Different standard solution concentrations of between 0 to 5 ppm were prepared from the above to obtain the equivalence. The absorbances, of the standards and samples were read on the fluorescent spectrophotometer at 460 nm wavelength. The amount of Vitamin B_2 in samples was calculated using the formula:

$$\text{Vitamin B2 (mg/100 g)} = \frac{\text{Meter Reading x Standard x Dilution factor of sample}}{\text{Weight of Sample}}$$

Niacin or Nicotinic acid (Vitamin B3)

Procedure: Five grams (5 g) of sample was blended and 100 ml of distilled water added to dissolve all nicotinic acid or niacin present. Five milliliters (5 ml) of this solution was drawn into 100 ml volumetric flask and made up to mark with distilled water. Then, 10 – 50 ppm of niacin stock solution was also prepared.

The absorbances of the diluted stock solutions, different concentrations of standard stock solutions and extract of sample were measured at a wavelength of 385 nm on a spectrophotometer to obtain the gradient factor.

The amount of niacin in the sample was calculated using the formula:

$$\text{mg/100 g Niacin} = \frac{\text{Absorbance x Dilution Factor x Gradient Factor of Stock Solution}}{10}$$

Thiamine (Vitamin B1)

Procedure: One gram (1 g) of sample was weighed into 100 ml volumetric flask; 25 ml of 0.1 M H_2SO_4 was added and mixed by careful swirling. Additional 25 ml of 0.1 M H_2S0_4 was added to rinse any adhering sample particle off the flask. The flask was set in a boiling water bath to ensure a complete dissolution of the sample in the acid. The flask was shaken frequently in the first 5 minutes and subsequently every 5 minutes for 30 minutes. Then, 5 ml of taka-diastase in 0.5 M sodium acetate solution was added and flask set in cold water to cool the content below 50^0C. The flask was stoppered and kept at 45-50^{o}C for 2 hours mixed and made up to 100 ml. The mixture was filtered through a No 42 Whatman filter paper, discarding the first 10 ml and keeping the remaining. Then 10 ml of the remaining filtrate was pipetted into a 50 ml volumetric flask and 5 ml of acid potassium chloride solution was added, shaking thoroughly to mix well. Standard thiamine solution of range 10 mg/ml to 50 mg/ml were prepared from 100 mg/ml stock and treated same way prepared from sample above. The absorbances of the sample as well as that of standards were read on a fluorescent UV spectrophotometer (Cecil A20 model) at a wavelength of 285 nm.

The concentration of vitamin B1 in mg/100 g was calculated using the formula:

$$\frac{\text{Absorbance x Ave.Gradient x Dilution Factor}}{\text{Weight of sample}}$$

Vitamin B9

Procedure: One gram (1 g) of the sample was weighed into a 250 ml volumetric flask. A volume, 100 ml of distilled water was added and shaken for 45 minutes and made up to mark with distilled water.

The sample mixture was filtered into another 250 ml beaker, discarding the first 20 ml that has been filtered. Another 20 ml filtrate was collected. To the filtrate, 5 ml of 1 % sodium dithionite solution was added to decolorize the yellow colour. Standard folic acid of range 0-10 µg/ml was prepared from stock folic acid. A sample blank made up with distilled water was also prepared. The absorbances of sample as well as standard were read at a wavelength of 445 nm on a spectronic 21D spectrophotometer. The concentration of the vitamin was calculated using the relationship:

$$\text{Vitamin B9} = \frac{\text{Absorbance of sample x Gradient Factor x Dil.Factor}}{\text{Weight of sample}}$$

Vitamin B6: Pyridoxine

One gram (1 g) of sample was weighed into a 100 ml beaker, 0.5 g of ammonium chloride, 45 ml of chloroform and 5 ml of absolute alcohol were added to extract all the pyridoxine. The mixture was thoroughly shaken in a separating funnel for 30 minutes. Exactly 5 ml of distilled water was added to the mixture in the separating funnel to partition the aqueous layer from the chloroform layer. The chloroform layer containing the pyridoxine was filtered into a 100 ml volumetric flask and made up to mark with chloroform. Then 0-10 ppm of vitamin B6 or pyridoxine standard were prepared from 100 ppm stock standards solution of pyridoxine and treated in a similar way as sample to obtain the gradient factor. The absorbance of a yellowish colour solution developed was measured on Cecil 505E Spectrophotometer at a wavelength of 415 nm. The concentration of vitamin B6 in mg/100 g was calculated using the formula:

$$\text{Vitamin B6} = \frac{\text{Absorbance of sample x Gradient Factor x Dilution Factor}}{\text{Weight of sample x 100}}$$

Vitamin E: Tocopherol

One gram (1 g) of sample was weighed into a 250 ml conical flask fitted with a reflux condenser. Then, 10 ml of absolute alcohol and 20 ml of 1M alcoholic sulphuric acid were added. The

condenser and flask were wrapped in aluminum foil and refluxed for 45 minutes and cooled for 15 minutes. Exactly 50 ml of distilled water was added to the mixture and transferred to a 250 ml separating funnel covered with aluminum foil. The unsaponifiable matters in the mixture were extracted with 5 x 30 ml dimethyl ether. The combined extracts were washed free of acid, dried by evaporating at a low temperature. The residues obtained were immediately dissolved in 10 ml absolute alcohol. Aliquots of solutions of the sample and standards (0.3-3.0 mg vitamin E) were transferred to a 20 ml volumetric flask, 5 ml absolute alcohol added, followed by a careful addition of 1 ml concentrated HNO_3. The flasks were placed on a water bath at 90^0C for exactly 3 minutes from the time the alcohol began to boil, cooled rapidly under running water and adjusted to volume with absolute alcohol. Measurement of the absorbance at 470 nm against a blank containing 5 ml absolute alcohol and 1 ml concentration was done. HNO_3 treated in a similar manner was read.

The concentration of vitamin E in mg/100 g was calculated using the formula:

$$\text{Vitamin E (µg/100 g)} = \frac{\text{Absorbances of sample x Gradient factor x Dil. Factor}}{\text{Weight of sample}}$$

Vitamin A

Two grams (2 g) of the powdered sample was weighed into a flat bottom reflux flask; 10 ml of distilled water was added and shaken carefully to form a paste. Then 25 ml of alcoholic KOH solution was added and a reflux condenser attached. The above mixture was heated in a boiling water bath for 1 hour with frequent shaking. The mixture was cooled rapidly and 30 ml of water was added. The hydrolysate obtained was transferred into a separating funnel. The solution was extracted three times with 250 ml quantities of chloroform. Afterwards 2 g anhydrous Na_2S0_4 was added to the extract to remove any traces of water. The mixture was then filtered into 100 ml volumetric flask and made up to mark with chloroform. Standard solutions of ß-carotene (vitamin A of range 0 – 50 µg/ml) were prepared by dissolving 0.003 g of standard ß-carotene in 100 ml of

chloroform. The above gradients of different standard solutions prepared were determined with reference to their absorbances from which average gradient was taken to calculate vitamin A (ß-carotene in µg/100 g). The absorbance of the sample and standards were read on the spectrophotometer (Metrohm Spectronic 21D Model) at a wavelength of 328 nm.

The concentration of vitamin A in µg/100 g was calculated using the formula:

$$\text{Vitamin A (µg/100 g)} = \frac{\text{Absorbance of sample x Dilution Factor}}{\text{Weight of Sample}}$$

Conversions:

6 µg of ß-carotene = 1 retinol equivalent

12 µg of other biologically active carotenoids = 1 retinol equivalent

1 retinol equivalent of vitamin A activity = 1µg of retinol.

1 retinol equivalent = 3 IU (International Unit).

3.2.4 Quantitative Determination of Amino Acids of *C. dolichopentalum* Leaves

The amino acid profile of the known sample was determined using the method described by Benitez (1984). The known sample as dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Technicon sequential Multi-Sample Amino Acid Analyzer (TSM).

Defatting sample

A small amount (200 mg) of ground sample was weighed, wrapped in Whatman (No.1) filter paper and put in the Kjeldhal digestion flask. Concentrated sulphuric acid (10 ml) was added. Catalyst mixture (0.5 g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added.

The flask was then put in Kjeldhal digestion apparatus for 3 hours until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100 ml in standard volumetric flask. An aliquot (10 ml) of the diluted solution with 10 ml of 45 % sodium hydroxide was put into the Markham distillation apparatus and distilled into 10 ml of 2 % boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70 ml of distillate was collected.

The distillate was then titrated with standardized 0.01 N HCl to grey coloured sample

$$\text{Percentage Nitrogen} = \frac{(a-b) \times 0.01 \times 14 \times V \times 100}{W \times C}$$

Where:

a. = Titre value of the digested sample

b. = Titre value of blank sample

v. = Volume after dilution (100 ml)

W. = Weight of dried sample (mg)

C. = Aliquot of the sample used (10 ml)

14. = Nitrogen constant in mg

Hydrolysis of the sample

A known weight of the defatted sample was weighed into a glass ampoule and 7 ml of 6 M HCl added. Oxygen was expelled by passing nitrogen into the ampoule (this was to avoid possible oxidation of some amino acids e.g methionine and cysteine during hydrolysis). The glass ampoule was sealed with Bunsen burner flame and heated in an oven at $105^0C \pm 5^0C$ for 22 hours. The ampoule was allowed to cool before opening at the tip and the content was filtered. It should be noted that tryptophan is destroyed by 6 M HCl during hydrolysis.

The filtrate was evaporated to dryness at 40^0C under vacuum, in a rotary evaporator. The residue was dissolved with 5 ml acetate buffer (pH 2.0) and stored in plastic specimen bottles kept in a freezer at 4^0C.

Loading of the hydrolysate into TSM analyzer

Ten (10) microlitre of the hydrolysate was loaded into the cartridge of the Technicon Sequential Multi-Sample Amino Acid (TSM) analyzer. The period of analysis lasted for 76 minutes. The TSM analyzer was designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate.

Calculation of the amino acid values from the chromatogram peaks

The net height of each peak produced by the chart recorder of TSM (each representing an amino acid) was measured. The half-height of the peak on the chart was found and the width of the peak on the half height was accurately measured and recorded. The approximate area of each peak was obtained by multiplying the height with the width at half-height. The norleucine equivalent (NE) for each amino acid in the standard mixture was calculated using the formula:

$$NE = \frac{\text{Area of Norleucine Peak}}{\text{Area of each amino acid}}$$

A constant, S was calculated for each amino acid in the standard mixture:

Where $S_{std} = NE_{std} \times \text{Molecular weight} \times \mu MAA_{std}$

Finally, the amount of each amino acid present in the sample was calculated in g/16 gN or g/100g protein using the formula:

$$\text{Concentration (g/100g protein)} = NH \times WNH/2 \times S_{std} \times C$$

$$\text{Where } C = \frac{\text{Dilution} \times 16}{\text{Sample Wt (g)} \times N\% \times 10 \times \text{Vol. loaded}} \div NH \times W\ (Nle)$$

Where: NH = Net height, W = Width at half height, Nle = Norleucine

3.2.5 Phytochemical Screening

By using standard procedures to identify constituents, chemical tests were carried out on the extracts and on the powdered specimen. This was to define their bioactive principles. The tests also

served as a template for elucidating their possible mechanism of action and the biochemical effects that were observed.

3.2.5.1: Qualitative phytochemical analysis

Test for the presence of saponins (Harbone, 1973)

i. Frothing Test: 1.0 ml of the extract was diluted with distilled water, shaken vigorously and observed on standing for 1 minute for stable froth which would not disappear when boiled.

ii. Blood Haemolysis Test: This is a confirmatory test for the presence of saponins. Blood got from an animal was placed on a slide and about 2 drops of sample was let onto the blood. Haemolysis is a positive result for the presence of saponins.

Test for the presence of tannins

i. To 1.0 ml of sample, was added 1.0 ml of $FeCl_3$. A blue colour was produced which gradually changed to olive green as more $FeCl_3$ was added.

ii. To 1.0 ml of the extract, 1.0 ml of aqueous ammonia was added and exposed to air. A green colour gradually developed, indicating the presence of chlorogenic acid (a class of tannins).

Test for the presence of flavonoids

i. To 1.0 ml of the sample, 1.0 ml of 5 % lead acetate was added. The formation of precipitates indicated the presence of flavonoids (anthocyanidins).

ii. To 2.0 ml of the sample, was added 1.0 ml of 2.0 M sodium hydroxide solution. Yellow colouration indicated the presence of flavonoids.

Test for the presence of alkaloids

i. Exactly 1.0 ml of sample was shaken with 5.0 ml of 2 % HCl on a steam bath and filtered.

ii. To 1.0 ml of filtrate, was added 0.5 ml of Wagner's reagent (2 g of iodine and 3 g of KI in 100 ml of distilled water). A reddish brown precipitate indicated alkaloid presence.

iii. To 1.0 ml of filtrate, was added 0.5 ml of Meyer's reagent (1.4 g of mercuric chloride and 4.5 g of KI in 100 ml of water). This was mixed and diluted to 100 ml with distilled water and observed for cream colour precipitate which indicated the presence of alkaloids.

Test for the Presence of cyanogenic glycosides (A.O.A.C., 1984)

Cyanogenic glycoside presence was determined by heating 20 ml of the sample in a conical flask for one hour. The vapour released turned alkaline picrate paper from yellow to orange and finally brick red. This indicated the presence of cyanogenic glycosides.

Oxalate

To 3.0 ml of aqueous extract of the powdered sample, drops of glacial acetic acid were added. A greenish black colouration indicated the presence of oxalate.

3.2.5.2 Quantitative Phytochemical Analysis

Saponin content

Saponin content was determined by the method of Harbone (1973) as reported by Obadoni and Ochuko (2001). Initially, 20 g of the powdered sample was boiled with 100 ml of 20.0 % v/v aqueous solution of ethanol for 4 hours at 55^{o}C with occasional stirring. The residue after filtration was re-extracted with fresh 100.0 ml of 20 % v/v ethanol solution as before. Both filtrates were combined and the volume reduced to about 50 ml on a water bath set at 90^{o}C. This was cooled and extracted with 20.0 ml of diethyl ether in a 250 ml separating funnel. The ether layer was discarded, and the lower layer was shaken with 50.0 ml of n-butanol and the two layers formed were allowed to separate. The organic layer was washed twice with 10.0 ml of 5 % w/v sodium chloride solution in a funnel. The washed organic layer was poured into a pre-weighed beaker and evaporated to dryness on a boiling water bath. This was cooled and weighed again.

Calculation: % saponin $= \frac{w_3 - w_2}{w_1} \times 100$

Where w_3 = weight of beaker and residue after evaporation to dryness

w_2 = weight of beaker alone

w_3 = weight of sample

Flavonoid content

Two grams of the powdered sample were mixed with 100 ml of 80 % v/v methanol solution. This was stirred on a magnetic stirrer for 3 hours and filtered using Whatman filter paper No 42. Re-extraction of the residue was repeated with a fresh 100 ml of 80 % v/v methanol. The filtrates from the double extraction were combined in a pre-weighed beaker and evaporated to dryness on a boiling water bath. This was cooled and weighed again.

Calculation: $\%\ \text{flavonoid} = \frac{w_3 - w_2}{w_1} \times \frac{100}{1}$

Where $w_3 - w_2$ = weight of flavonoid

w_1 = weight of sample

Alkaloid content

Alkaloid content was determined by the method of Harbone (1973). Two grammes of powdered sample in 100.0 ml of 20 % v/v ethanolic acid solution was allowed to stand for 3 hours while stirring on a magnetic stirrer. The residue was re-extracted with a fresh 100.0 ml of 20 % v/v ethanolic ethanoic acid solution. Both filtrates were combined and concentrated on a water bath to about 50.0 ml. This was cooled and concentrated. Concentrated ammonium hydroxide was added to the concentrate in drops till complete precipitation of the alkaloids occurred. This was left to cool completely and filtered using a pre-weighed filter paper.

Calculation: $\%\ \text{alkaloid} = \frac{w_3 - w_2}{w_1} \times 100$

Where $w_3 - w_2$ = weight of alkaloids

w_1 = weight of sample

Tannin content

Exactly 0.5 g of powdered sample and 50.0 ml of distilled water were stirred for 2 hours using a magnetic stirrer. This was filtered using a water-wet filter paper. The volume was made up to 50.0 ml. The following were added to 3 test tubes labelled water, sample filtrate and standard (tannic acid (2 mg/ml)); 1.0 ml of each 0.1 M hydrochloric acid, ferric chloride and potassium ferrocyanide solutions. This was mixed and absorbance taken at 720 nm using a spectrophotometer within ten minutes.

Calculation: $$\text{Tannin (mg/ml)} = \frac{ABS\ sample}{ABS\ std} \times \frac{conc.\ of\ standard}{1}$$

Cyanogenic glycosides

Two hundred millilitres (200 ml) of deionized distilled water was added to 1.0 g of powdered sample. This was allowed to stand for 2 hours. This setup was distilled until about 150.0 ml of the reaction mixture was collected in an Erlenmeyer's flask containing 20.0 ml of 2.5 % w/v sodium hydroxide solution. Measuring out 100 ml of the content in the receiver flask into another Erlenmeyer flask, 8.0 ml of 6.0 M ammonium hydroxide solution followed by 2.0 ml of 5.0 % KI was added to the content in the flask, this was mixed and titrated with 0.02 M silver nitrate solution from a burette until there was no further turbidity upon the addition of silver nitrate.

Calculation:

The cyanogenic glycoside concentration was obtained using the formula;

$$X = T \times \frac{108}{1} \times \frac{N}{1000} \times \frac{V_e}{V_a} \times \frac{100}{W}$$

Where, X % = % cyanogenic glycoside

T = titre value

108 = equivalent weight of silver nitrate

M = molarity of silver nitrate solution

1000 = scaling factor to obtain $AgNO_3$ content of 1.0 ml of $AgNO_3$ solution

V_e = extract volume

V_a = aliquot volume

W = weight of sample in grams

100 = scaling factor to convert value to percentage.

Oxalate content

After adding 20.0 ml of 0.3 M HCl solution to 5.0 g of dry powdered sample, it was stirred at 50°C for 1 hour and filtered. This process was repeated twice. The filtrates were combined and made up to 100.0 ml with distilled water. Three drops of phenolphthalein indicator was added to 20.0 ml of the filtrate followed by the addition of 5.0 M ammonium hydroxide in drops till the reaction mixture became alkaline. Glacial ethanoic acid was added in drops until the pink colouration just disappeared, a few drops more was added to make it acidic. Then 5.0 ml of 5 % $CaCl_2$ solution was also added to the mixture and allowed to stand for 3 hours. This was centrifuged at 300 rpm for 15 minutes and washed 3 times with hot water, using centrifugation technique. The resulting solution dissolved in 3.0 ml of 1.50 M H_2SO_4 acid with warming at 70–80°C was titrated with freshly prepared 0.01 N potassium permanganate solution till a permanent pink colouration that lasted for 30 seconds was obtained. This was done in duplicates. A blank titration was also done using 3.0 ml of H_2SO_4 acid.

Calculation:

This is a redox reaction represented by the equation

$$C_2O_4^{2-} + 8H^+ + MnO_4^- \longrightarrow 2CO_2 + 4H_2O + Mn^{2+}$$

Using the formula;

$$M_aV_a = M_bV_b$$

Where M_a = molarity of the oxalate solution

V_a = volume of the oxalate solution

M_b = molarity of potassium permanganate solution

V_b = volume of potassium permanganate solution

Using the relationship:

g/l = molarity × molar mass

The weight of oxalate in 1.0 dm^3 was obtained as X gram in 5.0 g sample.

Thus the weight of oxalate in 100 g of sample

$$\% X = \frac{X}{1} \times \frac{20_a}{1000} \times \frac{100_a}{20_b} \times \frac{100_b}{5}$$

Where

X = weight of oxalate obtained from (molarity × molecular mass)

20_a = volume of extract aliquot for analysis

1000 = reference volume for molar concentration

100_a = total volume of extract

20_b = volume of extract aliquot taken for analysis

100_b = scaling factor to convert to percentage

5 = weight of sample taken for analysis.

3.2.5.3 Phytochemical Determination Using Gas Chromatography (Kelly & Nelson, 2014)

Principle: Gas chromatography is based on the principle of partition of solute between a stationary liquid phase and gaseous mobile phase.

Procedure: The dried sample (20 g) was soaked for 72 hours in ethyl acetate. The filtrate through Whatman No.1 filter paper was concentrated under reduced pressure using rotary evaporator at a maximum temperature of 45°C to yield 1 g crude extract. The ethyl acetate extract (1 g) was subjected to thin layer chromatography (TLC), developed in ethyl acetate. The pure samples from the TLC were dissolved in ethyl acetate and 1.0 µl was subjected to GC analysis for phytochemical determination.

3.2.6 Extraction of Flavonoids, Alkaloids, Saponins and Tannins for GC Analysis.

Extraction of alkaloids: Extraction of alkaloids was done as outlined by Ngounou *et al.* (2005). One gram (1 g) of the pulverized sample was macerated in 10 ml of hexane for about 72 hours. The sample was filtered and the residue was air dried. This was initially treated with 10 % aqueous ammonia and finally macerated in chloroform for 24 hours. After filtration and subsequent evaporation at reduced pressure, the resultant crude extract was treated with 7.5 ml 5 % aqueous HCl. The aqueous phase was made alkaline with aqueous ammonia and extracted thrice with chloroform; the chloroform fraction was washed with water. The extract was concentrated and dried using anhydrous sodium sulphate before gas chromatography analysis.

The extract obtained was subjected to gas chromatography on a DB-5MS column capillary with column dimensions: 30 m × 0.25 mm × 0.25 µm. The inlet and detection temperatures were 250˚C and 320˚C respectively. The equipment was run on split injection, with 20:1 split ratio, and utilised nitrogen as the carrier gas. The hydrogen and compressed air pressures were 28 psi and 38 psi respectively. The oven temperature was run initially at 60˚C for 5 min. The first ramping was at 10˚C/min for 20 min and the second ramping at 15˚C/min for 4 min.

Extraction of flavonoids: Extraction of flavonoids was done as outlined by Millogo-Kone *et al.* (2009). Fifty gram (50 g) of the pulverized sample was weighed out and transferred to a stopperd flask and treated with ethanol until the powder was fully soaked. The flask was shaken every hour for the first six hours and then kept aside and later shaken after 24 hours. This process was repeated for three days and then the extract was collected and evaporated to dryness using nitrogen stream. Exactly 0.5 g of the concentrate was weighed into 250 ml conical flask capacity; `100 ml of de-ionized water was added and boiled for 10 minutes. The flavonoid extract was obtained by pouring 100 ml of boiling methanol- water (70:300 v/v) onto the materials. The homogenate was allowed to macerate for about 4 hours and was filtered through a Whatman No. 1 filter paper. The filtrate was then derivatized for volatility in gas chromatography analysis.

The extract obtained was subjected to gas chromatography on a HP INNOWax column with column dimensions, 30 m × 0.25 mm × 0.25 µm. The inlet and detection temperatures were 250˚C and 320˚C. The equipment was run on split injection, with 20:1 split ratio, utilising nitrogen as the carrier gas. The hydrogen and compressed air pressures were 22 psi and 35 psi respectively. The oven temperature was run initially at 50˚C. The first ramping was at 80˚C/min for 20 minutes and maintained for another 4 minutes. The second ramping was at 12˚C/min for 4 minutes and maintained for another 4 minutes.

Extraction of saponins: Extraction of saponin was done as outlined by Guo *et al.* (2009). The pulverized samples (10 g) of *C. dolichopentalum* were extracted thrice with 20 ml of methanol for 20 min with ultra-sonication. The combined extracts were concentrated to syrup under reduced pressure, and then suspended in water. The suspension was extracted successively, with petroleum ether, chloroform and 1-butanol saturated with water, to give the respective extracts after removal of the solvent. The 1-butanol-soluble part was subjected to a D101 macro-pourous absorption resin column chromatography successively eluted with distilled water and ethanol. The ethanol eluates were collected and some portions of the residues were concentrated to 1 ml in a vial for the gas chromatographic analysis.

One microliter (1 µL) was injected into the injection port of the gas chromatographic equipment. The extract obtained was subjected to gas chromatography on a capillary DB-225ms column with column dimensions: 30 m × 0.25 mm × 0.25 µm. The inlet and detection temperatures were 250˚C and 320˚C respectively. The equipment was run on split injection, with 20:1 split ratio, and nitrogen as the carrier gas. The hydrogen and compressed air pressures were 28 psi and 40 psi respectively. The oven temperature was run initially at 60˚C for 5 min. The first ramping was at 12˚C/min for 18 min. The second ramping was at 15˚C/min for 5 min.

Extraction of tannins: Extraction of tannins was done as outlined by Swain (1979). Exactly 0.2 g of the pulverized sample was measured into 50 ml borosilicate beaker. Then, 20 ml of 50 % methanol was added and covered with paraffin and placed in a water bath at 80 ^{o}C for 1 hour. The content was stirred with a glass rod to prevent lumping. The extract was quantitatively filtered with a doubled layered Whatman No.1 filter paper into a 100 ml volumetric flask rinsed with 50 % methanol. This was concentrated to 2 ml in the borosilicate vial for the gas chromatographic analysis. One microliter (1 µL) was injected into the injection port of the gas chromatographic equipment.

The extract obtained was subjected to gas chromatography on a HP-5 column with column dimensions: 30 m × 0.25 mm × 0.25 µm film. The inlet and detection temperatures were 250˚C and 320˚C respectively. The equipment was run on split injection, with 20:1 split ratio, and nitrogen as the carrier gas. The hydrogen and compressed air pressures were 28 psi and 40 psi respectively. The oven temperature was run initially at 120˚C for 5 min and ramped at 10˚C for 20 min. The second ramping was at 15˚C/min for 5 min.

3.2.6.1 Analysis of Extracted Samples by Gas Chromatography

The extracts were introduced into the carrier gas (nitrogen) using an injector. Liquid samples were vaporized before injection into the carrier stream. The gas stream passed through the packed column, through which the components of the sample moved at velocities that were influenced by the degree of interaction of each constituent with the stationary non-volatile phase. The substances having greater interaction with the stationary phase were retarded to a greater extent and consequently separated from those with smaller interactions. As each component left the column with the carrier, it passed through a detector which was placed at the exit from the column. The detector was electrically attached to a read out device that displayed the detector response as a function of time. The plot of detector response as a function of time was a chromatogram. Each separated component of the analyte appeared as a peak on the chromatogram.

Qualitative analysis was performed by comparing the time required for the component to pass through the column with the corresponding times for known substances. The interval between the instant of injection and the detection of the component is known as the retention time.

Quantitative analysis was done by preparing a working curve at a specific retention time by plotting the peak height (or peak area) of a series of standards (appendix 1) of known flavonoids, alkaloids, saponins and tannins as a function of the concentration of the component being assayed. The concentration of the component in the analyte was determined from the chromatographic peak height (or area) of the component and the working curve.

3.2.7 Preparation and Analysis of Samples by Gas Chromatography-Mass Spectrometry (GC-MS)

Chloroform extract of plant sample

Fifty grams (50 g) of the sample was soaked in absolute ethanol for 48 hours in various portions and repeatedly extracted with ethanol in various portions. The extracts were combined and were re-extracted using chloroform to obtain chloroform soluble extract which was stored in sample bottle for GC-MS analysis.

Column chromatography of plant sample

Eight hundred grams (800 g) of the ground sample was soaked in 95 % ethanol for 48 hours and filtered. The filtrates were concentrated using rotary evaporator regulated at 40^{o}C to get the ethanol extract. The crude extracts were partitioned between chloroform and water to obtain chloroform soluble fractions. Ten gram (10 g) of the chloroform extract was subjected to column chromatography over silica gel and eluted with100 ml petroleum ether, followed by petroleum ether-chloroform mixture. They were labelled as follows; (a) 100 ml petroleum ether A_{CD}, (b) 90/10 B_{CD}, (c)80/20 C_{CD} (d)70/30 D_{CD}, (e) 60/40 $E_{CD,}$ (f)50/50 F_{CD}, (g) 40/60 G_{CD}, (h) 30/70 H_{CD} (i)

20/80 I_{CD} (j) 10/90 J_{CD}. Further elution with chloroform and methanol mixture afforded the fractions 90/10 K_{CD}, 80/20 L_{CD} 70/30 M_{CD}, 60/40 N_{CD}, 50/50 O_{CD}, 40/60 P_{CD}, 30/70 Q_{CD}, 20/80 R_{CD} 10/90 S_{CD}.

Thin layer chromatography of column chromatography eluates

The eluates from column chromatography were subjected to thin layer chromatography using silica gel 60 G and iodine vapour for development. Fraction F_{CD} was obtained using petroleum ether-chloroform mixture (3:1) with their retention factor (Rf) value of 0.82. Fraction I_{CD} with Rf value 0.83 appeared as only one spot. D_{CD} with Rf value of 0.84, G_{CD} with Rf value of 0.85, J_{CD} with RF value of 0.86, K_{CD} with Rf value of 0.89 and M_{CD} with Rf value of 0.64 were indicated as single spot on the silica gel respectively.

GC-MS of chloroform extract and eluates of thin layer chromatography

GC- analyses were carried out with SHIMAZU Japan Gas Chromatography 5890-11 with a fused GC column OV 101 coated with polymethyl silicon (0.25 mm x 50 m) and the conditions were as follows: Temperature programming from 80 – 200^{o}C held at 80^{o}C for I minute, rate 5^{o}C/min and at 200^{o}C for 20 minutes, FID Temperature of 300^{o}C, injection temperature of 250^{o}C, carrier gas nitrogen at a flow rate of 1 cm^3/min, split ratio 1: 75. GC-MS Gas chromatography mass spectrum analyses were conducted using GC-MS QP 2010 Plus Shimazu Japan with injector temperature at 230^{o}C and carrier gas pressure of 100 kpa. The column length was 30 m with a diameter of 0.25 mm and the flow rate of 50 ml/min. The eluents were automatically passed into the mass spectrometer with a detector voltage set at 1.5 kV and sampling rate of 0.2 seconds. The mass spectrometer was also equipped with a computer fed mass spectra data bank, HERMCE Z 233 MZ centrifuge, Germany was used.

3.2.8 Preparation of Sample for Fourier Transform Infrared Spectroscopy (FTIR) Analysis

One gram (1 g) of the dried plant sample was mixed with 0.5 g of potassium bromate (KBr) and 1.0 ml of nujol oil (a solvent for preparation of sample by Buck 530 IR-spectrophotometer) was added with the aid of a syringe to form a paste. The paste was introduced into the instrument sample mould and allowed to scan at a wavelength range of 600-4000 nm to obtain the spectra. The eluates from the column chromatography were subjected to FTIR analysis; here 1.0 ml of the nujol oil was added to 1.0 ml of the chloroform fraction.

3.2.9 Extraction of Alkaloid, Saponin, Flavonoid and Tannin for *In Vivo* Studies

Alkaloid extraction

Fifty gram (50 g) quantity of the sample was weighed into a 1000 ml beaker and 500 ml of 29 % acetic acid in ethanol was added and allowed to stand for 6 hr. This was filtered and the filtrate was concentrated over a water bath to one quarter of the original volume. The alkaloid was precipitated out using concentrated ammonium hydroxide which was added drop by drop until precipitation was complete. The solution was allowed to settle and the precipitate was collected by filtration using Whatman No. 1 filter paper (Obadoni & Ochuko, 2001).

Saponin extraction

Fifty grams (50 g) of the sample was weighed into a 1000 ml beaker and 500 ml of 20 % ethanol was added and stirred using a glass rod. The mixture was heated over water bath for 4 hr with continuous stirring while the temperature was maintained at 55°C. The mixture was filtered and the residue was re-extracted with 500 ml of 20 % ethanol. The combined extract was reduced to 40 ml over water bath at 90°C. The concentrated extract was transferred into a 250 ml separation funnel and 50 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. This process was repeated thrice and 60 ml of n-butanol was

added. The mixture was washed twice with a 10 ml of 5 % sodium chloride. The remaining solution was heated over water bath and the residue dried to constant weight (Obadoni & Ochuko, 2001).

Flavonoid extraction

Fifty grams (50 g) of the plant sample was extracted repeatedly with 500 ml of 80 % aqueous methanol at room temperature. The solution obtained was filtered with Whatman no. 45 filter paper. The combined filtrates was later transferred into a crucible and evaporated to dryness over a water bath (Boham & Kocipai, 1974)

Tannin extraction

Fifty grams (50 g) of the plant sample was extracted with 500 ml of water. The aqueous extract was extracted thrice with ethyl acetate to eliminate neutral substances. The extract was brought to pH 2 by the addition of concentrated HCl and re- extracted with ethyl acetate. This was later evaporated to dryness.

3.2.10 Radical Scavenging/Antioxidant Studies

3.2.10.1 The Hydrogen peroxide scavenging ability (Wettashinge & Shaidi, 2000).

Principle: This method is based on comparison between the reduction of hydrogen peroxide (H_2O_2) by the sample and by a reference antioxidant.

Procedure: Flavonoids, alkaloids, saponins and tannins extracted from *C. dolichopentalum* were each dissolved in 3.4 ml of 0.1 M phosphate buffer (pH 7.4) and mixed with 600 µl of a 43 mM solution of hydrogen peroxide prepared with the same buffer. The final concentrations of the four extracts were graduated 0-25 µg/ml. The absorbance of all reaction mixtures were recorded after 30 minutes at 230 nm. For each concentration, a blank sample (without H_2O_2) was used for background subtraction.

Calculation: H_2O_2 scavenging capacities of samples were calculated using the following equation.

H_2O_2-scavenging capacity (%) = $\frac{100 - H_2O_2 \text{ Conc. of sample } X100}{H_2O_2 \text{ Conc. of control medium.}}$

3.2.10.2 Nitric oxide scavenging ability (Maroccoci *et al.*, 1994)

Principle: This test relies on a diazotization reaction originally described by Greiss (1879). The Greiss reagent system is based on the chemical reaction which uses sulphanilamide and N-1-naphthylethylene diamine dihydrochloride (NED) under acidic conditions.

The compound sodium nitroprusside (SNP) is known to decompose in aqueous solution at physiological pH producing nitric oxide ($NO^{\cdot}$). Under aerobic conditions NO reacts with oxygen to produce stable products; nitrate and nitrite, the quantities of which can be determined using Greiss reagent. Measuring nitrite (NO_2-), which is one of the two primary, stable and non volatile breakdown products of $NO^{\cdot}$ This is one of the means to investigate nitric oxide formation.

Procedure: To start, 4 ml of the extract of increasing concentrations were added to different test tubes containing 1 ml of SNP solution (25 mM); the tubes incubated at 29^{o}C for 2 hours. Then 2 ml aliquot of the incubated solution was removed and diluted with 1.2 ml Greiss reagent. The absorbance of the chromophore that formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthylethylene diamine dichloride was immediately read at 550 nm and referred to the absorbance of standard solution of sodium nitrite salt treated in the same way with Greiss reagent.

Calculation: The amount of nitrite produced was determined from a standard dose-response curve $y=-0.4289x^2 +1.1534x + 0.0093$ (correlation coefficient $R^2 = 0.9968$). X was calculated from the second order polynomial equation. Inhibition of nitrite formation by extract and the standard plant antioxidant (Quercetin) were calculated relative to the control.

$$\% \text{ Inhibition} = \frac{100-(\text{Test} \times 100)}{(\text{Control})}$$

3.2.10.3 Hydroxyl radical scavenging ability (Halliwell *et al.*, 1987).

Principles: Free radical dependent-2-deoxyribose degradation to malondialdehyde was studied using the Fenton oxidant reaction mixture of Fe^{3+}/ascorbic acid and H_2O_2.

Procedure: Hydroxyl radical scavenging ability was measured by studying the competition between deoxyribose and the test compounds for hydroxyl radical generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system. The reaction mixture contained the extract (0-2000 ug/l), $FeCl_3$ (0.1 mM), EDTA (0.1 mM), H_2O_2 (1mM) ascorbate (0.1 mM), KH_2PO_4.KOH buffer (20 mM, pH 7.4) and deoxyribose (2.8 mM) in a final volume of 1.0 ml. After incubation at 37^0C for 1hour, deoxyribose degradation was measured as TBARS by the method of Ohkawa *et al.*, (1979) as modified by Liu, *et* al., (1990).

Determination of thiobarbituric acid reactive substances (TBARS): To the incubation mixture were added, 1.5 ml of 20 % acetic acid (pH 3.5), 1.5 ml of 0.8 % thiobarbituric acid (TBA), 0.2 ml of 8.1 % sodium dodecyle sulphate (SDS). The mixture was heated at 100^0C for 1 hour and cooled. Then 2 ml of TCA was added. The mixture was vortexed vigorously and centrifuged at 300 x g for 10 minutes and the absorbance read at 532 nm

Calculation: Concentration of the thiobarbituric acid reactive substances (TBARS) was determined using the molar extinction coefficient of malondialdehyde. Inhibition of deoxyribose degradation which is a measure of hydroxyl radical scavenging ability was calculated by

$$\% \text{ OH Radical scavenging} = 100 - \frac{MDA_{test}}{MDA_{control}} \times 100$$

3.2.10.4 Hydrogen peroxide induced lipid peroxidation of rabbit brain homogenate (neuroprotection).

The ability of *C. dolichopentalum* extract to inhibit lipid peroxidation was evaluated by incubating rabbit brain homogenates treated with H_2O_2 at different concentrations of the extract (Muralikrishna *et al.,* 2008).

Principles: H_2O_2 induces lipid peroxidation in rabbit brain homogenates. Lipid peroxides react with thiobarbituric acid to form a pink product, thiobarbituric acid reacting substances (TBARS), measurable colourimetrically at 532 nm. The difference between the control and *C. dolichopentalum* extract treated sample is the measurement of decrease in TBARS formation.

Procedure: Whole rabbit brain was homogenized in phosphate buffered saline (10 % w/v). A measured volume, 200 µl of the rabbit brain homogenate, 0 to 800 mg/ml of *C. dolichopentalum* extract, 10 µl of hydrogen peroxide were incubated for 1 hour. TBARS were measured according to the method described by Liu *et al.* (1990). Briefly, to the incubation mixture were added 0.75 ml of 20 % acetic acid (pH 3.5), 0.75 ml of 1. 0 % thiobarbituric acid (TBA) and 0.2 ml of SDS (8.1%). The mixture was heated at 100^0C for 1 hour. Thereafter 2 ml of 10 % TCA was added and centrifuged at 6000 rpm for 5 minutes and optical density taken at 532 nm.

Calculation: $$\% \text{ Inhibition} = \frac{100 - \text{Test} \times 100}{\text{Control}}$$

3.2.10.5 Reducing power assay (Oyaizu, 1986).

Principles: This investigates the Fe^{3+}/Fe^{2+} transformation in the presence of the test compound. The reducing capacity of the extract may serve as a significant indicator of its potential antioxidant activity (Hsu *et al.,* 2006).

Procedure: Different concentrations (0- 1000 µg/ml) of the extract and standard (butylated hydroxyl toluene) in different test tubes were mixed thoroughly with 2.5 ml of 0.2 mM phosphate

buffer at pH 6.6 and 2.5 ml of 1 % $K_3Fe(CN)_6$. The mixture was incubated at 50^oC for 20 minutes, 2.5 ml of the upper layer of the solution was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1 % $FeCl_3$. The absorbance was read at 700 nm in a Spectronic 20 spectrophotometer.

Calculation: Reducing power was determined from the plot of optical density against concentration of extract. Reducing power (RP 0.5_{Au}) was taken as the concentration of extractable to give 0.5 absorbance reading.

3.2.10.6 Antimicrobial potentials of *C. dolichopentalum* extracts by total dehydrogenase activity (DHA) assay. Alisi *et al.* (2008) method with minor modifications was employed in total dehydrogenase assay.

Principle: 2,3,5- Triphenyl tetrazolium chloride (TTC), an artificial electron acceptor is reduced to the red – coloured triphenyl- formazan (TPF) by electrons from viable cells such as *E. coli, S. aureaus, S. typhi* and *S. pneumonia.*

Procedure: The assay was done in 4 ml volumes of nutrient broth-glucose- TTC medium supplemented in varying concentrations of extract.

The microbial cells were standardized in a spectrophotometer to an optical density of 0.70 at 420 nm. These standardized cell suspension were used as innoculum in the dehydrogenase assay. Portions (0.3 ml) of these bacterial suspensions were inoculated into triplicate glass tubes containing 2.5 ml of phosphate buffered (pH 6.8) nutrient broth- glucose medium amended with *C. dolichopentalum* extract (0-2000 µg/ml) and pre-incubated on a rotary incubator (150 rpm) at room temperature (28 ± 2^0C) for 30 minutes. Thereafter 0.1 ml of 1 % (w/v) TTC in de-ionized distilled water was added to each test tube. The final concentrations of nutrient broth, glucose and TTC in the medium were 2, 2, and 0.25 mg/ml, respectively.

The controls consisted of the isolates and the media without *C. dolichopentalum* extract. The reaction mixtures were further incubated at room temperature (28 ± 2^0C) for 8.0 hours. The TPF

formed were extracted in 4 ml amyl alcohol and determined spectrophotometrically at 500 nm (λmax). The amount of formazan produced was determined from a standard dose– response curve (0-20 µg/ml TPF in amyl alcohol, y = 0.487x, R^2= 0.997). Dehydrogenase activity was expressed as milligrams of TPF formed per mg dry weight of cell biomass per hour.

Calculation: Inhibition of dehydrogenase activity of the isolates by ethanol extract of *C. dolichopentalum* (EECD) was calculated relative to the control. The percentage inhibitions for organisms were plotted against the concentrations of the extracts using the Table 2D curve V 5.01 system software. The toxicity threshold concentrations (IC_{20}, $1C_{50}$, $1C_{70}$, $1C_{80}$ and $1C_{100}$) were then evaluated from the dose response plots. The total inhibitory concentrations ($1C_{100}$) values which were non-determinable from the simple inhibition plots were subjected to evaluation using a log transformation of % inhibition plots. Note that: Log % Inhibition = 2 = $1C_{100}$

3.2.11 Toxicological Studies

3.11.1 Ethanol extraction of plant

Fresh leaves of *C. dolichopentalum* were shreded, and air dried for 4 weeks, and reduced to a coarse powder in a grinding mill (Kenwood BL357). Three hundred grams of the powder was extracted with 1.75 L of 80 % ethanol by maceration for 48 hours, this was done in three separate cans and then pulled together. The sediment was removed by coarse filtration using a sieve followed by a Whatman No 1 filter paper. The extract was concentrated using a rotary evaporator under mild temperature and reduced pressure and labeled ethanol extract *of Combretum dolichopentalum* (EECD).

3.2.11.2 Animals

Wistar albino rats were purchased from the Animal House of the Department of Veterinary Medicine, University of Nigeria Nsukka, Enugu State, Nigeria. The animals were acclimatized for 7

days at room temperature in metal cages under 12/12 hour light and dark and were fed and maintained *ad libitum* on water and rat pellets (Pfizer Feeds, Aba, Nigeria). This study was conducted in accordance with laws and regulations for handling experimental animals as was approved by the Department of Biochemistry, FUTO.

3.2.11.3 Experimental design

First study

Fifty (50) rats weighing between 150 and 200 g were used for this prophylactic studies. The rats were separated into 5 groups of ten rats each after 7 days acclimatization. This study was designed as shown in Table 3.1.

Table 3.1: Experimental design for the first study

Groups	Group Identity	Treatments
I	Normal control (NC)	feed and water
II	Positive control (PC)	feed, water and CCl_4
III	Treated group (T_{250})	250 mg/kg body weight of EECD and CCl_4
IV	Treated group (T_{500})	500 mg/kg body weight of EECD and CCl_4
V	Sylimarin group	50 mg/kg body weight of Silymarin and CCl_4

All the groups received food and water *ad libitum* for 28 days. On day 29, 0.2 ml/kg body weight of CCl_4 in liquid paraffin (2:1) was administered intraperitoneally to all groups (except normal control). The CCl_4 was allowed to act on the animals for 48 hr. After overnight fast and light anaesthesia with dimethyltetrachloride, the rats were sacrificed and blood collected by cardiac puncture. Liver and kidney tissues of the animals were obtained, washed in 1.15 % KCl buffered solution and dabbed with paper, weighed and prepared for homogenization and histopathology.

Second study

Sixty five (65) Wistar albino rats were weighed and sorted into nine groups (Table 3.2) of seven animals each, so that their average weights were approximately equal. The animals were housed in metal cages. After 7 days acclimatization on rat pellets, they were weighed, and the weights used to calculate amount of extracts to be administered and other treatments to be done.

Table 3.2: Experimental design for the second study

Groups	Group Identity	Treatment
I	Normal control (NC)	feed and water
II	Positive control (PC)	feed, water and CCl_4
III	Treated group (T_{250})	250 mg/kg body weight of EECD and CCl_4
IV	Treated group (T_{500})	500 mg/kg body weight of EECD and CCl_4
V	Sylimarin group	50 mg/kg body weight of silymarin and CCl_4
VI	Flavonoid group	100 mg/kg body weight of flavonoid and CCl_4
VII	Saponin group	100 mg/kg body weight of saponin and CCl_4
VIII	Alkaloid group	100 mg/kg body weight of alkaloid and CCl_4
IX	Tannin group	100 mg/kg body weight of tannin and CCl_4

The EECD were administered daily by oral gavage, for 7 days. The dosage of administration of the extract was adopted from the acute toxicity studies carried out earlier. The animals were allowed food and water *ad libitum*. At the end of 7 days pre-treatment with EECD, flavonoids, saponins, alkaloids and tannins, the animals in all the groups (except normal control) were intoxicated with 0.4 ml/kg body weight $CC1_4$ in liquid paraffin (2:1). The CCl_4 was allowed to act on the animals for 48 hrs. After overnight fast the rats were subjected to a light anaesthesia with dimethyltetrachloride and blood collected by cardiac puncture. The livers and kidneys of the animals were obtained,

washed in 1.15 % KCl buffered solution and dabbed with paper and weighed. The livers and kidneys were prepared for biochemical and histopathology studies.

3.2.11.4 Preparation of liver homogenate

The kidneys and livers were homogenized in KCl (10 mM) phosphate buffer with ethylene diamine tetra acetic acid (EDTA; pH 7.4) and centrifuged at 12,000 x g for 60 minutes. The following enzyme activities: glutathione peroxidase, superoxide dismutase and catalase were assayed using the supernatant.

3.2.11.5 Blood collection

Blood samples of each animal were taken by cardiac puncture. Whole blood (2 ml) was drawn for haematological analysis, and the remaining allowed 45 minutes to clot at room temperature. After centrifugation at 600 x g for 15 minutes, the serum collected was used to assay various biochemical parameters.

3.2.12 Biochemical Assays.

3.2.12.1 Assay of aspartate aminotransferase activity: The assay method employed was that of Reitman & Frankel, (1957).

Principle: The method is based on the catalysis of the transfer of the amino group from L-aspartate to α-ketoglutarate resulting in the formation of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine.

$$\alpha\text{-ketoglutarate} + \text{L-aspartate} \xrightarrow{\text{AST}} \text{L-glutamate} + \text{Oxaloacetate}$$

Reagents: Reagent 1:100 mmol/l phosphate buffer, pH 7.4, 100 mmol/l L-aspartate and 2 mmol/l α–oxoglutarate, Reagent 2: 2 mmol/L, 2-4, dinitrophenylhydrazine.

Procedure: The reagents were pipetted into labelled test tubes as follows:

Reagents	Reagent Blank	Sample
Sample	-	0.1
Reagent 1	0.5 ml	0.5 ml
Distilled water	0.1ml	_
Mixed and incubated for 30 minutes at 37 ^{0}C		
Reagent 2:	0.5 ml	0.5ml
Mixed and incubated for 20 mins at 25 ^{0}C		
NaOH (0.4 M/l)	5.0 ml	5.0 ml

The test tubes were mixed thoroughly and absorbance read in a Turner 390 spectrophotometer against the reagent blank at 510 nm wave length after 5 minutes.

3.2.12.2 Assay of serum alanine aminotransferase (ALT) activity.

The assay method employed was that of Reitman & Frankel (1957).

Principle: ALT catalyzes the transfer of α-amino group from L-alanine to α- ketoglutarate resulting in the formation of pyruvate and L-glutamate. ALT activity is measured by monitoring the concentration of pyruvate formed with 2, 4–dinitrophenyl hydrazine.

Reagents: Reagent 1 (R1): 100 mmol/L phosphate buffer pH 7.4, 200 mmol/L L-alanine and 2.0 mmol/L α-oxoglutarate. Distilled water, NaOH, Reagent 2 (R2): 2.0 mmol/L 2,4- dinitrophenyl hydrazine.

Procedure: Reagents were pipetted into labelled test tubes as follows;

Reagents	Reagent Blank	Sample
Sample	–	0.1 ml
Solution R1	0.5 ml	0.5 ml
Distilled water	0.1 ml	–
Mixed and incubated for 30 min at 37^{0}C		

Solution R2	0.5 ml	0.5 ml
Mixed and incubated for 20 min at 25^0C		
NaOH	5.0 ml	5.0 ml

The test tubes were mixed thoroughly and absorbance read against the reagent blank at 510 nm wavelength after 5 minutes.

3.2.12.3 Determination of serum total bilirubin concentration

The concentration of serum total bilirubin was determined by the method of Zoppi *et al.* (1976).

Principle: Direct bilirubin in the sample reacts with diazotized sulphanilic acid forming a coloured complex that can be measured spectrophotometrically. Both 'direct'' and indirect bilirubin couple with diazotized sulphanilic acid in the presence of cetrimide. The term 'direct' and 'total' refer to the reaction characteristics of serum bilirubin in the absence or presence of solubilizing (accelerating) reagents. Direct bilirubin is only approximately equivalent to the conjugated and unconjugated fractions.

Reagents: Reagent AT (29 mmol/l suphanilic acid, 0.2 mmol/l HCl and 50 mmol/L cetrimidin, working reagent (11.6 mmol/l sodium nitrite, distilled water, bilirubin standard (4.3 g/dl)

Procedure: The reagents were pipetted into labelled test tubes as follows:

Reagents	Reagent blank	Sample blank	Sample	Standard
Distilled water	100 µl	–	–	–
Sample	–	100 µl	100 µl	–
Bilirubin standard	–	–	–	100µl
Reagent AT	–	1.0 ml	–	–
Working reagent	1.0 ml	–	1.0 ml	1.0 ml

The test tubes were mixed thoroughly and incubated at room temperature for 2 minutes. The absorbance of sample blank ($A_{sampleblank}$) was read at 540 nm against distilled water.

The absorbance of samples ($A_{samples}$) and standard ($A_{standard}$) were read against the reagent blank at 540 nm. The total bilirubin concentration was calculated as follows:

$$C_{Sample} = \frac{A_{sample} - A_{sample\ blank} \times C_{standard}}{A_{standard}}$$

3.2.12.4 Determination of total protein concentration

The concentration of protein was determined according to the method of Tietz (1995).

Principle: Protein peptide bonds interact with copper (II) ions in an alkaline medium resulting in the formation of a coloured complex.

Reagents: Standard (66 g/l) bovine serum albumin, Biuret solution: 6 mmol/l of Copper (II) acetate, 12 mmol/l of potassium iodide, 1.15 mmol/l of NaOH and detergent

Reagents	Reagent blank	Standard	Sample
Distilled water	0.02 ml	--	--
Standard	--	0.02 ml	--
Sample (serum)		--	0.02 ml
Biuret Reagent	1.0 ml	1.0 ml	1.0 ml

The test tubes were mixed thoroughly and incubated at room temperature. The absorbance of sample (A_{sample}) and standard ($A_{standard}$) were read against the reagent blank at 500 nm.

Calculations: The protein concentration in the sample was calculated as follows:

$$C_{Sample} = \frac{A_{sample} - A_{sample\ blank} \times C_{standard}}{A_{standard}}$$

3.2.12.5 Determination of serum albumin concentration

The concentration of albumin was determined according to the method of Doumas *et al.* (1971).

Principle: Albumin in the sample reacts with bromocresol green in an acid medium forming a coloured complex that can be measured spectrophotometrically.

Reagents: Albumin standard, Reagent A (bromocresol reagent: 100 mmol/l acetate buffer: 0.27 mmol/l bromocresol green, detergent), pH 4.1

Procedure: The reagents were pipetted into labelled test tubes as follows:

Reagents	Blank	Standard	Sample
Albumin standard	--	10 µl	--
Sample	--	--	10 µl
Reagent A	1.0 ml	1.0 ml	1.0 ml

The test tubes were mixed thoroughly and incubated at room temperature for 10 minutes.

The absorbance of samples ($A_{samples}$) and standard ($A_{standard}$) were read against the blank at 630 nm,

Calculations: The albumin concentration in the sample was calculated as follows:

$$C_{Sample} = \frac{A_{sample} - A_{sample\ blank} \times C_{standard}}{A_{standard}}$$

3.2.12.6 Determination of total cholesterol concentration

The concentration of cholesterol was determined according to the method of Allain *et al.* (1976).

Principle: Cholesterol esterase hydrolyses esterified cholesterol to free cholesterol. The free cholesterol is oxidized to form hydrogen peroxide which further reacts with phenol and 4-aminoantipyrine by the catalytic action of peroxidase to form a red coloured quinoneimine dye complex as shown in the reactions below:

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Chol. esterase}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + \tfrac{1}{2} O_2 + H_2O \xrightarrow{\text{Chol.oxidase}} \text{holestenone} + H_2O_2$$

$$2H_2O_2 + \text{4–aminoantipyrine} + \text{phenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + H_2O$$

Reagents: Biosystem cholesterol kit.

Reagents A: (35 mMo/l) Sodium cholate, phenol 28 mmol/l, Cholesterol esterase > 0.2 U/ml, Cholesterol oxidase > 0.4 U/l, peroxidase > 0.8 U/ml; 0.5 mmol/l, 4-aminoantipyrine, pH 7.0

Procedure: Three test tubes were prepared for the reactions as follows:

Reagents	Blank	Standard	Sample
Cholesterol	--	10 µl	--
Sample	--	-	10 µl
Reagent A	1.0 ml	1.0 ml	1.0 ml

The test tubes were mixed thoroughly and incubated for 10 minutes at room temperature.

The Absorbance of samples ($A_{samples}$) and standard ($A_{standard}$) were read against the reagent blank at 500 nm using a Turner$^{(R)}$ 390 spectrophotometer.

Calculations: Cholesterol concentrations in the sample were calculated as follows:

$$C_{Sample} = \frac{A_{sample} - A_{sample\ blank} \times C_{standard}}{A_{standard}}$$

3.2.10.7 Determination of triacylglycerides concentration (TG)

The concentration of triacylglycerides was determined according to the method of Young (1995).

Principle: Lipoprotein lipase hydrolyses triacylglycerides to glycerol and free fatty acids. The glycerol formed reacts with ATP in the presence of glycerol kinase forming glycerol-3-phosphate which is oxidized to form hydrogen peroxide. The hydrogen peroxide further reacts with phenolic compound (chlorophenol) and 4- aminoantipyrine by the catalytic action of a peroxidase to form a red coloured quinoneimine dye complex.

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{fatty acid}$$

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

$$\text{Glycerol-3-P} + O_2 \xrightarrow{\text{Glycerol-3-P-oxidase}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{4- chlorophenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4H_2O$$

Reagents: TG standard (glycerol 200 mg/dl)

Reagent A (45 mmol/L glycerol kinase > 4 Umol/l; peroxidase> 0.8 Umol/l, 4-aminoantipyrene, 0.75 mmol/L, ATP µmol/ml, pH 7.0

Procedure:

Reagents	Blank	Standard	Sample
TG standard	--	10 µl	--
Sample	--	--	10 µl
Reagent A	1.0 ml	1.0 ml	1.0 ml

The test tubes were mixed thoroughly and incubated for 15 minutes at room temperature.

The absorbance of samples ($A_{samples}$) and standard ($A_{standard}$) were read against the reagent blank at 500 nm in a Turner $^{(R)}$ 390 spectrophotometer.

Calculations: The cholesterol concentration of the sample was calculated as follows:

$$C_{Sample} = \frac{A_{sample} - A_{sample\ blank} \times C_{standard}}{A_{standard}}$$

3.2.12.8 Determination of high density lipoprotein (HDL)-cholesterol concentration

The concentration of HDL-cholesterol was determined according to the method of Grove (1979).

P**rinciple:** Very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) in the sample precipitate with phosphotungstate and magnesium ions. On centrifugation, all HDL present in the supernatant is measured spectrophotometrically by means of the coupled reactions described below;

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Chol. Esterase}} \text{Cholesterol} + \text{fatty acid}$$

$$\text{Cholesterol} + \tfrac{1}{2} O_2 + H_2O \xrightarrow{\text{Chol.oxidase}} \text{Cholestenone} + 4H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{Dichlorophenol Sulphanate} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4H_2O$$

Reagents; HDL cholesterol standard (S), Reagent B; 35 mmol/l phosphate buffer, 0.5 mmol/l sodium cholate, cholesterol esterase > 0.2 U/ml, cholesterol oxidase > 0.1U/L, peroxidise > 1.0 U/ml; 0.5 mmol/l 4-aminoantipyrine, 4.0 mmol/l dichlorophenol sulphanate, pH 7.0

Reagent A – Phosphotungstate 0.4 mmol/l and 20 mmol/l Magnesium chloride.

Procedure: Reagents were pipetted into labelled test tubes as follows:

Sample (serum) 0.2 ml

Reagent A 0.5 ml

The test tubes were mixed thoroughly and stood at room temperature for 10 minutes. After centrifugation for 10 minutes at 4000 rpm, supernatants were carefully collected into labelled test tubes as follows:

Reagents	Blank	Standard	Sample
Distilled water	50 ul	--	--
HDL cholesterol standard (S)	--	50 ul	--
Sample supernatant	--	--	50 ul
Reagent B	1.0 ml	1.0ml	1.0 ml

The test tubes were mixed thoroughly and incubated at room temperature for 30 minutes. The absorbance of samples ($A_{samples}$) and standard ($A_{standard}$) were read against the reagent blank at 500 nm in a Turner 390 spectrophotometer.

Calculations: Cholesterol concentration in the sample (C_{sample}) was calculated as follows.

$$C_{Sample} = \frac{A_{sample} - A_{sample\ blank} \times C_{standard}}{A_{standard}}$$

3.2.12.9 Determination of low-density lipoprotein (LDL)-cholesterol concentration

The concentration of LDL-cholesterol was determined by the method of Assman *et al.* (1984).

Principle: LDL in the sample precipitate with polyvinyl sulphate. LDL-cholesterol concentration is calculated from the difference between the total cholesterol and the cholesterol in the supernatant after centrifugation. The cholesterol is measured spectrophotometrically by means of the coupled reactions described below:

$$\text{Chol. ester} + H_2O \xrightarrow{\text{Chol. esterase}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + \tfrac{1}{2} O_2 + H_2O \xrightarrow{\text{Chol. Oxidase}} \text{Cholestenone} + H_2O_2$$

$$H_2O_2 + \text{4-aminoatipyrine} + \text{phenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4H_2O_2$$

Reagents: Reagent A (3 g/l polyvinyl sulphate, 3 g/l polyethylene glycol), Reagent B (35 mmol/l sodium cholate, 28 mmol/l phenol, cholesterol esterase >0.2 U/ml cholesterol oxidase >0.4 U/l, peroxidase > 0.8 U/ml, 0.5 mmol/l 4-aminoantipyrine),

Procedure: Reagents were pipetted into labelled test tubes as follows:

Sample	0.4 ml
Reagent A	0.2 ml

The test tubes were centrifuged at 4000 rpm for 15 minutes after mixing thoroughly and stood for 15 minutes at room temperature.

The supernatants were carefully collected into labelled test tubes as follows:

Reagents	Blank	Standard	Sample
Distilled water	20 µl	-	-
Cholesterol standard	-	20 µl	-
Sample supernatant	-	-	20 µl
Reagent A (pH 7.0)	1.0 ml	1.0 ml	1.0 ml

The test tubes were mixed thoroughly and incubated at room temperature for 30 minutes.

The absorbance of samples ($A_{samples}$) and standard ($A_{standard}$) were read against the reagent blank at 500 nm in a Turner $^{(R)}$ 390 spectrophotometer.

Calculations: The cholesterol concentration ($C_{supernatant}$) in the sample was calculated as follows:

$$C_{supernatant} = \frac{A_{sample} - A_{sample} \times C_{standard} \times DIL_{factor}}{A_{standard}}$$

LDL-cholesterol = T. Chol – $C_{supernatant}$

3.2.13 Determination of Oxidative Stress Parameters in Rats Treated with *C. dolichopentalum.*

3.2.13.1 Assay of superoxide dismutase activity in liver homogenates

The activity of SOD was determined according to the method of Xin *et al.* (1991).

Principle: This method studies the activity of SOD as it converts superoxide to hydrogen peroxide in the presence of a detector iodonitrozolium violet. Superoxide is generated by xanthine oxidase reaction as shown below:

$$\text{Xanthine} + O_2 \longrightarrow \text{Uric acids} + O_2^{\cdot -}$$

$$O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

$$O_2 + \text{Violet} \longrightarrow \text{oxidized detector}$$

SOD activity measured is equivalent to 50 % inhibition of the detector reaction.

Procedure: A quantity of 0.9 ml of distilled water and 0.1 ml of sample were pipetted into a test tube. Then 0.1 ml of the diluted sample was mixed with 0.9 ml of carbonate buffer, and 75 U/l of xanthine oxidase added. The absorbance of the mixture was determined at 500 nm for 3 minutes at 20 seconds interval. The changing rate of absorbance was used to determine the superoxide dismutase activity. The result was expressed in Units/1.

3.2.13.2 Assay of catalase activity

The activity of catalase was determined according to the method of Aebi (1984).

Principle: The rate of decomposition of hydrogen peroxide (H_2O_2) by catalase is the basis of the test. The absorbance of H_2O_2 at 240 nm decreases with time. Catalase activity can be measured from this decrease.

Procedure: A quantity, 2.5 ml of phosphate buffer, 2 ml of H_2O_2 and 0.5 ml of sample were pippeted into a test tube. To 1.0 ml portion of the reaction aliquot, 2 ml of dichromate acetic acid reagent was added and mixed thoroughly. The absorbance of the mixture was determined at 240 nm at one minute intervals. Catalase activity was calculated using the equation below:

$$\text{Catalase activity (Unit/1)} = \frac{0.23 \times \log \left[\frac{\text{Abs1}}{\text{Abs2}} \right]}{0.0069}$$

Where Abs1= initial absorbance, Abs2 = final absorbance

3.2.13.3 Determination of glutathione (GSH) concentration

The concentration of GSH was determined according to the method of Raja *et al.* (2007).

Principle: GSH reacts with Ellman's reagent (5,5-dithiobis (2-nitrobenzoic acid (DTNB) to form a stable yellow colour, O-nitro-5-thiobenzoic acid. When Ellman's reagent is added to the sulphydryl compounds, it results in the development of a yellow colour which can be measured at 412 nm.

Procedure: A quantities, 1 ml of sample homogenate and 4 ml 10 % trichloroacetic acid were mixed and centrifuged at 3000 rpm for 10 minutes to separate the proteins. To 0.01 ml of this supernatant, 2 ml of phosphate buffer (pH 8.4), 0.5 ml of 5,5-dithiobis 2-nitrobenzoic acid and 0.4 ml double distilled water were added. This mixture was vortexed and the absorbance read within 15 minutes.

Calculations: The absorbance of glutathione was calculated from the standard calibration curve (y = mx) prepared by plotting the absorbance of standard glutathione against standard concentrations when subjected to the same experimental conditions.

3.2.13.4 Assay of glutathione peroxidase (GPx) activity

The concentration of GSH was determined according to the method of Paglia & Valentine (1967).

Principle: GPx catalyzes the oxidation of GSH by hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidized (GSSG) glutathione is immediately converted to the reduced form with concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance of NADPH at 340 nm is measured in a spectrophotometer.

$$2GSH + ROOH \xrightarrow{GPx} ROH + GSSG + H_2O$$

$$GSSG + NADPH + H^+ \xrightarrow{GR} NADP^+ + 2GSH$$

Procedure: Test tubes were prepared and aligned in a test tube rack. Then 3 ml of phosphate buffer, 0.55 ml of guaiacol, 0.03 ml of H_2O_2 and 0.1 ml of sample were added into the test tubes and appropriately mixed. The absorbance of the mixture was taken at 436 nm for 2 minutes at 30 seconds interval. The glutathione peroxidase activity was determined from the absorbance reading obtained and the activity was expressed in U/l.

3.2.13.5 Determination of malondialdehyde concentration

The concentration of MDA was determined according to the method of Wallin *et al.* (1993).

Lipid peroxidation is determined spectrophotometrically by measuring the concentration of the lipid peroxidation product – malondialdehyde (MDA).

Principle: Malondialdehyde (MDA) reacts with thiobarbituric acid (TBA) to form a red or pink coloured complex which in acid solution, absorbs maximally at 532 nm.

$$MDA + 2\ TBA \longrightarrow MDA{:}\ TBA\ adduct + H_2O$$

Procedure: Test tubes were prepared and aligned in a rack, then 0.1 ml of sample, 0.9 ml of distilled water, 0.5 ml of 25 % trichloroacetic acid (TCA) and 0.5 ml of 17 % TBA in 0.3 % NaOH were pipetted into the test tubes. The mixture was incubated at 95 ^{0}C for 40 minutes and cooled in water after incubation. Afterwards, 0.1 ml of 20 % sodium dodecyl sulphate was added to the mixture. The absorbance of the mixture was determined at 532 and 600 nm against a blank. The concentration of malondialdehyde was determined from the absorbance read from the mixture.

3.2.14 Renal Function Test

3.2.14.1 Determination of serum urea concentration

The concentration of urea was determined according to the method of Chaney & Marbach (1962).

Principle: Urea is hydrolyzed by urease to produce ammonia (NH_3) and CO_2. In a modified Berthelot reaction, the ammonium ion reacts with hypochlorite and salicylate in the presence of nitroprusside to form a coloured complex that can be measured at 576 nm.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_2$$

$$NH_4^+ + \text{salicylate} + NaClO + \xrightarrow{\text{nitroprusside}} \text{Indophenol}$$

1. Reagents
   - Reagent 1
   - Phosphate buffer (pH. 7.0) 120 mmol/L
   - Sodium salicylate 60 mmol/L
   - Sodium nitroprusside 5 mmol/L
   - EDTA 1 mmol/L
2. Reagent 2
   - Phosphate buffer (pH.7.0) 120 mmol/L
   - Hypochlorite ≈0.6 g/l
3. Enzyme
   - Urease >500 KU/L
4. Enzyme reagent

Enzyme working reagent was prepared by mixing the enzyme content with reagent 1.

5. Standard Reagent
   - Urea 13.3 mmol/l

- Sodium 0.095 %

Procedure: The following were pipetted as recommended in the kit

	Standard	Sample	Blank
Specimen	--	10 µl	--
Standard reagent	10 µl	--	--
Enzyme Reagent	1000 µl	1000 µl	1000 µl

Test tubes contents were each mixed and incubated at 37^0C for 3 minutes.

Reagent 2	1000 µl	1000 µl	1000 µl

The test tubes and their contents were mixed and incubated at 37^0C for 5 minutes. The Absorbance of sample and standard were measured against reagent blank at 576 nm.

Calculation: Urea concentration (mmol/L) = $\frac{\text{Absorbance sample}}{\text{Absorbance standard}} \times 13.3$

3.2.14.2 Determination of serum sodium concentration

The concentration of sodium was determined according to the method of Tietz (1976).

Principle: Sodium is precipitated as triple salt, sodium magnesium uranyl acetate, the excess uranium reacts with ferricyanide producing a chromophore whose absorbance varies inversely as the concentration of Reagents.

Procedure: They were pipetted as follows:

	Blank	Standard	Test
Distilled water	50 µl	--	--
Serum	--	--	50 µl
Sodium standard	-	50 µl	--
Reagent	1.0 ml	1.0 ml	1.0 ml

The test tube contents were mixed and centrifuged (1500 rpm) for 10 minutes and absorbance taken.

Calculation:

$$\text{Concentration of sodium mEq/L} = \frac{\text{Absorbance sample} - \text{Absorbance blank}}{\text{Absorbance standardd} - \text{Absorbance blank}} \times \text{conc. of standard}$$

3.2.14.3 Determination of serum potassium concentration

The concentration of potassium was determined according to the method of Henry *et al.* (1974).

Principle: Potassium was estimated by using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension, whose turbidity is proportional to potassium concentration.

Procedure: The following were pipetted into the respective test tubes.

	Blank	Standard	Test
Distilled water	10 µl	--	--
Sample			10 µl
Potassium standard	--	10 µl	--
Reagent	1.0 ml	1.0 ml	1.0 ml

The contents of the test tubes were mixed thoroughly and left to stand at room temperature for 3 minutes.The absorbances were read spectrophotometrically against the reagent blank at 500 nm.

$$\text{Concentration of potassium (mEq/L)} = \frac{\text{Absorbance sample x conc. of standard}}{\text{Absorbance standardd}}$$

3.2.14.4 Determination of serum chloride concentration

The concentration of chloride was determined according to the method of Tietz (1976).

Principle: Chloride ions displace thiocyanate from non-ionized mercuric thiocyanate. The released thiocyanate reacts with ferric ions to form a coloured complex that absorbs light at 480 nm whose intensity is directly proportional to the chloride concentration.

$$Hg(SCN)_2 + 2Cl^- \longrightarrow HgCl_2 + 2SCN^-$$

$$3SCN + Fe^{3+} \longrightarrow 4Fe(SCN)_3 \text{ red complex.}$$

Procedure: The reagents were brought to room temperature and then pipetted into labelled test tubes as follows:

Reagents	Blank	Standard	Sample
Chloride reagent	1.5 ml	1.5 ml	1.5 ml
Standard (100mEq/L NaCl)	--	0.01 ml	--
Sample (Serum)	--	--	0.01 ml
Distilled water	0.01 ml	--	--

The contents of the test tubes were mixed thoroughly and incubated at room temperature for 5 min.The absorbance of samples ($A_{samples}$) and standard ($A_{standard}$)were read against the reagent blank at 480 nm in a spectrophotometer (Turner® 390).

3.2.14.5 Determination of serum bicarbonate concentration

The concentration of serum bicarbonate was determined according to the method of Tietz *et al.* (1987)

Principle: Serum bicarbonate (Total CO_2) concentration was determined by the use of a CO_2 gas electrode (ASTRA CO_2 apparatus; Beckman Instruments, USA). First the sample was acidified to produce gaseous CO_2 from bicarbonate. The released gaseous CO_2 is determined by a Pco_2 electrode that is set in the reaction chamber of the CO_2 module. The rate of change of pH of the buffer inside the membrane of the measuring electrode is taken as the measure of total CO_2 present in the sample.

Procedure: A standard solution of sodium bicarbonate (25 mmol/l) was first used to calibrate the instrument. Then, a measured volume (10 µl) of the sample was aspirated into reaction vessel of the

apparatus, acidified automatically with lactic acid, mixed and the CO_2 liberated was determined by the PCO_2 electrode set in the reaction chamber of the CO_2 module.

3.2.14.6 Determination of iron and zinc concentrations.

The concentrations of iron and zinc were determined with atomic absorption spectrophotometer (AAS)

Principle: This technique relies on the Beer Lambert's law. It requires known standard concentration to establish the relation between measured absorbance and the analyte concentration. This was done after ashing the supernatant and treating the ash with 10 % HCl.

Reagent: Commercial kits and products of Randox Laboratories Ltd, Austin and Teco diagnostic.

Procedure

Preparation of Ash: Clean platinum dish was ignited at 500^0C for 30 minutes in a furnace. The dish was cooled in a desiccator and weighed until a constant weight was obtained. Two gram (2 g) of liver homogenate was accurately weighed into the dish slightly opening the cover for escape of gases at 500^0C (loss of chloride due to vulcanization tends to occur above 500^0C. A whitish residue remains in the platinum dish after ashing.

Calculations:

$$\% \text{ Ash Content} = \frac{\text{Weight of Ash}}{\text{Weight of sample}} \times 100$$

Five millilitres (5 ml) of 10 % HCl solution were added to the ash and warmed on a water bath to dissolve the ash. A measured quantity was transferred into a 20 ml standard volumetric flask. The solution was aspirated into the Atomic Absorption spectrophotometer which gave the absorbance of the individual metals (zinc and iron).

Calculation $A = \text{Iog } (I_0/I)$

Where, Io is intensity of incident light, I = intensity of transmitted light

3.2.15 Vitamins

3.2.15.1 Determination of vitamin C concentration

The concentration of serum vitamin C was determined by the method of Omaye *et al.* (1979)

Principle: Ascorbic acid is oxidized by copper to form dehydroascorbic acid which reacts with 2,4-dinitrophenylhydrazine to form 2,4-dinitrophenyl hydrazone. This complex can be measured spectrophotometrically at 520 nm.

Ascorbic acid + $CuSO_4$ ⟶ Dehydroascorbic acid

Dehydroascorbic acid + DNPH ⟶ Bis-2,4-dinitrophenylhydrazone ascorbate

Ascorbic acid was converted to dehydroascorbic acid by shaking with cupric sulphate solution and then coupled with 2, 4-dinitrophenylhydrazine in the presence of thiourea as a mild reducing agent. Sulphuric acid converts dinitrophenylhydrazone into a red coloured compound, which is assayed colorimetrically. Thiourea is added to prevent oxidation of dinitrophenylhydrazine reagent by interfering substances.

Reagents:

i. 5 % Trichloroacetic acid (TCA)

ii. DTC reagent: 3 g of 2,4-dinitrophenylhydrazine, 0.4 g thiourea and 0.05 g of copper sulphate is dissolved in 100 ml of 9 N Sulphuric acid

iii. 65 % sulphuric acid (ice cold)

iv. Standard ascorbic acid

Procedure: To 0.5 ml of plasma, 0.5 ml of water and 1 ml of TCA were added, mixed thoroughly and centrifuged. Thereafter 1 ml of the supernatant, 0.2 ml of DTC reagent was added and incubated at 37^{o}C for 3 hours. Then 1.5 ml of sulphuric acid was added, mixed well and the solution read at 520 nm in a spectrophotometer. The concentration of vitamin C was expressed as mg/dl of plasma.

3.2.15.2 Determination of vitamin E concentration

The concentration of serum vitamin E was determined by the method of Palan *et al.* (1973).

Principle: This method involves the conversion of ferric ions to ferrous ions by α-tocopherol and the formation of red coloured complex with 2,2-dipyridyl. Absorbance of the chromophore is measured at 520 nm in a spectrophotometer.

Reagents:

i. 2 % 2,2-dipyridyl solution

ii. 5 % ferric chloride solution

iii. Standard : 100 mg of α-tocopherol in 1.0 % ethanol

iv. n-Butanol

Procedure: To 0.5 ml of serum, 1.5 ml of ethanol was added, mixed and centrifuged. The supernatant was dried at 80^{o}C for 3 hours. To this 0.2 ml of 2,2-dipyridyl solution and 0.2 ml of ferric chloride solution were added, mixed well and 4 ml of butanol was added. The colour that developed was read at 520 nm in the spectrophotometer. Values were expressed as mg/dl of serum.

3.2.16 Haematological Analyses

An automated haematology analyzer machine (Mindray BC 2300, USA) was used for the haematological analyses.

Principle: The instrument performs blood cell count by direct count (DC) detection method and haemoglobin analysis by non-cyanide haemoglobin analysis method.

Procedure: Blood sample was aspirated, measured to a predetermined volume, diluted at the specified ratio, and then fed into each transducer. The transducer chamber has a minute hole, the aperture. On both sides of the aperture, there are electrodes between which flows a direct current. Blood cells suspended in the diluted sample pass through the aperture, causing direct current

resistance to change between the electrodes. As direct current resistance changes, the blood cell size is detected as electric pulses.

Blood cell count was calculated by counting the pulses, and a histogram of blood cell sizes was plotted automatically by the machine by determining the pulse sizes. Also analysing the histogram makes it possible to obtain various analysis data.

1. White Blood Cell/ Haemoglobin Analysis Flow

i. Blood was aspirated into the sample rotor valve from the sample probe.

ii. Exactly 6 µl of blood measured by the sample rotor valve was transferred to the WBC transducer chamber along with 1.994 ml of diluents. At the same time, 1.0 ml of WBC/HB - lyse reagent was added to prepare 1:500 dilution sample. The solution was allowed to stand for 10 seconds so that RBCs are haemolysed and platelets shrink while WBC membrane remains intact. At the same time, haemoglobin is converted into red coloured met-haemoglobin.

iii. Of the diluted/haemolysed sample in the WBC transducer chamber, approximately 1 ml was transferred to the HB flow cell. Out of this 500 µl was aspirated through the aperture. The pulses of the blood cells when passing through the aperture are counted by the DC detection method.

iv. In the HB flow cell, 555 nm wavelength beam irradiated from the light emitting diode (LED) was applied to the sample in the HB flow cell. The concentration of this sample was measured as absorbance. This absorbance was compared with that of the diluents alone that was measured before addition of the sample, thereby calculating HB value.

2. Red Blood Cell/Platelet (RBC/PLT) Analysis Flow

i. Blood was aspirated from the sample probe into the sample rotor valve.

ii. Exactly 4 µl of the blood measured by the sample rotor valve was diluted into 1:500 with 1.966 ml of diluents and brought to the mixing chamber as diluted sample (1st step dilution).

iii. Out of the 1:500 dilution sample 40 µl was measured by the sample rotor valve, diluted into 1:25000 with 1.960 ml of diluents and then transferred to the RBC/PLT transducer chamber (2nd Step dilution).

iv. Then, 250 µl of the sample in the RBC/PLT transducer chamber is aspirated through the aperture. At this time, RBC and PLT are counted by the DC detection method. At the same time, PCV (haematocrit value) was calculated by RBC pulse height detection method.

3. Calculation of Red Blood Cell Constants

The RBC constants were calculated automatically from RBC, HB and PCV.

i. Mean cell volume (MCV, fl) = $\frac{PCV\ (\%)}{RBC\ (\frac{x10^6}{\mu l})} \times 10$

ii. Mean cell haemoglobin (MCH, pg) = $\frac{HB\ (\frac{g}{dl})}{RBC\ (\frac{x10^6}{\mu l})} \times 10$

iii. Mean cell haemoglobin concentration (MCHC, g/dl) = $\frac{HB\ \left(\frac{g}{dl}\right)}{PCV\ (\%)} \times 100$

3.2.17 Histopathology of the liver and kidney

The method described by Okoro (2002) with minor modifications was used for the histology of the liver and kidney.

Principle: Histopathology is the study of diseased tissues. It is proper to infer organ injury or inflammation from the evaluation of routine biochemical parameters correlating such findings with histopathological changes of the organ/organs in question.

Procedure: Basic histological or pathological technique involves the following:

1. Tissues collection

2. Fixation
3. Processing
4. Sectioning
5. Staining and mounting

Tissue collection: Tissue was collected immediately after sacrifice to prevent post-mortem autolysis and decomposition.

Fixation: Tissue samples from the liver and kidney were fixed in a large quantity of fixative. Fixation is the preservation after death of shape, structure and chemical constituents of cells and tissues with buffered formalin etc.

Processing: This included dehydration, clearing, infiltration and embedding.

Dehydration: The tissue was passed through ascending grades of alcohol (30 %, 50 %, 70 %, 90 % and absolute alcohol) 1 hr, 2 hr and 3 hr each. Dehydration is important because removal of water facilitates the subsequent impregnation with embedding media. The best agent for dehydration is ethyl alcohol (ethanol). To check for complete dehydration, a small amount of white anhydrous copper sulphate is added; if it turns blue then water is present.

Clearing or de-alcoholisation: After dehydration, alcohol was removed from the tissues by immersing them in Xylene for 3 hours. Clearing agent has the property of making tissues transparent.

Embedding or impregnation: Tissues were transferred from the clearing agent to a bath of molten paraffin wax in the embedding oven. During this stage, the clearing agent was eliminated from the tissue by diffusion into the surrounding wax. Embedding involves impregnation of tissues with a medium that will fill all cavities, spaces and interstices and then solidify them to form a firm consistency that can give considerable support during cutting of sections without alteration of the cellular architecture or constituents. Once the tissues were infiltrated with paraffin wax, it is placed

in the Lenckhart embedding boxes (mould) covered with molten paraffin and then was allowed to harden for 30minutes, forming a paraffin block containing the tissue. This allows for cutting of sections. The mould was transferred to a container of cold water gently, where it was allowed to harden for 30 minutes.

Trimming: The excess wax was removed at this point carefully and attached to a holder. Trimming is important to ensure uniform sections and prevent the block from cracking.

Sectioning: The tissues were sectioned with a rotary microtome. This was effected by the vertical rise and fall of the object against the knife edge.

Staining and mounting: Paraffin sections were placed on glass slides. A section adhesive was used for firm attachment and then stained by water-soluble stains that permit differentiation of various structural compounds. Before staining, the sections were treated thus:

i. Dewaxed and hydrated by placing the sections on a hot plate briefly at temperature $10^{o}C$ above the melting point (deparaffination).
ii. Immersed in xylene for 30 minutes.
iii. Transferred to absolute alcohol for 30 seconds to remove xylene and ensure that it is not carried over to the lower grade alcohols.
iv. Slides were transferred from absolute alcohol to 90 % alcohol for 30 seconds.
v. Slides were transferred again to 70 % alcohol for 30 seconds.
vi. Washed thoroughly in distilled water.
vii. Immersed in Harris haemotoxylin for 30 minutes.
viii. Washed thoroughly in running tap water.
ix. Differentiated in solution until only the cell nuclei retained the stain.
x. It was blued with scotch in running tap water for 5 minutes.
xi. It was counter-stained with eosin for 1 minute.

xii. It was dehydrated in ascending grades of alcohol.

xiii. It was cleared in xylene and mounted with DPX (dibutylpthalate, polystyrene, xylene).

Haematoxylin is a nuclear stain and stains the nucleus blue black/purple depending on the haematoxylin used. Eosin is a cytoplasmic counterstain, with at least 3 different shades of pink depending on cell part or tissue type.

3.2.18 Statistical Analysis

Data generated were presented as simple percentages and some were analyzed by one-way ANOVA with the aid of computer based Power Anaysis Software (PASW) version 18.0 and Graphpad prism 5.3. Multiple comparisons for the ANOVA were done using least significant difference (LSD) to determine the statistical significance of the differences among different experimental groups. $P<0.05$ was considered statistically significant. The plant extract free radical inhibition data (mean values from triplicate determinations) were fitted into kinetic equation- logistic-dose-response model and sigmoid abcd model, using Levenberg-Marquardt algorithm (Table curve 2D SYSTAT USA) Marquardt (1964).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Proximate composition of *C. dolichopentalum* leaves

Table 4.1 shows the result of proximate composition of *C. dolichopentalum* leaves

Table 4.1: Proximate composition of *C. dolichopentalum* leaves

Macronutrient	% Composition
Moisture	7.64±0.01
Protein	6.84±1.01
Carbohydrate	63.35±4.93
Ash	6.18±0.98
Lipid	10.55±0.45
Fibre	6.07±0.45

Values are means ± standard deviation of triplicate determinations

The result (Table 4.1) of proximate studies on the *C. dolichopentalum* leaves showed a high concentration of carbohydrate and appreciable amounts of protein, lipid and ash.

4.1.2 Mineral Composition of *C. dolichopentalum* Leaves

Table 4.2 shows the result of mineral composition of *C. dolichopentalum* leaves

Table 4.2: Mineral composition of *C. dolichopentalum* leaves

Minerals	Concentration (mg/kg)
Sodium	193.8±10.05
Potassium	223.24±13.66
Magnessium	0.06±0.01
Calcium	0.05±0.01
Phosphorus	0.52±0.07
Zinc	0.12±0.02
Iron	0.88±0.41

Values are mean± standard deviation of triplicate determination

The values obtained in the study of mineral composition of *C. dolichopentalum* leaves (Table 4.2) shows that potassium had the highest concentration followed by sodium. It contains appreciable amountof iron, phosphorus, zinc magnesium and calcium in increasing order of presentation.

4.1.3 Amino acid profile of *C. dolichopentalum* leaves

Table 4.3 shows the result of amino acid composition of *C. dolichopentalum* leaves

Table 4.3: Amino acid profile of *C. dolichopentalum* leaves

Amino Acids	Concentration g/100g protein
Essential amino acids	
Lysine (Lys)	2.89±0.02
Histidine (His)	2.02±0.07
Arginine (Arg)	4.06±0.03
Valine (Val)	3.91±0.08
Methionine (Met)	0.91±0.03
Isoleucine (Ileu)	3.11±0.08
Leucine (Leu)	5.84±0.14
Phenylalanine (Phe)	3.63±0.08
Tryptophan (Trp)	ND
Non essential amino acids	
Aspartic Acid (Asp)	7.32±0.03
Threonine (Thr)	2.24±0.05
Serine (Ser)	3.01±0.04
Glutamic acid (Glu)	8.33±0.04
Proline (Pro)	NC
Glycine (Gly)	3.84±0.11
Glutamine (Gln)	NC
Alanine (Ala)	3.68±0.02
Cysteine (Cys)	0.79±0.06
Tyrosin (Try)	2.22±0.08

ND = Not detected, NC = Not computed. Values are mean± standard deviation of triplicate determination

Table 4.3 shows that there were appreciable amounts of essential and non essential amino acids. Leucine and arginine were the most abundant essential amino acids, while glutamic and aspartic acids were the most abundant non essential amino acids in the plant extract. The table further shows that tryptophan was not detected in the sample.

4.1.4 Total amino acids and amino acid groups (g/100g protein

Table 4.4 shows the result of total amino acids and amino acid groups of *C. dolichopentalum* leaves

Table 4.4: Total amino acids and amino acid groups (g/100g protein) in leaves of *C. dolichopentalum*

Parameter	Concentration
Total amino acids and amino acid groups (g/100g protein)	
Total amino acids (TAA)	57.8
Total non essential amino acid (TNEAA)	33.25
Total essential amino acid including His	24.55
Total essential amino acid without His	22.53
Total neutral amino acids (TNAA)	25.63
Total acidic amino acids (TAAA)	15.65
Total basic amino acid (TBAA)	6.95
Total sulphur-containing amino acids (TSAA)	1.7
Total aromatic amino acids (TArAA)	5.85
Percentages of amino acid groups and amino acids	
% Total non essential amino acids (%TNEAA)	57.8
% Total essential amino acids (%TEAA)with His	42.47
% Total essential amino acids (%TEAA) without His	38.98
% Total neutral amino acid (%TNAA)	44.34
% Total acidic amino acids (%TAAA)	27.08
% Total basic amino acids (%TBAA)	12.02
% Total sulphur-containing amino acid (%TSAA)	2.94
% cys in TSAA	46.47
% Total aromatic amino acid (%TArAA)	10.12
% tyr in TArAA	37.95
Ratio of amino acids and amino acid grouping	
Leu/lle ratio	1.88
TBAA/TAAA ratio	0.44
TEAA/TAA ratio with His	0.42
TEAA/TAA ratio without His	0.39

Table 4.4 shows *C. dolichopentalum* contains more acidic amino groups than basic amino groups. It also reveals that *C. dolichopentalum* contains more non essential amino acids than essential amino acids. The table futher shows that the ratio of leucine to isoleucine in *C. dolichopentalum* leaves is greater than 1, indicating higher leucine content**.**

4.1.5 Vitamin composition of *C. dolichopentalum* leaves

Table 4.5 shows the vitamin composition of *C. dolichopentalum*

Table 4.5: Vitamin composition of *C. dolichopentalum* leaves

Sample	Concentration (mg/ml)
Vitamin A	3.14±0.13
Vitamin B1	1.79±0.10
Vitamin B2	0.14±0.01
Vitamin B3	2.26±0.07
Vitamin B6	0.47±0.01
Vitamin B7	0.29±0.02
Vitamin B9	0.16±0.01
Vitamin E	0.57±0.3

Values are means ± standard deviation of triplicate determinations

The Vitamin A (3.14±0.13 mg/ml) and vitamin B3 (2.26±0.07 mg/ml) as shown in Table 4.5 are the most abundant. Appreciable amounts of vitamin B1, vitamin B2, vitamin B6, vitamin B7, vitamin B9 and vitamin E were also obtained.

4.1.6 Phytochemical composition of *C. dolichopentalum* leaves

Table 4.6 shows the phytochemical composition of *C. dolichopentalum* leaves

Table 4.6: Phytochemical composition of *C. dolichopentalum* leaves

Phytochemicals (Quantitative)	% Concentration
Alkaloids	14.24±2.24
Flavonoids	17.00±2.00
Tannins	6.09±0.32
Saponins	4.19±0.69
Cyanogenic Glycosides	2.89±0.22
Oxalate	2.56±0.56
Phytate	0.10±0.01

Values are means ± standard deviation of triplicate determinations

Table 4.6 reveals that the quantitative phytochemical analyses of the ethanol extract of *C. dolichopentalum* leaves indicated the presence of flavonoids, alkaloids, saponins, tannins, cyanogenic glycosides, oxalate, and phytate. The analyses carried out on the sample revealed high concentrations of flavonoids, alkaloids, saponins and tannins.

4.1.7 GC aided identity of the phytochemical constituents of *C. dolichopentalum* leaves

Table 4.7 shows the result GC aided identity of the phytochemical constituents of *C. dolichopentalum* leaves.

Table 4.7: GC aided identity of the phytochemical constituents of *C. dolichopentalum* leaves. (Chromatogram shown in appendix 4D)

Phytochemicals using GC	Concentration (µg/ml)
Spartein	0.002
Oxalate	1.179
Anthrocyanin	0.585
Tannin	16.048
Lunamarine	5.989
Epicatechin	6.516
Rutin	263.249
Kaempferol	25.156

The result GC aided identification of the phytochemical constituents of *C. dolichopentalum* leaves (Table 4.7) shows appreciable presence of flavonoids such as kaempferol, lunamarine and rutin, alkaloids such as sparteine. The table further shows that kaempferol was higher among flavonoids; Lunamarine was higher among alkaloids and also confirms a high concentration of tannins. See appendix 4.1a for the GC spectrum.

4.1.8 Flavonoid constituent of *C. dolichopentalum* leaves obtained using GC

Table 4.8 shows the flavonoid constituents of *C. dolichopentalum* leaves obtained using GC

Table 4.8: Flavonoid constituents of the leaves of *C. dolichopentalum* obtained using gas chromatography (Chromatograms shown in appendices 4.1b and 4.2)

Names of Identified			mg/100g $x10^{-4}$	
Flavonoids	1	2	Mean	STDEV
Catechin	0.021807	0.035761	0.03	0.01
Resvaratrol	1.0884	1.7968	1.44	0.50
Apigenin	33586	34067	33826.5	340.12
Daidzein	1.29822	2.1132	1.71	0.58
Butein	2.0742	3.3609	2.72	0.91
Naringenin	6.1529	9.9984	8.08	2.72
Biochanin	3.7986	6.2431	5.02	1.73
Luteolin	26502	22136	24319	3087.23
Kaemferol	308798	283032	295915	18219.31
(-)-Epicatechin	5.9374	9.6932	7.82	2.66
(-)-Epicatechin-3-gallate	0.1043	0.10726	0.11	0.00
Gallocatechin	1.4664	2.399	1.93	0.66
Quercetin	62359	51328	56843.5	7800.09
Isorhamnetin	55433	44075	49754	8031.32
Myricetin	835.16	43.291	439.23	559.94
Naringin	0.7983	1.2046	1.00	0.29
Kaempferol-3-arabinoside	0.58587	0.92309	0.75	0.24
Quercitrin	0.9921	0.45662	0.72	0.38
Isoquercetin	310.06	488.97	399.52	126.51
Orientin	0.48125	0.72309	0.60	0.17
Isoorientin	8.713	432.18	220.45	299.44
Total	487856.7	435641.5	461749.11	36921.75

The result shown in Table 4.7 reveals the presence of 21 flavonoids (46.17± 3.7 mg/100g) Flavonoids such as quercetin, isorhamnetin, apigenin and keampferol showed the highest concentration and catechin recorded the least concentration.

4.1.9 Saponin constituents of the leaves of *C. dolichopentalum* obtained using GC

Table 4.9 shows the saponin constituents of *C. dolichopentalum* leaves obtained using GC

Table 4.9: Saponin constituents of the leaves of *C. dolichopentalum* obtained using gas chromatography (Chromatograms shown in appendices 4.3 and 4.4)

			mg/100g $x10^{-2}$	
Names of Identified Saponin	1	2	Mean	STDEV
Hispogenin	0.037225	0.031888	0.03	0.00
Solagenin	1.6927	1.4675	1.58	0.16
Diosgenin	3.16466	2.7297	2.95	0.31
Tigogenin	1.80633	1.5832	1.69	0.16
Neochlorogenin	9.49701	8.1961	8.85	0.92
Hecogenin	136.193	182.95	159.57	33.06
Sapogenin	6579.754	5727.77	6153.76	602.44
Tribuloin	29.0494	24.4102	26.73	3.28
Yanogenin	10.619	9.084	9.85	1.09
Conyzorgin	0.10844	0.22847	0.17	0.08
Saponine	2968.03	2569.18	2768.61	282.03
Total	9739.952	8527.631	9133.79	857.24

Table 4.9 shows saponins content of *C. dolichopentalum* leaves. Saponins such as sapogenin, saponine, and hecogenin respectively were relatively more abundant while conyzorgin and hispogenin indicated the lowest concentration. See appendices 4.3, 4.4, 4.13 and 4.14 for the GC profile and external standard report.

4.1.10 Alkaloids in the leaves of *C. dolichopentalum* obtained using GC

Table 4.10 shows the alkaloids constituents of *C. dolichopentalum* leaves obtained using GC

Table 4.10: Alkaloids constituents of the leaves of *C. dolichopentalum* obtained using gas chromatography. (Chromatograms shown in appendices 4.5 and 4.6)

			mg/100g $x10^{-3}$	
Names of Identified Alkaloids	**1**	2	Mean	STDEV
9. Octadecenamide	3.0514	4.9179	3.99	1.32
Dihydro-oxo-demethoxyhaemanthamine	3.7166	2.1746	2.95	1.09
Augustamine	0.79432	0.81176	0.80	0.01
Oxoassoanine	6.4266	7.4229	6.93	0.70
Crinane-3alpha-ol	13.579	16.804	15.19	2.28
Buphanidrine	29.116	56.339	42.73	19.25
Powelline	6.07674	10.415	8.25	3.07
Undulatine	5.3104	7.9602	6.64	1.87
Ambelline	6.61421	9.4801	8.05	2.03
6-hydroxybuphanidrine	3.9351	4.0891	4.01	0.11
Hydroxypowelline	7.8645	10.47	9.17	1.84
Crinamidine	31.567	38.955	35.26	5.22
6-hydroxyundulantine	9.4319	18.182	13.81	6.19
1 beta, 2 beta-Epoxyambelline	9.2786	3.7761	6.53	3.89
Epoxy-3, 7 dimethoxycrinane-11-one	9.27721	11.933	10.61	1.88
Akuamidine	42.399	40.505	41.45	1.34
Mitrsaphylin	4.4844	4.6347	4.56	0.11
Voacangine	23.948	18.6013	21.28	3.78
Total	216.871	267.4717	242.17	35.78

Table 4.9 shows that alkaloids such as akuamidine, crinamidine, and voacangine were relatively greater than augustamine and 9-octadecenamide which recorded the least concentration. See appendices 4.5, 4.6, 4.10 and 4.11 for the GC profile and external standard report.

4.1.11 Tannin constituents of the leaves of *C. dolichopentalum* obtained using GC

Table 4.11 shows the Tannin constituents of *C. dolichopentalum* leaves obtained using GC

Table 4.11: Tannin constituents of the leaves of *C. dolichopentalum* obtained using gas chromatography (Chromatograms shown in appendices 4.7 and 4.8)

			mg/100g	
Name of Tannin	**1**	2	Mean	STDEV
Tannic acid	55.72334	47.31545	51.52	5.95
Total	55.72334	47.31545	51.52	5.95

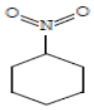
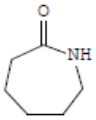
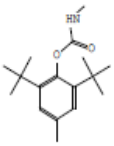
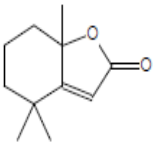
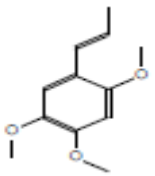
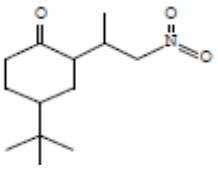
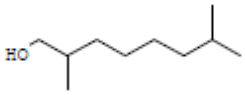
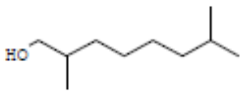
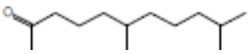
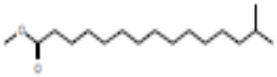
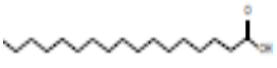
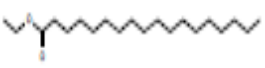
Screening of *C. dolichopentalum* as shown in table 4.10 for Tannins revealed the presence of tannic acid in large quantity (51.52 mg/100g).

See appendices 4.7, 4.8, 4.15 and 4.16 for the GC profile and external standard report.

4.1.12 GC-MS analysis of ethanol extract of *C. dolichopentalum* leaves

Table 4.12a shows the molecular weights, formula and structures of compounds of EECD

Table 4.12a: Molecular weights, formula and structures of compounds identified from the GC-MS of ethanol extract of *C. dolichopentalum* Leaves (chromatogram in appendix 4.17)

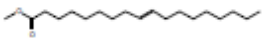
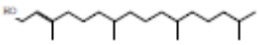
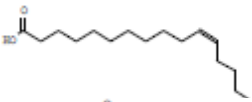
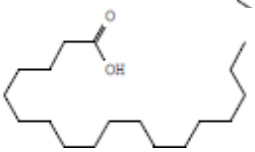
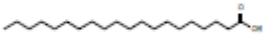
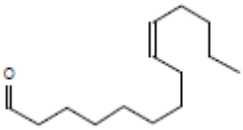
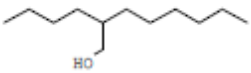
S/n	Molecular Weight	Weight	Formula	Molecular Structure
1	Nitrocyclohexane	129	$C_6H_{11}NO_2$	
2	Amino caproic lactam	113	$C_6H_{11}NO$	
3	Phenol-2,6-bis (1,1-dimethyl)-4 -methyl,methyl carbamate	277	$C_{17}H_{27}NO_2$	
4	Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	180	$C_{11}H_{16}O_2$	
5	(E)-2,4,5. -trimethoxypropenyl benzene -	208	$C_{12}H_{16}O_3$	
6	4-t-Butyl-2-(1-methyl-2-nitroethyl) cyclohexanone	241	$C_{13}H_{23}NO_3$	
7	2,7- dimethyl-1-octanol	158	$C_{10}H_{22}O$	
8	1-Tridecyne	180	$C_{10}H_{22}O$	
9	6,10-dimethyl-2-undecanone	198	$C_{13}H_{26}O$	
10	Hexadecane	226	$C_{16}H_{34}$	
11	Methyl 14-methylpentadecanoate	270	$C_{17}H_{34}O_2$	
12	Heptedecanoic acid	270	$C_{17}H_{34}O_2$	
13	Ethyl octadecanoate	312	$C_{20}H_{40}O_2$	

The results of GC-MS study (Table 4.12a) revealed the presence of cyclohexane, primary alcohols, esters, alkanoic acids as well as epoxide and haloalkanes. (Chromatograms shown in appendix 4.9)

4.1.13 GC-MS analysis of ethanol extract of *C. dolichopentalum* leaves

Table 4.12 shows the molecular weights, formula and structures of compounds identified from the GC-MS of ethanol extract of *C. dolichopentalum*

Table 4.12b: Molecular weights, formula and structures of compounds identified from the GC-MS of ethanol extract of *C. dolichopentalum*

S/n	Molecular Weight	Mole Weight	Molecular Formula	Molecular Structure
14	Methyl trans-9-ctadecanoate	296	$C_{19}H_{36}0_2$	
15	3,7,11,15-tetramethyl-2-hexadecen-1-ol	296	$C_{20}H_{40}O$	
16	11-Hexadecanoic acid	254	$C_{16}H_{30}O_2$	
17	Octadecanoic acid	284	$C_{18}H_{36}O_2$	
18	1-Flourodecane	160	$C_{10}H_{12}F$	
19	Eicosanoic acid	312	$C_{20}H_{40}O_2$	
20	Eicosane	282	$C_{20}H_{42}$	
21	9-tetradecenal	210	$C_{14}H_{26}O$	
22	2-Buthyl-1-octanol	186	$C_{12}H_{26}O$	
23	Cyclododecane epoxide	182	$C_{12}H_{22}O$	
24	Tetratetracotane	618	$C_{44}H_{90}$	

The results of GC-MS study (Table 4.12b) revealed the presence of cyclohexane, primary alcohols, esters, alkanoic acids as well as epoxide and haloalkanes.

4.1.14 FTIR spectrophotometry of of *C. dolichopentalum* leaves

Figure 4.1 shows the FTIR absorption spectra of crude *C. dolichopentalum* leaves

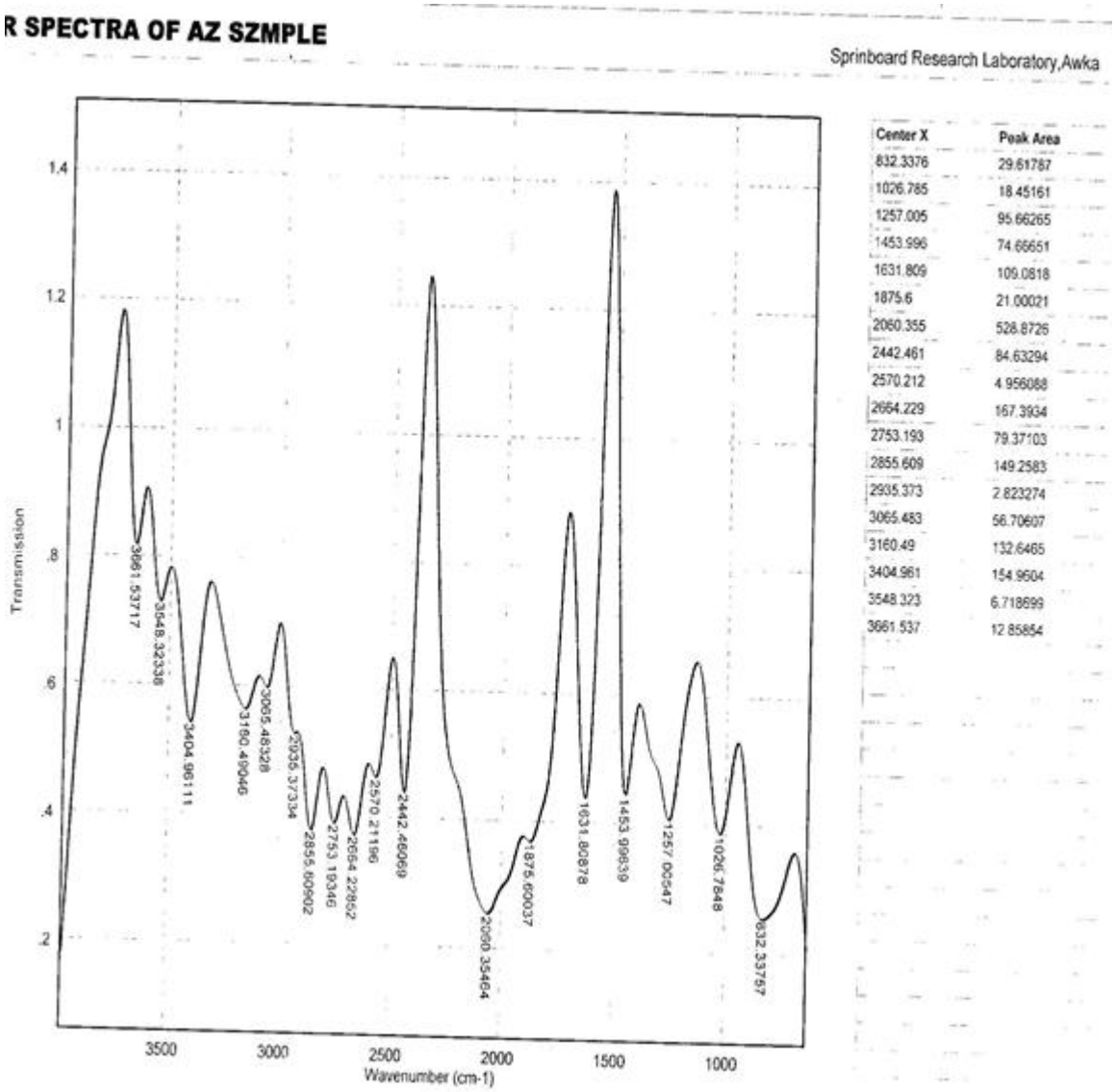


Figure 4.1: FTIR spectra of *C. dolichopentalum* leaves showing absorption peaks of different functional groups

The spectra characteristics of *C. dolichopentalum* leaves such as wavelength, functional groups and possible compounds indicated by the peaks (Figure 4.1) are shown in Table 4.12

4.1.15 FTIR spectrophotometry of *C. dolichopentalum* leaves

Table 4.12 shows the FTIR spectra characteristics of *C. dolichopentalum* leaves

Table 4.12: FTIR Spectra Characteristics of *C. dolichopentalum* leaves

S/N	Wavelength (cm^{-1})	Functional Group	Compounds
1	832.5578	C-Cl	Chloro compound C-Cl stretch
2	1026.785	R-O-P	Ether CO stretch
3	1257.005	RNH_2	1^0 amine NH stretch
4	1453.996	CH_3	Methyl CH stretch
5	1631.809	RNH_2	2^0 amine NH stretch
6	1875.6	C-O-C	Cyclic esther CO stretch
7	2060.355	RCOOH	Carboxylic acid COO stretch
8	2442.461	R-C $\equiv$ N	Nitriles CN anti-symmetric stretch
9	2570.212	CH_2SH	Thiol SH stretch
10	2664.229	CH_2SH	Thiol SH stretch
11	2753.193	CH_2	Methylene CH stretch
12	2855.609	CH_2	Methylene CH stretch
13	2935.373	R-S-C $\equiv$ N	Thiocyanate SCN anti-symmetric stretch
14	3065.483	RCHOH	1^0 alcohol OH stretch
15	3160.49	RCHOH	1^0 alcohol OH stretch
16	3404.961	RNH	2^0 amine NH stretch
17	3548.323	R_2CHOH	3^0 alcohol OH stretch
18	3661.537	R_3N	3^0 amine NH stretch

Table 4.12 details the characteristics of the peaks presented in Figure 4.1. It shows the wavelengths, functional groups and possible compounds present in *C. dolichopentalum* leaves

4.1.16 FTIR spectrophotometry of *C. dolichopentalum* leaves

Figure 4.2 shows the FTIR spectra of eluate-Dcd from column chromatography of EECD

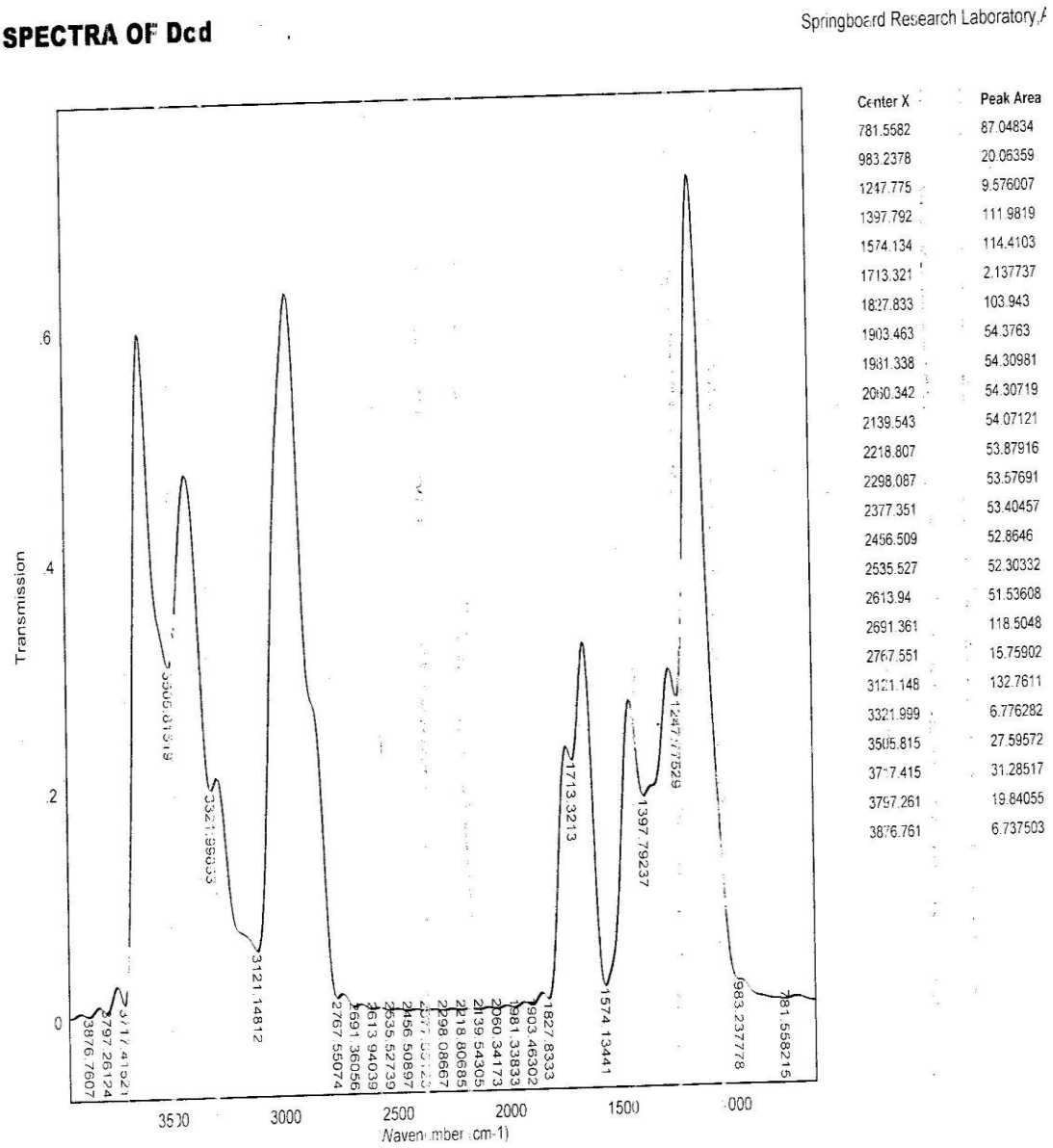


Figure 4.2: FTIR spectra of eluate-Dcd from column chromatography of EECD

Figure 4.2 shows a normal absorbtion at 1713 cm^{-1} indicating the C=O stretch. The two strong bands between 1600-1500 cm^{-1} and 1390-1300 cm^{-1} indicate a nitro compound. C≡C stretch is near 2150 cm^{-1}. The absorption spectra indicate the eluate as 4-t-butyl-2-(1-methyl-2-nitroethyl) cyclohexanone – $C_{13}H_{23}NO_3$.

4.1.17 FTIR spectrophotometry of *C. dolichopentalum* leaves

Figure 4.3 shows the FTIR spectra of eluate-Fcd from column chromatography of EECD

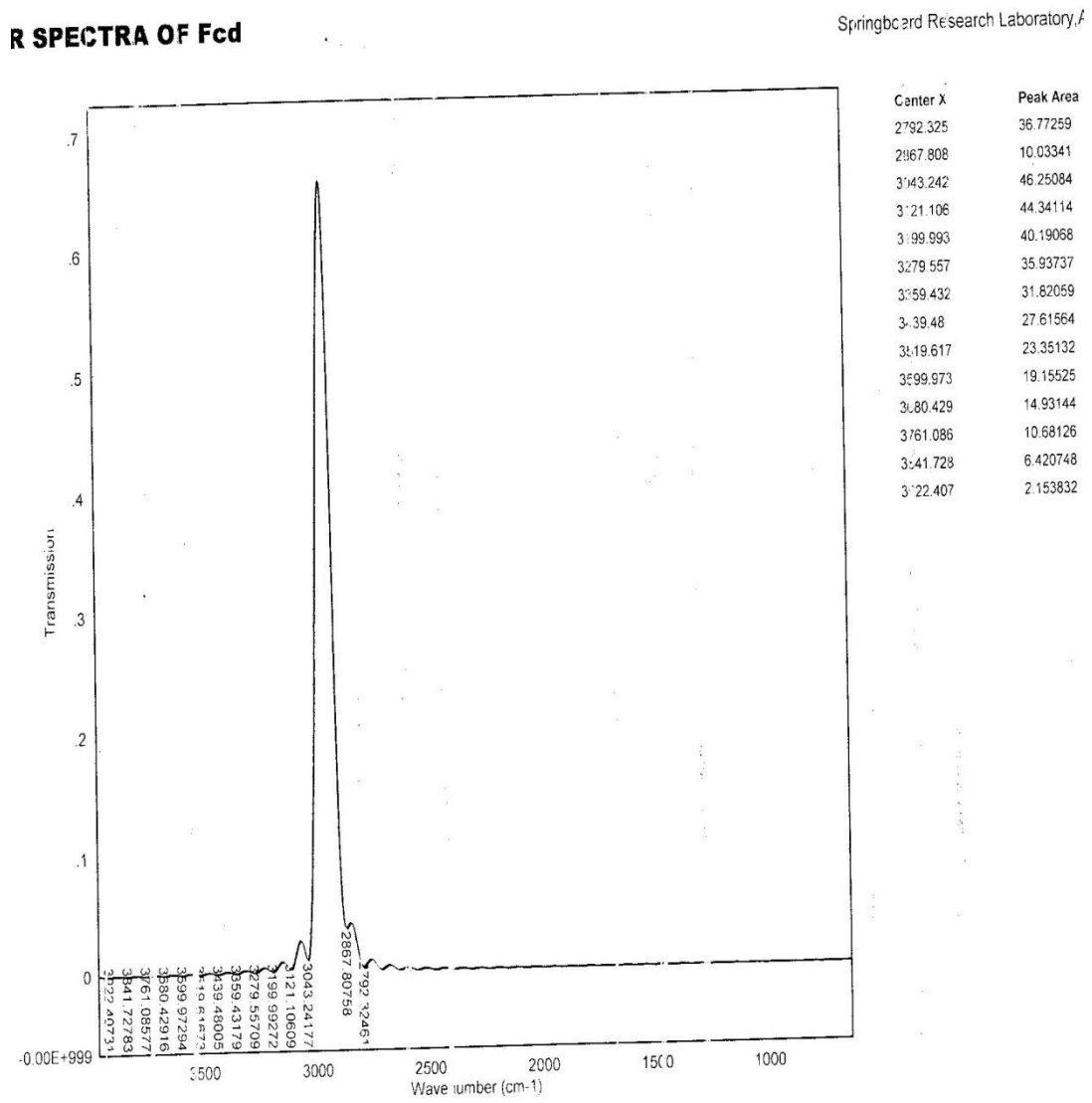


Figure 4.3: FTIR spectra of eluate-Fcd from column chromatography of EECD

In alkanes absorption always occur to the right of 3000 cm^{-1}. The absorption stretch from 1350-960 cm^{-1} indicates the halide C-F. Therefore the absorption spectra in Figure 4.3 indicate the eluate as 1-fluorodecane- $C_{10}H_{21}F$.

4.1.18 FTIR spectrophotometry of *C. dolichopentalum* leaves

Figure 4.4 shows the FTIR spectra of eluate-Gcd from column chromatography of EECD

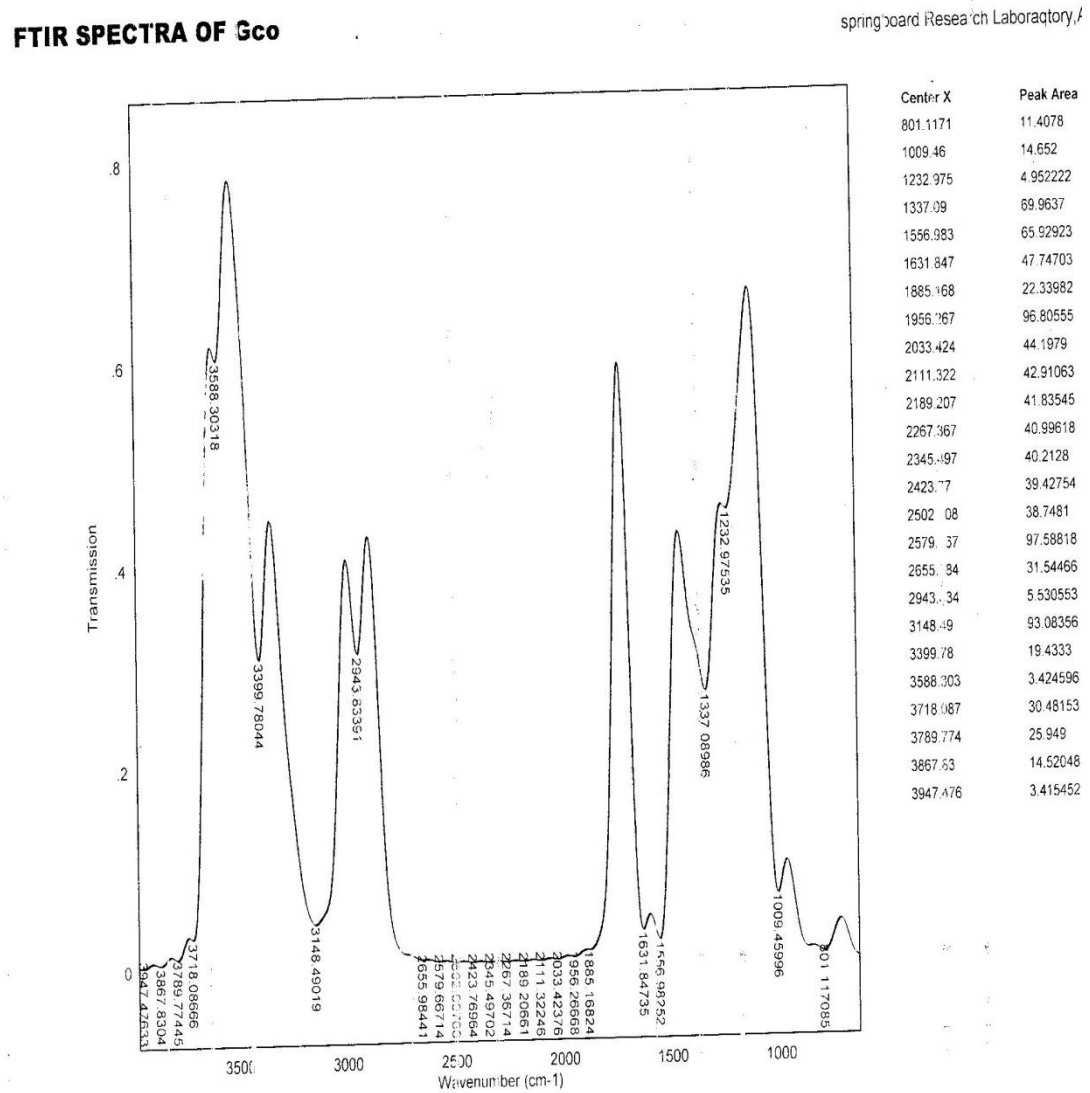


Figure 4.4: FTIR spectra of eluate-Gcd from column chromatography of EECD

Figure 4.4 shows a strong bands of N=O stretch between 1600-1500 cm^{-1} and 1390-1300 cm^{-1}. The sharp absorptions that occured in pairs around 1631 cm^{-1} on the left of 1337 cm^{-1} are characteristics of an aromatic ring. The weak absorptions that appeared approximately around 2000 cm^{-1} indicates that the aromatic ring is di-substituted.The absorption spectra indicated the eluate as phenol-2,6-bis - (1,1-dimethylethyl) - 4 - methyl methyl carbamate - $C_{17}H_{27}NO_2$.

4.1.19 FTIR spectrophotometry of *C. dolichopentalum* leaves

Figure 4.5 shows the FTIR spectra of eluate-Icd from column chromatography of EECD

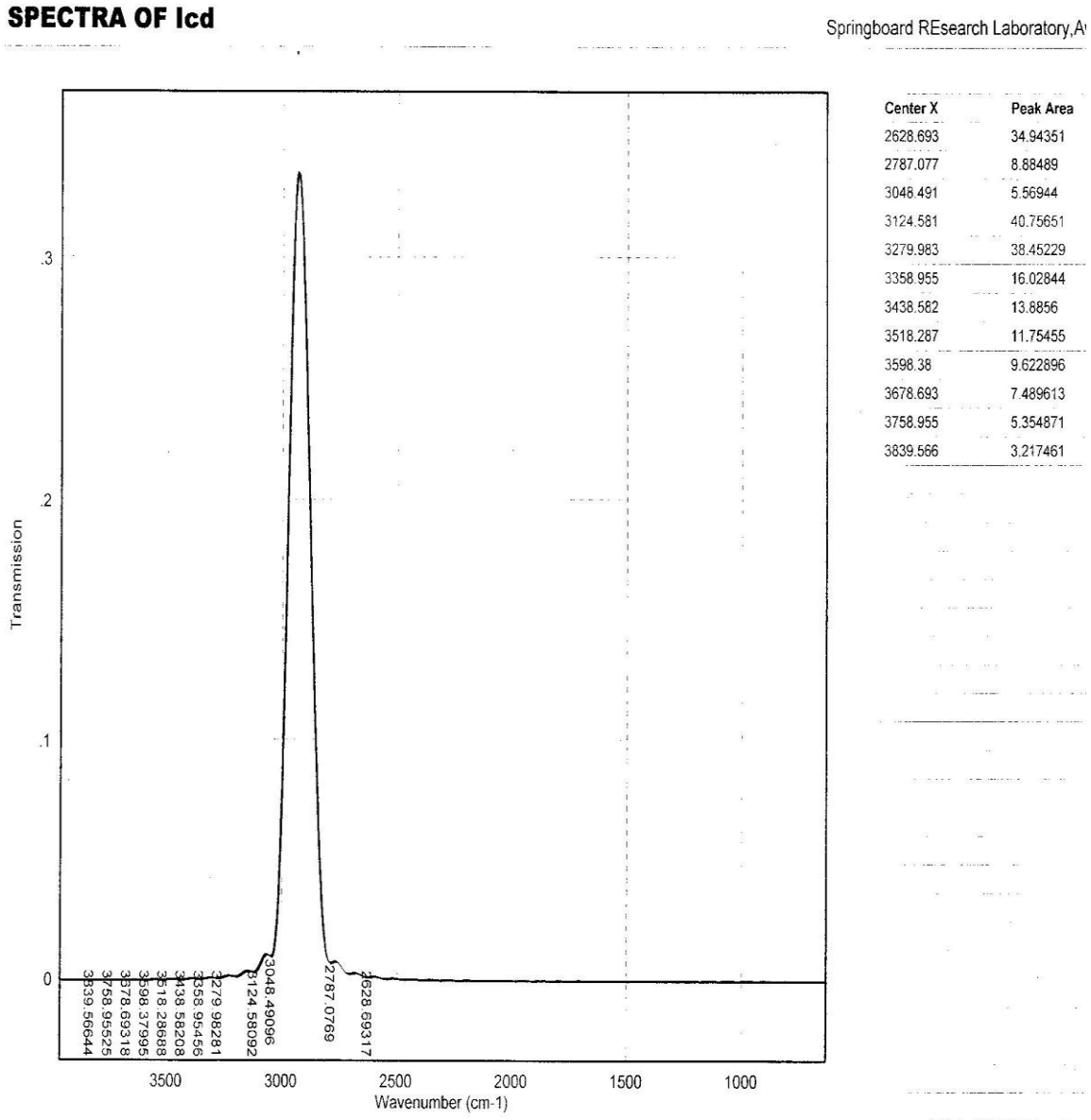


Figure 4.5: FTIR spectra of eluate-Icd from column chromatography of EECD

The C-H stretch of alkanes occur around 3000 cm^{-1}. The absorption peaks between 2787 cm^{-1} and 3048 cm^{-1} indicates an alkane. This implies that the absorption spectra shown in figure 4.5 indicate the eluate as eicosane – $C_{20}H_{42}$.

4.1.20 FTIR spectrophotometry of *C. dolichopentalum* leaves

Figure 4.6 shows the FTIR spectra of eluate-Jcd from column chromatography of EECD

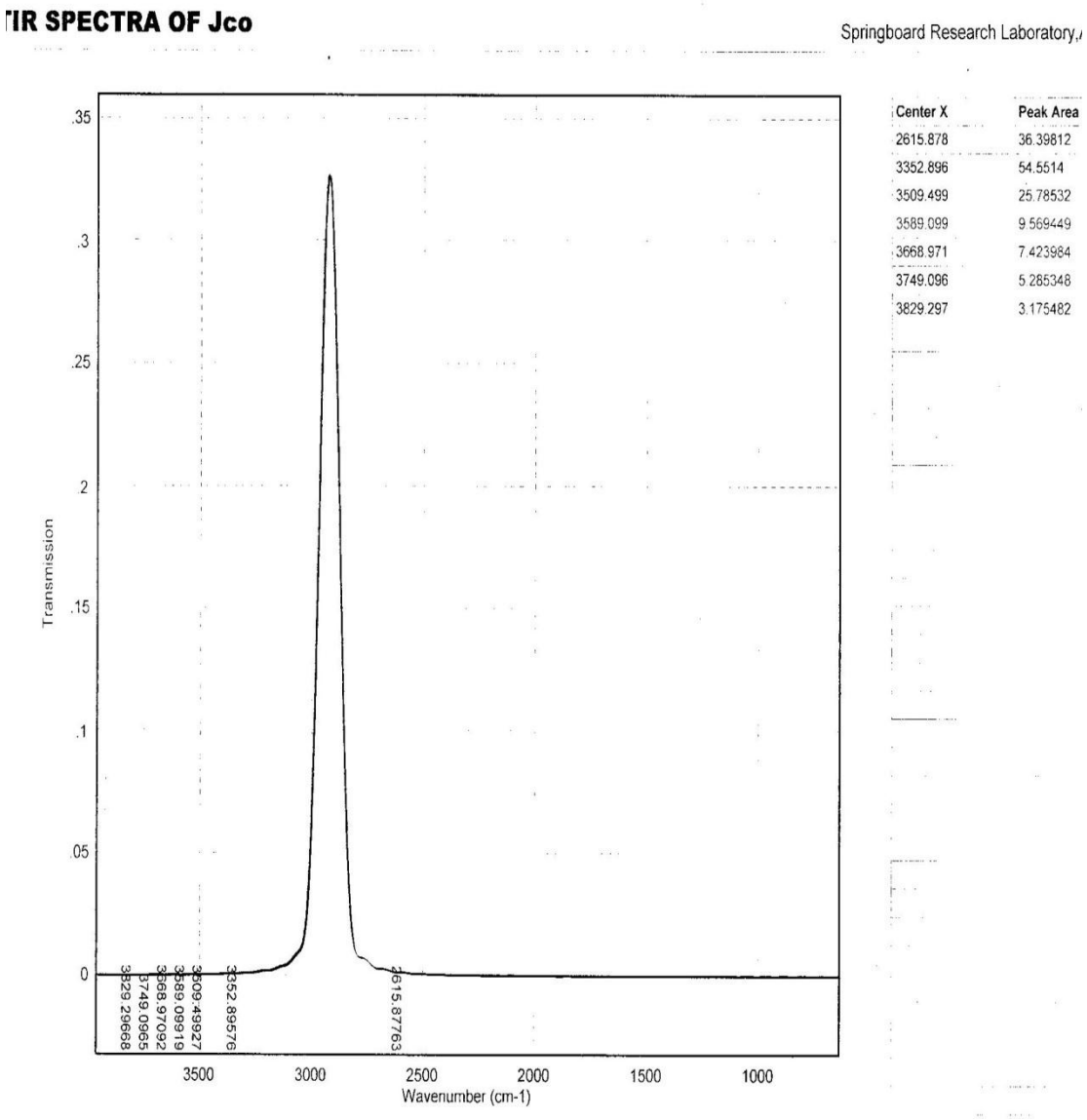


Figure 4.6: FTIR spectra of eluate-Jcd from column chromatography of EECD

The sharp peak and the broad absorptions which occur around 3500-3200 cm^{-1} overlapping the C-H stretch are reveals presence of alcohol or OH compound. The absorption spectra shown in Figure 4.6 indicate the eluate as 2,7-dimethyl-1-octanol- $C_{10}H_{22}O$.

4.1.21 FTIR spectrophotometry of *C. dolichopentalum* leaves

Figure 4.7 shows the FTIR spectra of eluate-Kcd from column chromatography of EECD

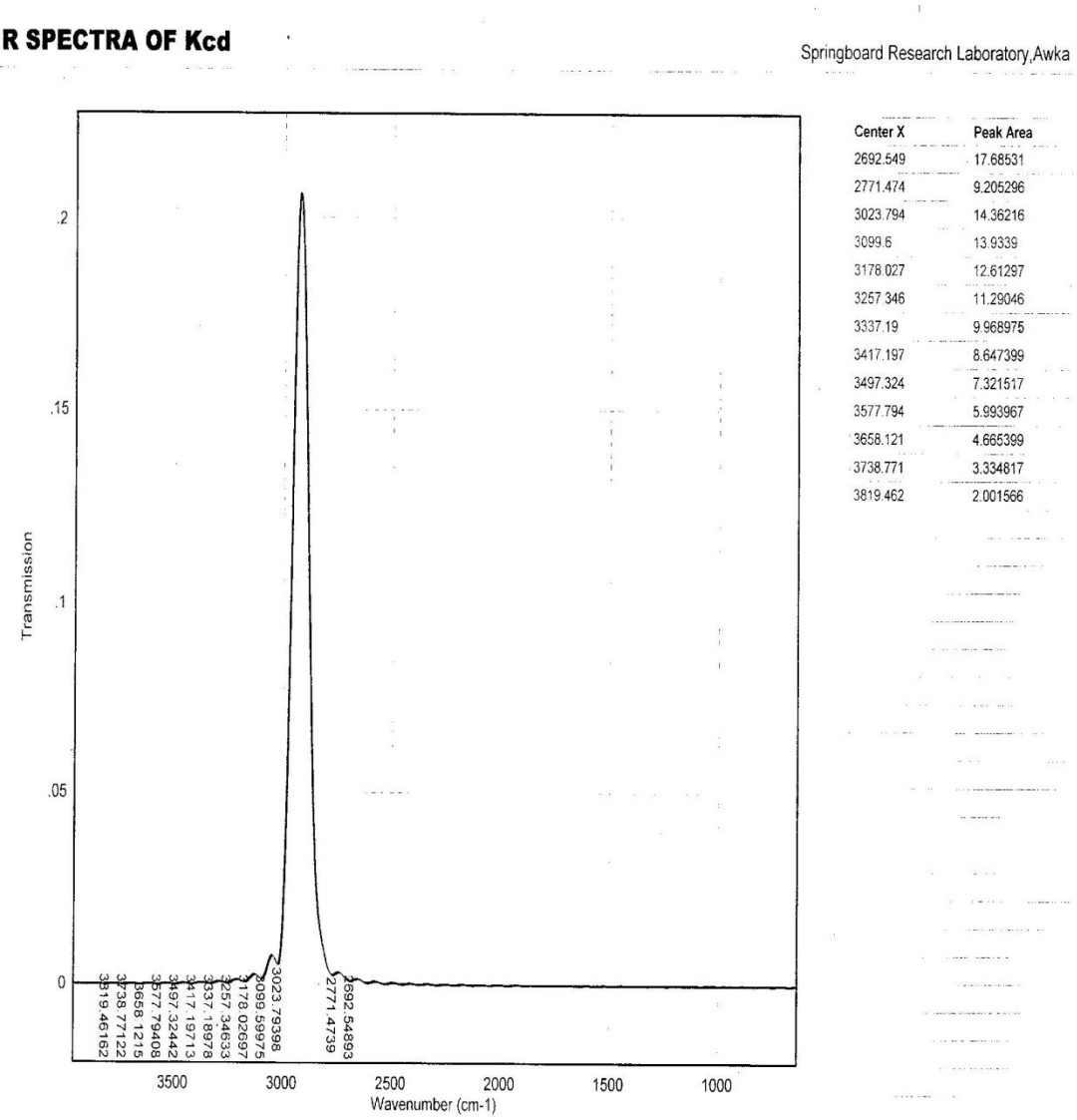


Figure 4.7: FTIR spectra of eluate-Kcd from column chromatography of EECD

The absorption spectra in figure 4.7 indicated the eluate as hexadecane – $C_{16}H_{32}$. The absorbtion stretch at 2771 cm^{-1} and 3023 cm^{-1} indicates the methylene groups and the compound as an alkane.

4.1.22 FTIR spectrophotometry of *C. dolichopentalum* leaves

Figure 4.8 shows the FTIR spectra of eluate-Mcd from column chromatography of EECD

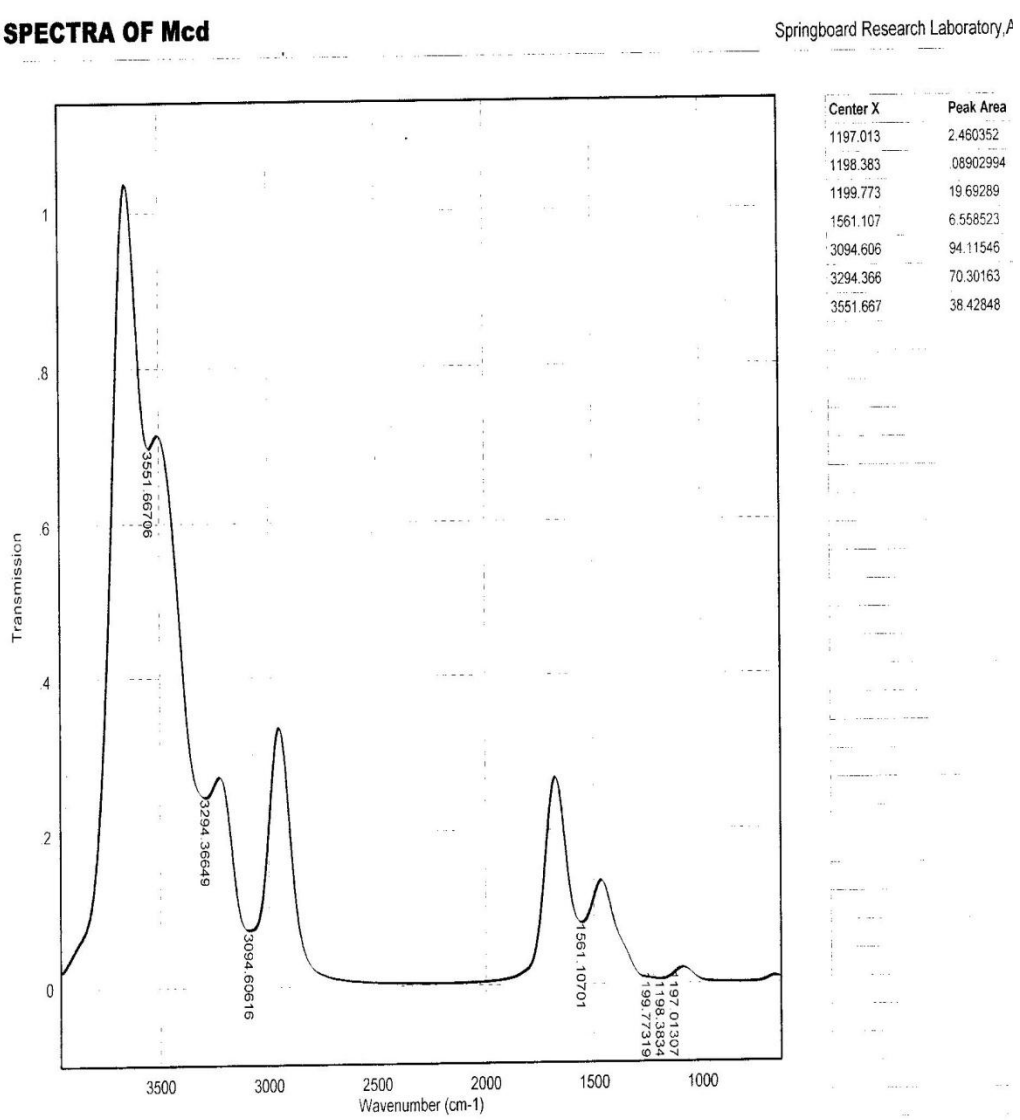


Figure 4.8: FTIR spectra of eluate-Mcd from column chromatography of EECD

The absorption spectra in figure 4.8 indicated the eluate as 2-butyl-1-octanol- $C_{12}H_{26}O$. The absorbtion spectra shows peaks at 3551 cm^{-1} for OH group, 3294 cm^{-1} for ≡C-H and 3094 cm^{-1} for C-H stretch.

4.1.23 *In vitro* radical-scavenging potentials of EECD

Figure 4.9 shows the nitric oxide ($NO^{\cdot}$) scavenging potentials of *C. dolichopentalum*.

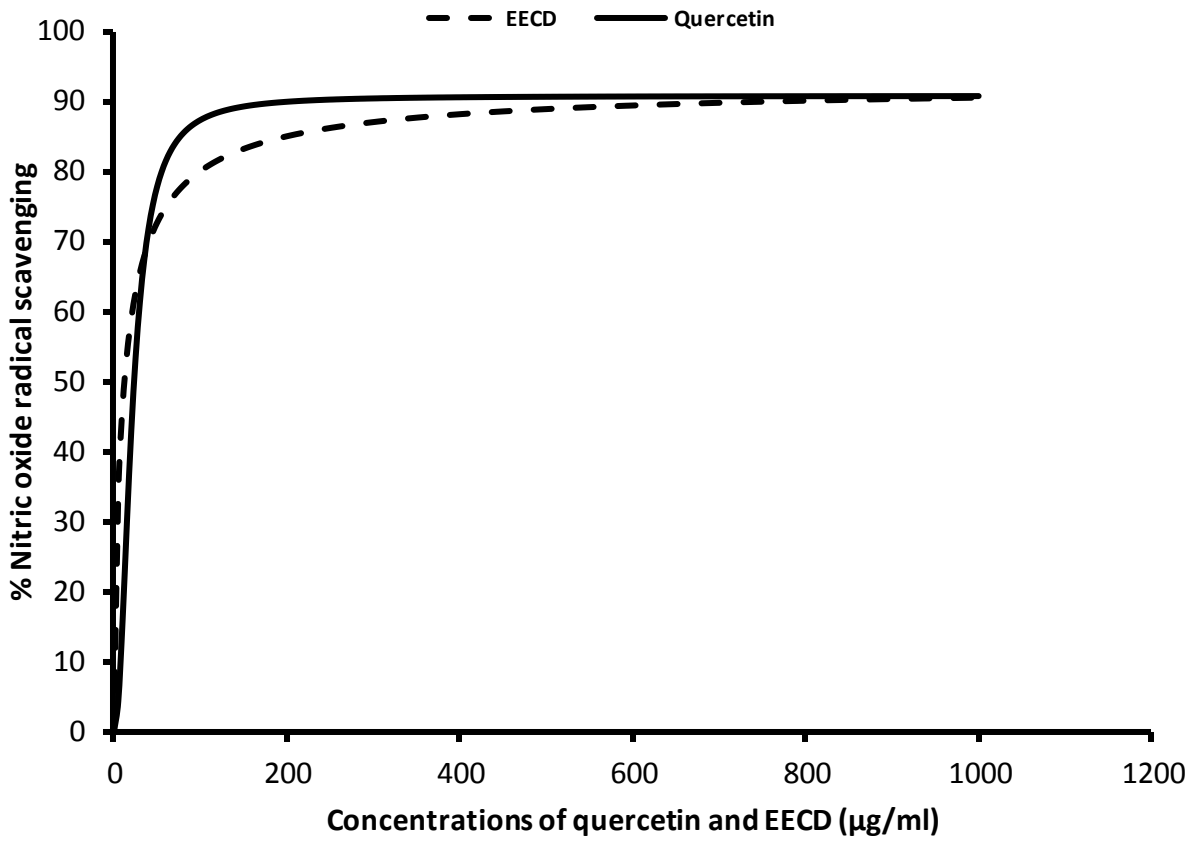


Figure 4.9: Nitric oxide ($NO^{\cdot}$) scavenging potentials of *C. dolichopentalum*.

Figure 4.9 shows the result of $NO^{\cdot}$ radical scavenging potentials of EECD. The investigation showed EECD as a better $NO^{\cdot}$ scavenger compared to the plant standard quercetin. The threshold inhibitory concentration/scavenging power of EECD (mg/ml) are shown in appendix 4A

4.1.24 *In vitro* radical-scavenging potentials of EECD

Figure 4.10 shows the reducing power of the ethanol extract of *C. dolichopentalum*.

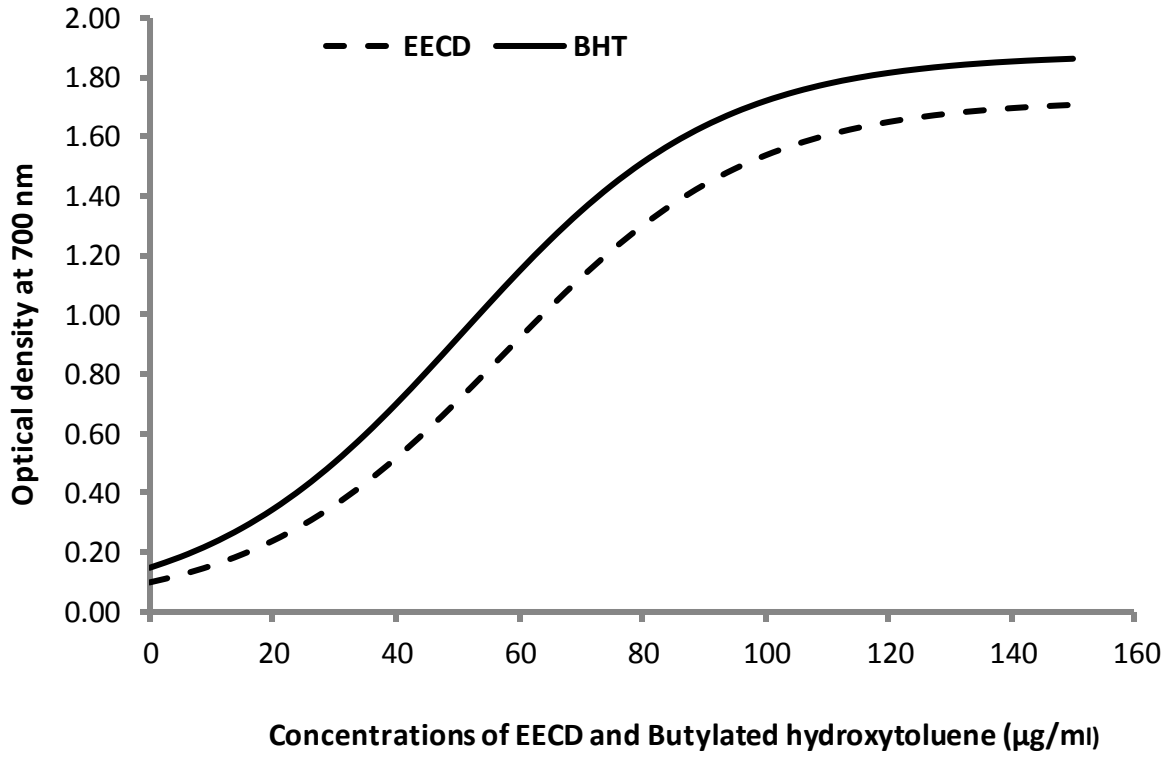


Figure 4.10: Reducing power of the ethanol extract of *C. dolichopentalum*.

The result of reducing power potential of EECD is shown in Figure 4.10. It revealed that EECD dose dependently transformed Fe^{3+} to Fe^{2+} indicating strong reducing power. The concentration of extract able to give 0.5 absorbance unit was found to be 39.11 µg/ml for EECD, whereas BHT was $RP_{0.5}$ = 29.95 μg/ml. The threshold inhibitory concentration/scavenging power of EECD (mg/ml) are shown in appendix 4C

4.1.25 *In vitro* radical-scavenging potentials of EECD

Figure 4.11 shows the lipid peroxidation inhibition by *C. dolichopentalum* in rabbit brain homogenate.

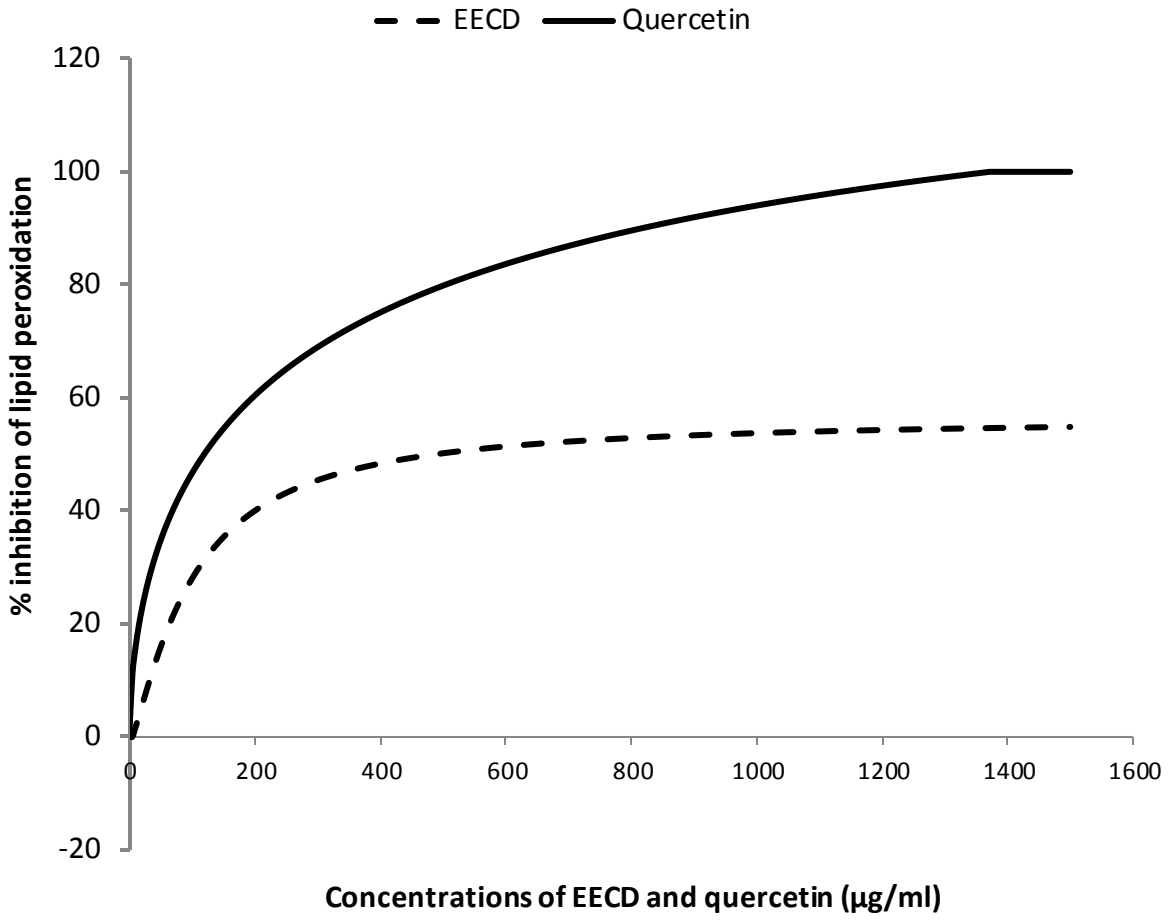


Figure 4.11: Lipid peroxidation inhibition by *C. dolichopentalum* in rabbit brain homogenate.

Inhibition of lipid peroxidation in rabbit brain homogenate by EECD (Figure 4.11) showed an inhibition concentration less than that of the plant standard quercetin. The threshold Inhibitory concentration/scavenging power of EECD (mg/ml) are shown in appendix 4A

4.1.26 *In vitro* radical-scavenging potentials of EECD

Figure 4.12 shows the hydroxyl radical ($OH^{\cdot}$) scavenging ability of *C. dolichopentalum*

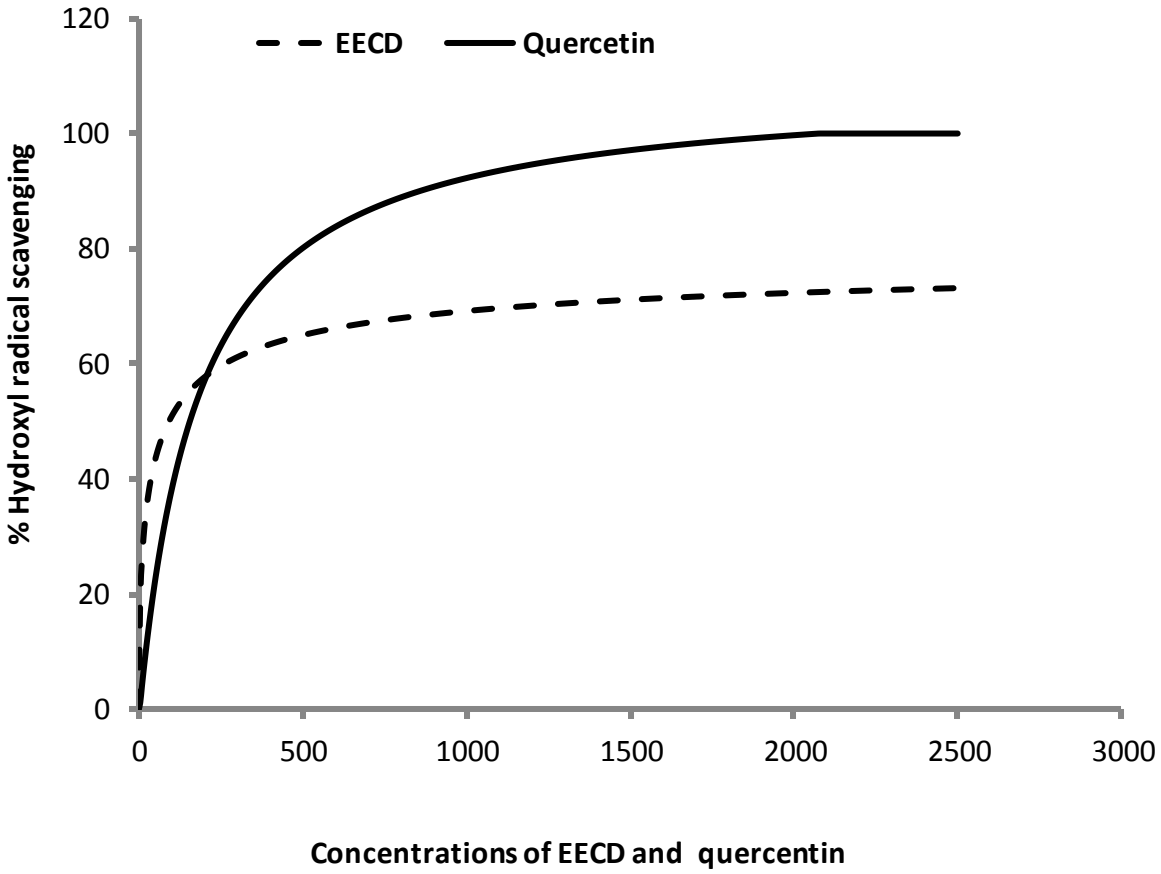


Figure 4.12: Hydroxyl radical ($OH^{\cdot}$) scavenging ability of *C. dolichopentalum*

Figure 4.12 shows the hydroxyl radical ($OH^{\cdot}$) Scavenging potentials of *C. dolichopentalum* leaves. Ethanol extract of *C. dolichopentalum* caused a dose dependent threshold inhibitor concentration with an IC_{50} of 92.39 µg/ml. The threshold Inhibitory concentration/scavenging power of EECD (mg/ml) are shown in appendix 4A

4.1.27 *In vitro* radical-scavenging potentials of *EECD*

Figure 4.13 shows the effect of ethanol extract of *C. dolichopentalum* on inhibition of total dehydrogenase activity against *E. coli*.

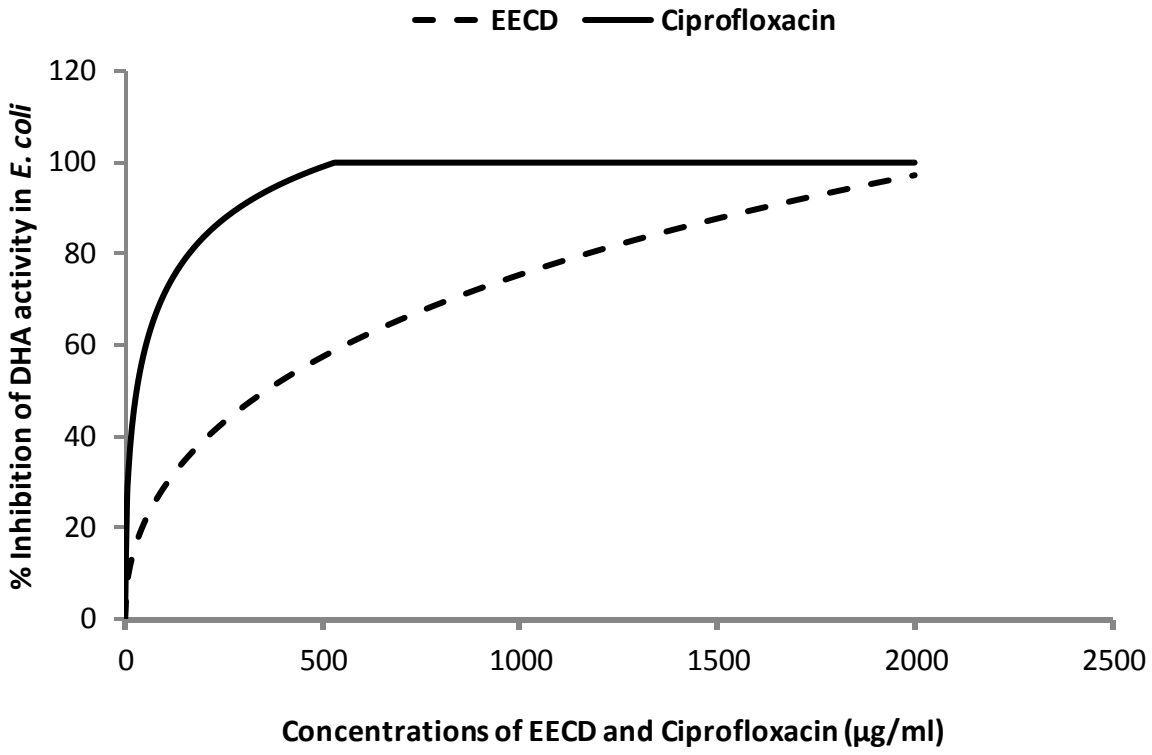


Figure 4.13: Effect of ethanol extract of *C. dolichopentalum* on inhibition of total dehydrogenase activity against *E. coli*.

The inhibition of total dehydrogenase activity against *E. coli* by EECD is shown in Figure 4.13. The extract dose dependently inhibited Total dehydrogenase activity in *E. coli* following the logistic dose response curve although this was less than the inhibition caused by the standard drug ciprofloxacin. The threshold inhibitory concentrations are as shown in appendix 4B

4.1.28 *In vitro* radical-scavenging potentials of EECD

Figure 4.14 shows the effect of ethanol extract of *C. dolichopentalum* on inhibition of total dehydrogenase activity against *S. aureus*.

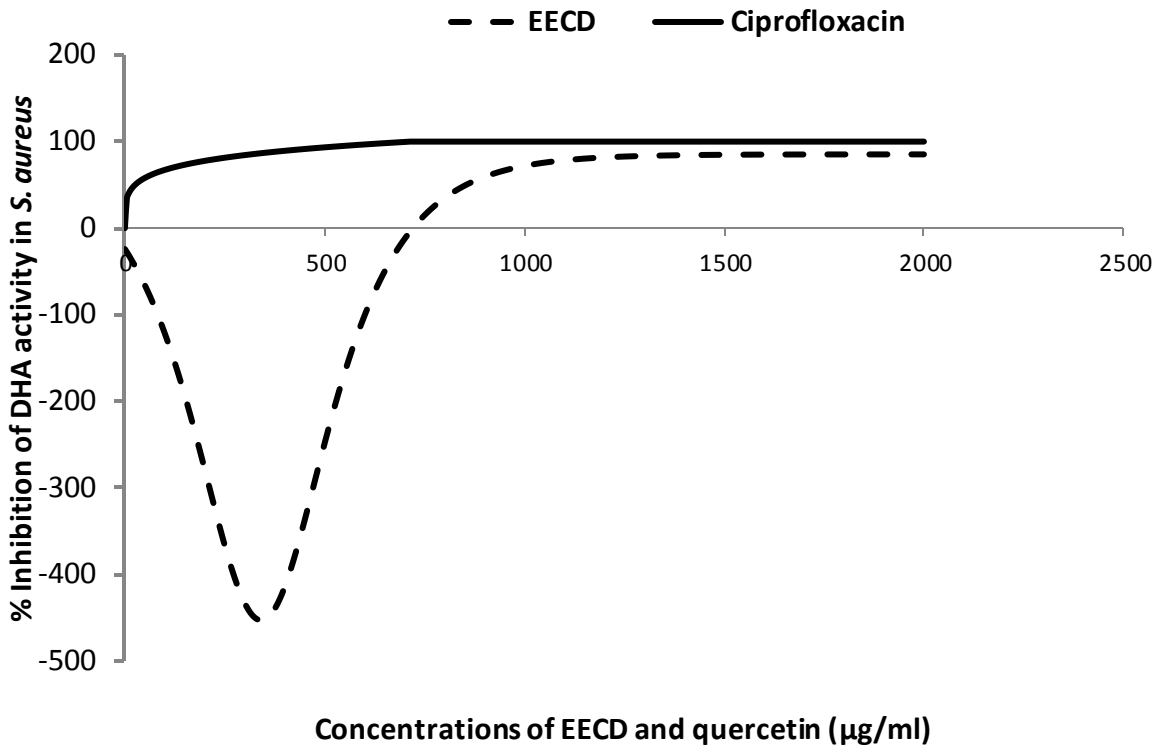


Figure 4.14: Effect of ethanol extract of *C. dolichopentalum* on inhibition of total dehydrogenase activity against *S. aureus*.

Figure 4.14 shows the inhibition of total dehydrogenase activity against *S. aureus* by EECD.The extract dose dependently inhibited total dehydrogenase activity in *S. aureus* following the logistic dose response curve at IC less than that of the standard drug. Threshold inhibitory concentrations are as shown in appendix 4B

4.1.29 *In vitro* radical-scavenging potentials of *EECD*

Figure 4.15 shows the effect of ethanol extract of *C. dolichopentalum* on inhibition of total dehydrogenase activity against *Streptococcus pneumonia*.

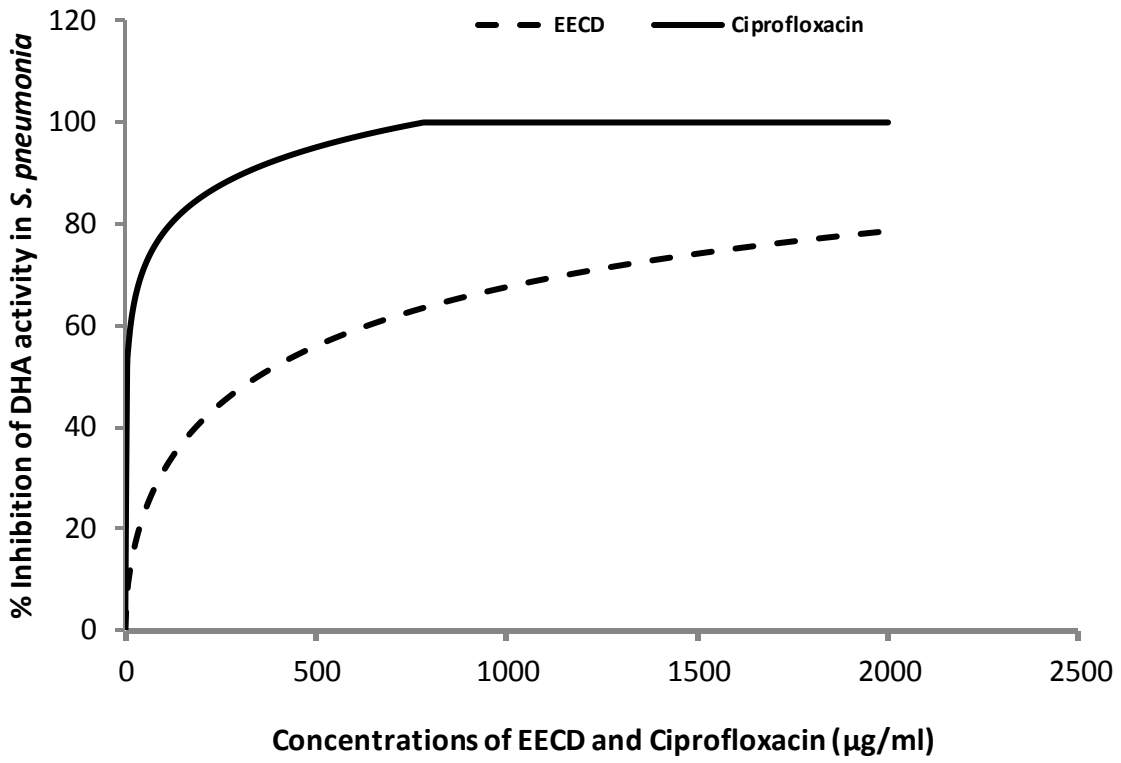


Figure 4.15: Effect of ethanol extract of *C. dolichopentalum* on inhibition of total dehydrogenase activity against *S. pneumonia*.

Figure 4.15 shows the inhibition of total dehydrogenase activity against *S. pneumonia* by EECD. The extract dose dependently inhibited Total dehydrogenase activity in *S. pneumonia* following the logistic dose response curve as shown in figure 4.15 with an IC less than that recorded by the standard drug ciprofloxacin. Threshold inhibitory concentrations are as shown in appendix 4B

4.1.30 *In vitro* radical-scavenging potentials of EECD

Figure 4.16 shows the effect of ethanol extract of *C. dolichopentalum* on inhibition of total dehydrogenase activity against *S. typhi*.

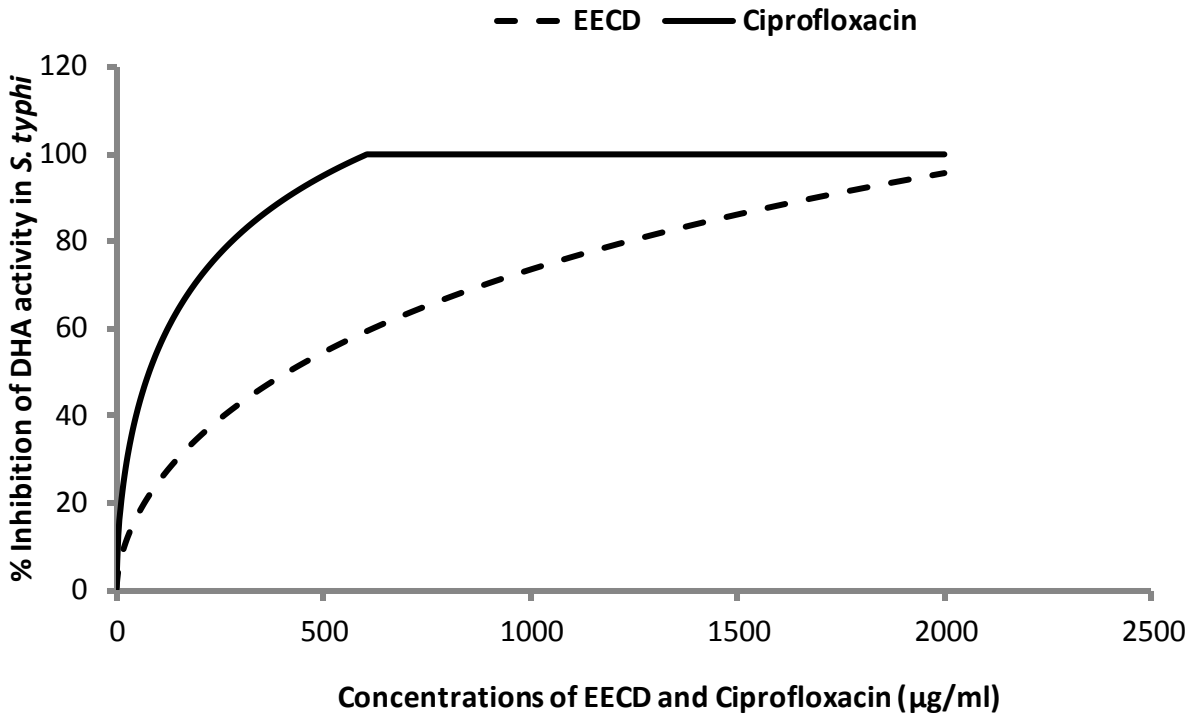


Figure 4.16: Effect of ethanol extract of *C. dolichopentalum* on inhibition of total dehydrogenase activity against *S. typhi*.

Figure 4.16 shows that EECD dose dependently inhibited total dehydrogenase activity in *S. typhi* following the logistic dose response curve in a manner less than that of the standard drug ciprofloxacin. The threshold inhibitory concentrations are as shown in appendix 4B

4.1.31 *In vitro* radical-scavenging potentials of EECD

Figure 4.17 shows the hydrogen peroxide radical scavenging activity of flavonoid, saponin, alkaloid and tannin extracted from *C. dolichopentalum*

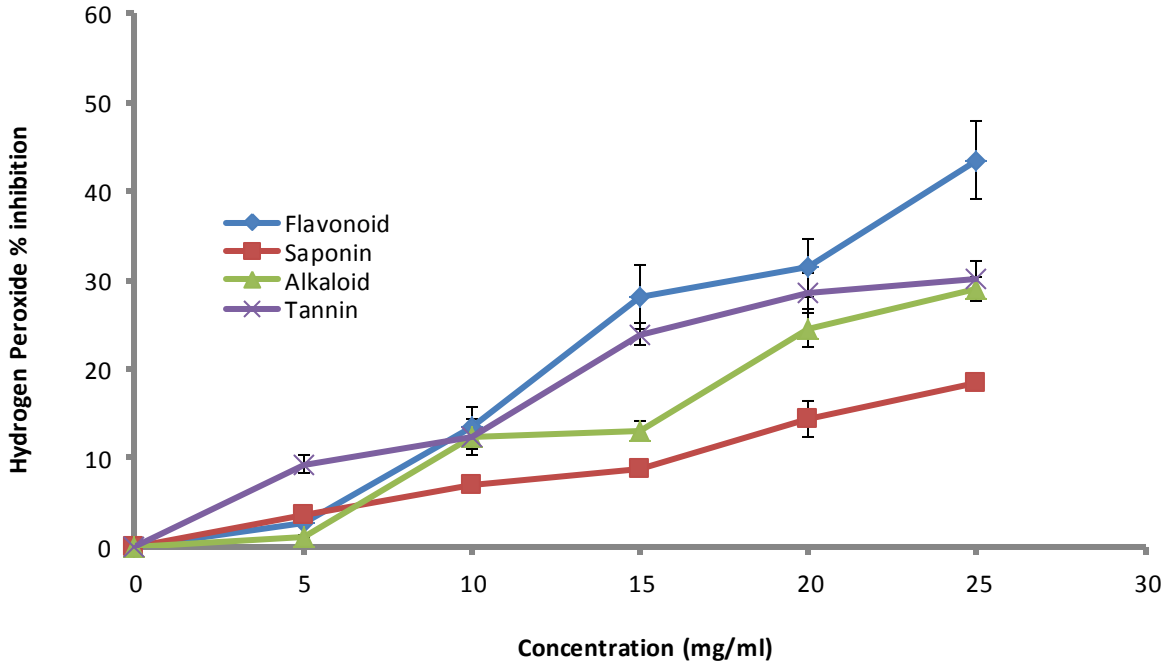


Figure 4.17: Hydrogen peroxide radical scavenging activity of flavonoid, saponin, alkaloid and tannin extracted from *C. dolichopentalum*

Figure 4.17 shows that flavonoid, saponin, alkaloid, and tannin scavenging abilities increased with increased extract concentration. However, flavonoid □ tannin □ alkaloid □ saponin, showed better scavenging activity, with an IC_{50} of 36.10 mg/ml, r^2=0.94; saponin had 126.25 mg/ml, r^2 = 0.97, while alkaloid had 61.18 mg/ml, r^2=0.94 and tannin had 55.56 mg/ml, r^2=0.97

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4.1.32 *In vivo* radical-scavenging potentials of ethanol extract *C. dolichopentalum* leaves

Figure 4.18 shows Effect of EECD on malondialdehyde (MDA) concentrations in CCl_4- induced oxidative stress.

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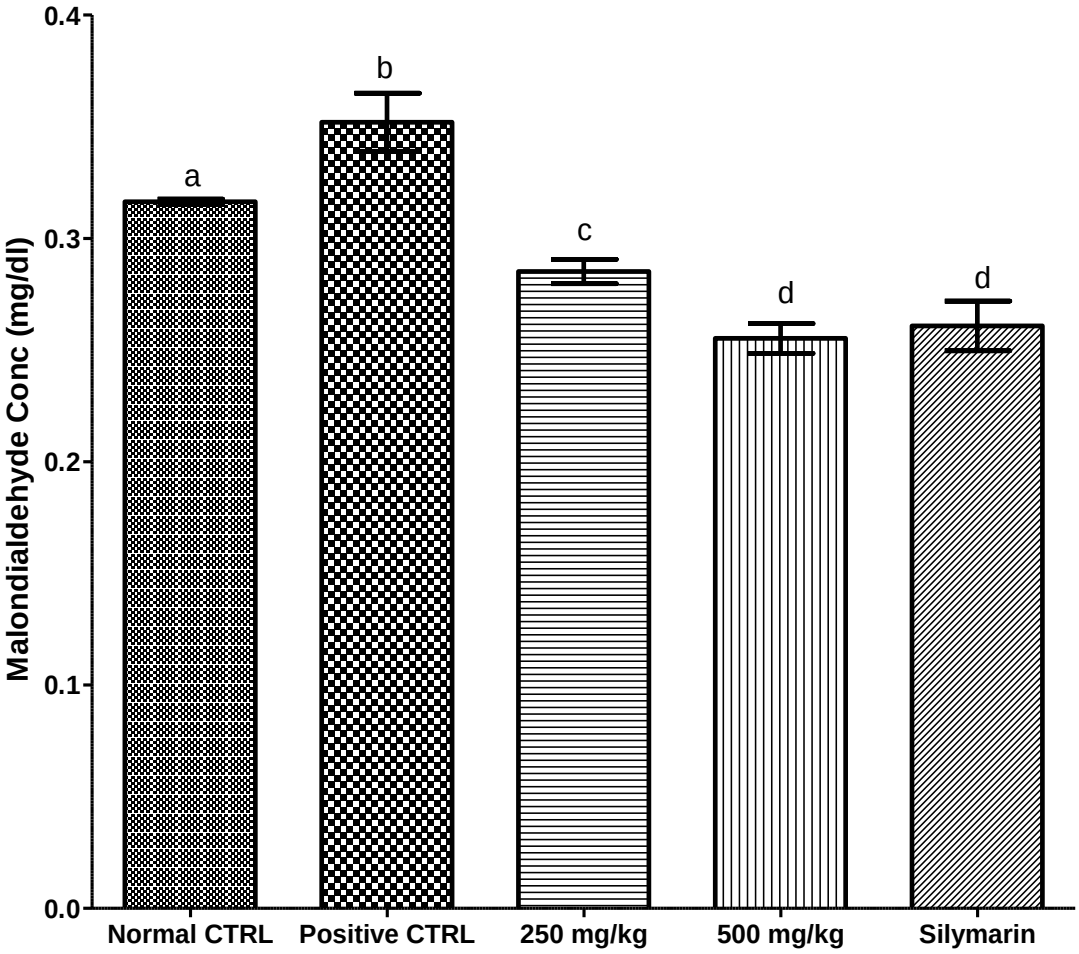


Figure 4.18: Effect of EECD on malondialdehyde (MDA) concentrations in carbon tetrachloride-induced oxidative stress. Bars bearing different letters are statistically significant ($p<0.05$)

Figure 4.18 shows a significant ($p \square 0.05$) increase in MDA concentrations in the positive control group compared to the normal and the groups treated with 250 mg/kg b.w and 500 mg/kg b.w EECD. However there was no significant difference ($p \square 0.05$) between the 500 mg/kg b.w and the Silymarin treated groups

4.1.33 *In vivo* radical-scavenging potentials of ethanol extract *C. dolichopentalum* leaves

Figure 4.19 shows the effect of EECD on malondialdehyde (MDA) concentration in CCI_4 -induced oxidative stress

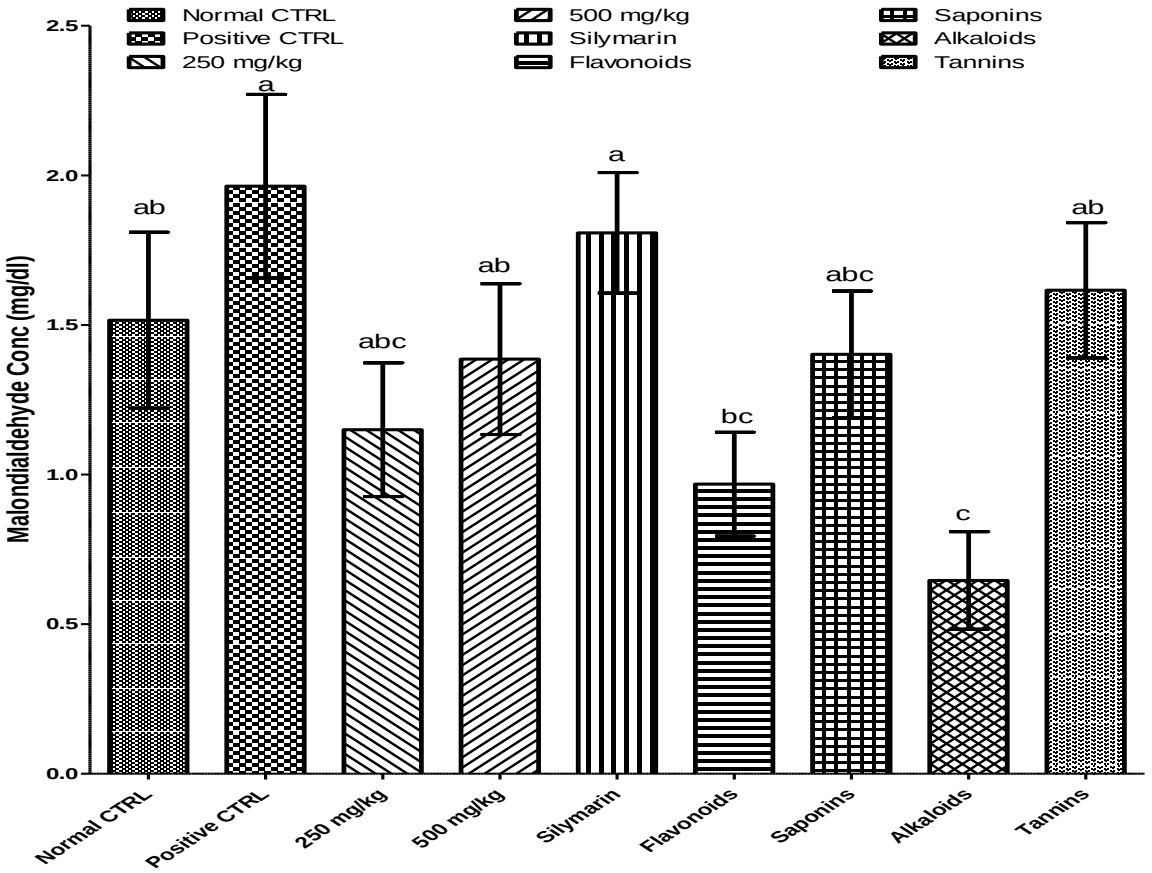


Figure 4.19: Effect of EECD on malondialdehyde (MDA) concentration in CCl_4 -induced oxidative stress. Bars bearing different letters are statistically significant ($p<0.05$).

The evidences in the result as shown in figure 4.19, there was a significant ($p \square 0.05$) increase in the positive control group compared to the normal. A significant ($P<0.05$) decrease in the flavonoid and alkaloid control were also observed. Nevertheless, there was no significant difference ($p \square 0.05$) among the normal, 500 mg/kg b.w EECD and tannin groups. There was also no significant difference ($p \square 0.05$) between the 250 mg/kg b.w EECD and the saponin treated groups.

4.1.34 *In vivo* radical-scavenging potentials of ethanol extract *C. dolichopentalum* leaves

Figure 4.2 shows the effect of EECD on glutathione (GSH) in carbon tetrachloride -induced oxidative stress.

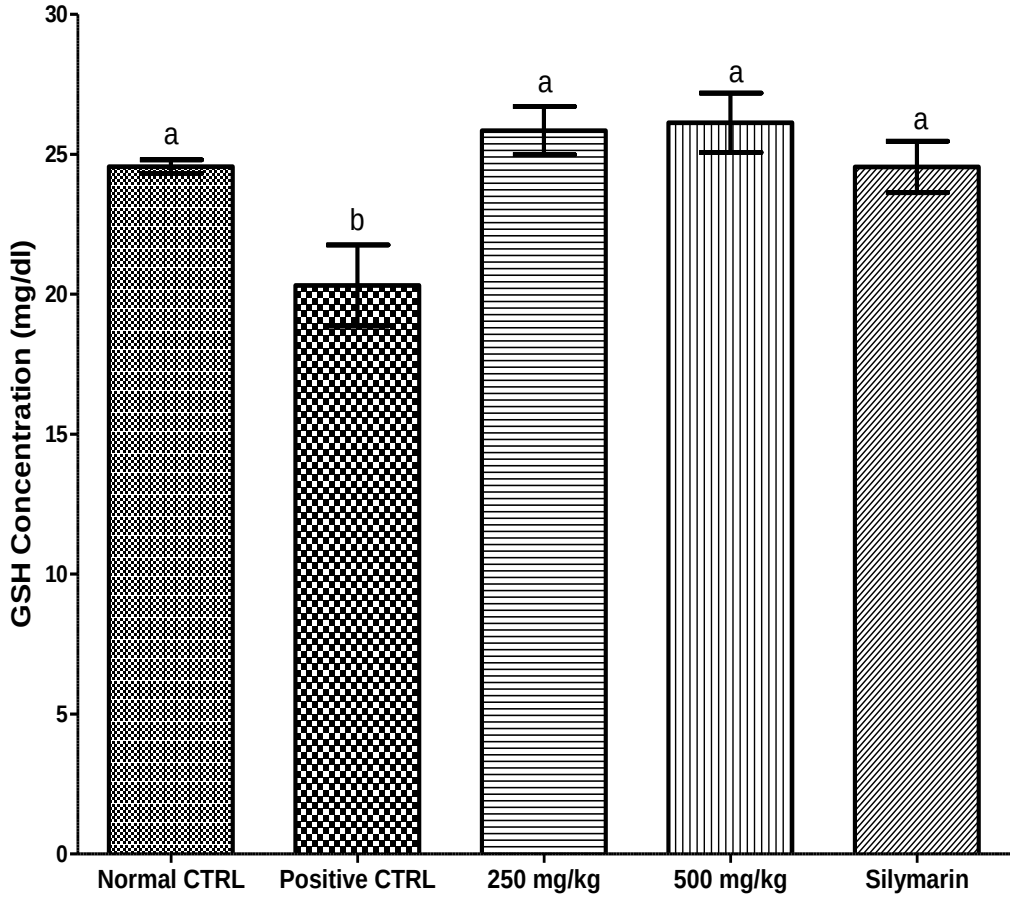


Figure 4.20: Effect of EECD on glutathione (GSH) in carbon tetrachloride-induced oxidative stress. Bars bearing different letters are statistically significant ($p<0.05$)

This Figure 4.20 shows that there was a significant ($p \square 0.05$) reduction in GSH concentration of the positive control compared to the normal, similarly it reveals that the treated groups showed a greater GSH concentration compared to the positive control. Nevertheless, there was no significant difference among the normal, 250 mg/kg, 500 mg/kg b.w EECD, and the silymarin treated groups

4.1.35 *In vivo* radical-scavenging potentials of ethanol extract *C. dolichopentalum* leaves

Figure 4.21 shows the effect of EECD on glutathione peroxidase (GPx) activity in carbon tetrachloride- induced.

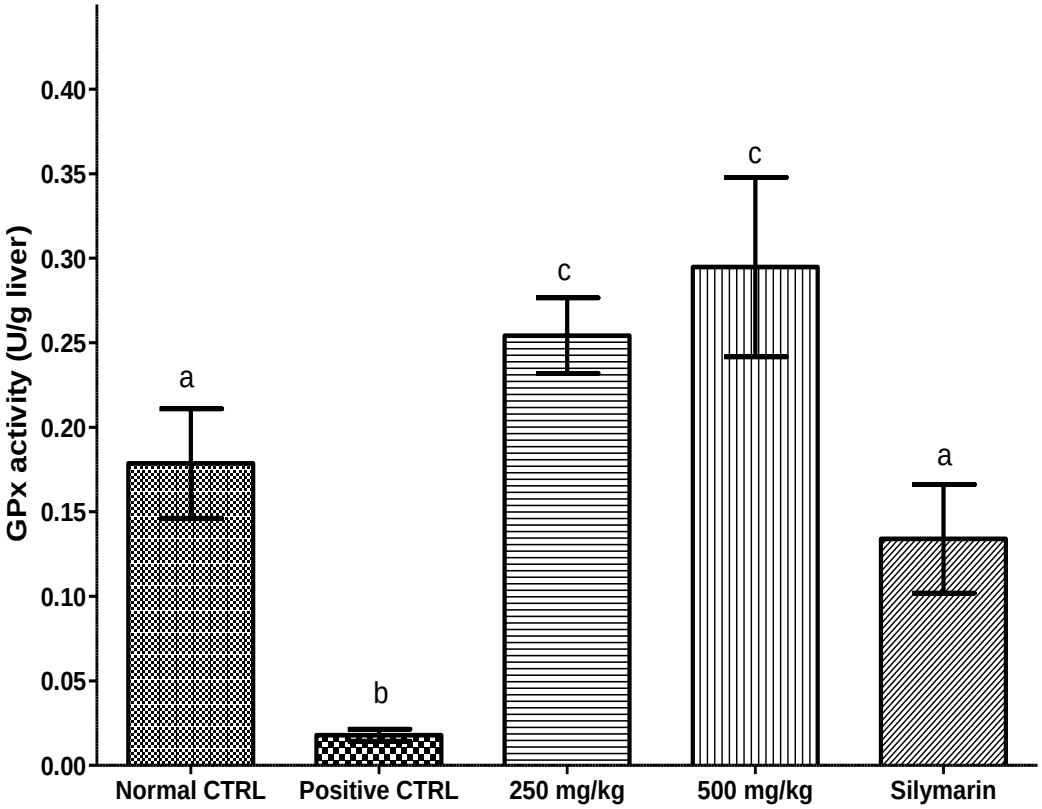


Figure 4.21: Effect of EECD on glutathione peroxidase (GPx) activity in carbon tetrachloride - induced oxidative stress. Bars bearing different letters are statistically significant ($p<0.05$)

The figure 4.21 shows that GPx activity of the positive control was reduced significantly (p □0.05P) when compared to the normal, EECD treated groups. However there was no significant difference (p □0.05) between the 250 mg/kg b.w and 500 mg/kg b.w of the extract.

4.1.36 *In vivo* radical-scavenging potentials of ethanol extract *C. dolichopentalum* leaves

Figure 4.22 shows the effect of EECD on glutathione peroxidase (GPx) activity of CCl_4 -induced oxidative stress.

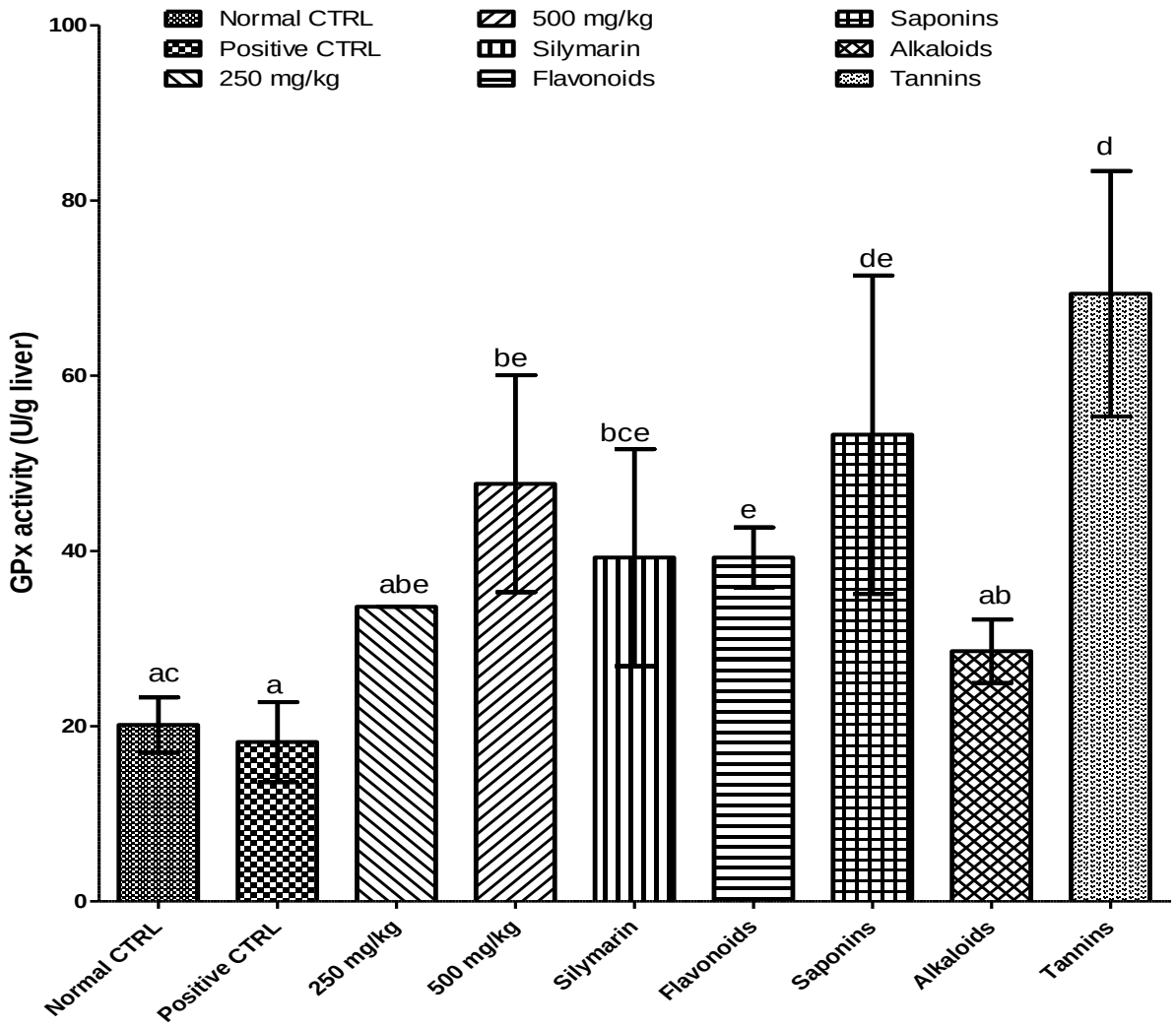


Figure 4.22: Effect of EECD on glutathione peroxidase (GPx) activity of CCl_4 -induced oxidative stress. Bars bearing different letters are statistically significant ($p<0.05$).

The figure 4.22 shows a significant ($P<0.05$) decrease in the positive control group compared to the normal and the groups treated with EECD (250 and 500 mg/kg b.w), Silymarin, flavonoids, saponins as well as tannins.

4.1.37 *In vivo* radical-scavenging potentials of ethanol extract *C. dolichopentalum* leaves

Figure 4.23 show the effect of EECD in superoxide dismutase (SOD) activity in carbon tetrachloride -induced oxidative stress.

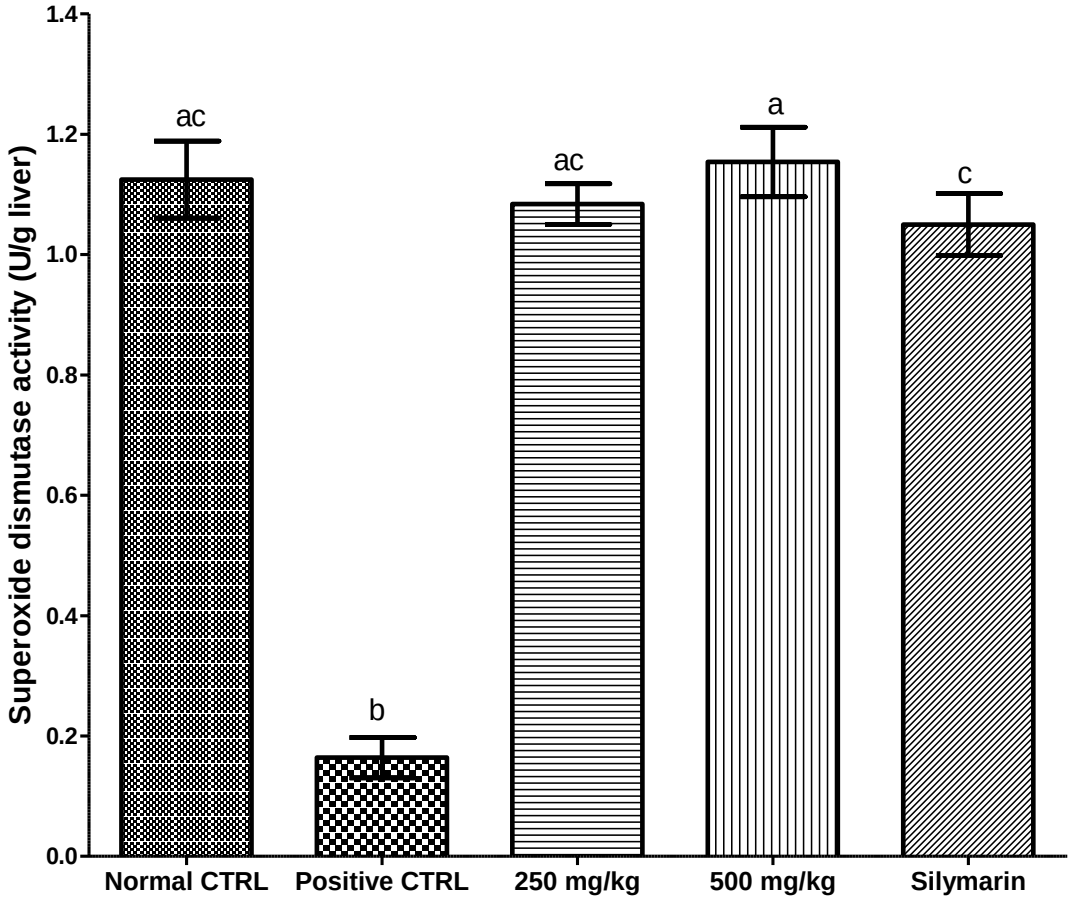


Figure 4.23: Effect of EECD in superoxide dismutase (SOD) activity in carbon tetrachloride - induced oxidative stress. Bars bearing different letters are statistically significant ($p<0.05$)

Figure 4.23 shows that in the positive control group, the activity of SOD reduced significantly ($P<0.05$) when compared to those of the normal control, 250 mg/kg and 500 mg/kg b.w EECD treated group. Also revealed was the non significant difference ($p \square 0.05$) between the normal and 250 mg/kg b.w EECD treated group.

4.1.38 *In vivo* radical-scavenging potentials of ethanol extract *C. dolichopentalum* leaves

Figure 4.24 shows the effect of EECD in superoxide dismutase (SOD) activitiy in CCl_4 -induced oxidative stress.

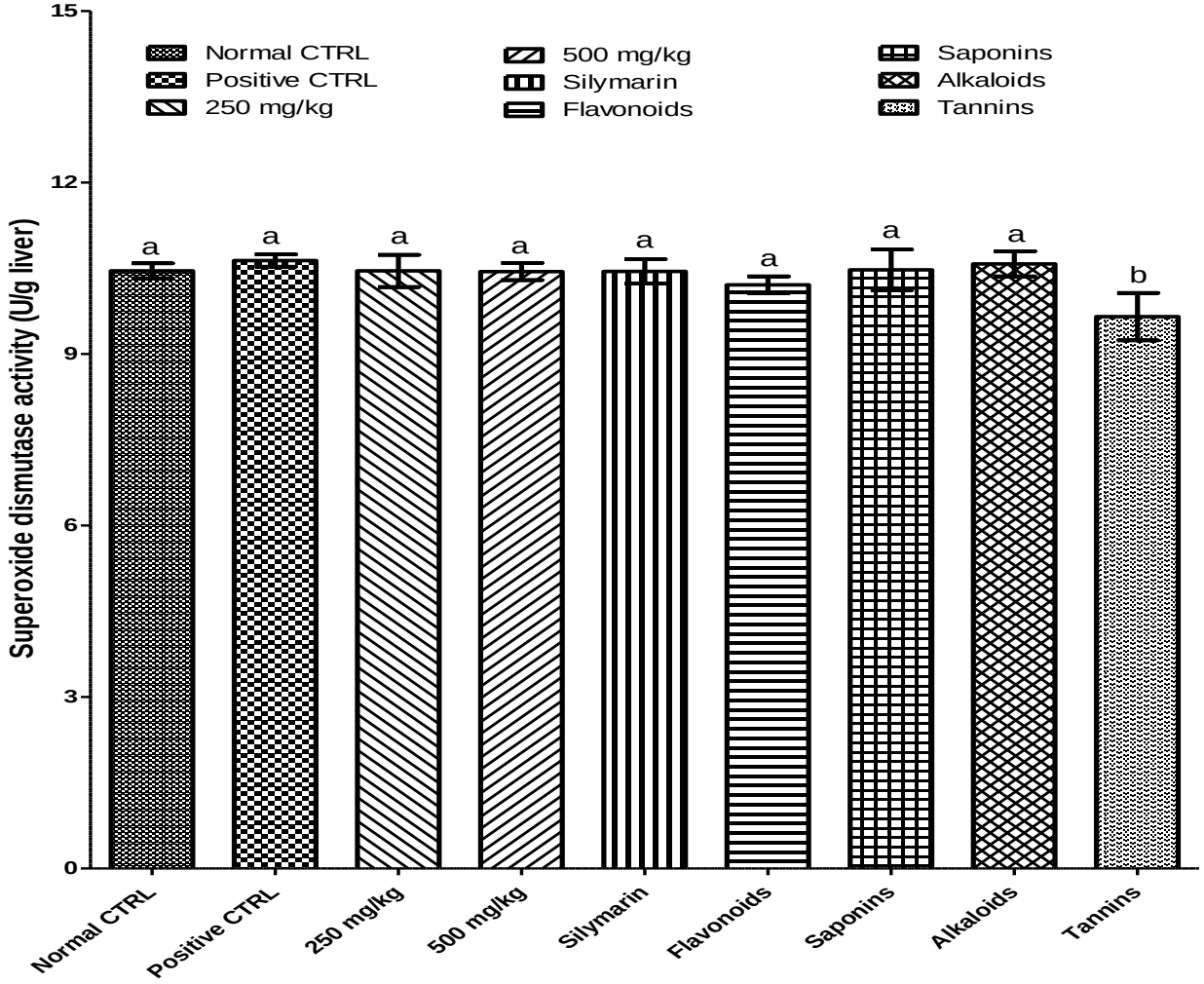


Figure 4.24: Effect of EECD in superoxide dismutase (SOD) activitiy in CCl_4 -induced oxidative stress. Bars bearing different letters are statistically significant ($p<0.05$)

This figure shows a significant ($P<0.05$) difference only in the Tannin control compared to the positive control. A non-significant difference among the positive control and the other groups such as the normal, silymarin, flavonoid, saponin, alkaloid, EECD at 250, and 500 mg/kg b.w were also observed.

4.1.39 *In vivo* radical-scavenging potentials of ethanol extract *C. dolichopentalum* leaves

Figure 4.25 shows the effect of EECD on catalase activity in carbon tetrachloride intoxicated rats.

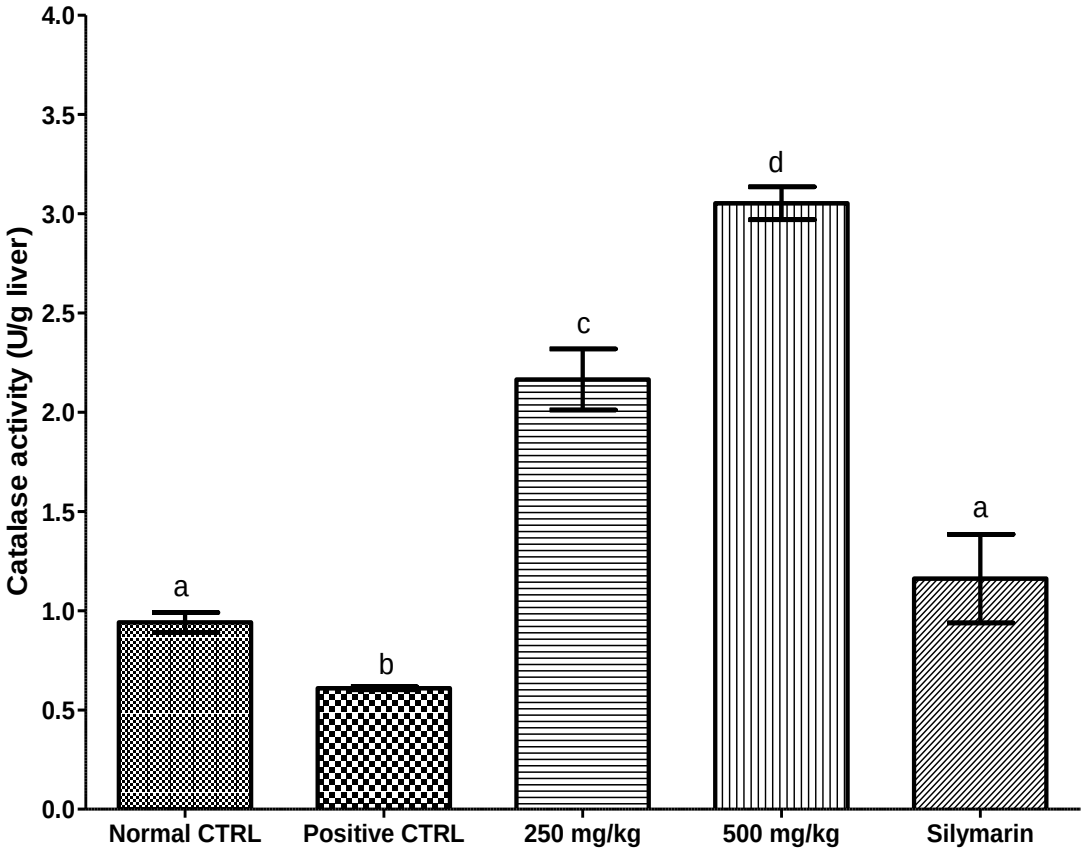


Figure 4.25: Effect of EECD on catalase activity in carbon tetrachloride intoxicated rats. Bars bearing different letters are statistically significant ($p<0.05$)

This Figure (4.25) shows that catalase activity in the positive control animals was reduced significantly ($p \square 0.05$) when compared to normal control, silymarin, 250 mg/kg and 500 mg/kg b. w EECD treated groups. There was no significant difference ($p \square 0.05$) between the normal and the silymarin treated groups.

4.1.40 *In vivo* radical-scavenging potentials of ethanol extract *C. dolichopentalum* leaves

Figure 4.26 shows the effect of EECD in catalase activity in CCl_4 -induced oxidative stress.

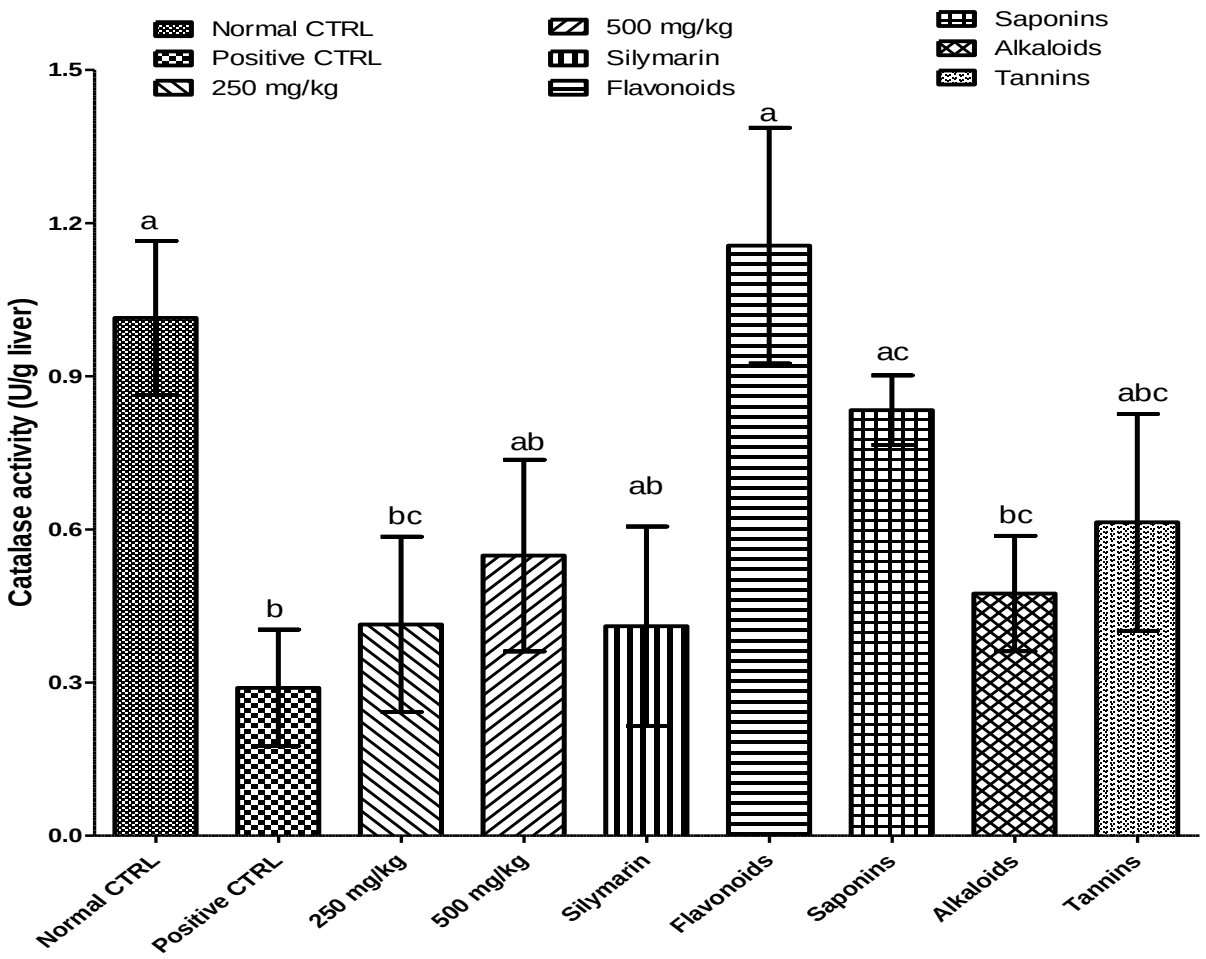


Figure 4.26: Effect of EECD in catalase activity in CCl_4 induced -oxidative stress. Bars bearing different letters are statistically significant ($p<0.05$).

Figure 4.26 shows a significant ($P<0.05$) decrease in catalase activity of the positive control group compared to the normal, silymarin, flavonoid, saponin, alkaloid, and tannin control groups, as well as the 250 mg/kg, and 500 mg/kg b.w EECD treated groups. However there was no significant difference ($p\square0.05$) between the EECD treated group at 250 mg/kg and the alkaloid control, the 500 mg/kg b.w EECD treated and silymarin groups as well as the normal and the flavonoid control groups.

4.1.41 Antioxidant studies of ethanol extract of *C. dolichopentalum* leaves

Figure 4.27 shows the effect of EECD on vitamin C concentration in carbon tetrachloride- induced hepatotoxicity.

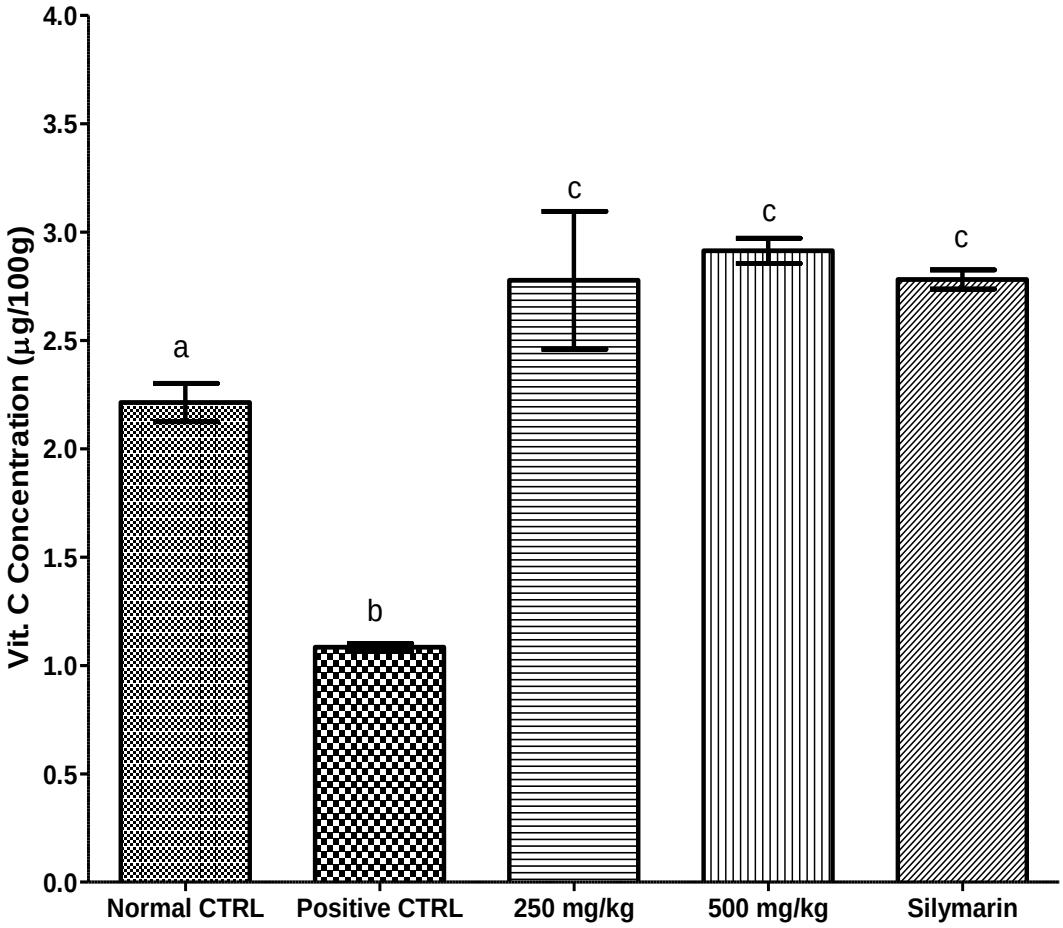


Figure 4.27: Effect of EECD on vitamin C concentration in carbon tetrachloride- induced hepatotoxicity. Bars bearing different letters are statistically significant ($p<0.05$)

Figure 4.27 indicates that vitamin C concentration of the positive control was reduced significantly ($p\square 0.05$) when compared to the normal, silymarin, 250 mg/kg and 500 mg/kg b.w EECD treated group. Also observed was the non significant difference ($p\square 0.05$) among the silymarin, the 250 and 500 mg/kg b.w EECD treated groups.

4.1.42 Antioxidant studies of ethanol extract of *C. dolichopentalum* leaves

Figure 4.28 shows the effect of EECD in vitamin E concentration in carbon tetrachloride- induced hepatotoxicity

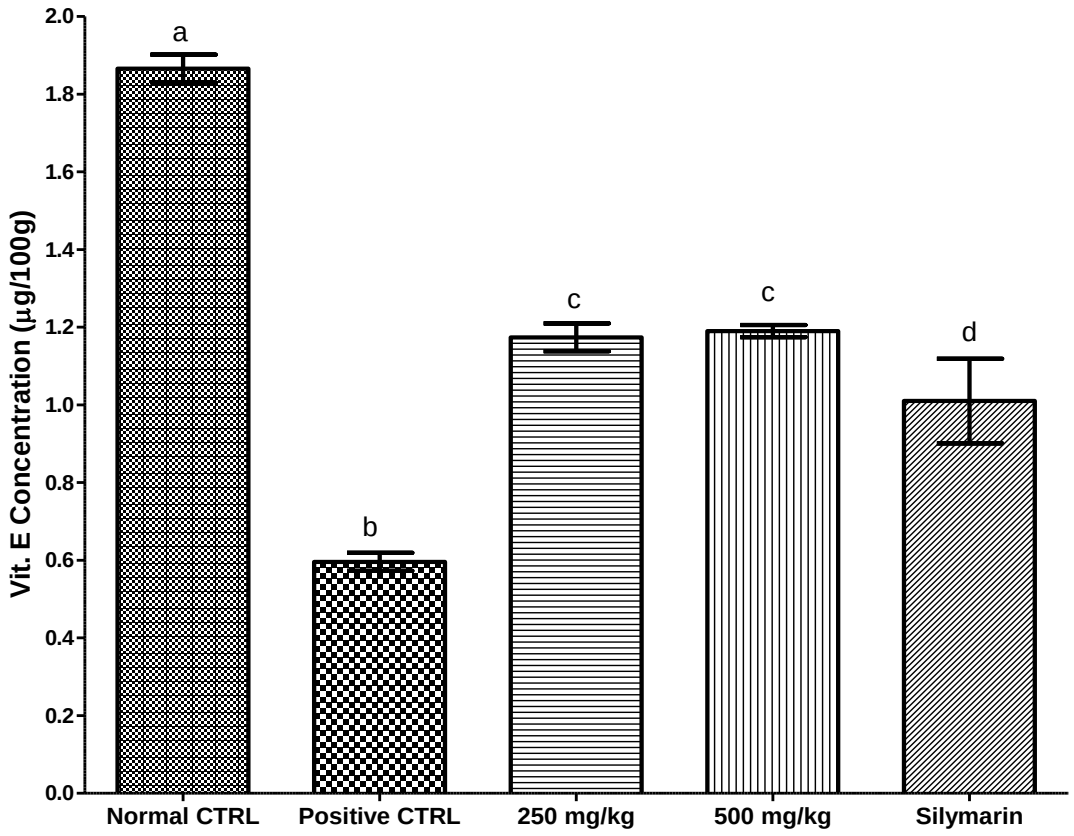


Figure 4.28: Effect of EECD in Vitamin E concentration in carbon tetrachloride- induced hepatotoxicity. Bars bearing different letters are statistically significant ($p<0.05$)

Figure 4.28 show that vitamin E concentration of the positive was significantly ($p \square 0.05$) reduced when compared to the normal control, 250 mg/kg and 500 mg/kg b.w EECD treated rats. Observation of the non significant difference ($p \square 0.05$) between the 250 and 500 mg/kg b.w EECD treated groups was also made.

4.1.44 Effect of EECD on some liver function parameters

Figure 4.29 shows the effects of EECD on serum alanine amino transferase (ALT) activity in carbon tetrachloride -induced hepatotoxicity.

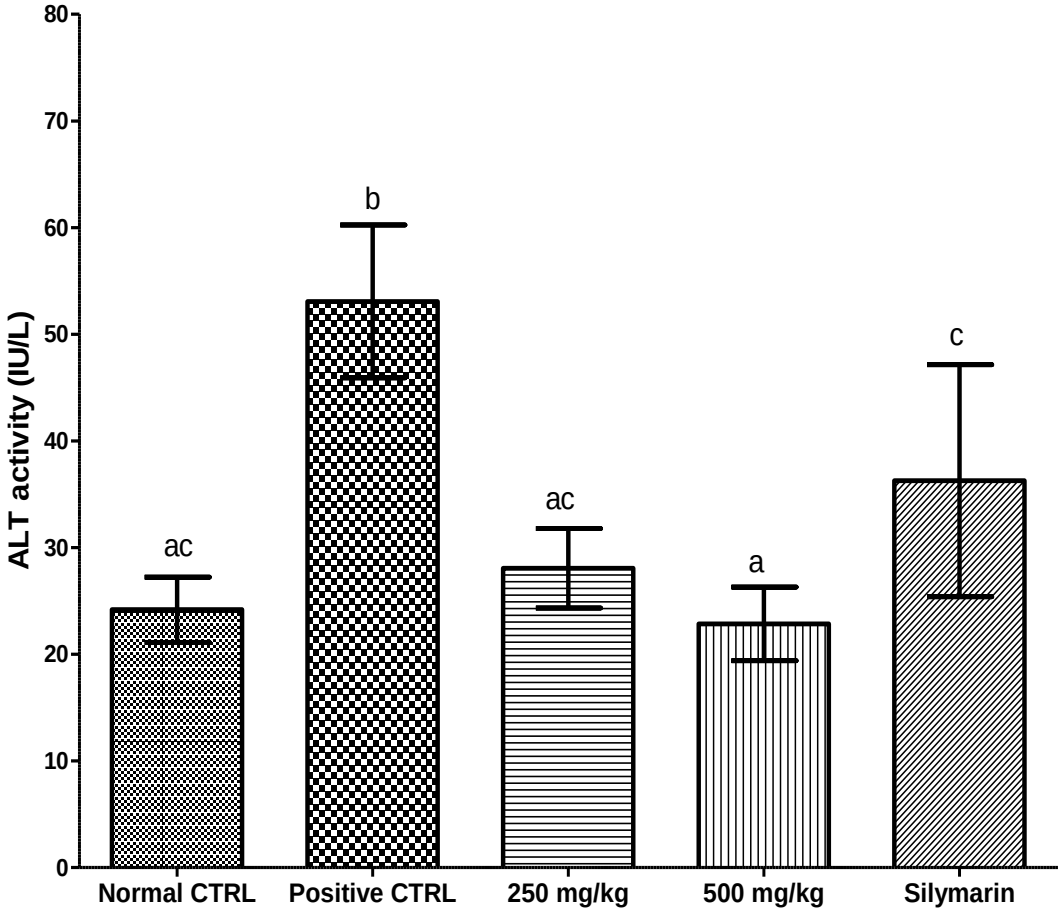


Figure 4.29: Effects of ethanol extracts of C. *dolichopentalum* on serum alanine amino transferase (ALT) activity in carbon tetrachloride- induced hepatotoxicity. Bars bearing different letters are statistically significant ($p<0.05$)

Figure 4.29 shows that ALT activity in the positive control were elevated significantly ($p \square 0.05$) when compared to normal control, EECD treated group at 250 mg/kg b.w. and 500 mg/kg b. w as well as the silymarin groups. A non significant difference ($p \square 0.05$) between the normal and 250 mg/kg b.w EECD treated groups was also observed.

4.1.45 Effect of EECD on some liver function parameters

Figure 4.30 shows the effect of EECD on alanine aminotransferase (ALT) activity in CCl_4 -induced hepatotoxicity.

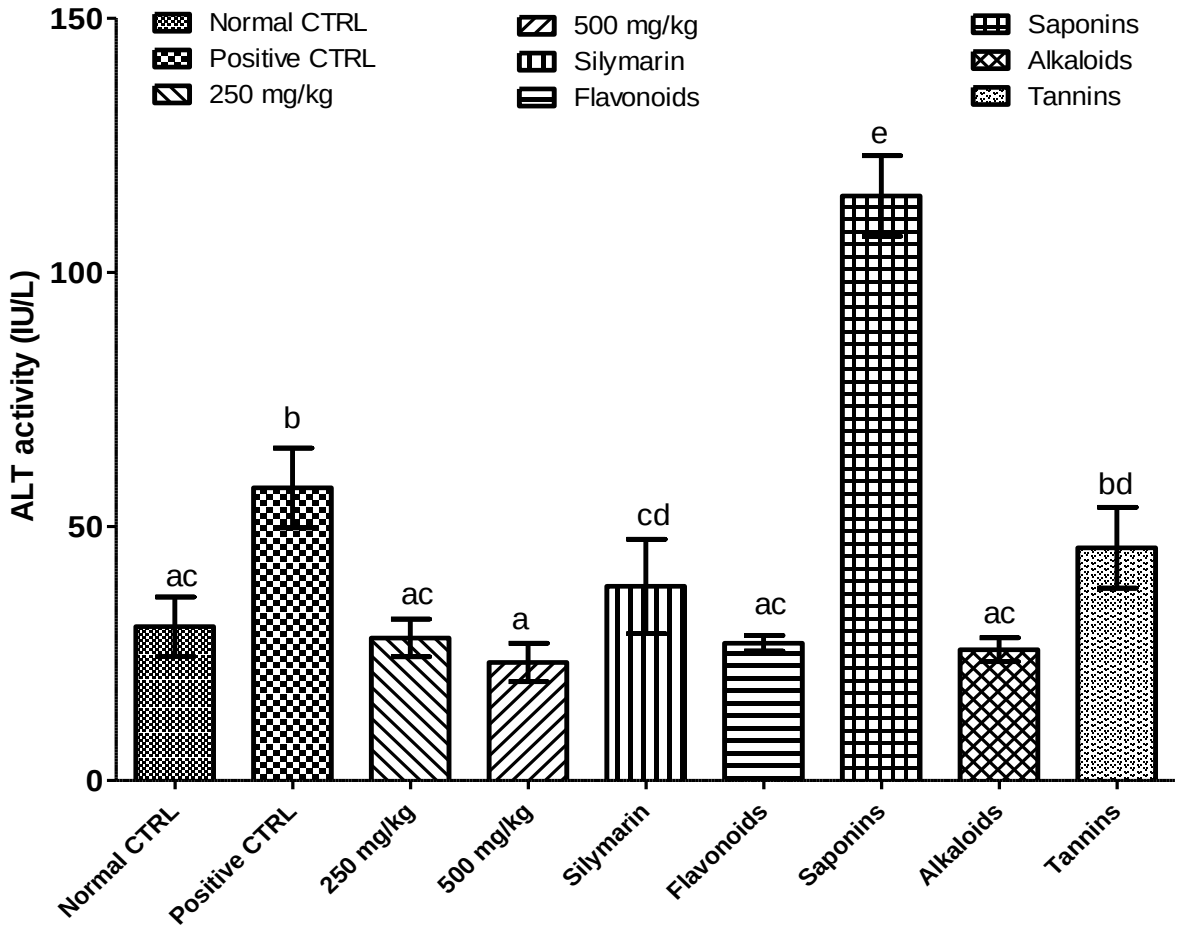


Figure 4.30: Effect of EECD on alanine aminotransferase (ALT) activity in CCl_4 - induced hepatotoxicity. Bars bearing different letters are statistically significant ($p<0.05$)

Fiugure 4.30 shows a significant ($P<0.05$) increase in the ALT activity of the positive control group compared to the normal, and all the treated groups. However there was no significant difference ($p \square 0.05$) among the normal, EECD treated group at 250 mg/kg b.w as well as flavonoid and alkaloid treated groups. We also observed a significant increase in the saponin control compared to the positive control groups.

4.1.46 Effect of EECD on some liver function parameters

Figure 4.31shows effect of EECD on serum aspartate amino transferase (AST) activity in carbon tetrachloride- induced hepatotoxicity.

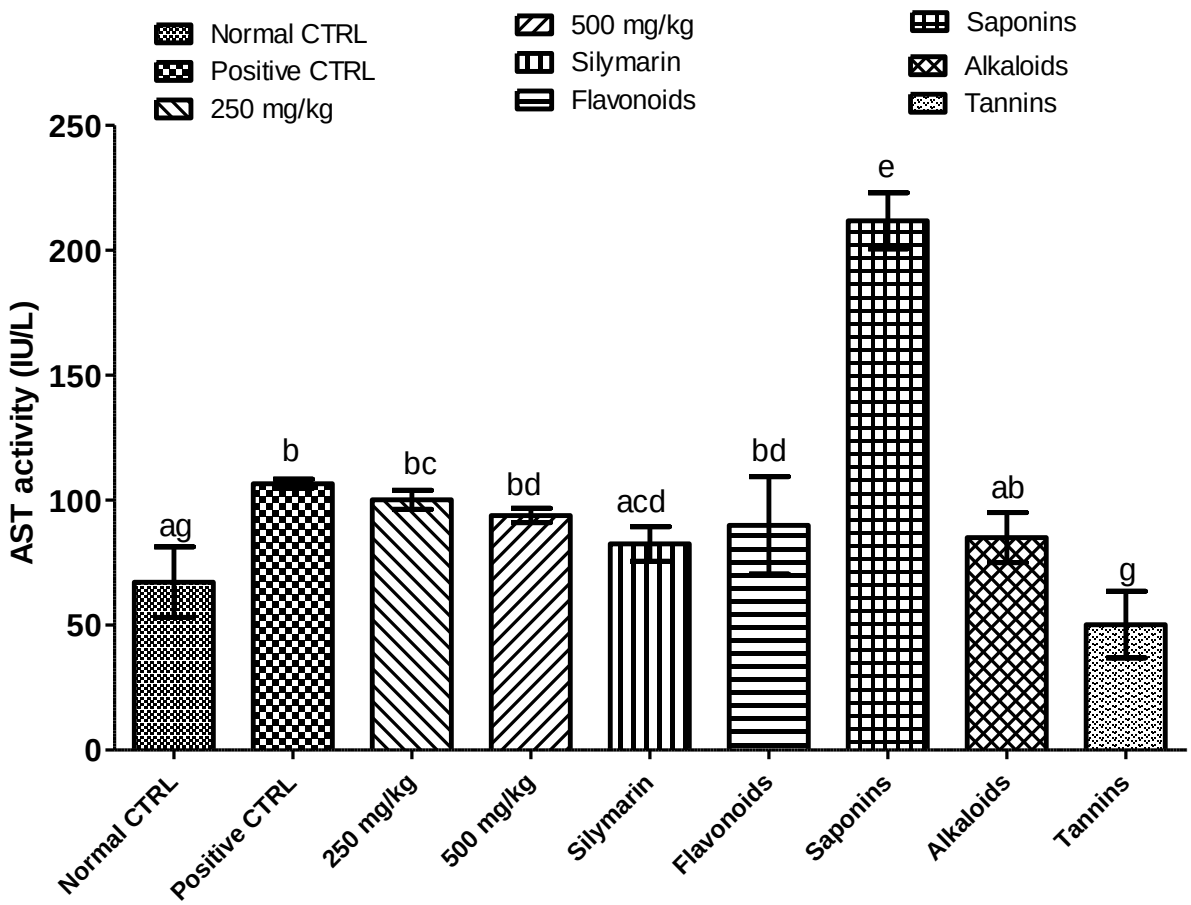


Figure 4.31: Effect of EECD on serum aspartate amino transferase (AST) activity in carbon tetrachloride- induced hepatotoxicity. Bars bearing different letters are statistically significant ($p<0.05$)

Figure 4.31 shows that AST activities in the positive control were elevated significantly ($p \square 0.05$) when compared to the normal control, silymarin, and the EECD treated group at 250 mg/kg b.w 500 mg/kg b. w. However there was no significant difference ($p \square 0.05$) between the normal and the 500 mg/kg b.w EECD treated groups.

4.1.47 Effect of EECD on some liver function parameters

Figure 4.32 shows the effect of EECD on serum aspartate aminotransferase (AST) activity in CCl_4-induced hepatotoxicity.

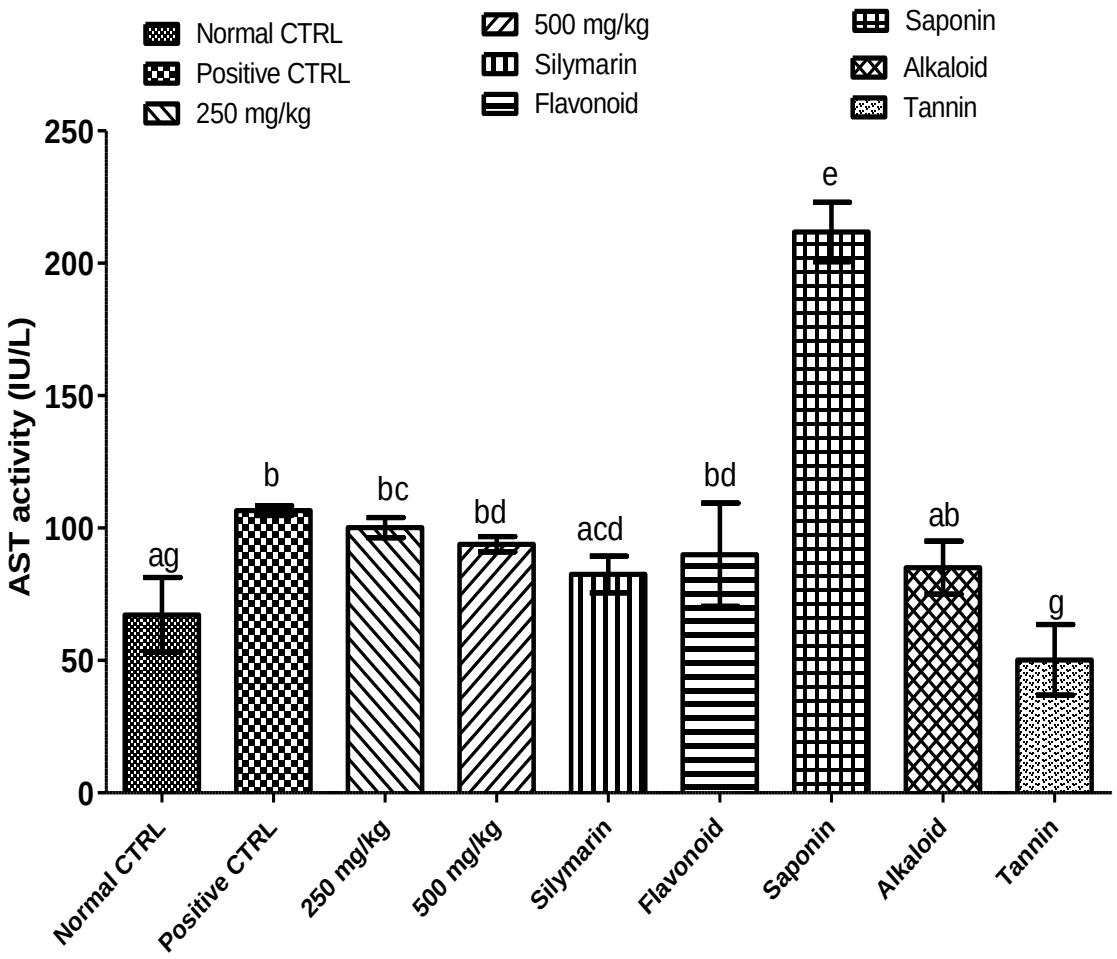


Figure 4.32: Effect of EECD on serum aspartate aminotransferase (AST) activity in CCl_4 -induced hepatotoxicity. Bars bearing different letters are statistically significant ($p<0.05$)

This result as shown in figure 4.32 revealed a significant ($P<0.05$) increase in the intoxicated group compared to the normal and other intoxicated but treated groups. Also revealed was the non significant difference ($p \square 0.05$) between the 500 mg/kg b.w EECD and the flavonoid group.

4.1.48 Effect of EECD on some liver function parameters

Figure 4.33 shows the effect of EECD on total protein concentration in carbon tetrachloride-induced hepatotoxicity.

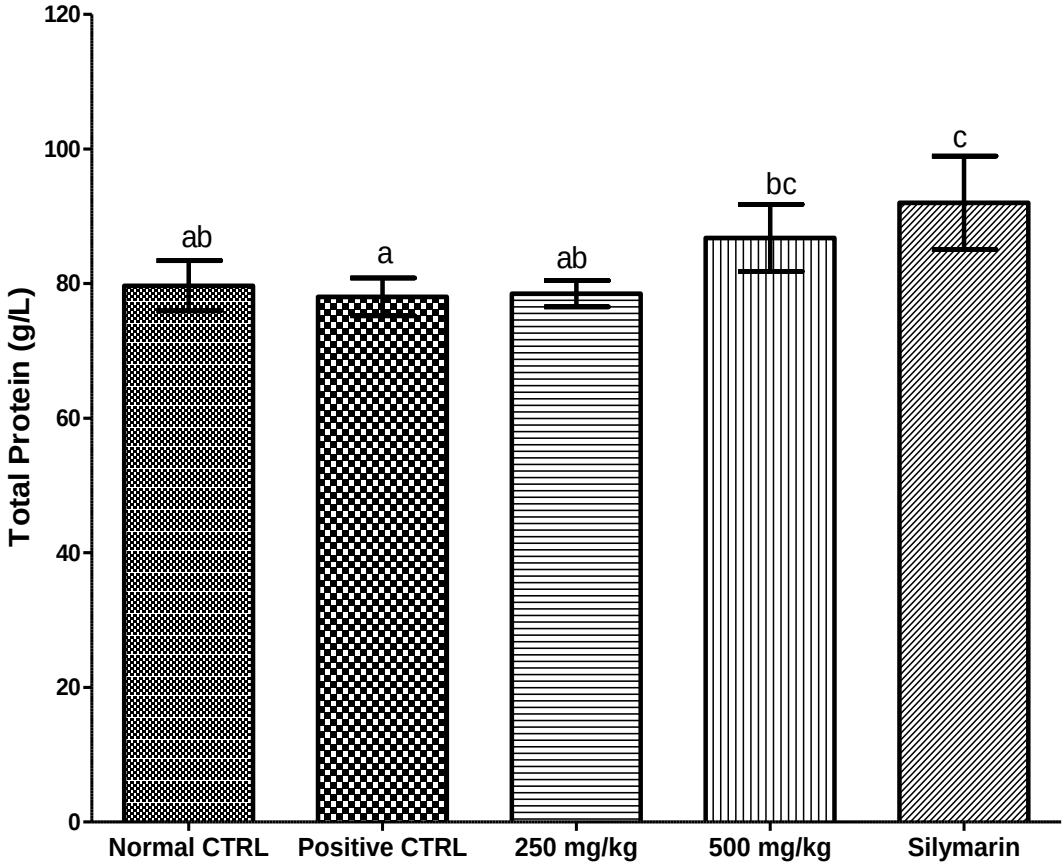


Figure 4.33: Effect of EECD on total protein concentration in carbon tetrachloride-induced hepatotoxicity. Bars bearing different letters are statistically significant ($p<0.05$)

This result as shown in Figure 4.33 shows that total protein concentration of the positive control reduced non significantly ($p \square 0.05$) when compared to the normal control and 250 mg/kg b. w treated group. There was also no significant difference ($p \square 0.05$) between the normal and the 250 mg/kg b.w EECD treated groups. Treatment with EECD at 500 mg/kg b.w and the silymarin showed significantly ($p \square 0.05$) elevated total protein concentrations.

4.1.49 Effect of EECD on some liver function parameters

Figure 4.34 shows the effect of EECD on serum total protein in CCl_4 -induced hepatotoxicity.

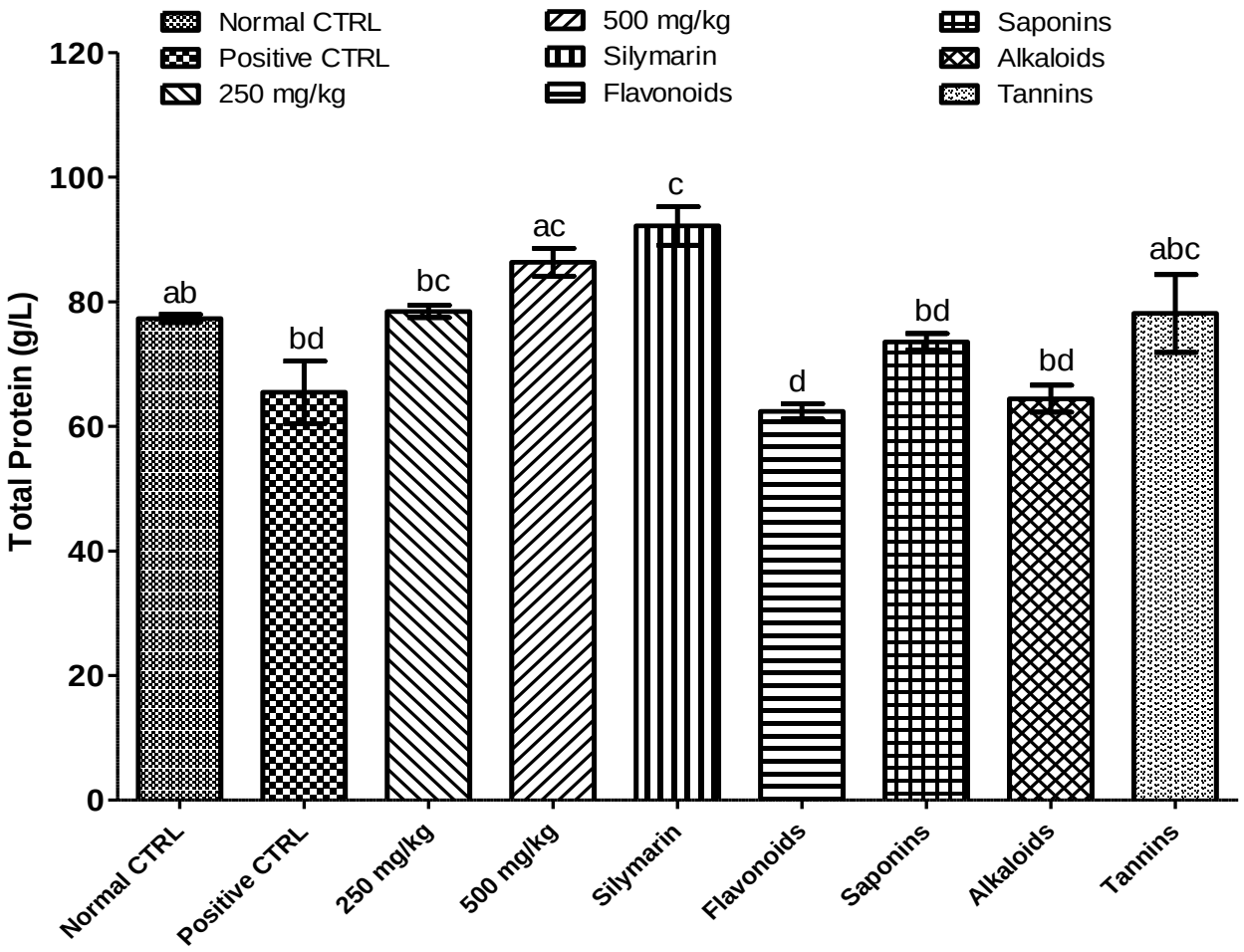


Figure 4.34: Effect of EECD on serum total protein in CCl_4 -induced hepatotoxicity. Bars bearing different letters are statistically significant ($p<0.05$).

Figure 4.34 shows a significant ($P<0.05$) decrease in serum total protein concentration of the positive control compared to the normal and other treated groups, except the alkaloid control. However there was no significant difference ($p \square 0.05$) among the positive control, saponin and alkaloid control groups.

4.1.50 Effect of EECD on some liver function parameters

Figure 4.35 shows the effect of EECD on serum albumin concentration in CCl_4 –induced hepatotoxicity.

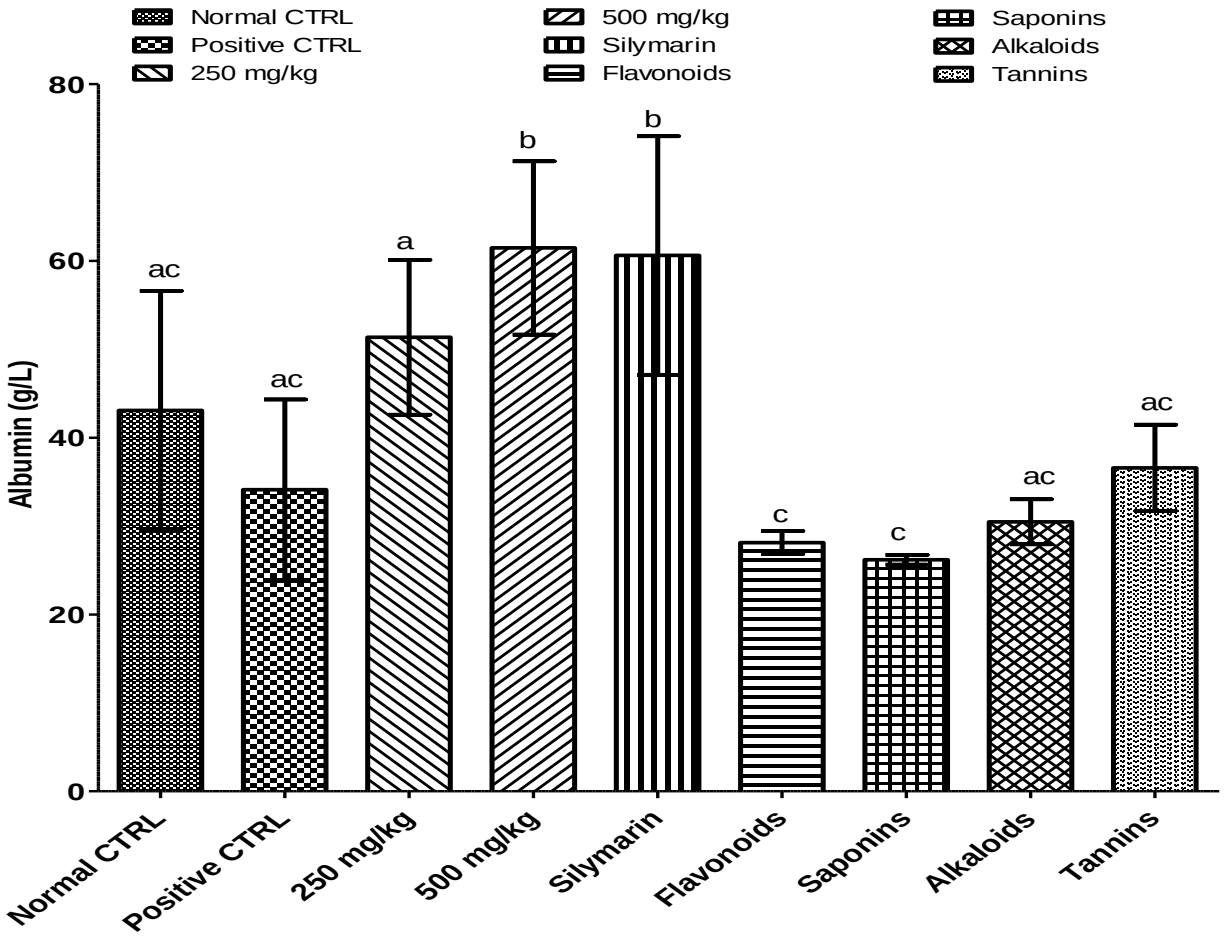


Figure 4.35: Effect of EECD on serum albumin concentration in CCl_4 –induced hepatotoxicity. Bars bearing different letters are statistically significant ($p<0.05$)

Figure 4.35 shows a non-significant ($p\square0.05$) drop in albumin concentration in the positive control group compared to the normal, the alkaloid and tannin groups. There was no significant difference ($p\square0.05$) between the 500 mg/kg b.w EECD and the silymarin groups, there was also no significant difference ($p\square0.05$) between the flavonoid and saponin groups, as well as between the alkaloid and tannin control. However a significant ($P<0.05$) difference was observed in the positive control compared to the silymarin, the 250 and the 500 mg/kg b.w EECD treated groups.

4.1.51 Effect of EECD on some liver function parameters

Figure 4.36 shows effect of EECD on serum globulin concentration in CCl_4 –induced hepatotoxicity.

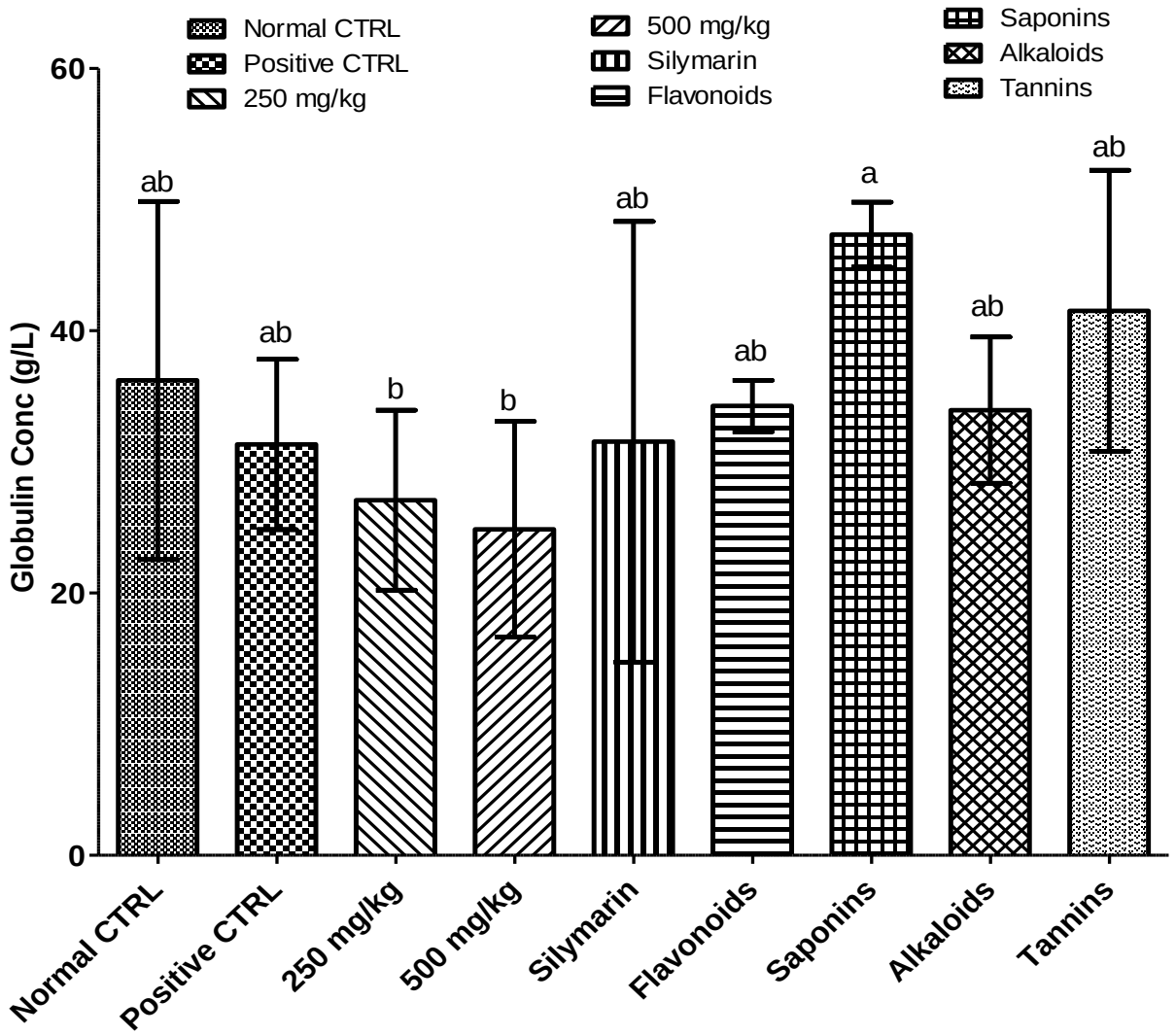


Figure 4.36: Effect of EECD on serum globulin concentration in CCl_4 –induced hepatotoxicity. Bars bearing different letters are statistically significant ($p<0.05$).

Figure 4.36 shows a non-significant ($p\square 0.05$) reduction in globulin concentration in the positive control group compared to the normal, silymarin, flavonoid, alkaloid and tannin groups. There was also no significant difference ($p\square 0.05$) between the alkaloid and tannin control, the silymarin and flavonoid control as well as between the 250 and 500 mg/kg b.w EECD treated groups. However a significant ($P<0.05$) difference was observed in the EECD treated group and saponin control.

4.1.52 Effect of EECD on some liver function parameters

Figure 4.37 shows the effect of EECD on albumin globulin ratio in carbon tetrachloride - induced hepatotoxicity.

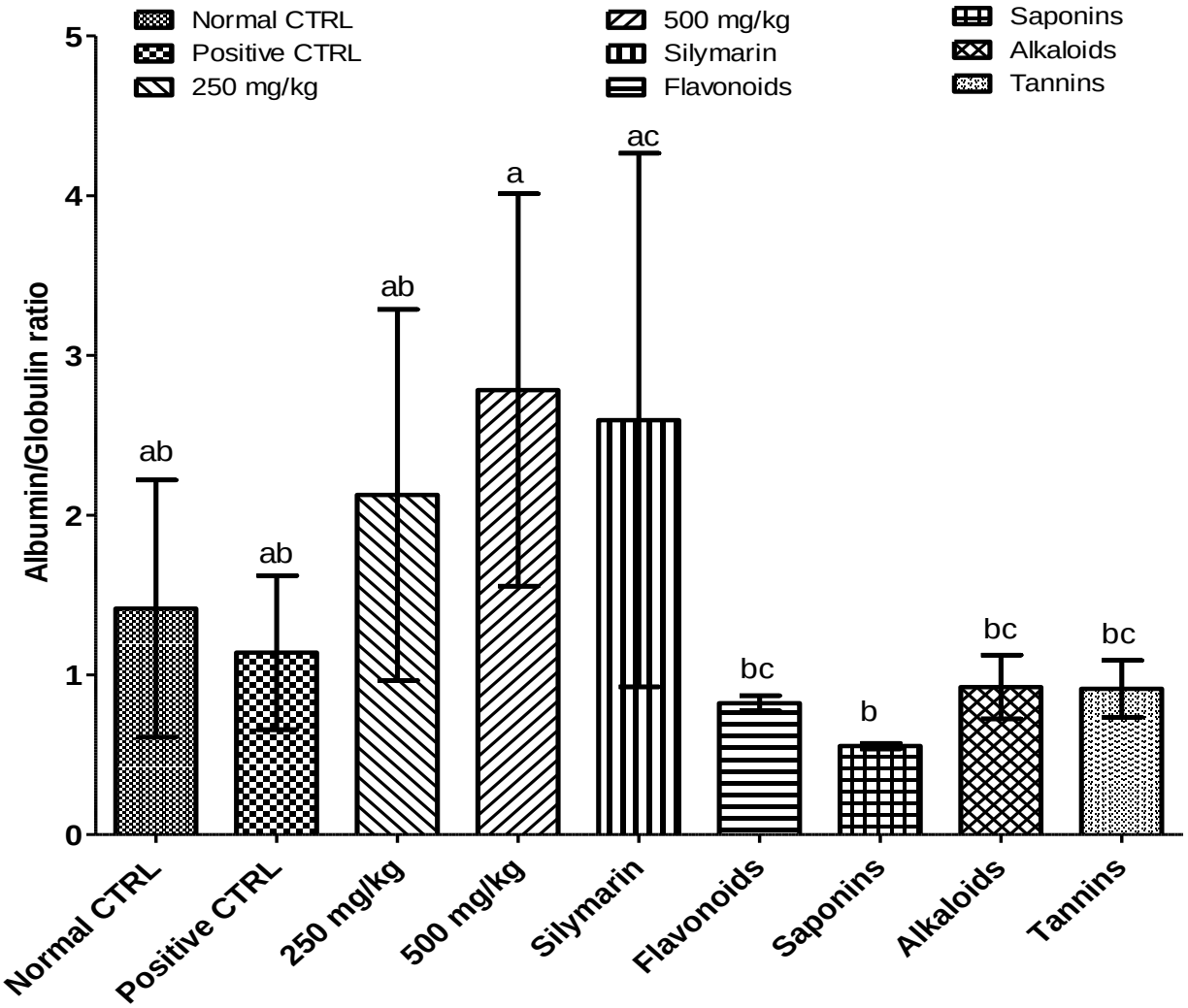


Figure 4.37: Effect of EECD on albumin globulin ratio in carbon tetrachloride- induced hepatotoxicity. Bars bearing different letters are statistically significant ($p<0.05$).

Figure 4.37 shows a non-significant ($p \square 0.05$) decrease in A/G ratio in the positive control group compared to the normal and the 250 mg/kg b.w EECD. However, a significant ($P<0.05$) difference was observed with 500 mg/kg EECD treated group, flavonoid, saponin control, alkaloid and tannin. There was no significant difference ($p \square 0.05$) among the flavonoid, alkaloid and the tannin control.

4.1.53 Effect of EECD on some liver function parameters

Figure 4.38 shows the effect of EECD on serum total bilirubin in CCl_4 -induced hepatotoxicity.

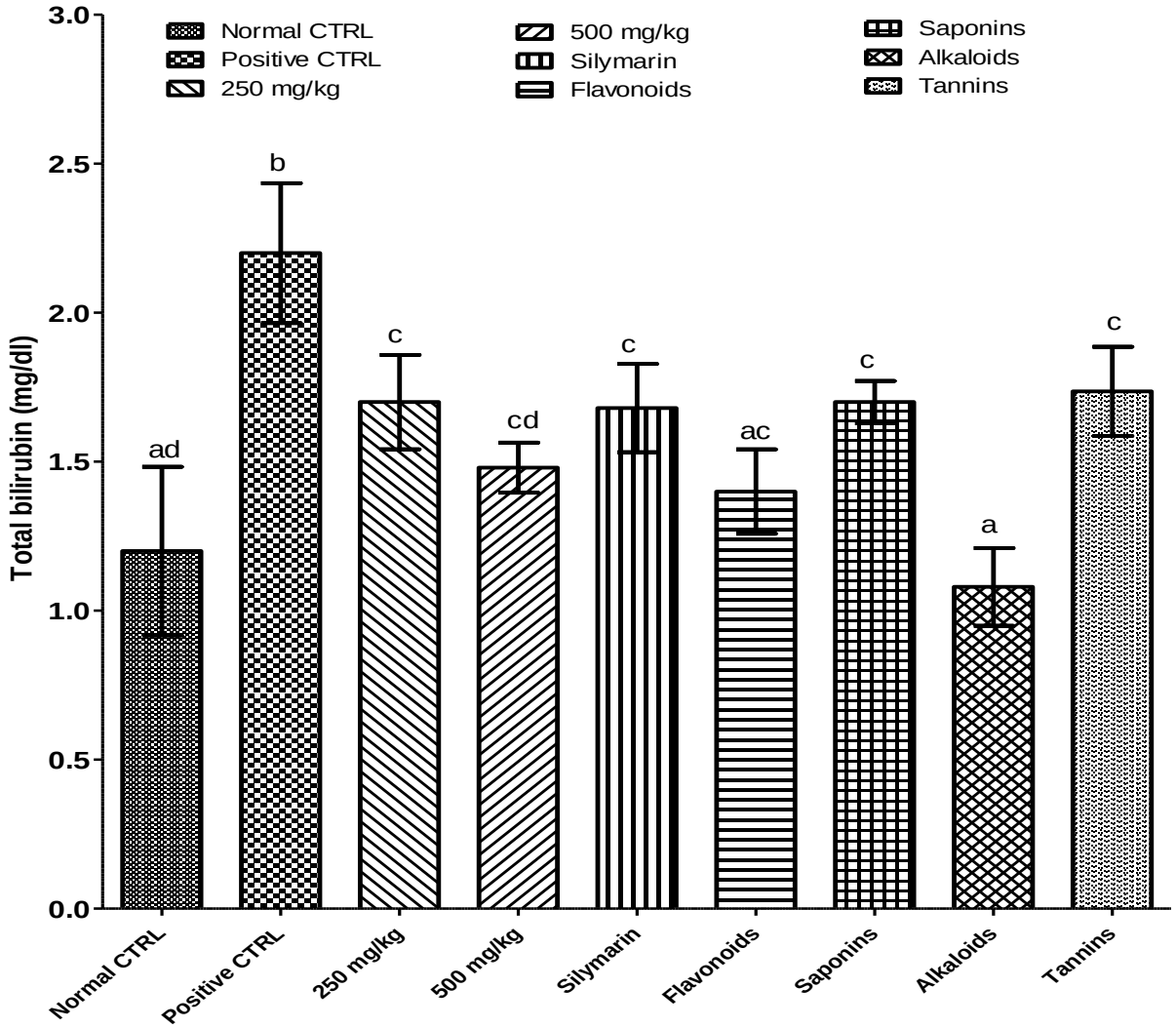


Figure 4.38: Effect of EECD on serum total bilirubin in CCl_4 -induced hepatotoxicity. Bars bearing different letters are statistically significant ($p<0.05$)

Figure 4.38 shows significant ($P<0.05$) increases in total bilirubin concentration of the positive control compared to the normal and other intoxicated but treated groups. However, there was no significant difference ($p \square 0.05$) among the 250 mg/kg b.w EECD, silymarin, saponin, and the tannin control.

4.1.54 Effect of EECD on serum lipids

Figure 4.39 shows the effect of EECD on serum triacylglyceride concentration in carbon tetrachloride- induced hepatotoxicity.

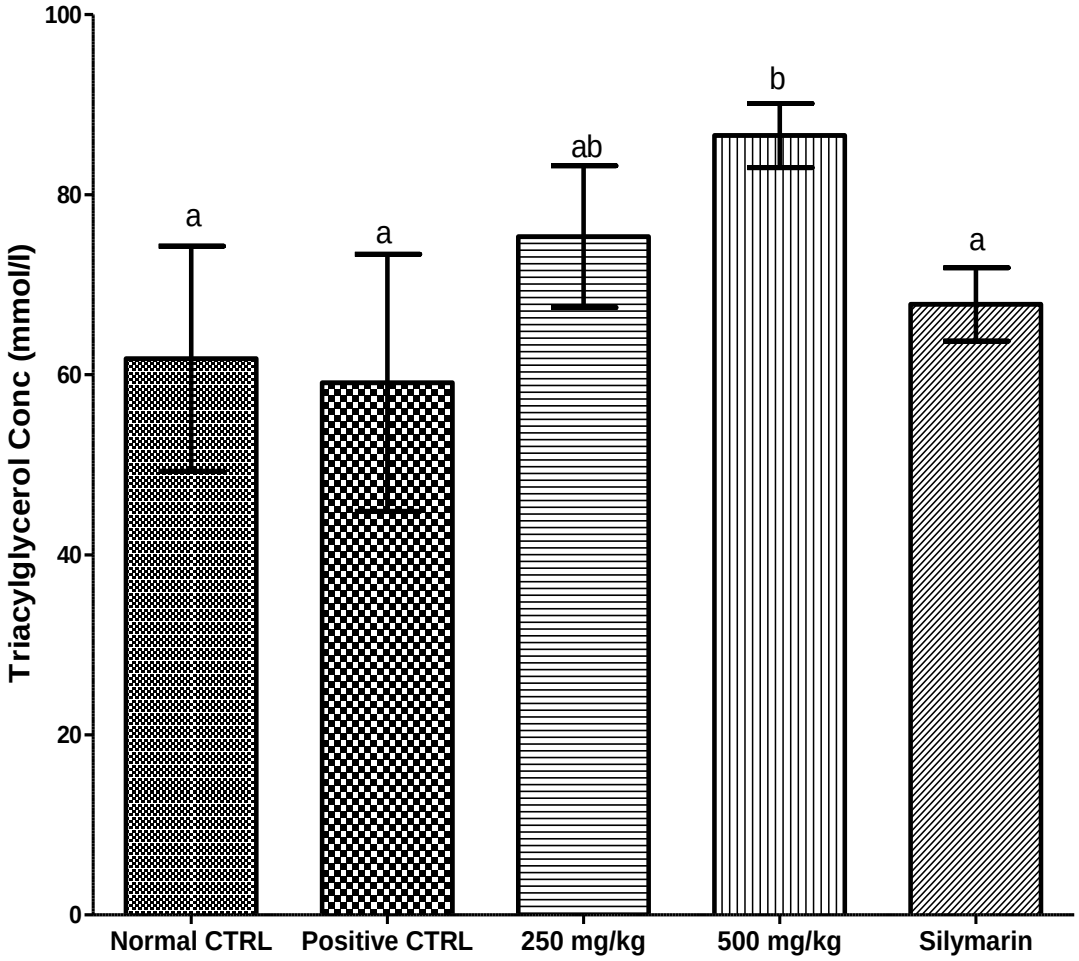


Figure 4.39: Effect of EECD on serum triacylglyceride concentration in carbon tetrachloride- induced hepatotoxicity. Bars bearing different letters are statistically significant ($p<0.05$)

Figure 4.39 shows a non-significant ($p \square 0.05$) reduction in triacylglyceride concentration of the positive control compared to the normal control and the silymarin treated group. Nevertheless, there was a significant ($p \square 0.05$) difference between the 250 and 500 mg/kg b.w EECD treated groups.

4.1.55 Effect of EECD on serum lipids

Figure 4.40 shows the effect of EECD on serum triacylglyceride in CCl_4 -induced hepatotoxicity.

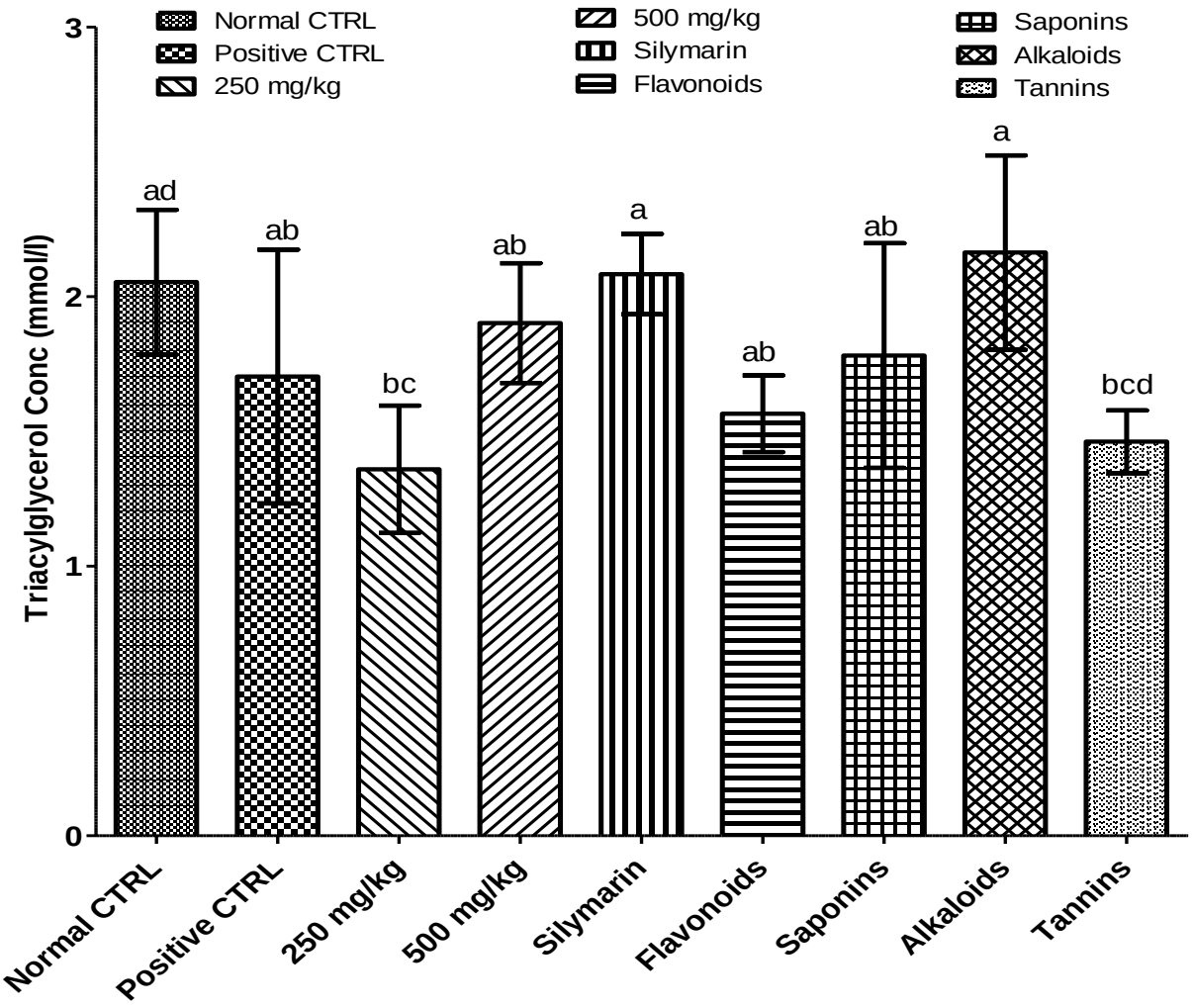


Figure 4.40: Effect of EECD on serum triacylglyceride in CCl_4 -induced hepatotoxicity. Bars bearing different letters are statistically significant ($p<0.05$).

Figure 4.40 shows a significant ($P<0.05$) reduction in serum triacylglyceride concentration of the positive control group compared to the normal, 250 mg/kg b.w EECD, the silymarin, the alkaloid and tannin treated groups. However there was no significant difference ($p \square 0.05$) among the positive control, the saponin, flavonoid and the 500 mg/kg b.w EECD treated groups.

4.1.56 Effect of EECD on serum lipids

Figure 4.41 shows the effect of EECD on serum very low density lipoprotein (VLDL) in CCl_4 -induced hepatotoxicity.

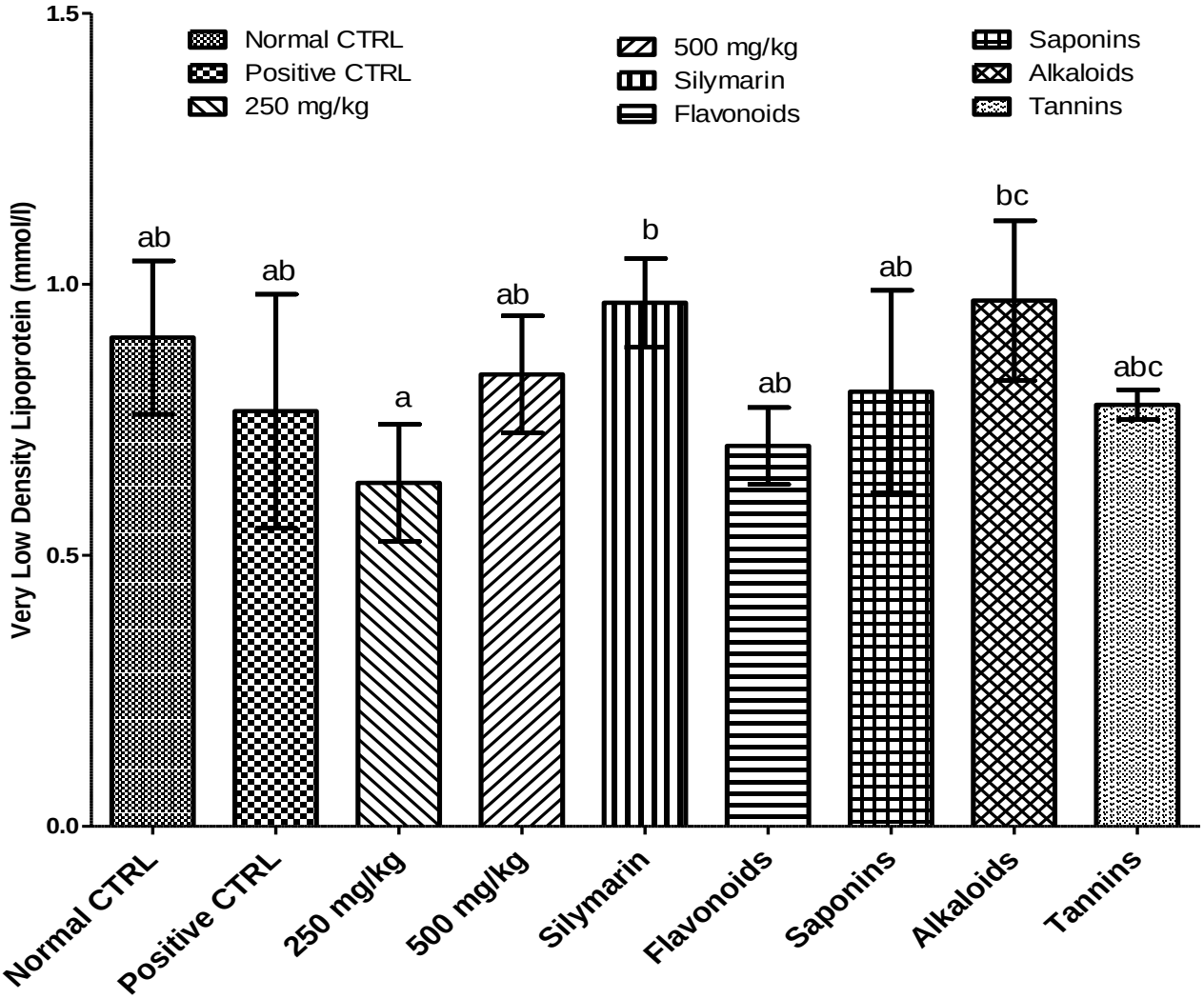


Figure 4.41: Effect of EECD on serum very low density lipoprotein (VLDL) in CCl_4 -induced hepatotoxicity. Bars bearing different letters are statistically significant ($p<0.05$)

Results as shown in figure 4.41 indicate a non-significant ($p\square0.05$) reduction in the concentration of VLDL in the positive control group compared to the normal, the 500 mg EECD, flavonoid and saponin treated groups. However, a significant difference was observed with the 250 mg EECD, silymarin, alkaloids and tannins groups.

4.1.57 Effect of EECD on serum lipids

Figure 4.42 shows effect of EECD on serum low density lipoprotein (LDL) concentration in CCl_4 - induced hepatotoxicity.

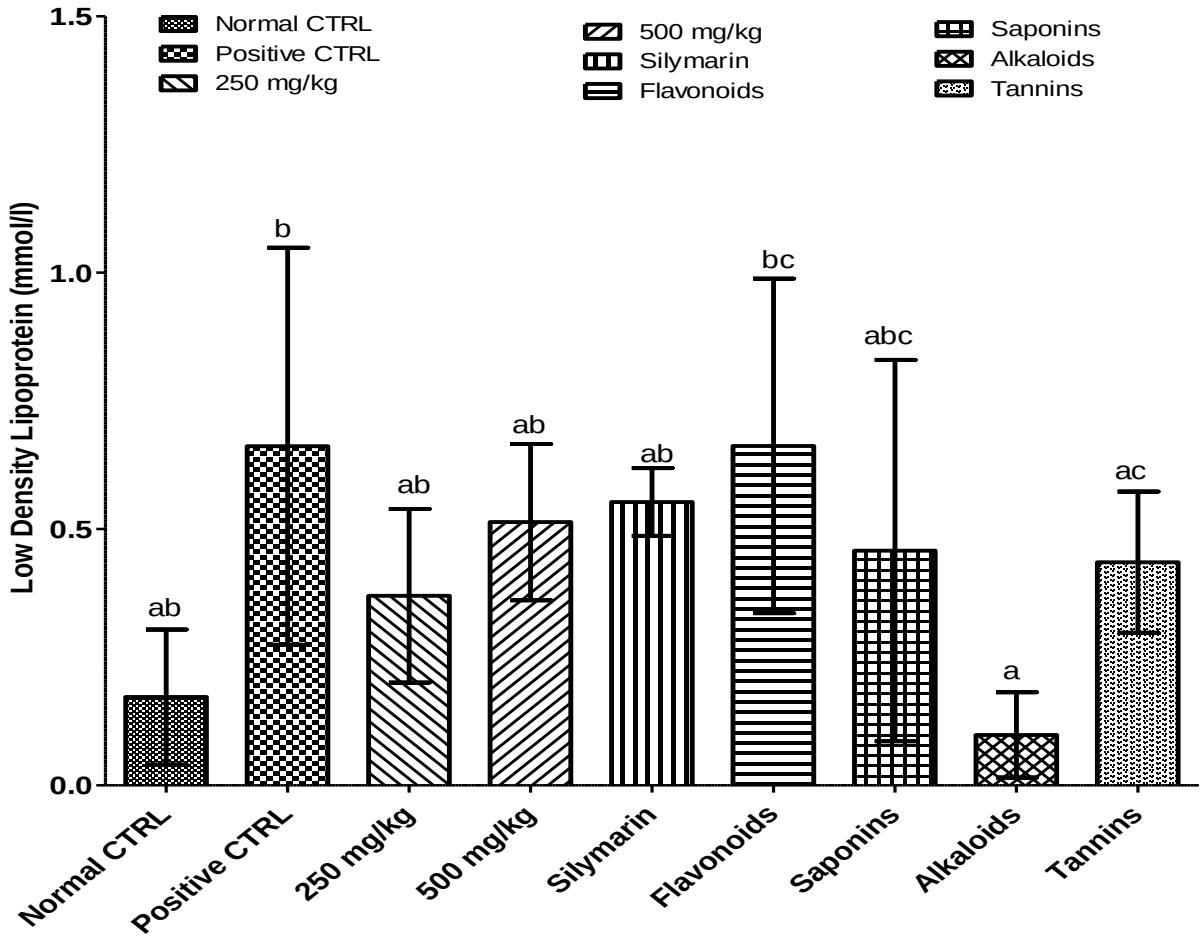


Figure 4.42: Effect of EECD on serum low density lipoprotein (LDL) concentration in CCl_4 -induced hepatotoxicity. Bars bearing different letters are statistically significant ($p<0.05$).

Figure 4.42 shows a non-significant (P□0.05) increase in LDL concentration in the of the positive control group compared to the normal, the EECD and silymarin treated groups. However, a significant ($P<0.05$) reduction was observed in the flavonoid, saponin, alkaloid and tannin groups.

4.1.58 Effect of EECD on serum lipids

Figure 4.43 shows the effect of EECD on LDL- cholesterol in CCl_4 -intoxicated rats.

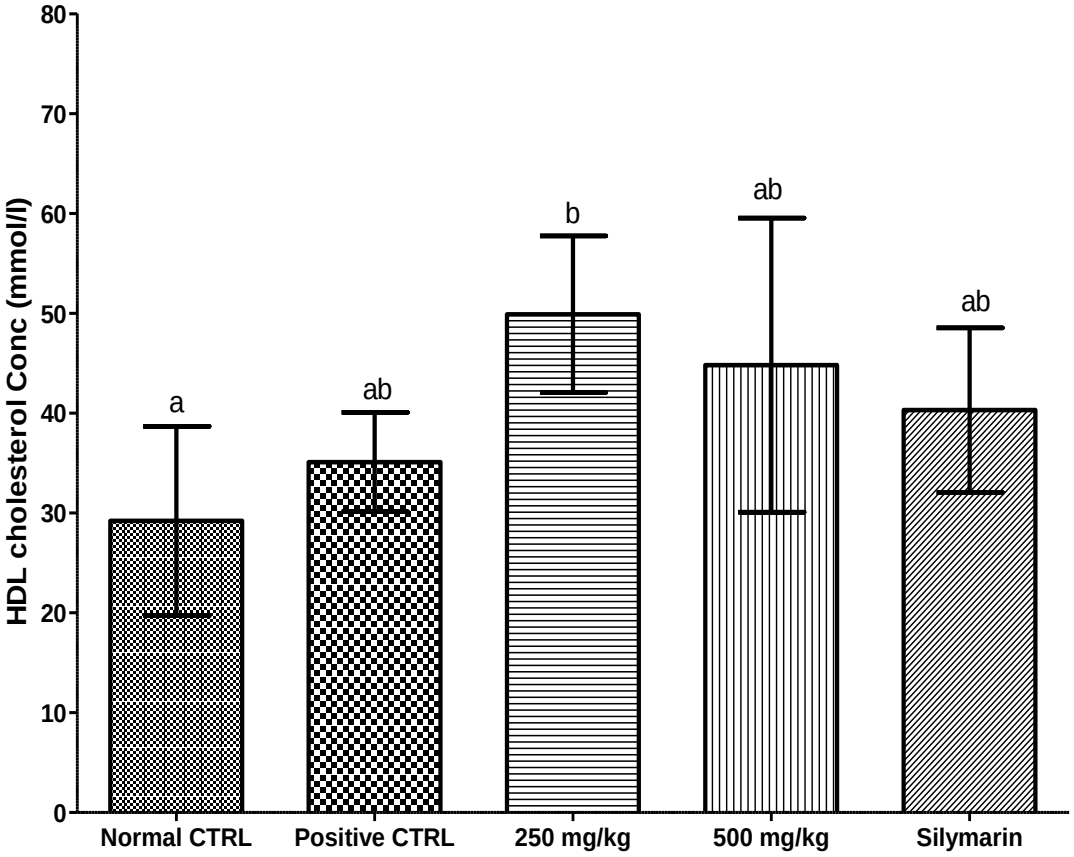


Figure 4.43: Effect of EECD on LDL- cholesterol in CCl_4 -intoxicated rats. Bars bearing different letters are statistically significant ($p<0.05$).

Figure 4.43 shows a non-significant ($p \square 0.05$) difference in high-density lipoprotein cholesterol concentration in the positive control group compared to the EECD treated at 500 mg/kg b.w and the silymarin groups. However there was a significant difference between the normal and the EECD treated group at 250 mg/kg b. w EECD.

4.1.59 Effect of EECD on serum lipids

Figure 4.44 shows the effect of EECD on serum HDL-cholesterol concentration in CCl_4 – induced oxidative stress.

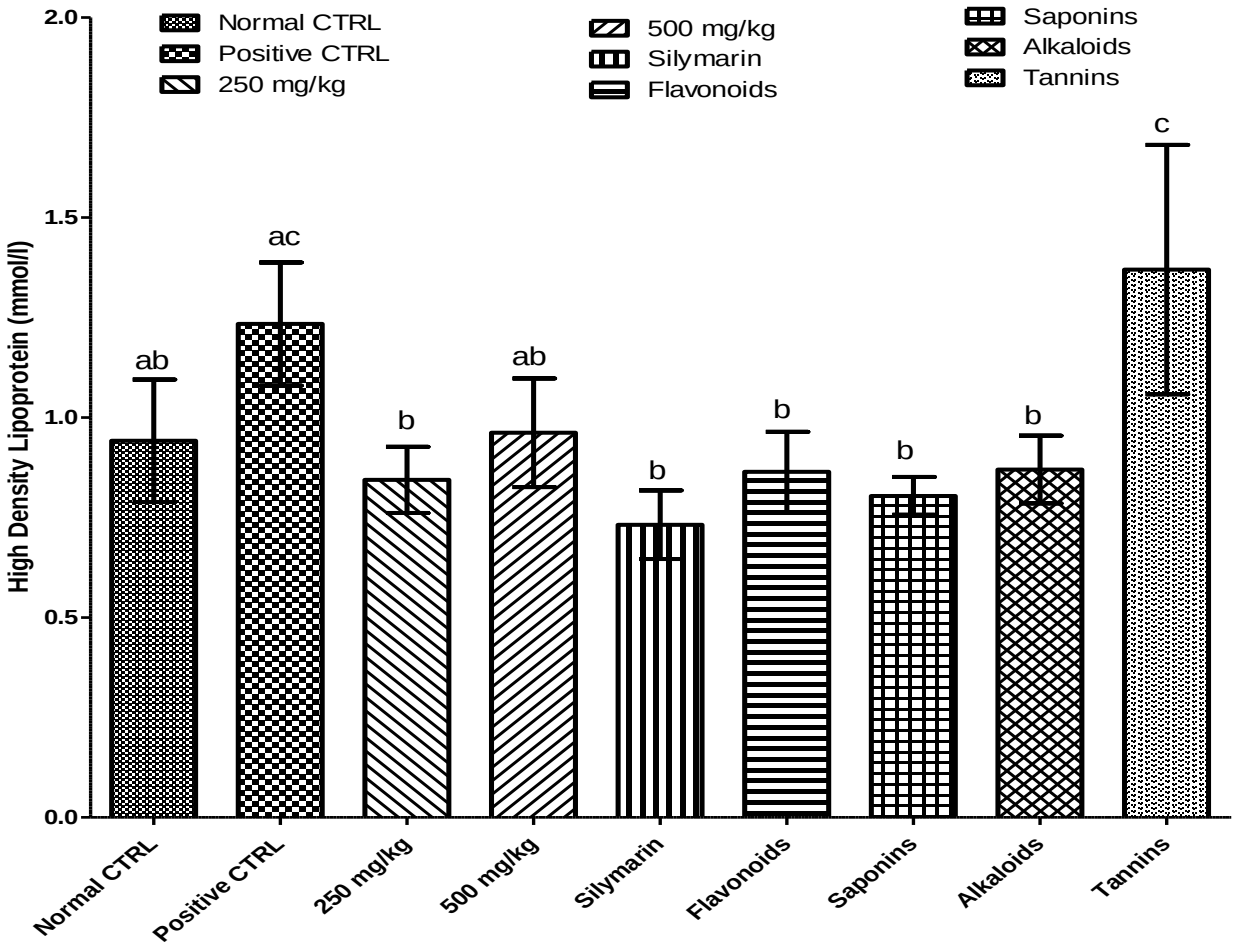


Figure 4.44: Effect of EECD on Serum HDL-cholesterol concentration in CCl_4 –induced oxidative stress. Bars bearing different letters are statistically significant ($p<0.05$)

Figure 4.44 shows a significant ($P<0.05$) increase in HDL levels of the positive control group compared to the EECD group treated at 250 mg/kg as well as the silymarin, flavonoid, saponin, and alkaloid. However, there was no significant difference ($p \square 0.05$) among the EECD treated at 250 mg/kg b.w, the silymarin, flavonoid, saponin, and the alkaloid control.

4.1.60 Effect of EECD on serum lipids

Figure 4.45 shows the effect of EECD on LDL/HDL ratio in CCl_4 -induced hepatotoxicity.

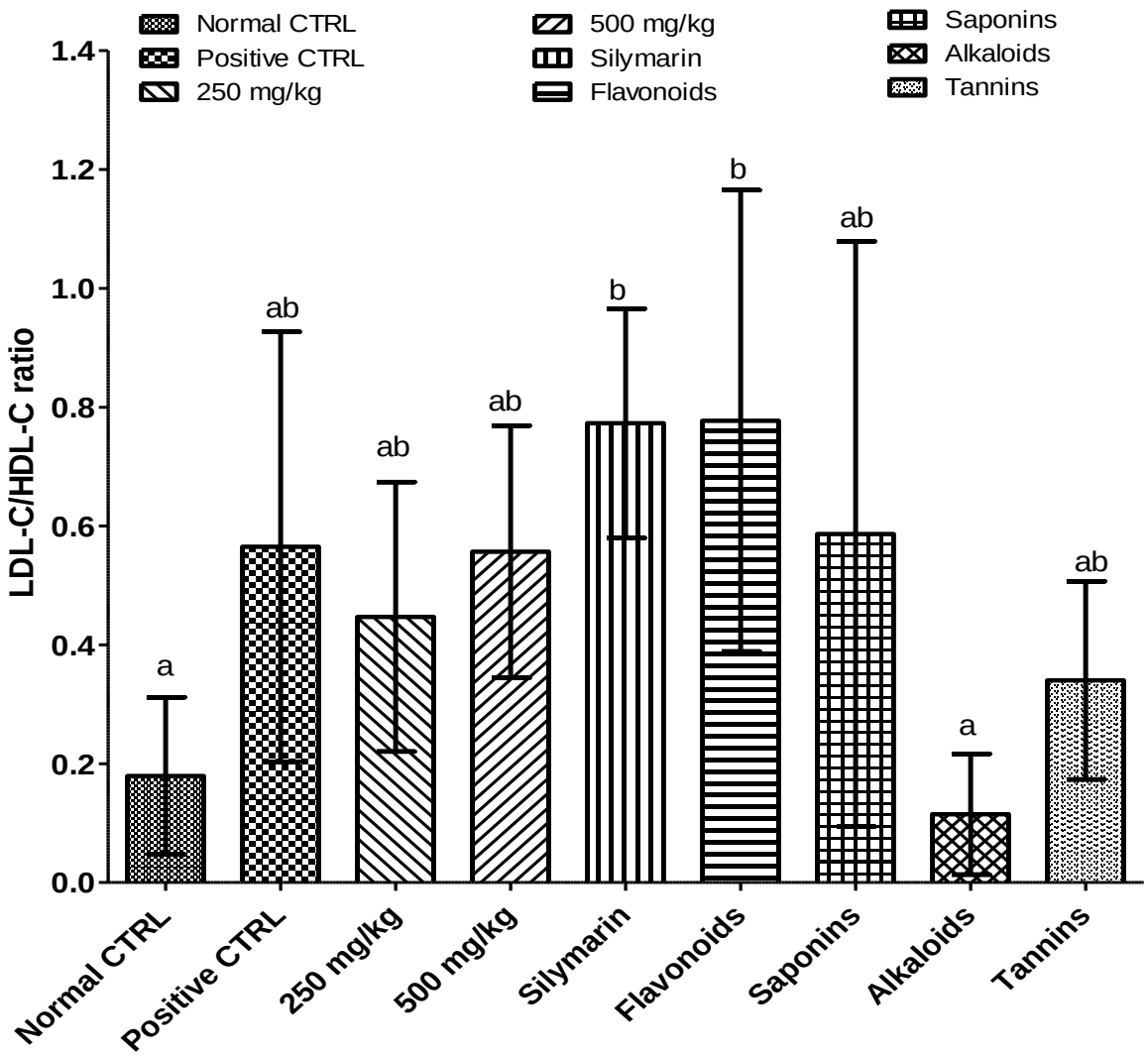


Figure 4.45: Effect of EECD on LDL/HDL ratio in CCl_4 -induced hepatotoxicity. Bars bearing different letters are statistically significant ($p<0.05$)

Figure 4.45 shows a significant ($P<0.05$) increase in the LDL/HDL ratio of the positive control group compared to the normal, and the alkaloid and non significantly ($p \square 0.05$) compared to the EECD treated, saponin as well as the tannin groups. There was also no significant difference ($p \square 0.05$) between the normal and the alkaloid control and between the silymarin and flavonoid control.

4.1.61 Effect of EECD on serum lipids

Figure 4.46 shows the effect of EECD on serum total cholesterol concentration in CCl_4 -induced hepatotoxicity.

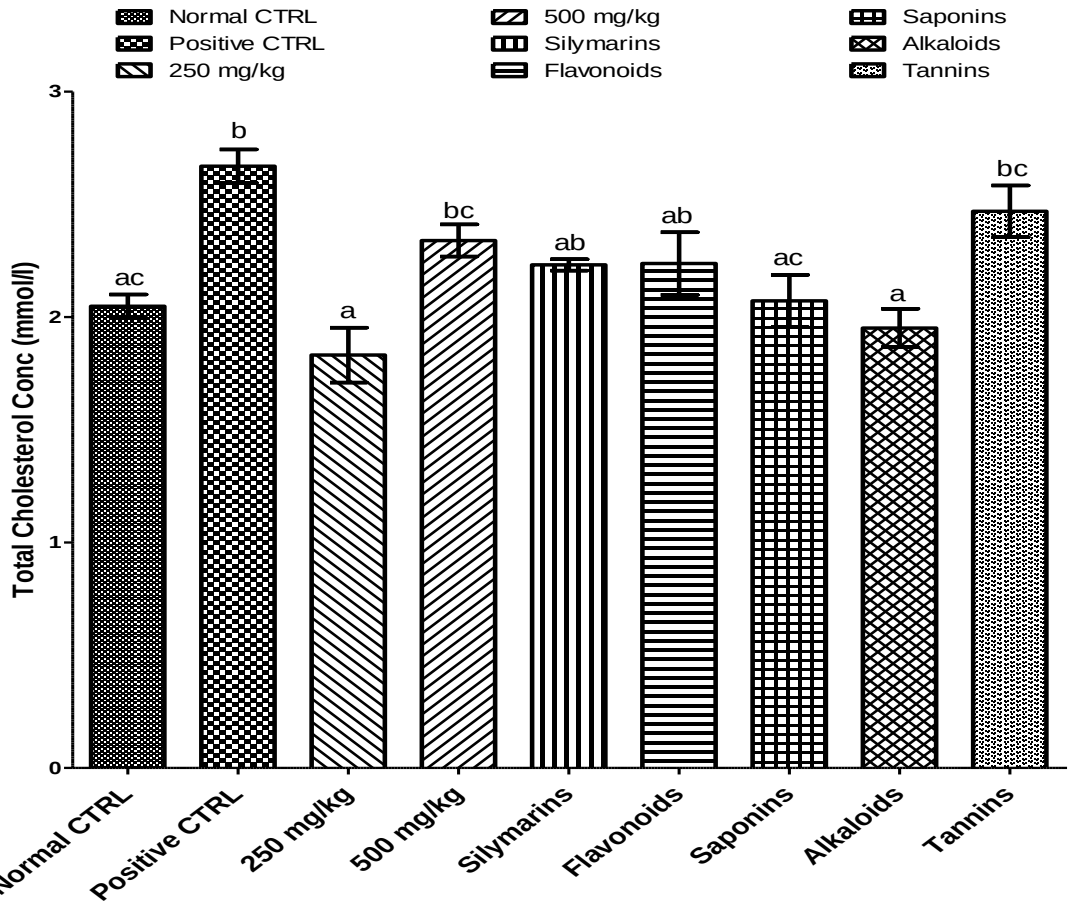


Figure 4.46: Effect of EECD on serum total cholesterol concentration in CCl_4 -induced hepatotoxicity. Bars bearing different letters are statistically significant ($p<0.05$)

Figure 4.46 shows a significant ($P<0.05$) increase in serum total cholesterol of the positive control group compared to the normal and the EECD (250 mg/kg), saponin, and alkaloid treated groups. However there was no significant ($p \square 0.05$) difference between the normal and the saponin control, and between the silymarin and the flavonoid control.

4.1.62 Effect of EECD on serum lipids

Figure 4.47 shows the effect of EECD on total non-high density lipoprotein cholesterol (TnHDL) in CCl_4-induced hepatotoxicity

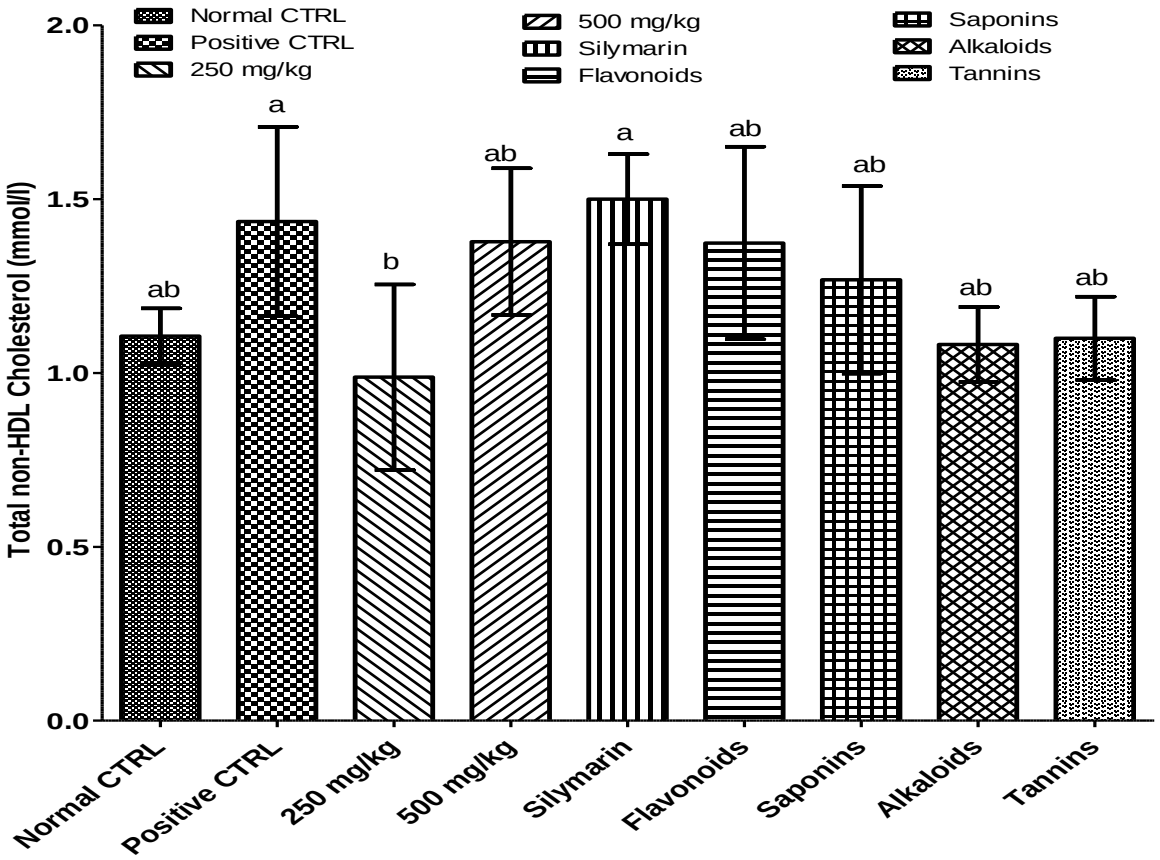


Figure 4.47: Effect of EECD on total non-high density lipoprotein cholesterol (TNHDL) in CCl_4-induced hepatotoxicity. Bars bearing different letters are statistically significant ($p<0.05$)

Figure 4.47 shows a significant ($P<0.05$) increase in the TnHDL cholesterol in the positive control group compared to the normal and the treated groups, except the silymarin treated group. However there was no significant ($p \square 0.05$) difference among the normal, EECD treated at 500 mg/kg b.w, flavonoid, saponin, alkaloid and tannin control.

4.1.63 Effect of EECD on kidney and electrolytes parameters

Figure 4.48 shows the effect of EECD on serum urea concentration in CCl_4 -induced nephrotoxicity.

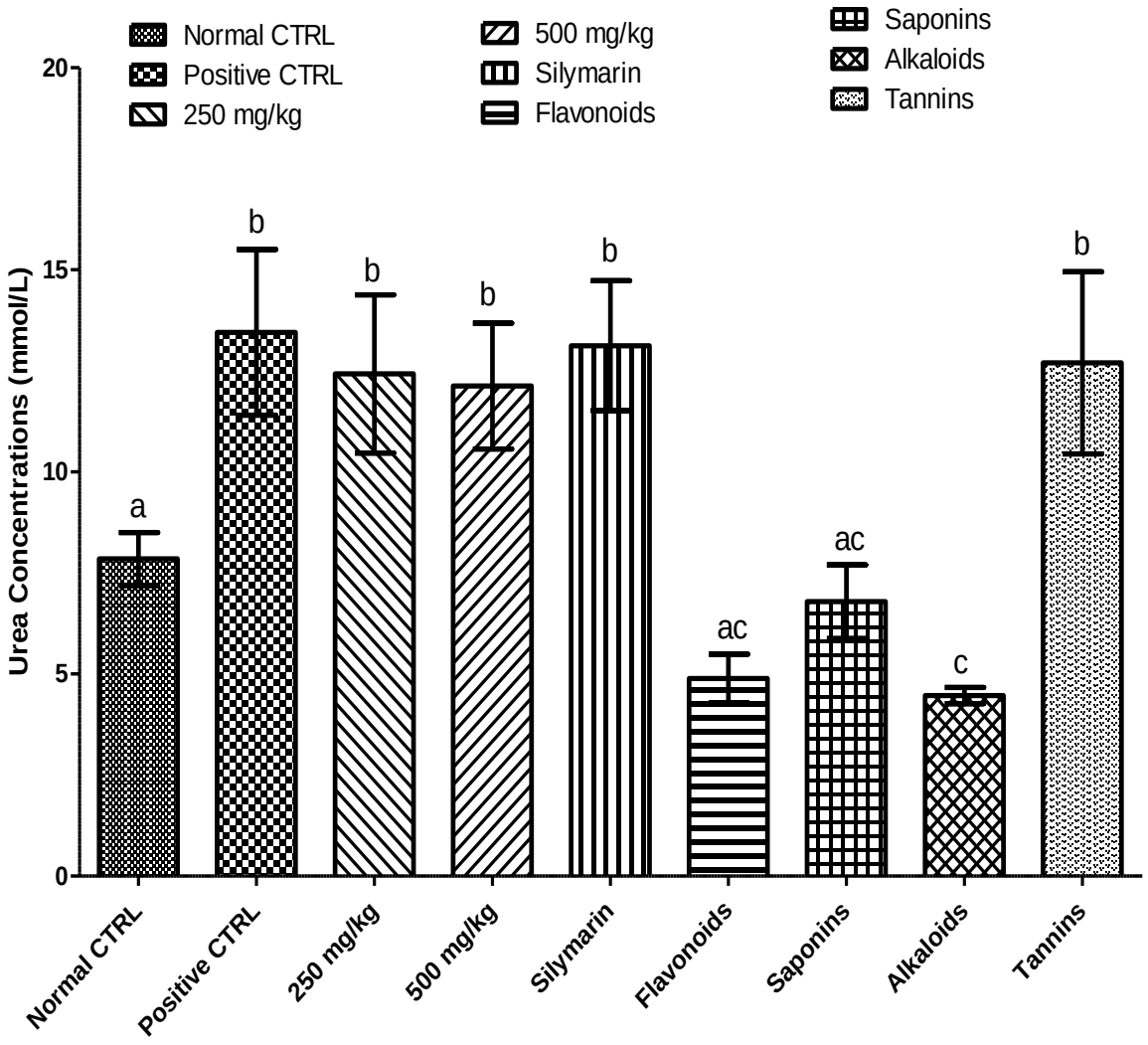


Figure 4.48: Effect of EECD on serum urea concentration in CCl_4 induced nephrotoxicity. Bars bearing different letters are statistically significant ($p<0.05$)

Figure 4.48 shows a significant ($p \square 0.05$) increase in the positive control group compared to the normal, the flavonoid, the saponin, and the alkaloid groups. However, there was no significant ($p \square 0.05$) difference among the positive control, the EECD treated groups, the silymarin as well as the tannin groups.

4.1.64 Effect of EECD on some kidney and electrolytes parameters

Figure 4.49 shows the effect of EECD on sodium concentration in CCl_4 -induced nephrotoxicity.

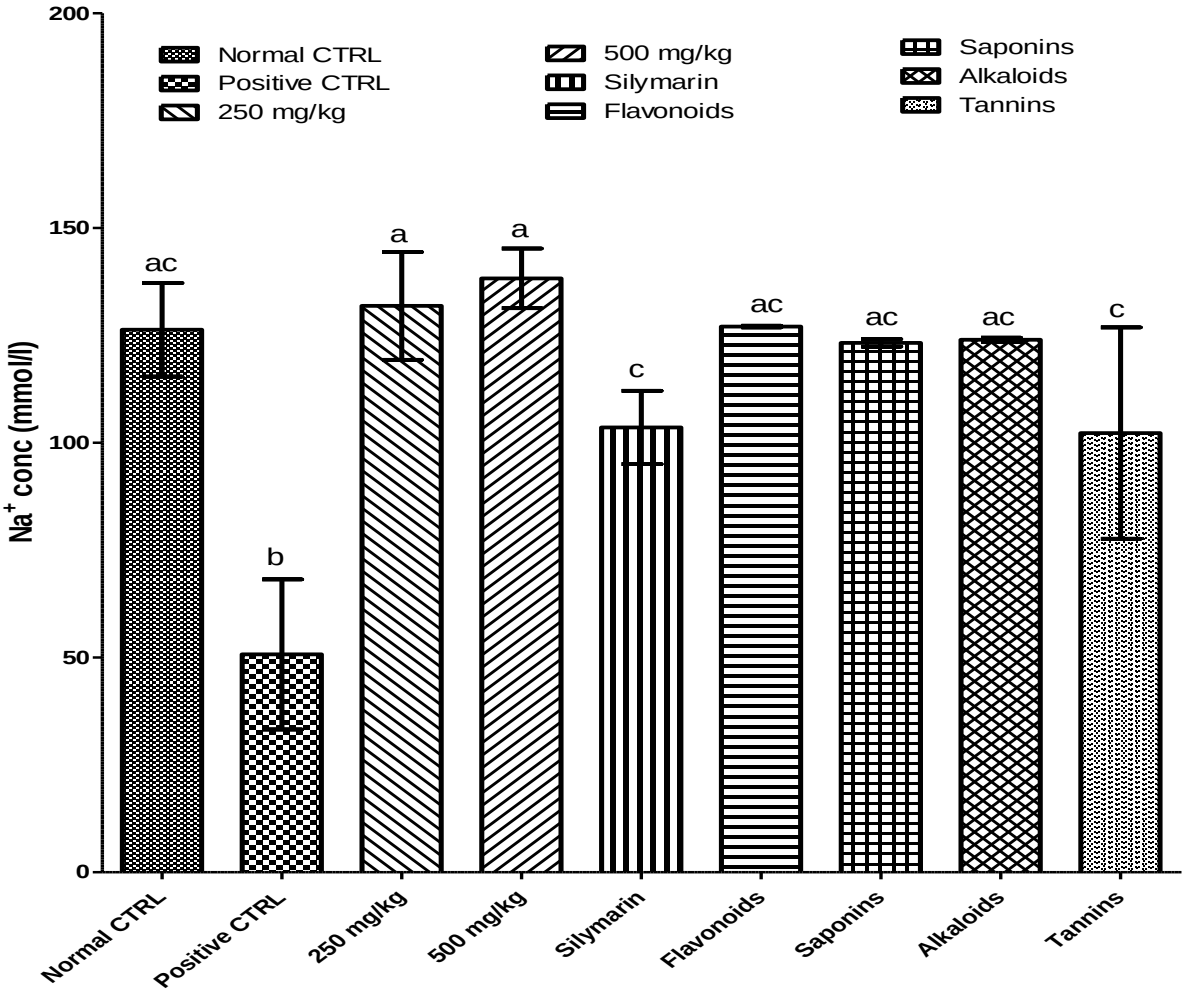


Figure 4.49: Effect of EECD on sodium concentration in CCl_4 -induced nephrotoxicity. Bars bearing different letters are statistically significant ($p<0.05$)

Figure 4.49 shows a significant ($P<0.05$) decrease in sodium concentration of the positive control group compared to the normal and all the treated groups including the silymarin group. Also observered was a non significant ($p \square 0.05$) difference among the normal, the flavonoid, saponin, and tannin control. There was also a non significant ($p \square 0.05$) difference between the silymarin and the tannin groups.

4.1.65 Effect of EECD on some kidney and electrolytes parameters

Figure 4.50 shows the effect of EECD on potassium concentration in CCl_4 -induced nephrotoxicity.

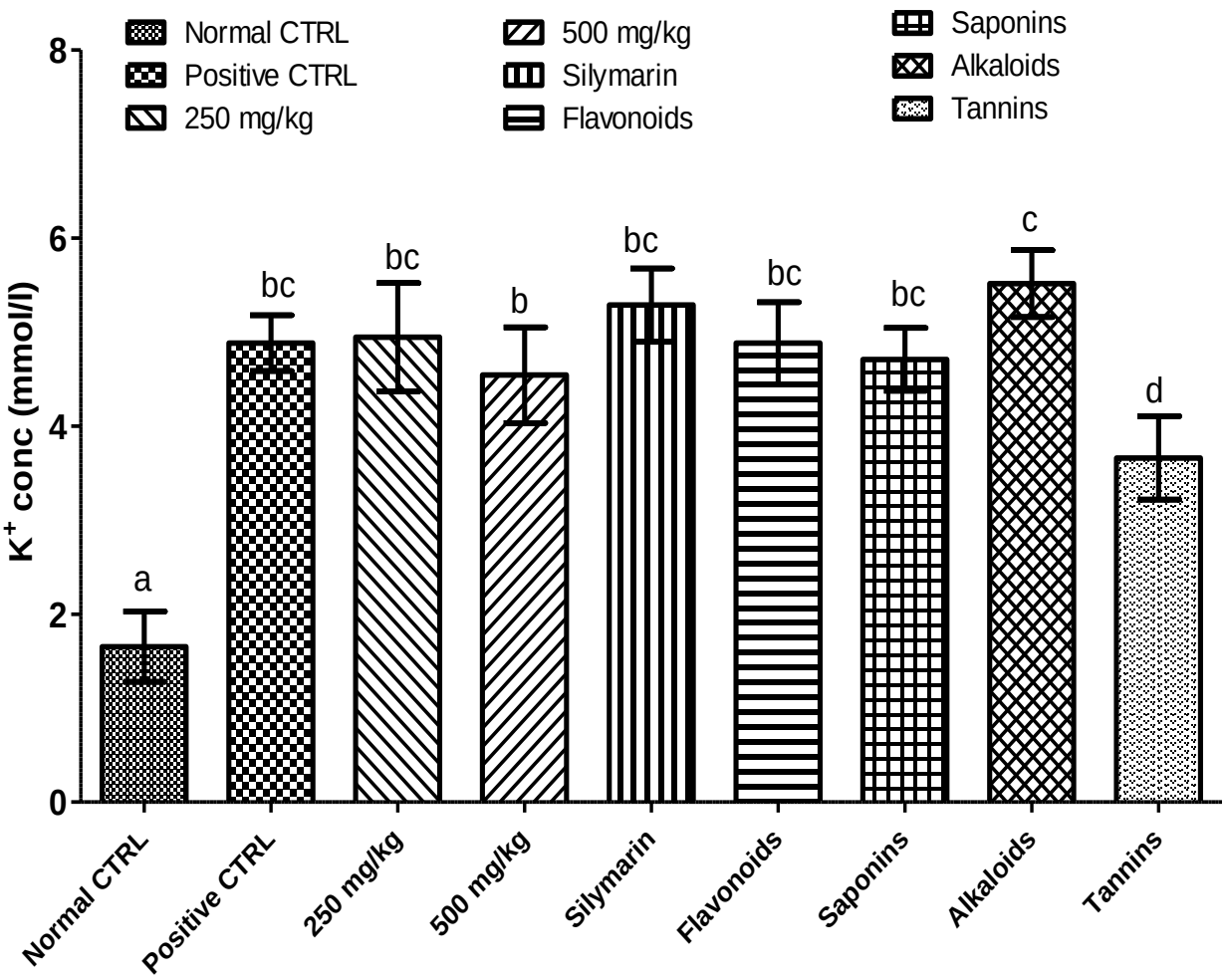


Figure 4.50: Effect of EECD on potassium concentration in CCl_4- induced nephrotoxicity. Bars bearing different letters are statistically significant ($p<0.05$).

Figure 4.50 shows a significant ($P<0.05$) increase in potassium concentration of the positive control group compared to the normal group. A significant difference was also observed between the positive control group, the alkaloid and the tannin groups. However a non significant ($p\square0.05$) difference was observered between the positive control and the EECD at 250 mg/kg, the silymarin, the flavonoid as well as the alkaloid control.

4.1.66 Effect of EECD on some kidney and electrolytes parameters

Figure 4.51 shows effect of EECD on chloride concentration in CCl_4 -induced oxidative stress.

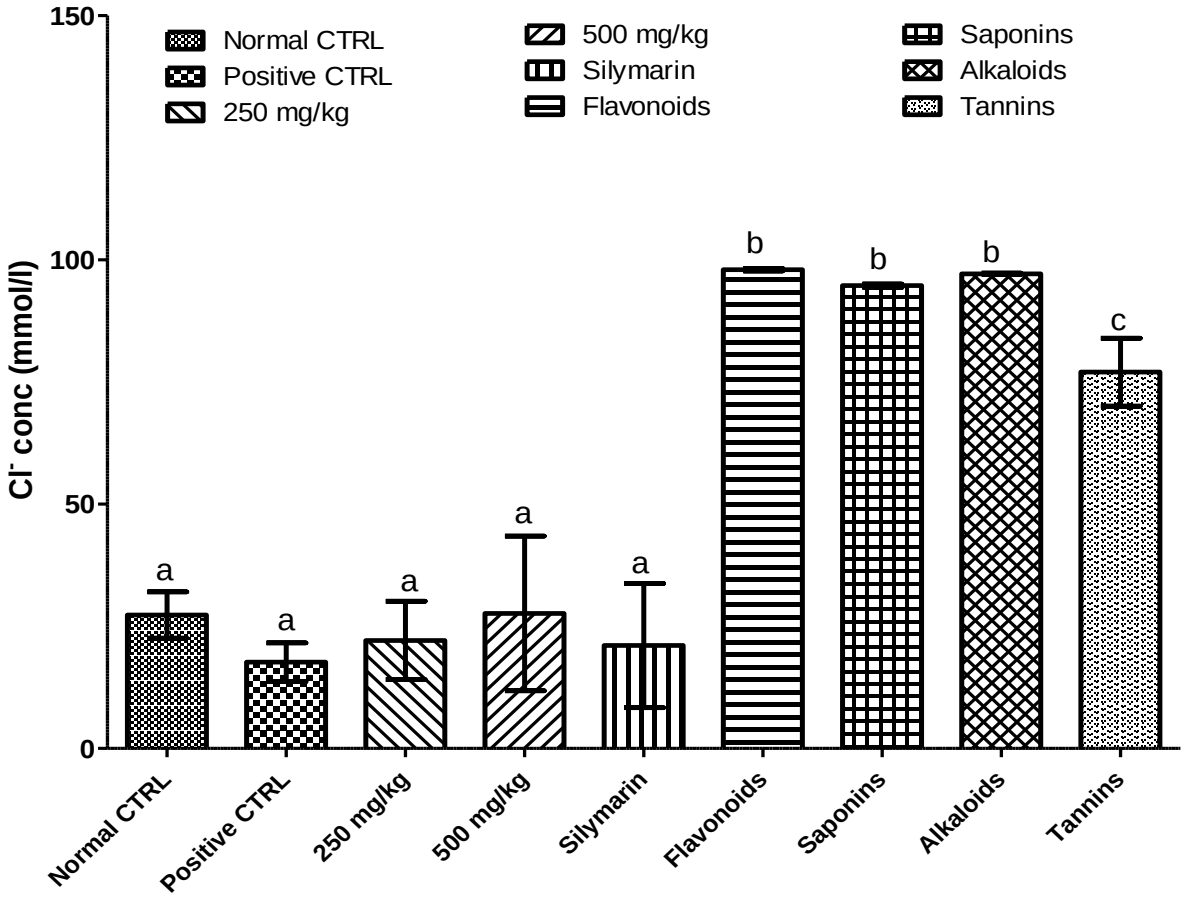


Figure 4.51: Effect of EECD on chloride concentration in CCl_4 -induced oxidative stress. Bars bearing different letters are statistically significant ($p<0.05$)

The result as shown in figure 4.51 reveals a significant ($P<0.05$) increase in chloride concentration of the flavonoid, saponin, alkaloid and tannin groups compared to the positive control group. There was no significant ($p \square 0.05$) difference among the normal, the positive control, the EECD treated groups as well as the silymarin groups.

4.1.67 Effect of EECD on some kidney and electrolytes parameters

Figure 4.52 shows the effect of EECD in serum bicarbonate (HCO_3) in CCl_4 -induced oxidative stress.

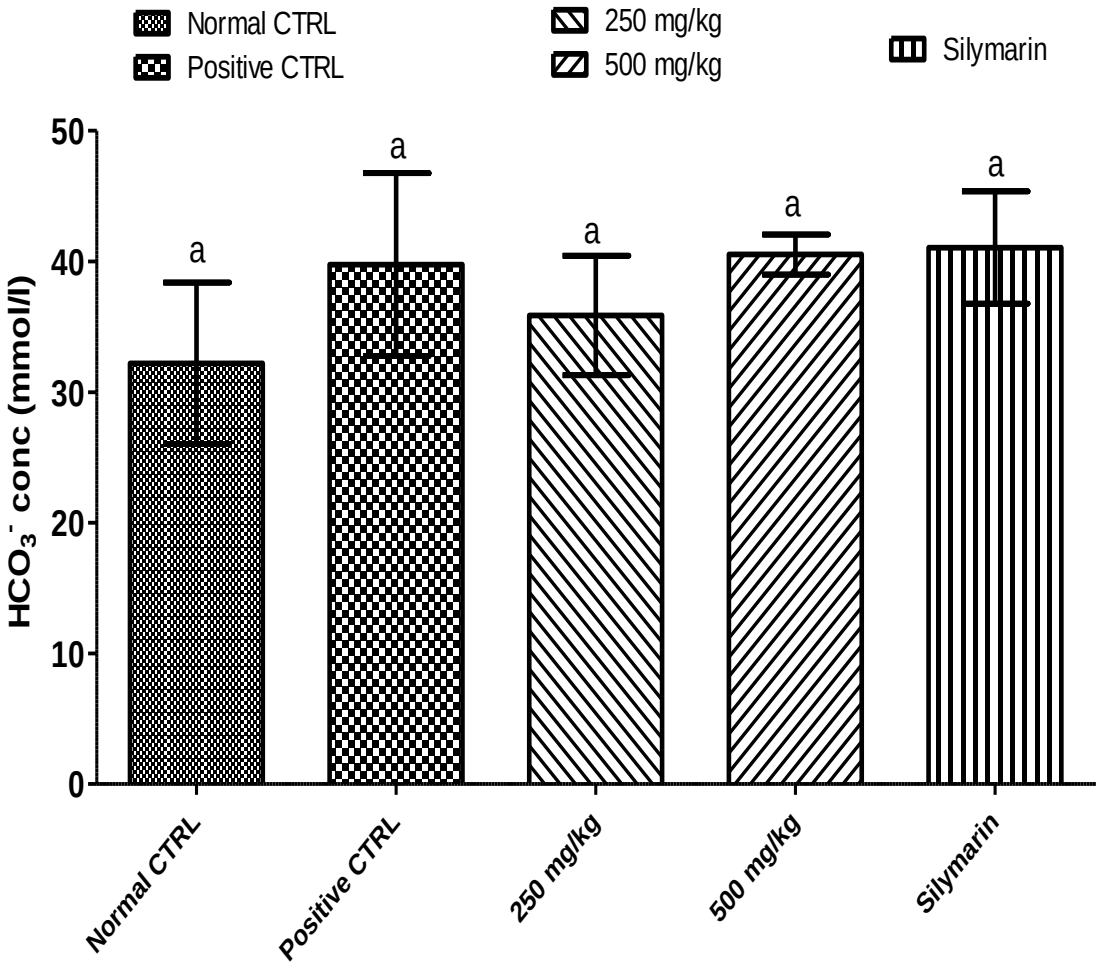


Figure 4.52: Effect of EECD in serum bicarbonate (HCO_3) in CCl_4 -induced oxidative stress. Bars bearing different letters are statistically significant ($p<0.05$)

Figure 4.52 shows a non-significant ($p\square0.05$) increase in the serum bicarbonate concentration of the positive control group compared to the normal. Also observered was a non significant ($p\square0.05$) difference between the positive control and both the EECD and silymarin treated groups.

4.1.68 Effect of EECD on selected serum minerals

Figure 4.53 shows the effect of EECD on iron concentration in the liver of rats intoxicated with carbon tetrachloride.

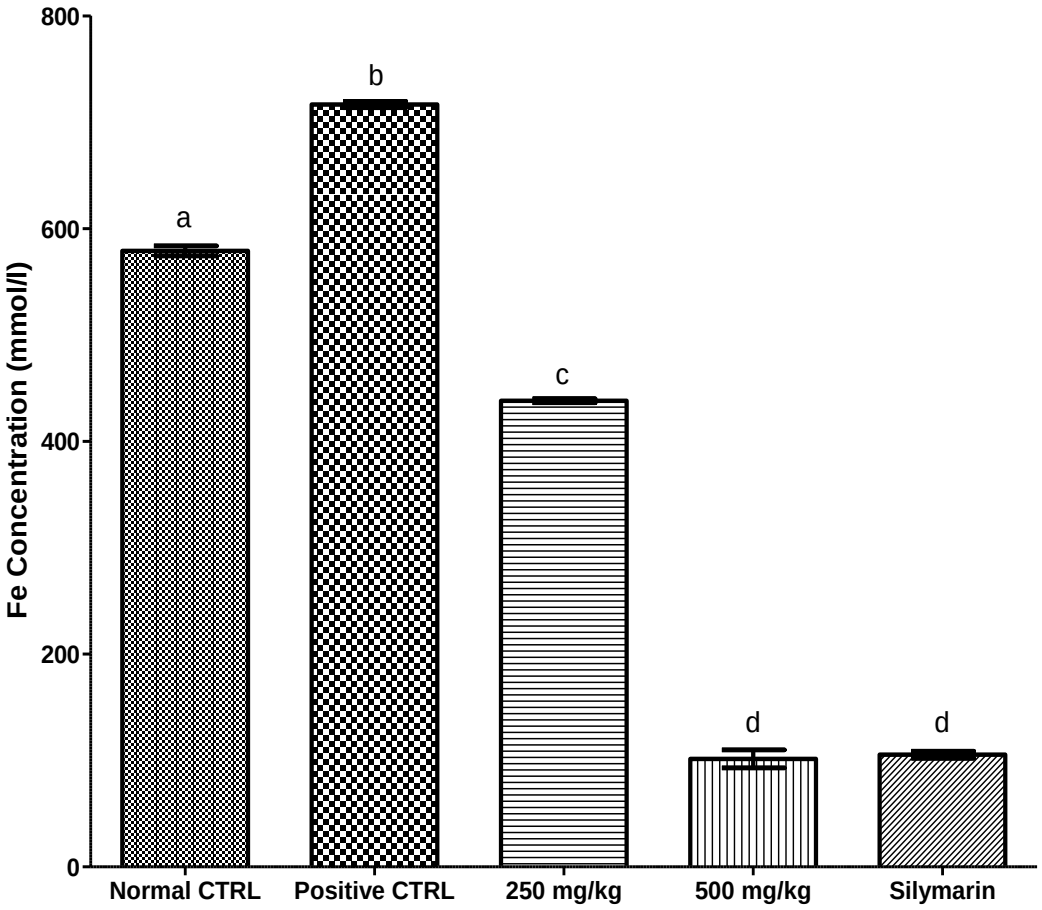


Figure 4.53: Effect of EECD on iron concentration in the liver of rats intoxicated with carbon tetrachloride. Bars bearing different letters are statistically significant ($p<0.05$).

This result as shown in figure 4.53 reveals a significant ($p \square 0.05$) increase in iron concentration of the positive control compared to the normal and both the EECD and silymarin treated groups.

4.1.69 Effect of EECD on selected serum minerals

Figure 4.54 shows the effect of EECD zinc concentration on the liver in carbon tetrachloride intoxicated rats.

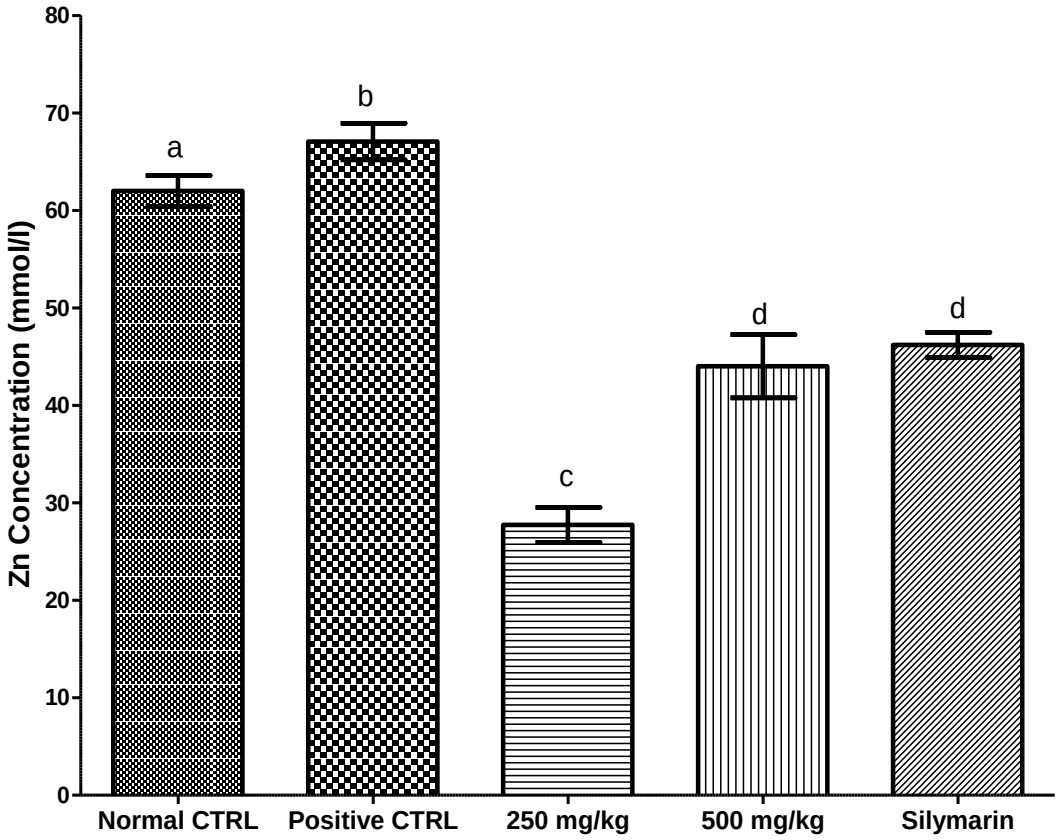


Figure 4.54: Effect of EECD zinc concentration on the liver in carbon tetrachloride intoxicated rats. Bars bearing different letters are statistically significant ($p<0.05$).

Figure 4.54 shows that zinc concentration was significantly (p□0.05) elevated in the positive control group compared to the normal and the treated groups at 250 mg/kg b.w and 500 mg/kg b. w including the silymarin group. However a non significant (p□0.05) difference was obsevered between the 250 mg/kg and 500 mg/kg b.w EECD treated groups.

4.1.70 Effect of EECD on some haematological parameters

Figure 4.55 shows the effect of EECD on red blood cell (RBC) in CCl_4 -induced oxidative stress.

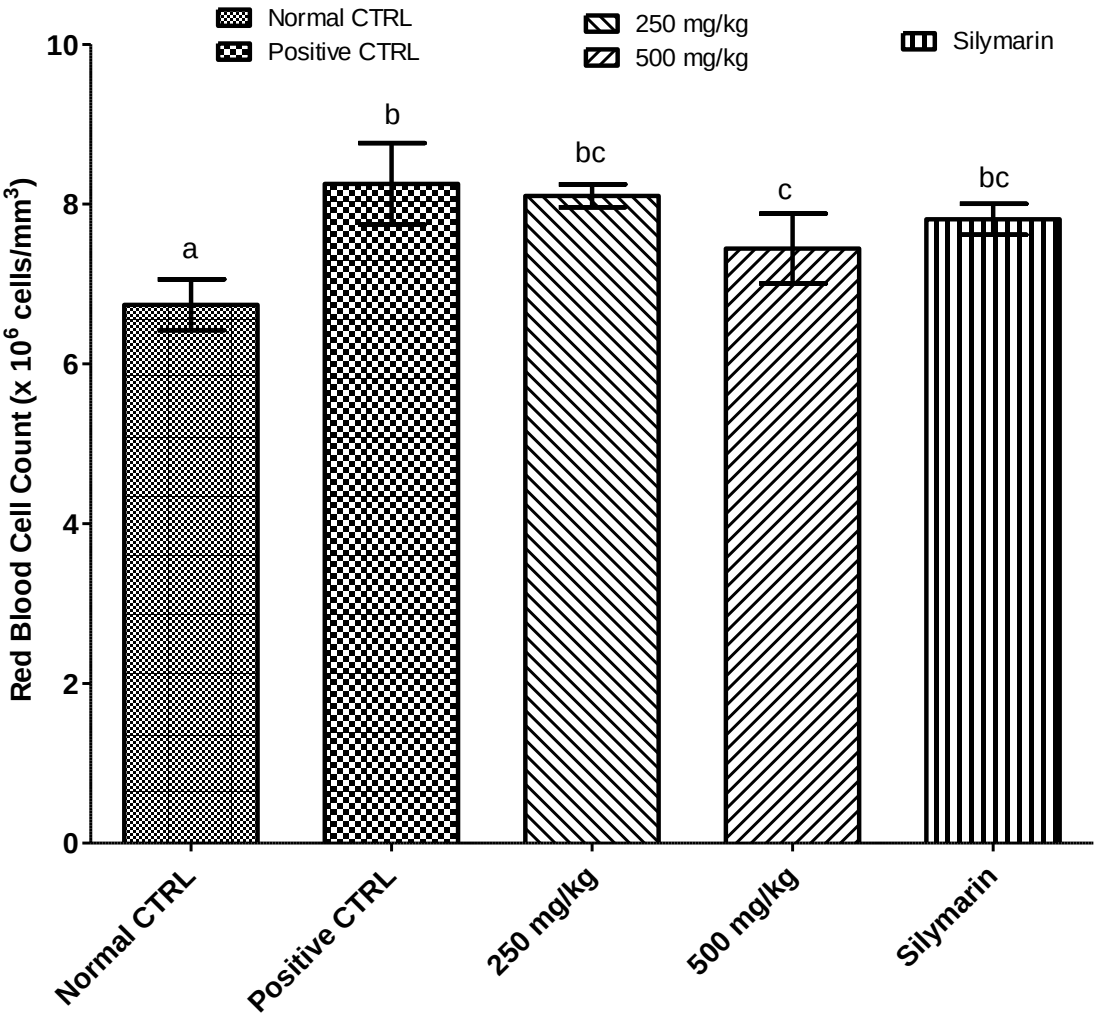


Figure 4.55: Effect of EECD on red blood cell (RBC) in CCl_4 -induced oxidative stress. Bars bearing different letters are statistically significant ($p<0.05$)

Figure 4.55 shows a significant ($P<0.05$) increase in the RBC count of the positive control group compared to the normal, and the 500 mg/kg EECD treated group. However, there was no significant ($p \square 0.05$) difference among the positive control, 250 mg/kg EECD treated and silymarin groups.

4.1.711 Effect of EECD on haematological parameters

Figure 4.56 shows the effect of EECD on haemoglobin concentration in CCl_4 - induced oxidative stress.

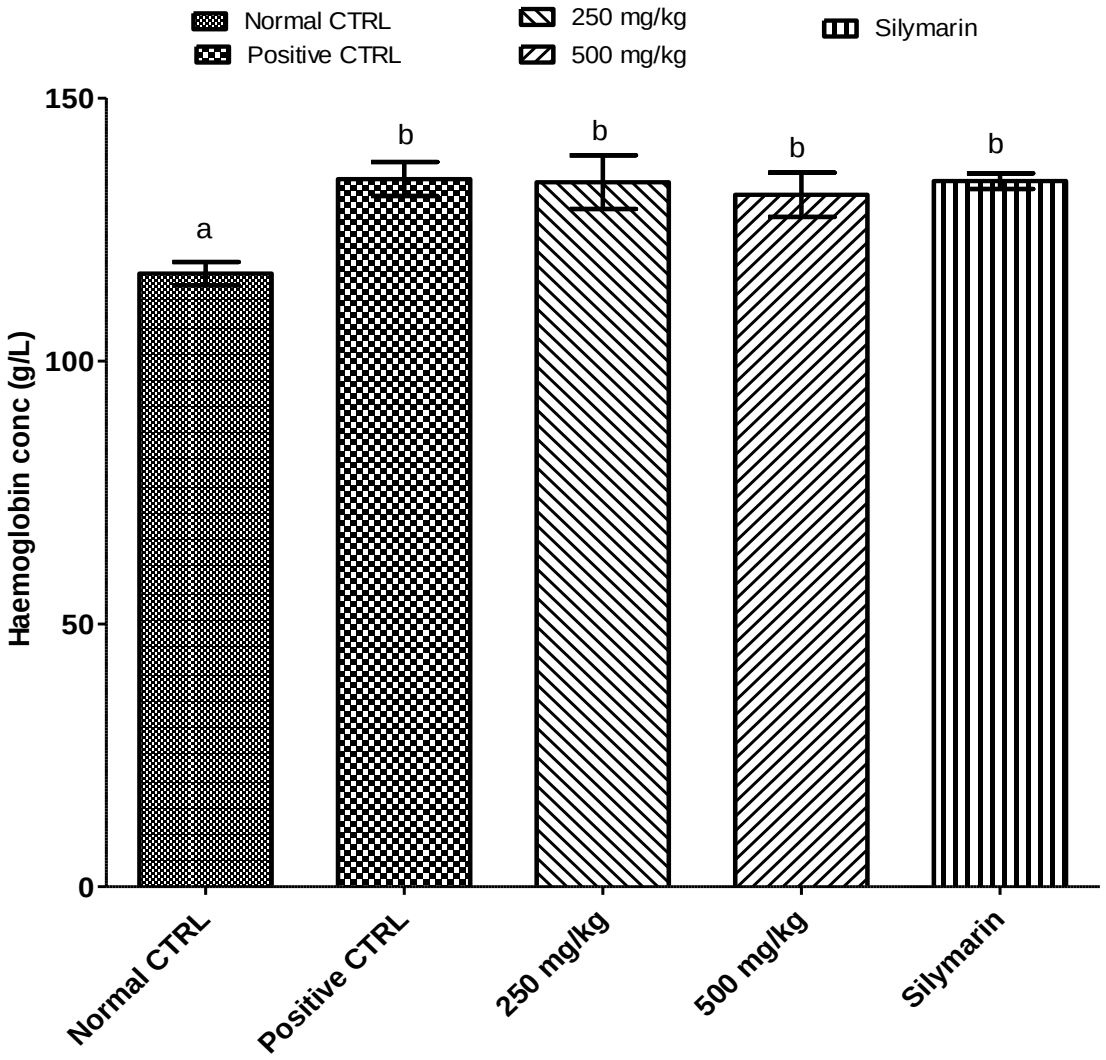


Figure 4.56 Effect of EECD on haemoglobin concentration in CCl_4 -induced oxidative stress. Bars bearing different letters are statistically significant ($p<0.05$)

Figure 4.56 shows a significant ($P<0.05$) increase in the haemoglobin concentration of the positive control, 250 and 500 mg/kg EECD and silymarin treated groups compared to the normal. A non significant ($p \square 0.05$) difference was also observed between the positive control, 250 and 500 mg/kg EECD and silymarin treated groups.

4.1.72 Effect of EECD on haematological parameters

Figure 4.57 shows the effect of EECD on the haematocrit (PCV) concentration in CCl_4-induced oxidative stress.

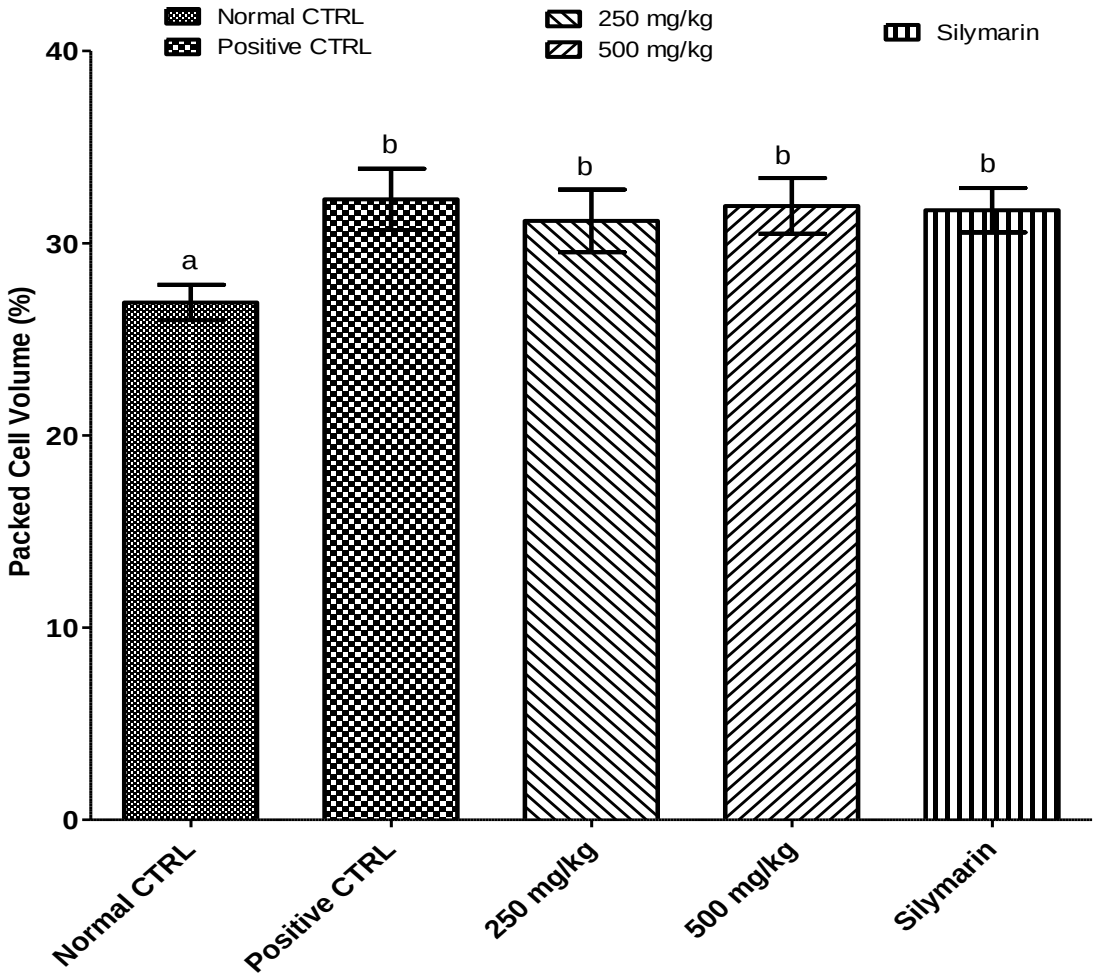


Figure 4.57: Effect of EECD on the haematocrit (PCV) concentration in CCl_4-induced oxidative stress. Bars bearing different letters are statistically significant ($p<0.05$).

Figure 4.57 shows a significant ($P<0.05$) increase in the haematocrit concentration of the positive control, 250 mg/kg, 500 mg/kg EECD and silymarin treated groups compared to the normal. Also a non significant (p□0.05) difference was observed among the positive, 250 mg/kg, 500 mg/kg EECD and the silymarin treated groups.

4.1.73 Efect of EECD on haematological parameters

Figure 4.58 shows the effect of EECD on mean cell haemoglobin (MCH) in CCl_4-induced oxidative stress.

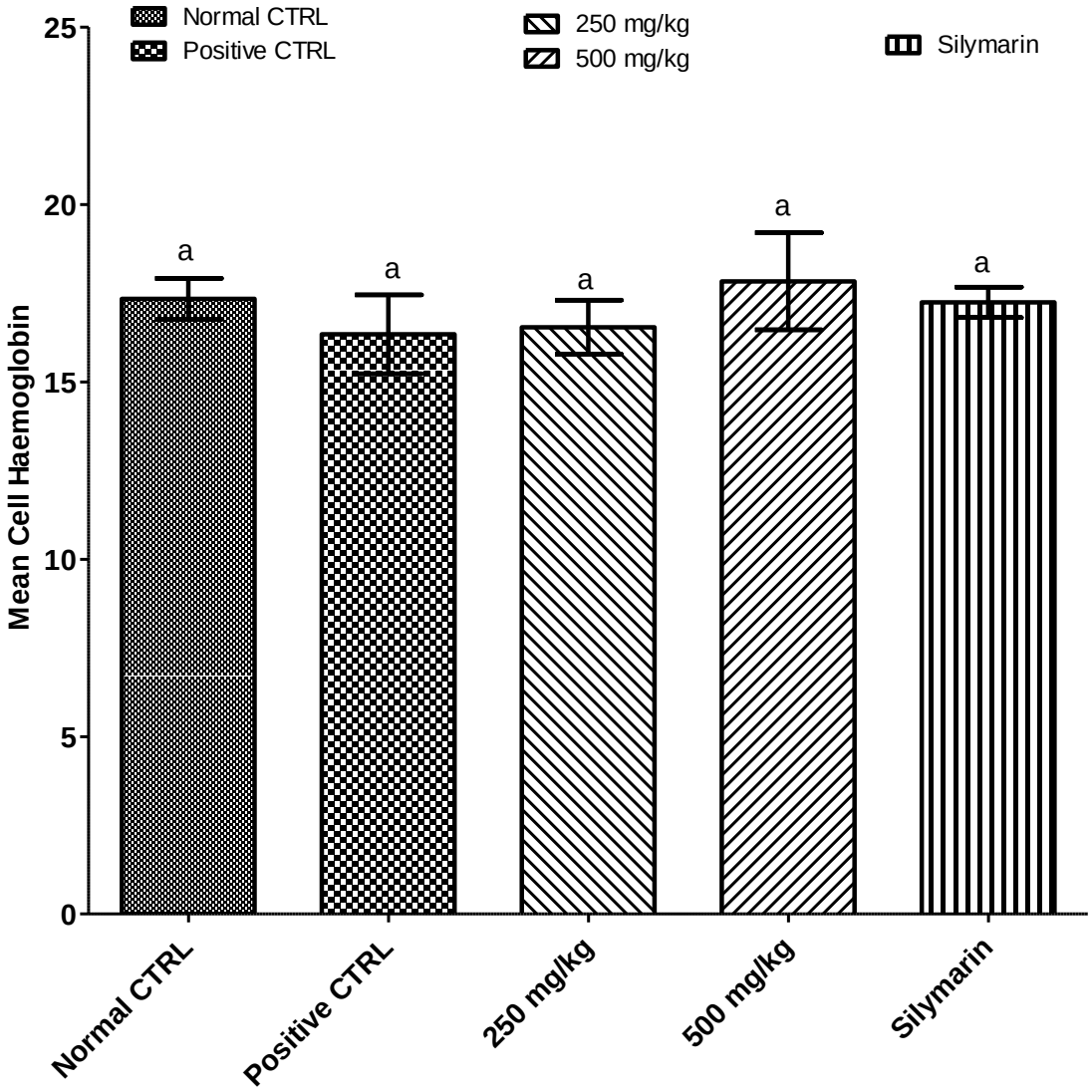


Figure 4.58: Effect of EECD on mean cell haemoglobin (MCH) in CCl_4-induced oxidative stress. Bars bearing different letters are statistically significant ($p<0.05$).

Figure 4.58 shows a non-significant ($p\square0.05$) reduction in the concentration of MCH of the positive control group and 250 mg/kg EECD treated groups compared to the normal. Similarly a non significant ($p\square0.05$) fluctuation in MCH concentration was observed between the normal control group 500 mg/kg EECD and silymarin treated groups.

4.1.74 Effect of EECD on haematological parameters

Figure 4.59 shows the effect of EECD on mean cell haemoglobin concentration (MCHC) in CCl_4 -induced oxidative stress.

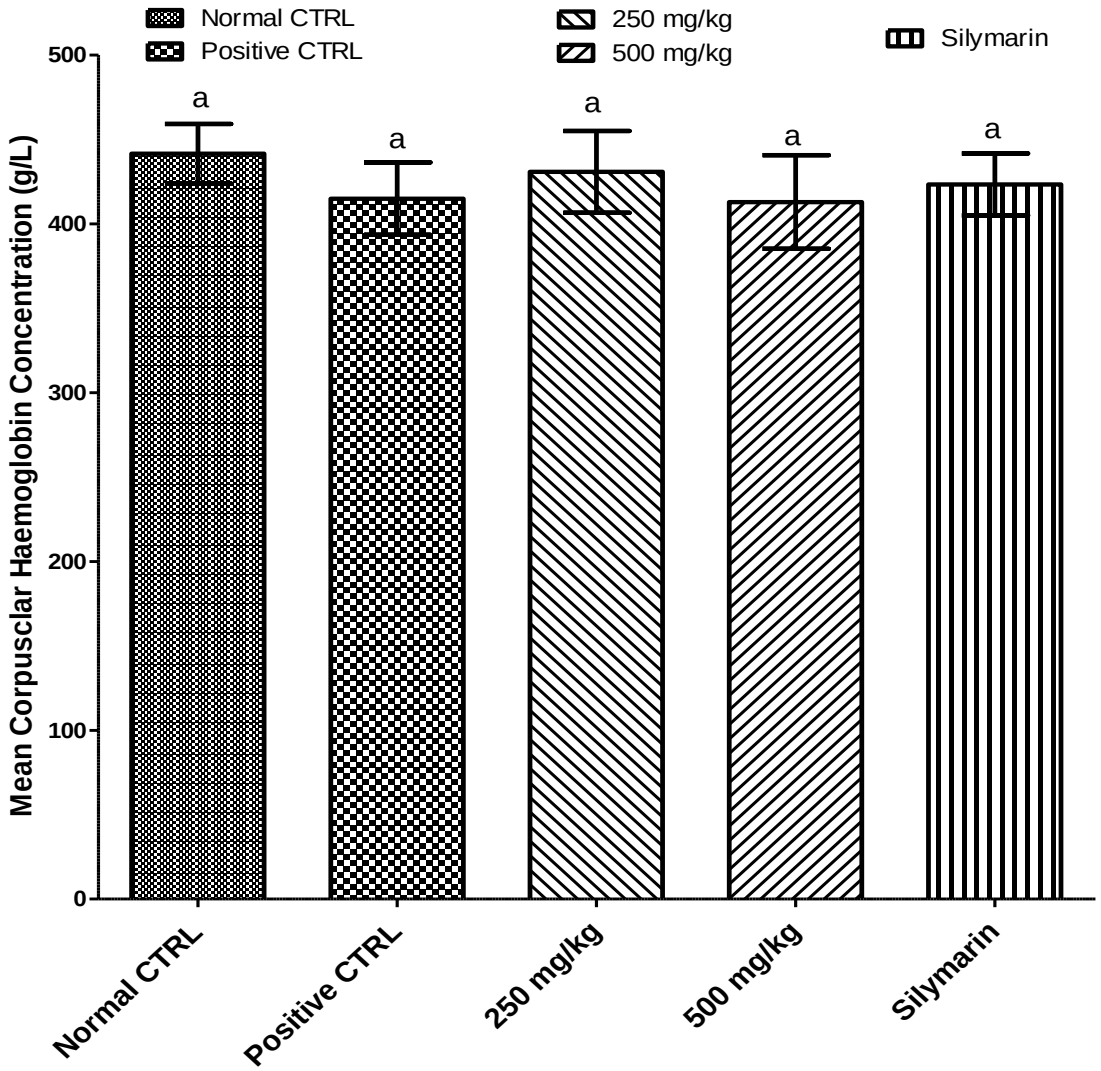


Figure 4.59: Effect of EECD on mean cell haemoglobin concentration (MCHC) in CCl_4 - induced oxidative stress. Bars bearing different letters are statistically significant ($p<0.05$)

Figure 4.59 shows a non-significant ($p \square 0.05$) reduction in the MCHC concentration of the positive group compared to the normal and a non significant ($p \square 0.05$) difference was observed among the EECD at 250, 500 mg/kg b.w and the silymarin treated groups.

4.1.75 Effect of EECD on haematological parameters

Figure 4.60 shows the effect of EECD on mean cell volume (MCV) in CCl_4 -induced oxidative stress.

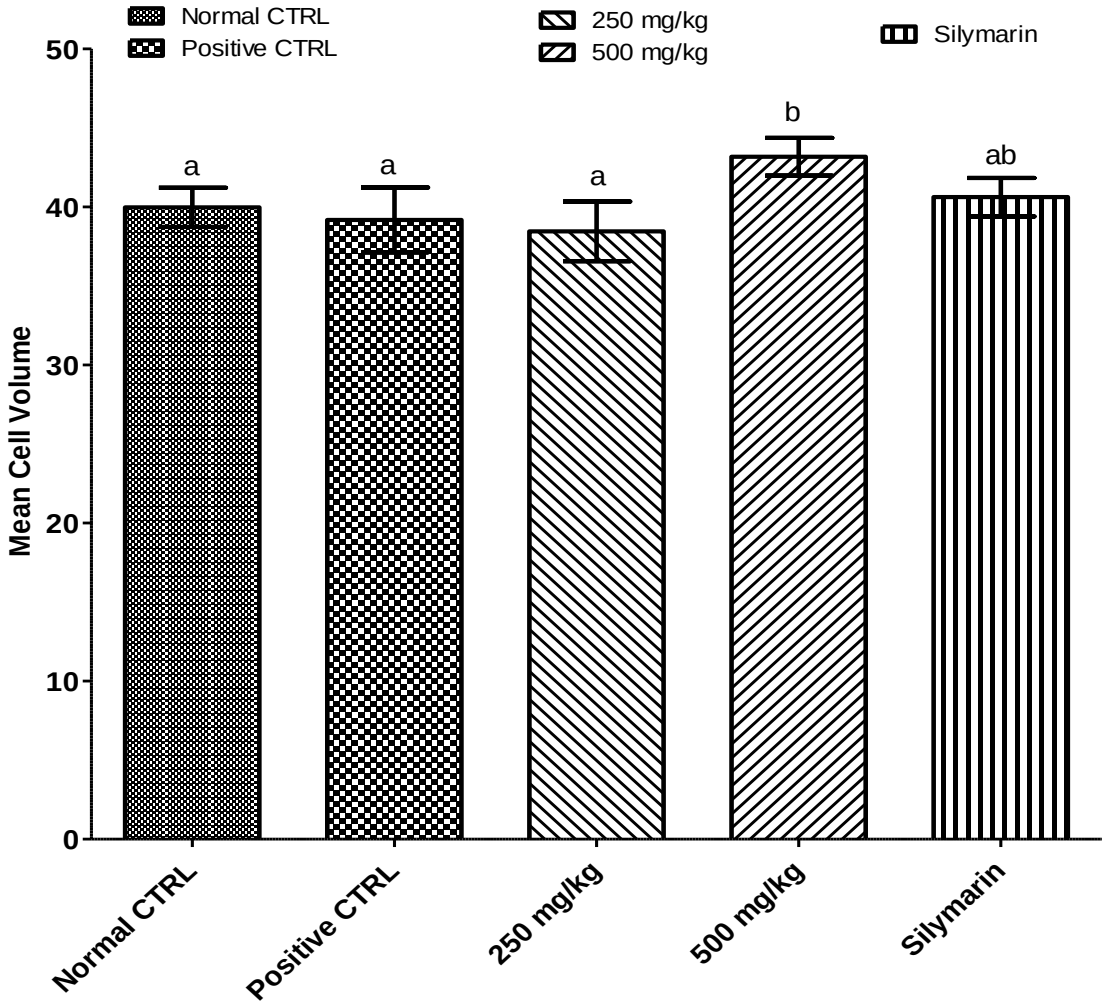


Figure 4.60: Effect of EECD on mean cell volume (MCV) in CCl_4 -induced oxidative stress. Bars bearing different letters are statistically significant ($p<0.05$).

Figure 4.60 shows a non-significant ($p \square 0.05$) drop in the MCV levels of the positive control group compared to the normal and the 250 mg/kg EECD treated group. However, a significant ($P<0.05$) increase was observed in the 500 mg/kg EECD treated group compared to the positive control group.

4.1.76 Effect of EECD on haematological parameters

Figure 4.61 shows the effect of EECD on white blood cell count (WBC) in CCl_4 -induced oxidative stress.

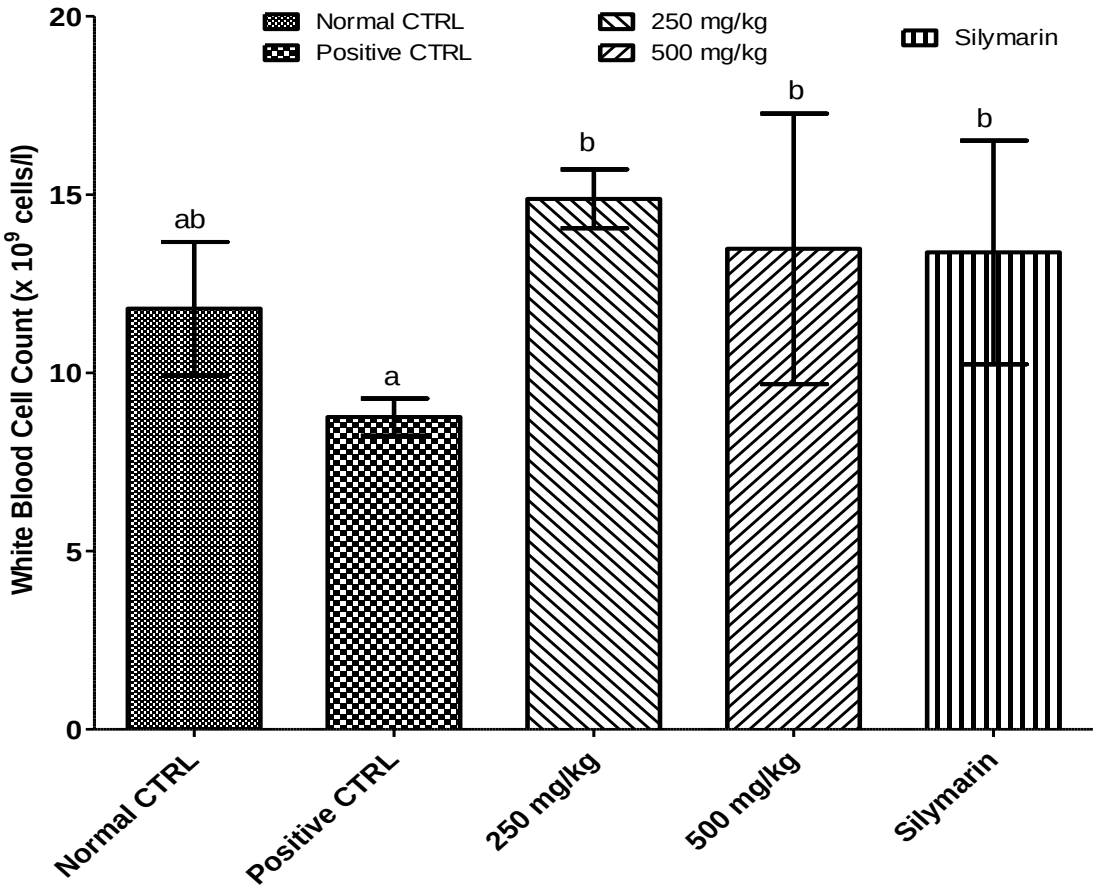


Figure 4.61: Effect of EECD on white blood cell count (WBC) in CCl_4 -induced oxidative stress. Bars bearing different letters are statistically significant ($p<0.05$)

Figure 4.61 shows a significant ($P<0.05$) reduction in WBC count of the positive control group compared to the normal, the EECD at 250 and 500 mg/kg as well as the silymarin treated groups. However, there was a non significant ($p\square0.05$) difference among the EECD treated at 250 mg/kg, the 500 mg/kg and the silymarin groups.

4.1.76 Effect of EECD on haematological parameters

Figure 4.62 shows the effect of EECD on platelet levels in CCl_4- induced oxidative stress.

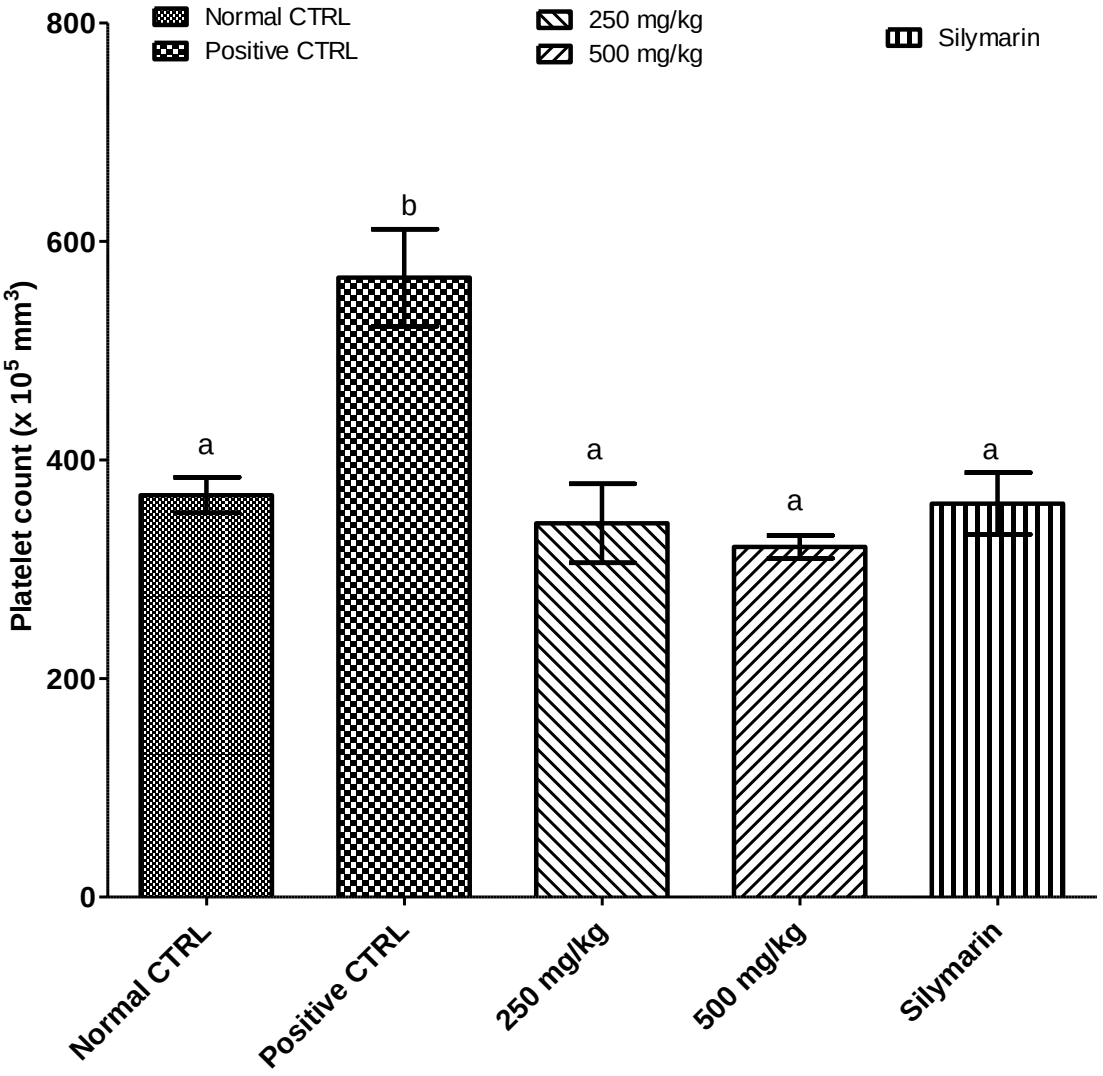


Figure 4.62: Effect of EECD on platelet levels in CCl_4- induced oxidative stress. Bars bearing different letters are statistically significant ($p<0.05$)

Figure 4.62 shows a significant ($P<0.05$) increase in the platelet level of the positive control group compared to the normal, both the EECD and the silymarin treated groups. However, there was a non significant ($p \square 0.05$) difference among the normal, the silymarin, the 250 and 500 mg/kg b.w EECD treated groups.

4.1.77 Histological studies on liver samples

A

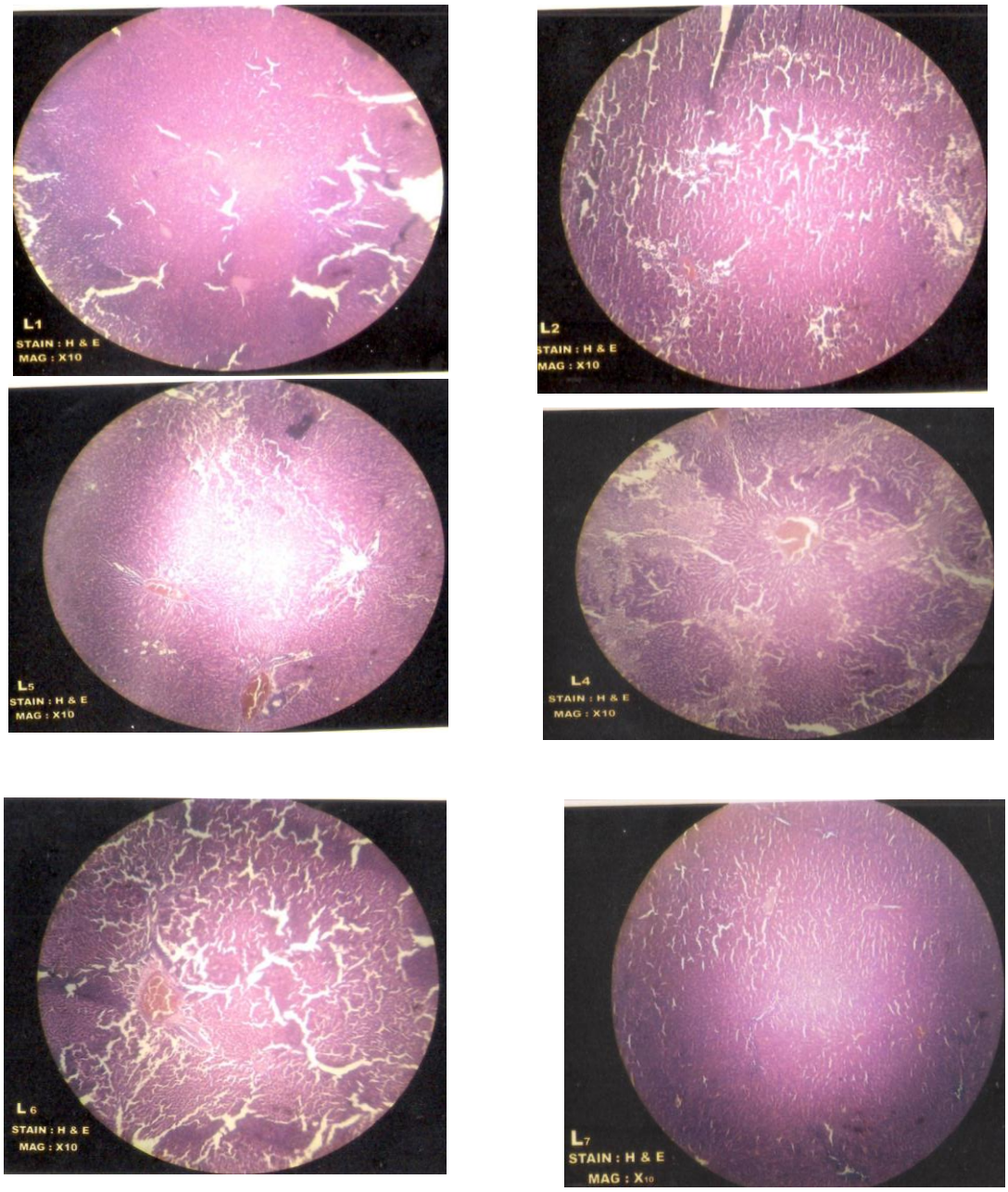


Figure 4.63: Light microphotographs of HE-stained sections of rat livers. L1=normal, L2= positive control, L4= 250 mg/kg, L5= 500 mg/kg, L6=saponin L7=Silymarin

Figure 4.63 reveals massive fatty change and centrilobular necrosis in most areas of the liver in the positive control compared to the normal control with normal hepatocytes, portal triads and vasculature. Also seen are mononuclear cell infiltration mostly macrophages and lymphocytes around the central vein and in the portal areas in the positive control group. Abnormal hepatocytes more severe than that found in the positive control group were obsevered in the saponin control. However, normal hepatocytes, portal triads and vasculature were obsevered in the 500 mg/kg b.w EECD and silymarin treated groups.

4.1.78 Histological studies on kidney samples

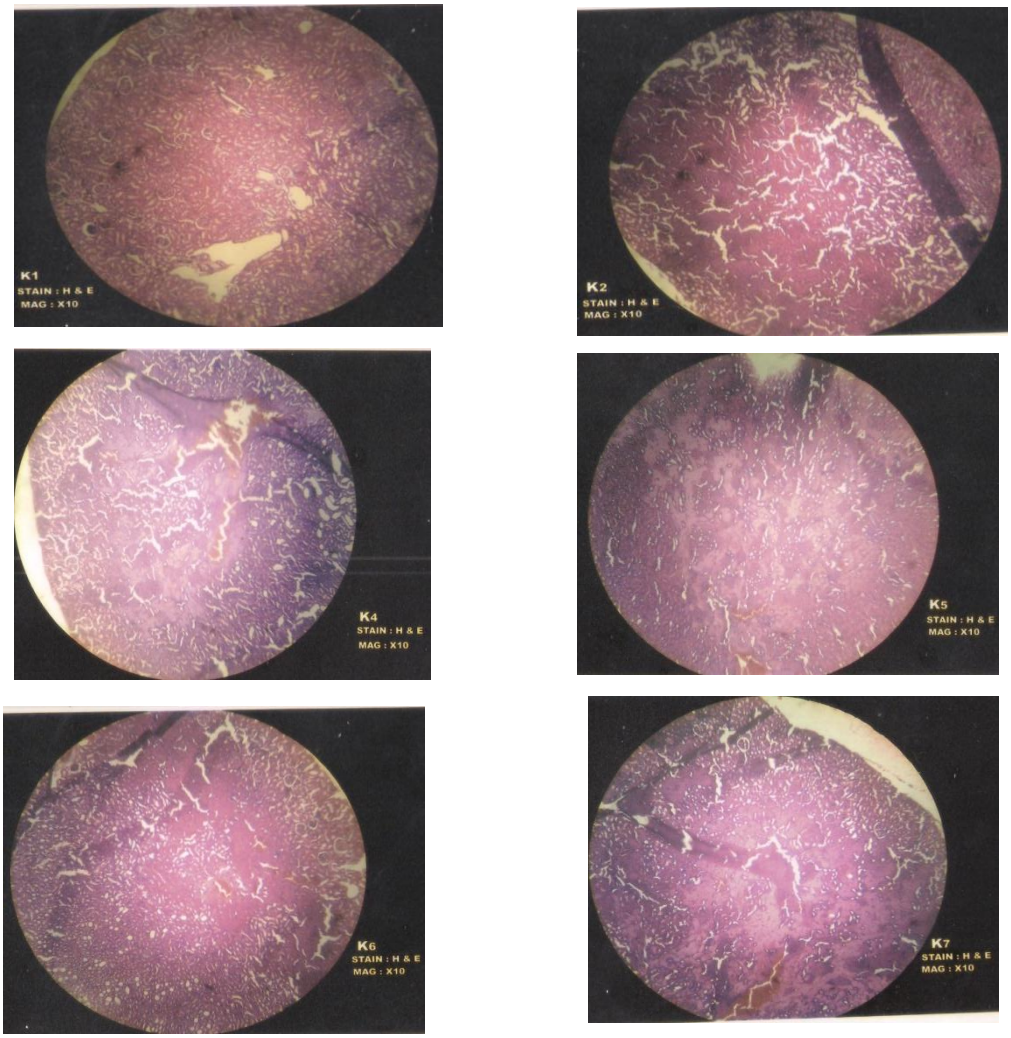


Figure 4.64: Light microphotographs of HE-stained sections of rat kidneys. K1=Normal, K2= positive control, K4= saponin, K5= 250 mg/kg, K6= 500 mg/kg, K7= Silymarin

The kidney section (Figure 4.64) of the normal group shows normal appearance of tubules, glomeruli, tubular and interstitial cells. However the kidney section of rat in positive control indicates glomerular and tubular degenerations, interstitial inflammation and oedema, congestion of the tubular cell and marked collagen deposition. Also shown was acute tubular necrosis. The saponin group indicated a more severe damage to the glomerular and tubular cells compared to the positive. However, normal glomeruli and tubular cells were obsevered in the kidneys of EECD treated rats with 500 mg/kg b.w EECD showing better protection.

4.2 DISCUSSION

The proximate, phytochemical, amino acid, vitamin and mineral compositions of *C. dolichopentalum* were determined. The *in vitro* and *in vivo* free radical scavenging and antimicrobial potentials of the plant leaves were also evaluated. Also the hepatoprotective, antinephrotoxic and antioxidant properties of the plants on CCl_4 induced toxicity in Wistar albino rats were determined. Furthermore, studies on the haematological and histological effects of the plants were used to evaluate the possible protection offered by ethanol extract of *C. dolichopentalum* (EECD) in Albino Wistar rats challenged with CCl_4.

4.2.1 Nutritional Composition of *C. dolichopentalum* leaves

Proximate composition

The nutritional composition of *C. dolichopentalum* leaves showed the presence of carbohydrates, protein, lipids, crude fibres, ash and moisture at appreciable percentages (Table 4.1). *C. dolichopentalum* contains fairly good quantities of carbohydrates, proteins and lipids than *Boerhavia diffusa* (10.56 %, 2.26 % and 1.16 % respectively) and *Commelina nudiflora* (5.67 %, 1.69 % and 1.44 % respectively) (Ujowundu *et al.,* 2008), as well as *Phyllanthus amarus* (45.52 %, 6.10 % and 6.03 % respectively) (Igwe *et al.,* 2007). The good distribution of nutrients in *C. dolichopentalum* was recommended to domestic animals (Ujowundu *et al.,* 2008). However, this is not so with *C. dolichopentalum* leaves, because domestic animals have been observed to avoid it. This may be as a result of its high alkaloid and saponin contents. Saponin has been observed to protect plants from protozoa and molluscs and other predator species (Lacaille – Dubois & Wagner, 2000). The plant leaves have a higher ash content (6.15 %) compared to *Spondias mombin* leaves (0.09±0.01 %), but less fibre and moisture content when compared to *S. mombin* leaves with 10.51±0.84 % and 15.13±0.57 % respectively. Ash content of a material is the residue

remaining after ignition at 500 – 600^{0}C for 2-4 hours. Thus it is a reflection of the mineral content of a plant.

Mineral composition

Minerals are known to play important metabolic roles in the living cells (Enechi & Odonwodo, 2003). Sodium and potassium were the major macroelements found in *C. dolichopentalum* leaves (Table 4.2) and are higher than those found in *S. mombin* leaves (Igwe *et al.*, 2010). Many functions of sodium and potassium are carried out in association with each other. Both sodium and potassium influence muscular activity and, are involved in acid-base balance, neuromuscular irritability and nerve conduction process. They are also important in cardiac function. Potassium is also needed as cofactor by some enzymes such as pyruvate kinase. However, magnesium, calcium, phosphorus, zinc and iron contents of the leaves of *C. dolichopentalum* were low when compared to some edible vegetables mentioned above. Iron, zinc and manganese strengthen the immune system possibly as either antioxidants or apoenzymes of enzymatic antioxidants. Other minerals identified include phosphate and chloride. Phosphorus plays a role in the formation of the structure of the bone and tooth. It is a component of membrane phospholipid, ATP, DNA and RNA. It is also intimately involved in energy metabolism, storage, regulation and as an intracellular buffering element.

Amino acid composition

Apart from being monomeric constituents of proteins and peptides, amino acids serve variety of functions. The total amino acid content of *C. dolichopentalum* leaves was 57.8 g/100g (Table 4.3). The R. chains of alanine, valine, leucine and isoleucine tend to cluster together within proteins; stabilizing protein structure by means of hydrophobic interactions. The hydroxyl group of tyrosine form hydrogen bonds. It is an important functional group in some enzymes. The amount of

tyrosine present in *C. dolichopentalum* is less than that of the aromatic amino acid, phenylalanine. Tyrosine forms thyroid hormones, epinephrine, norepinephrine and melanin.

Cysteine forms disulphide bonds which play a special role in the structure of many proteins by forming covalent links between parts of a protein molecule or between different polypeptide chains (Nelson & Cox, 2005). Both cysteine and methionine (1.70 g/100g) are also sources of sulphur. Glutamate, cysteine and glycine are used in the synthesis of glutathione. Acidic amino acids such as glutamate and aspartate (15.65 g/100g) are used for the synthesis of pyrimidines and purines. Glycine and cysteine are used as detoxicants of specific substances. Methionine in S – adenosylmethionine (active methionine) by transmethylation transfers methyl group to various substances.

Essential amino acids are not synthesised by the body and thus must be taken in diet, thus *C dolichopentalum* can be a good source of essential amino acids. *C. dolichopentalum* leaf was also observed to contain basic amino acids (6.95 g/100g). The most hydrophilic R groups are those that are either positively (basic) or negatively (acidic) charged. In many enzyme catalysed reactions, histidine residues facilitate the reaction by serving as a proton donor/acceptor. Histidine also in this capacity act as a buffer in plasma (Nelson & Cox, 2005).

Vitamin composition of the leaves

Results from the vitamin study showed the presence of vitamins A, E and the B-vitamins (B1, B2, B3, B6, B7, and B9) in the leaves in appreciable quantities (Table 4.4). Vitamins play important role as coenzymes in several energy yielding chemical reactions. Most water soluble vitamins are generally lost from the body rapidly because of the limited capacity of the system to store them. In the light of this, it is biochemically important for these vitamins to be consumed daily in diet. *C. dolichopentalum* leaves are rich in B-vitamins. All B-vitamins function as coenzymes in energy metabolism. Vitamin B1 (thiamine) acts as coenzyme to the enzymes: pyruvate dehydrogenase, α–

ketoglutarate dehydrogenase and transketolase in translocation reaction in hexose monophosphate pathway (HMP). Deficiency of vitamin B1 results in beriberi (Wardlaw *et al.*, 2004).

Vitamin B2 (riboflavin) acts as coenzymes in redox reactions, transferring hydrogen in various metabolic reactions that involve enzymes such as succinate dehydrogenase and glutathione reductase. Deficiency of vitamin B2 causes glossitis, heilosis, seborrheic dermatitis, stomatis and inflammation of the throat, eye nervous system disorders and confusion (Berg *et al.*, 2002).

Vitamin B3 (niacin) has two co-enzymes forms; nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$). NAD^+ and $NADP^+$ are active participants in oxidation-reduction reactions especially those involved in ATP generation. Examples can be seen in alcohol dehydrogenase, lactate dehydrogenase (LDH), malate dehydrogenase (MDH), G-3-P dehydrogenase, pyruvate dehydrogenase and α-ketoglutarate dehydrogenase reactions. Vitamin B3 deficiency produces a disease called pellagra, characterised by dermatitis, diarrhoea and dementia (FNB, 1998).

Vitamin B6 is actually a family of three compounds: pyridoxal, pyridoxine and pyridoxamine. Its active form pyridoxal phosphate (PLP) acts as coenzyme to enzymes involved in amino acid metabolism e. g aminotransferase, decarboxylases. PLP also plays a role in glycogenolysis. Deficiency leads to microcytic hypochromic anaemia.

Vitamin B7 (biotin) functions as the co-enzyme for carboxylases, which catalyse CO_2 fixation (carboxylation) in biological systems e.g acetyl CoA carboxylase, propionyl CoA carboxylase and pyruvate carboxylase. Deficiency is rare, but results in hair loss, skin rash, convulsions, neurological disorders and impaired growth.

Vitamin B9 (folate) exists in its active form called tetrahydrofolate which functions as a co-enzyme. It is concerned with one carbon metabolism, involving the transfer and utilization of one carbon moiety either as methyl ($-CH_3$), formyl (-CHO), formate (H-COOH), forminino group (-

CH=NH) or hydroxylmethyl (-CH_2OH) group. Deficiency results in megaloblastic (or macrocytic) anaemia and, neural tube defects in fetuses (Giovannucci, 2002; Green, 2002).

The plant leaf has an ample content of vitamin A, whose biochemical actions are involved in vision, growth and development of many types of tissues (mucus forming cells) and immunity. Its deficiency leads to night blindness, diminished neutrophils and follicular hyperkeratosis (Byers *et al.,* 2002).

Vitamin E (Tocopherol) was also found in appreciable amounts in *C. dolichopentalum* leaves. This indicates that treatment with extracts of the leaves can prevent peroxidation, help in reproduction in male organisms. Studies have shown that deficiency of vitamin E leads to loss of motility of sperms and impaired spermatogenesis in rats (Aitken & Roman, 2008).

4.2.2 Phytochemical Composition of *C. dolichopentalum*

Phytochemicals provide health benefits further than those attributed to macronutrients and micronutrients (Hasler & Blumberg 1999). Appreciable amounts of saponins, alkaloids, flavonoids and tannins were found in the leaves of *C. dolichopentalum* (Table 4.5). Saponins showed high concentration of tricogenin, sapogenin, tribuloin and saponine besides others. Saponins are known to exert anticholesterolemic and hypoglycaemic effect through intra-lumenal physiochemical interaction or other yet unidentified activity (Price *et al.,* 1987). Saponins have also been observed to protect plants from protozoans and molluscs and also to act as antifungal and antiviral agents (Lacaille Dubois & Wagner, 2000; Traore *et al.*, 2000).

The plant leaves contain flavonoids including apigenin, luteolin, kaempferol, isorhamnetin and cunercatin in appreciable amounts (Tables 4.6 and, 4.7; Appendices 4.1-4.2). At cellular levels, flavonoids have been found to exert a variety of biological effects (Middleton *et al.,* 2000), presumably mediated by specific interaction with molecular targets (Kanakis *et al.,* 2005; Williams *et al.,* 2004; Calabro *et al.,* 2005). The capacity of flavonoids to act as antioxidant

depends on their molecular structure. The position of OH groups and other features in flavones (apigenin and luteolin) and flavonols (kaempferol, isorhamnetin and quercetin) are important for their antioxidant and free radical scavenging activities. Apigenin and luteolin like most flavonoids are known to have antioxidant, anti-inflammatory and antitumour properties.

Luteolin and apigenin activate the dopamine transporter (Zhao *et al.*, 2010; Zhang *et al.*, 2010). Some studies have focused on the ability of isorhamnetin to attenuate diabetes complications, such as diabetic cataract, lipid peroxidation and high blood glucose levels (Bohm & Tod, 2007). *In vitro* studies have shown that apigenin may be toxic to red blood cells (Zbidah *et al.*, 2012). Like various other flavonoids, apigenin has been found to possess affinity for the opoid receptors, acting in the nanomolar range, as a non-selective antagonist of all three opoid receptors (Katavic *et al.*, 2007). Therefore, they can be employed to counter the effects of the three opoids during overdose.

Kaempferol is considered as a possible cancer treatment (Kim & Choi, 2003; Calderon-Montario *et al.*, 2011; Donnapea *et al.*, 2014). Kaempferol can help fight against cancer because it reduces the resistance of cancer cells to anti-cancer drugs such as vinblastine and paclitaxel (Batra & Sharma, 2013). Quercetin, a strong antioxidant, may induce insulin secretion by activation of L-type calcium channels in the pancreatic β-cells (Trauchimand *et al.*, 2010; Bardy *et al.*, 2003).

The physiological effects of alkaloids have made them important compounds in medicine. They have been used as pain killers (morphine), stimulants (caffeine), muscle relaxers (cocaine), tranquilizers (curare), anti-cancer (vincristine, Vinplastine), anaesthetics (cocaine), autiarrythmias (quanidine), Vaso-constrictors (ergonovine, ephedrine), antimalarial (quinine), poisons (tobocurarine, coniine, strychinine), pupil expander (atropine) and hallucinogenic drugs (mescaline) (Russo *et al.*, 2013; Kittakoop *et al.*, 2014; Cushine *et al.*, 2014; Qui *et al.*, 2014).

C. dolichopentalum leaves contain such alkaloids as akuamidine, crinanindine, voacangine, buphranidine and undulatine in relatively large quantity besides others (Table 4.8; Appendices

4.5-4.6). The concentration of alkaloids increase just prior to seed formation and then drops off when the seed is ripe, suggesting that alkaloids may play a role in this process.

Tannic acid (Table 4.10; appendices 4.7-4.8) was indentified in the plant leaves in high concentration. Tannins are known to tar the outermost layer of the mucosa (TCMD, 1993) and thereby render it less permeable and more resistant to chemical and mechanical injury or irritation. According to TCMD, (1993) tannins are used as astringent or antidote for various poisons and as a tropical haemostatic.

Phytate and oxalate were found in low concentrations in the plant leaves. Phytate is a hexaphosphate ester of inositol that is widely distributed in vegetables. It is considered an antinutrient because of the possibility of its interference with proteolytic digestion, in addition to the fact that the phosphorus in it is not nutritionally available to monogastric animals (Oboh *et al.*, 2005). It is considered an antinutritional factor because it complexes with nutritionally essential divalent cations like Ca^{2+}, Fe^{2+}, Mg^{2+} and Zn^{2+}, thus rendering them unavailable from the diet. It may therefore be advisable to use the leaves with mineral supplements. Results also showed that phytate concentration in the plant leaves was higher than that found in *Sphenostylis stenocarpa* (0.42 %); *Citrullus colocythis*, (0.64 %), *Pentochethra macrophylla*, (0.36 %); *Muanna flagellipes*, (0.33 %) (Ojiako *et al.*, 2010).

Oxalate like phytate, binds some divalent metal ions such as Ca^{2+} and Mg^{2+}, thereby interfering with their bioavailability. Ingestion of an excessive amount of oxalate could cause hypocalcaemia, muscular weakness or paralysis, development of urinary calculi, blockage of the renal tubules by calcium oxalate crystals and gastrointestinal irritation.

4.2.3 *In vitro* Free Radical-Scavenging Potentials of *C. dolichopentalum* Leaves

One of the key players in the production of oxidoreductive stress is reactive oxygen species.

Nitric oxide ($NO^{\cdot}$)

The results obtained in the *in vitro* nitric oxide scavenging potentials indicate that EECD is a better scavenger of nitric oxide radicals when compared to quercetin used as the standard compound (Figure 4.9) with a high correlation coefficient (R^2=0.9953). EECD nitric oxide scavenging ability followed a dose dependent manner, even at lower concentrations. $NO^{\cdot}$ radical is implicated in the inactivation and nitration of human superoxide dismutase. Nitrosative stress may lead to nitrosylation reactions that can alter the structure of proteins and so inhibit their normal function. Cells of the immune system produce both superoxide ($O_2^{\cdot -}$) and nitric oxide ($NO^{\cdot}$) during the respiratory burst triggered by inflammation processes. Under this condition, nitric oxide and $O_2^{\cdot -}$ may react to produce significant amount of oxidatively active molecule- peroxynitrite anion ($ONOO^{\cdot}$) which is a potent oxidising agent that can cause DNA fragmentation and lipid oxidation (Carr *et al*., 2000; Klatt & Lamas, 2000, Richnour *et al.*, 2004).

At IC_5, IC_{10}, IC_{20} and IC_{50}, EECD scavenged $NO^{\cdot}$ better at the respective concentrations (0.27 µg/ml, 0.069 µg/ml, 1.95 µg/ml and 12.04 µg/ml), compared to the standard quercetin at the following concentrations 5.35 µg/ml, 7.69 µg/ml, 11.45 µg/ml and 23.21 µg/ml respectively. $NO^{\cdot}$ scavenging effect may be due to the presence of such flavonoids as kaemferol, quercetin, catechin, isorhamnetin etc.

Reducing power potential

The study of Fe^{3+}/Fe^{2+} transformation in the presence of EECD leaves (Figure 4.10) proved that the EECD has a good reducing power (R.P 0.5_{AU}=29.95 µg/ml), compared to extracts of *Chromoleana odorata* (RP 0.5_{AU}=210.22 µg/ml) (Alisi *et al.*, 2008). RP 0.5_{AU} is the amount of extract to give 0.5 absorbance unit. The reducing power of EECD was dose dependent with a high correlation coefficient (R^2=0.9932). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Hsu *et al.*, 2006). Most antioxidant drugs

used in the treatment of oxidative stress related diseases have strong reducing powers (Dalton *et al.,* 2004).

Neuroprotection

Inhibition of lipid peroxidation in rabbit brain is analogous to neuroprotection. Transmembrane transporters (such as P-glycoprotein) are important components of the blood-brain-barriers or blood testis-barrier. Loss of function of the barriers can cause xenobiotics to cross the 'checkpoint' and induce undesired toxic effects in these otherwise well-shielded organs (Matheny *et al.,* 2001; Ayrton and Morgan, 2001; Arya *et al.,* 2006). Quercetin used as the standard phytochemical, inhibited lipid peroxidation in rabbit brain homogenate better than EECD leaves (Figure 4.11). Nevertheless, EECD prevented peroxidation in rabbit brain homogenate at 64.70 µg/ml (IC_{50}). The brain is particularly vulnerable to oxidative damage because of its high oxygen utilization, high content of oxidizable polyunsaturated fatty acids and the presence of redox-active metals (Cu, Fe). Polyphenols cross the blood-brain barrier and can its exert antioxidant and iron chelating properties in the brain (Levites *et al.,* 2001). A study by Moreira *et al.* (2005) showed that oxidative stress plays an important role in the neurodegeneration and progression of Alzheimer's disease. Therefore EECD may play a role in the treatment and management of neurodegenerative diseases.

Hydroxyl radicals (OH^-)

Hydroxyl radical (OH^-) scavenging effects of *C. dolichopentalum* extract were studied *in vitro*. EECD significantly ($P<0.05$) inhibited Fe^{3+}/ascorbate/EDTA/H_2O_2 system-induced deoxyribose sugar oxidation. At 92.39 µg/ml of EECD, the extract scavenged 50 % (IC_{50}) of OH^- radicals generated. This value compared better than the standard plant derived phenolic antioxidant quercetin (Figure 4.12). OH^- is formed by Fenton and Haber Weiss reaction and decomposition of peroxynitrite. Hydroxyl radical has a high positive activity, making it a very dangerous radical

with a short *in vivo* half life of approximately 10^{-9} seconds (Packer *et al.,* 2001). OH^- is one of the most damaging radicals in the body with the ability to attack and destroy DNA, proteins and lipids (Valko *et al.,* 2005). The OH^- scavenging capacity of the extracts of *C. dolichopentalum* was high enough to consider it as a potential antioxidant compound. The inhibition of deoxyribose degradation by EECD was dose dependent with high correlation coefficient (R^2=0.9922).

Dehydrogenase assay (DHA)

DHA is an effective primary test for the assessment of ecotoxicological impacts of environmental substrates such as toxicity of metals to planktonic (Nweke *et al.,* 2006), heterotrophic (Nweke *et al.,* 2007), bacteria and pathogenic organisms in wounds (Alisi & Onyeze, 2008; Ibegbulem, 2008; Alisi *et al.,* 2011). The viability of microorganisms is representeded by the total dehydrogenase activity, thus a reduction in total dehydrogenase activity is an indication of the bactericidal effect of the plant extract on the microorganisms. The toxicity of ethanol extract of *C. dolichopentalum* to isolates from degenerated wound, isolated stool and high vaginal swab were determined. Our result (Figures 4.13- 4.16) show that EECD leaves were very effective antimicrobial agents against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Streptococcus pneumonia* isolates. Threshold inhibitory concentrations of the extracts showed that EECD inhibited dehydrogenase activity in all the organisms in a dose dependent manner. Although the standard drug ciprofloxacin was more bactericidal. However, at 355.78 µg/ml, EECD achieved an IC_{50} against *E. coli* when compared to *Euphobia hyssopifolia* at 492.46 µg/ml. While at 2270.68 µg/ml EECD eliminated 100 % *S. typhi* compared to *E. hyssopifolia* which needed a concentration as high as 41047.0 µg/ml to achieve 100 % inhibiting concentration (Alisi & Abanobi, 2012).

Hydrogen peroxide (H_2O_2) scavenging ability of flavonoids, saponins, alkaloids and tannins

Hydrogen peroxide (H_2O_2) is an important reactive oxygen species, because of its ability to penetrate biological membranes and its ability to form hydroxyl radicals in cells. It reacts with

Fe^{++} or Cu^{++} ions (Gulchin *et al.,* 2003) in a reaction called Fenton-Haber Weiss reaction. However, the flavonoids, saponins, alkaloids and tannins precipitated from leaves of *C. dolichopentalum* scavenged H_2O_2 in a dose dependent manner (Figure 4.17). The scavenging ability of flavonoids was higher compared to those of saponins, alkaloids and tannins from *C. dolichopentalum*. Polyhydroxylated compounds such as flavonoids are known to possess high antioxidant activity. This activity could be due to flavonoid ability to absorb, neutralize and scavenge free radicals (Duh *et al.,* 1999). The presence of hydroxyl groups on the aromatic ring structure of flavonoid, also confer radical scavenging ability. The presence of kaempferol, quercetin, isorhamnetin, luteolin, and apigenin might have brought about the scavenging ability of H_2O_2 (Calderon-Montario *et al.,* 2011). This effect indicates the ability of *C. dolichopentalum* to minimize oxidative damage to vital tissues in the body. The low level of scavenging ability of alkaloid compared to flavonoids and tannins precipitated from *C. dolichopentalum* may be due to the solubility of alkaloids in the test medium and substrate used may influence the ability of a compound to scavenge different radicals (Yu *et al.,* 2002).

GC-MS analysis of bioactive constituents of *C. dolichopentalum* leaves.

Knowledge of the chemical constituents of plants is desirable not only for the discovery of new therapeutic agents, but also disclosing new sources of economic phytocompounds for the synthesis of complex chemical substances and determining the actual significance of folkloric remedies (Milne *et al.*,1993). The GC-MS analysis was used to indentify compounds with varying molecular weights, molecular formular and structures in the ethanol extract of *C. dolichopentalum* (Table 4.11a-b*)*. These compounds (cyclohexamine, caprolactam, phenol-2,6-bis(1,1-dimethylethyl)-4-methyl carbamate etc) have numerous medicinal properties:

Cyclohexamine belongs to an aliphatic amine class. It is used as an intermediate in the synthesis of other organic compounds such as sulphenamide: a base reagent used as an accelerator for

vulcanization. Cyclohexamine is also used as a building block for several pharmaceuticals e.g mucolytics, analgesics, and bronchodilators (Karsten *et al.,* 2005).

Caprolactam is a colourless cyclic amide of caproic acid. Almost all caprolactam produced goes into the manufacture of nylon-6. Nylon-6 is widely used in fibers and plastics (Josef *et al.,* 2005).

Phenol-2,6-bis(1,1-dimethylethyl)-4, methyl carbamate could be used to synthesize phenol-4-[2-(aminomethyl)-4-thiazolyl]-2,6-bis(1,1-dimethylethyl) monohydrochloride which is used for the treatment of Huntington's disease (Committee for Orphan Medicinal Products, 2015). 1-(4-Bromobutyl)-2-piperidinone is a chemical compound classified as lactam (a cyclic amide), used as an intermediate in the preparation of other chemicals.

FTIR spectra of *C. dolichopentalum* leaves

The spectra wavelength serves as a characteristic medium to elucidate the inherent functional groups and organic compounds in the extracts (Table 4.12, Figure 4.1). The peak value at 832.5578 $cm^{-1,}$ was assigned to C-Cl stretch of chlorine compound and peak height at 1026.785cm^{-1} was assigned CO stretch of ether compounds. The medium bands at 1257.005cm^{-1}, 1631.809cm^{-1}, 3404.961cm^{-1} were assigned NH stretch of amine compounds.

The weak bands at 1875.60cm^{-1}, and 2060.355cm^{-1} were assigned to CO stretch of unsaturated ester and carboxylic compounds. The peak values at 1453.996cm^{-1}, 2753.193cm^{-1}, 2855.609cm^{-1}, 2570.212cm^{-1} and 2664.229cm^{-1} were assigned to CH and SH symmetric stretch of methylene and thiol compounds. The broad band at 3065.483cm^{-1}, 3160.49cm^{-1}, and 3548.323cm^{-1} were assigned to OH stretch of primary (1^{o}) and tertiary (3^{o}) alcohol compounds.

Infrared (IR) detects transitions between vibrational levels of molecules, thus only selected frequencies (energies) of IR are absorbed by a molecule. On account of the different frequencies, information may be gathered about the nature of vibration (torsion, translation, valence or deformation vibration), about the participating atoms as binding partners (C-O, C-N, and C-H),

and about the nature of bonds (single, double, or triple bonds). This allows conclusions to be drawn on the molecular structure (Pavia *et al.*, 1982).

The IR spectra of eluates from column chromatography of EECD revealed important compounds (Figures 4.2 – 4.8). The interpretation of the IR spectra data gave D_{cd} eluate as 4-t-butyl-2(1-methyl-2-nitroethyl) cyclohexanone, with Rf value of 0.84, a molecular weight of 234 and an empirical formular $C_{13}H_{23}NO_3$. N-H stretch occurs in the range 3500-3300cm^{-1}(Figure 4.2)

Infrared spectrum of eluate F_{cd} indicated flurodecane (Figure 4.3). Infrared spectrum of alkanes is usually simple with few peaks. C-H occurs around 3000cm^{-1}. In alkanes (except strained ring compounds) absorption always occurs to the right of 3000cm^{-1}. Eluate G_{cd} was identified as phenol-2,6- bis (1,1-dimethyl ethyl)-4, methyl carbamate with the empirical formular $C_{17}H_{27}NO_2$ (Figure 4.4). Eluates I_{cd} and Jcd were identified as eicosane ($C_{20}H_{42}$) (Figure 4.5) and 2,7-dimethyl-1-1octanol ($C_{10}H_{22}O$) (Figure 4.6) respectively. Eluates K_{cd} and Mcd contained hexadecane ($C_{16}H_{34}$) (Figure 4.7) and 2-butyl-1-octanol ($C_{12}H_{26}O$) (Figure 4.8) respectively.

4.2.4 *In Vivo* Studies Using Ethanol Extract of *C. dolichopentalum* Leaves

The liver is the most important organ which plays pivotal role in regulating various physiological processes in the body. It is involved in metabolism, secretion and storage, detoxication and synthesis of metabolites. Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequences (Shahani, 1999). Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages (Dianzani, 1993). The liver is the major target organ of CCl_4 toxicity owing to its high content of cytochrome P-450 (Sodergren *et al.*, 2001). CCl_4 is converted through hepatic microsomal cytochrome P-450 into trichloromethyl free

radicals ($\cdot CCl_3$ or $\cdot CCl_3OO$) (Preethi & Kuttan, 2009), which in turn initiate lipid peroxidation process (Adewole *et al.*, 2007; Adewole *et al.*, 2012).

Due to the reactivity of carboxylic products, particularly α, β –unsaturated carbonyls of lipid peroxidation, are implicated in various types of cell damage, including tissue injuries, cell membrane destruction, depletion of glutathione, protein modification, disturbance of calcium homeostasis, retardation of respiration, enzyme inhibition and decreased DNA, RNA and protein synthesis (Esterbauer *et al.*, 1991; Schaur, 2003; Eckl, 2003; Zarkovic, 2003).

This study observed significant increase ($P<0.05$) in the concentration of MDA in rat group intoxicated with CCl_4 (positive control). This shows that CCl_4 was able to penetrate hepatocytes, cytosol, mitochondrial and the endoplasmic reticulum (ER) of hepatocytes which contain xenobiotic reducing enzymes (electron transfer chain and NADPH dependent reductase catalysed reaction) where it was metabolised. Numerous studies have shown that metabolism of CCl_4 is required for toxicity (Tomasi *et al.*, 1987; Kamal *et al.*, 2011). Early report of pathological effects of CCl_4 showed that the mitochondria were altered after CCl_4 poisoning of rat (Christie & Judah, 1954; Albano *et al.*, 1985). Two important features characterising lipid peroxidation and distinguishing it from the oxidation of other cellular components are, firstly, propagation of a chain reaction, which spreads the damage across biomembranes. Secondly, products arising from lipid peroxidation (toxic aldehydes) can be equally reactive as the original ROS themselves, causing damage to cells by additional mechanisms (Boelsterli, 2007).

The group of rats intoxicated with CCl_4 and pretreated with EECD showed decreased MDA concentrations, this is in line with the work done by Ujowundu *et al.* (2011). The reduction of CCl_4 induced oxidative stress by the anti-lipid peroxidative activity of the EECD, might be due to the extract ability to prevent the penetration of CCl_4 into the liver cells, thus preventing the activation of the toxicants, since activation of CCl_4 is required for lipid peroxidation to occur

(Kamal *et al.*, 2011). The appreciable concentration of tannins in EECD may be responsible for this action. Tannins are known to 'tar' the outermost layer of the mucosa (Asuzu & Onu, 1990), rendering it less permeable and resistant to chemical or mechanical injury or irritation. The effect of pre-treatment of the animals with various phytochemicals (flavonoids, alkaloids, saponins, tannins (FAST)) precipitated from *C. dolichopentalum* leaves indicate that alkaloids and flavonoids extracts showed better inhibitory effect against the activation of CCl_4. Pre-treatment with these precipitates might have prevented lipid peroxidation in the liver of the test organism, reducing MDA concentration in these groups.

Glutathione (GSH), together with its coupled enzyme is one of the most important antioxidant defence lines in the body. Upon need, the level of GSH can become up regulated; this is indicated by an increase in NADPH generation. The importance of GSH becomes particularly evident when the redox balance is disrupted due to excessive GSH consumption as shown in the positive control; this greatly facilitated the development of toxicity caused by pro-oxidant xenobiotics (Bellomo *et al.*, 1992). Lipid peroxidation response to glutathione depletion was alleviated significantly ($P<0.05$) by the ethanol extract of *C. dolichopentalum* in a dose dependent manner just as the standard drug (silymarin). This may be attributed to the ability of the EECD to keep the cellular thiol redox status in the reduced form.

Glutathione peroxidase (a selenium-containing enzyme) is the most important enzyme for the extraperoxisomal inactivation of H_2O_2 (Kaplowitz *et al.*, 1991). A reduction in GSH will in turn reduce the activity of GPx as is evident in the intoxicated but untreated group (positive control). This is because GSH is a substrate for GPx as it provides the electrons needed to reduce H_2O_2 or hydroperoxides (Boelsterli, 2007). Treatment with the plant extract was able to correct this anomaly, possibly by providing scavengers for ROS. Ujowundu *et al.* (2012; 2014) also reported a restoration of GPx activity after intoxication with CCl_4 and subsequent treatment with plant

extract. The tannin group showed a more significant (P□0.05) effect in increasing the activity or expression of GPx compared to the flavonoid, saponin and alkaloid control groups.

Several lines of evidence support the assumption that removal of superoxide by SOD is indeed a detoxication reaction (Gunawardhana *et al.*, 1993). Basically, the cytosolic form (Cu, Zn - SOD) is more susceptible to the preoxidants (Wheeler *et al.*, 2001).

$$O_2^{\cdot -} + O_2^{\cdot -} \xrightarrow[SOD]{2H^+} H_2O_2 + O_2$$

While the mitochondrial form of SOD can be induced under oxidative stress, the activities of SOD and catalase decrease significantly ($P<0.05$) in the group of rats intoxicated with CCl_4 only. This decrease in the antioxidant enzyme activity might be attributed to the exhaustion of these antioxidants enzymes. In enzyme catalysed reaction, a short initial phase (pre-steady state or burst), where the enzyme – substrate complex is formed and free enzyme decreases, the turnover rate (enzyme activity) is low in this phase, according to the principle of mass conservation. Total enzyme is the summation of the bound and free enzyme complex (Bisswanger, 2008). As free enzyme reduces, the enzyme activity drops. This may explain the observed decrease in the activities of SOD, CAT, and GPx in oxidatively stressed system.

The pretreated groups showed increases in SOD and CAT activities probably due to the presence of flavoniods such as isorhamnetin, apigenin, luteolin, kaemferol and quercetin in EECD; these are hydroxylated compounds that act as scavengers of ROS and RNS. The capacity of flavonoids to act as antioxidant depends upon their molecular structure. The position of hydroxyl group and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities.

The non significant increase in SOD activity in the positive control group observed in the one week study might be as a result of induction of mitochondrial SOD in oxidative stress (Wheeler *et*

al., 2001). The alkaloid control group expressed higher SOD activity compared to the flavonoid, saponin and tannin control groups. The significant (P□0.05) increase in catalase activity in the flavonoid control group may be due to the presence of kaemferol, which has been shown to induce the expression of catalase (Calderon-Montario *et al.*, 2011).

Rats intoxicated with CCl_4 showed significant ($P<0.05$) reduction in vitamin C and E concentrations. Although, a vitamin to man, guinea pigs and monkeys, vitamin C is not a vitamin to rats since rats possess the enzyme L-gluconolactone oxidase needed to convert ketogluconolactone to ascorbic acid in the uronic acid pathway. Its deficiency is manifested in scurvy, fragile capillaries, poor dentine formation in children and delayed wound healing due to deficient formation of collagen (Chatterjea & Shinde, 2007). Decrease in vitamin C and E concentrations can be linked to depletion of the antioxidants as a result of oxidative stress caused by CCl_4.

Vitamin C and E concentrations were restored in the rat groups pre-treated with EECD before intoxication with CCl_4. This evidently shows the antioxidant properties of the extract against ROS. Vitamin C functions synergistically with other antioxidants. Alpha-tocopherol consists of a phenolic group which can reduce lipid peroxyl radicals. It has been claimed to be the most important lipid – soluble antioxidant. Its hydrophobic nature dictates its location exclusively in the cell membrane and that it protects membranes from oxidation by reacting with lipid peroxyl radicals (Harrera & Barbas, 2001; Traber & Atkinson, 2007). This reaction produces oxidised α – tocopheroxyl radicals that can be recycled back by ascorbate, which in turn is kept in its reduced state by GSH (Zinggy & Azzi, 2004; Azzi, 2007; Boelsterli, 2007).

Alanine aminotransferase (ALT) has been considered liver specific. In rat liver, ALT is found almost exclusively in periportal hepatocytes, and this is consistent with the enzyme role in

gluconeogenesis. Although, aspartate aminotransferase (AST) is less specific for liver injury compared to ALT, it is also expressed in the liver and considered as a maker for hepatic parenchymal injury induced by xenobiotics (Amacher, 1998). CCl_4 intoxicated rats showed a significant ($P<0.05$) increase in aminotransferases, compared to the normal group. After damage to hepatic parenchymal cells, the enzyme leaks into the serum and peak activities are found 24 to 48 h after a toxic insult (Mukherjae, 2002; Igwe *et al.,* 2008, Alisi *et al.,* 2008; Ujowundu *et al.,* 2011). Decrease in activities of the transaminases of EECD pre-treated rats compared to untreated group (PC) indicates stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl_4. This effect may be as a result of the tannin content of the plant, which renders the membrane less permeable to chemical injury (Asusu & Onoh, 1988). This effect is in agreement with the commonly accepted view that serum levels of transaminases return to normal with healing of hepatic parenchymal and the regeneration of hepatocytes (Thrabrew *et al*., 1987).

Saponin control showed a significant ($P\square0.05$) elevation in ALT activity compared to positive control. It appears that the saponin precipitate disrupted the plasma membrane before intoxication with CCl_4, thus a greater damage was exerted on the liver by saponin from *C. dolichopentalum*. This could be the reason animals do not graze on *C. dolichopentalum* leaves. However, treatment with flavonoid and alkaloid from *C. dolichopentalum* stabilized the plasma membrane as well as prevented hepatic tissue damage caused by the toxicant. Tannins from *C. dolichopentalum* protected against AST infiltrating the peripheral blood.

The extent of liver damage was further assessed by estimating serum levels of total protein (T.P), albumin, globulin, total bilirubin (T.B) and total cholesterol (T.C). Result showed a significant ($P<0.05$) decrease in T.P, albumin, and globulin concentrations, and increase in T.C and T. B in CCl_4 intoxicated rats (PC) when compared with the control (NC). A fall in protein and albumin concentrations has been reported in severe parenchymal liver damage, especially from poisoning

with CCl_4 (Saad *et al.*, 2014; Sarhan *et al.*, 2012). This reflects synthetic incapability of the liver of rats intoxicated with CCl_4. Thus this will alter the liver's ability to synthesis plasma protein that will be actively involved in fluid exchange, binding and transport function, buffering action and enzymatic activities (Chatterjea & Shinde, 2007).

The result of this study showed that rats in the tannin group showed a higher concentration of total protein compared to the flavonoid, saponin and alkaloid controls. It was also observed that total protein concentration increased dose dependently with the EECD. Decrease in albumin concentration, leads to oedema formation. Although tannin control gave a higher concentration of albumin compared to the other phytochemicals, it was however observed that the EECD in a dose dependent manner produced a higher albumin concentration.

A decrease in globulin concentration was observed in the positive control group compared to the normal group. Decrease in globulin concentration could be a response to toxic insult. Alpha-globulins are glycoproteins. They probably serve to carry needed carbohydrate constituents to the site of tissue repair following hepatic or nephrotic injury. α-Globulin like cearuloplasmin is a copper containing protein. It mainly functions as a ferroxidase and helps in oxidation of Fe^{2+} to Fe^{3+} which can be incorporated into transferritin. This can be a mechanism to reduce the bioavailability of Fe^{2+} concentration, thereby preventing Fe^{2+} from catalysing the production of hydroxyl radical which would enhance the already deranged system. Also, hemopexin, a β-globulin binds and removes circulating heme which is formed in the body, from the breakdown of haemoglobin, myoglobin or catalase (Chatterjea & Shinde, 2007).

An increase in globulin concentration was observed in the saponin control compared to the normal and positive controls. Globulin concentration increases in inflammatory processes (Chatterjea & Shinde, 2007). Also hemopexin a β-globulin binds and removes circulating heme which is formed in the body from the breakdown of haemoglobin, myoglobulin or catalase (Chaterjea & Shinde,

2007). Pretreatment with EECD caused a reversal of an upward increase in total protein and albumin concentrations and a restoration of globulin level.

A drop in the albumin – globulin ratio reflects hypoproteinemia as observed in the intoxicated group compared to the normal group. This could be as a result of losing albumin in urine in nephrotic syndrome, decreased synthesis of albumin due to cirrhosis of the liver or non availability of the precursors for albumin synthesis. Treatment with EECD like that of silymarin caused a rise in the A:G ratio, showing protection of the liver from toxic assault probably owing to its phytochemical constituent.

The higher A:G ratio of the EECD compared to the flavonoid, saponin, alkaloid, and tannin controls confirms the fact that protein hemostasis can be maintained better with the crude extract than with the individual phytochemicals.

There was a significant increase in the concentration of total bilirubin in rats treated with CCl_4 only compared to normal control. The increase in total bilirubin concentration reflected the observed increase in the breakdown of haemoglobin or other heme containing proteins such as myoglobin, catalase and cytochrome. It is also indicative of the liver's inability to remove bilirubin by glucoronidation due to inhibition of binding, conjugation and excretory capacity of hepatocytes (Deepak *et al.*, 2000; Mankani *et al.,* 2005). Failure of a damaged liver to conjugate bilirubin may be due to inactivation of the enzyme glucoronyl transferase which catalyzes the transfer of glucoronic acid from UDP – glucoronic acid to bilirubin. However, pretreatment with the extract protected heme containing proteins from oxidative damage. The reduction suggests that EECD possesses liver protective activities. This reduction is in line with the work of Rosalinda *et al.* (2009) and Tsala *et al.* (2010) who showed that plant extracts can cause a reduction in total bilirubin by protecting heme containing proteins. The EECD perhaps prevented the destruction of haemoglobin or aided the liver by enhancing bilirubin uptake and conjugation by the liver and

subsequent secretion into the bile ducts. Also, the alkaloids precipitated from *C. dolichopentalum* better prevented the destruction of heme containing proteins as well as enhanced bilirubin uptake, conjugation and subsequent excretion by the liver compared to the flavonoid, saponin and tannin groups.

An example of β-globulins is lipoprotein such as chylomicron which transports exogenous triglyceride (TG), phospholipids, cholesterol-esters, cholesterol and fat soluble vitamins from the intestine to the liver. Very low density lipoprotein (VLDL) also acts as carrier of endogenous TG in hepatic cells from the liver to the extrahepatic tissues for storage. β-Globulins (LDL) transport and deliver cholesterol to extrahepatic tissues and scavenge the body's cholesterol and blood vessel wall cholesterol by reverse cholesterol transport. 'Mature' HDL moves cholesterol to liver where cholesterol is catabolised. Thus a fall in β–globulins or hypolipoproteinemia could lead to inability of the liver to package and transport TG and VLDL-cholesterol as indicated by the reduced concentrations of TG and VLDL. The main apo-protein in HDL-cholesterol is apo-protein A and C, while in VLDL-cholesterol is apo-protein B100.

Normal levels of lipids in the liver are the result of maintenance of a proper balance between factors that tend to increase and decrease the fat content of the liver. CCl_4 is involved in the production of fatty liver as shown by the result of TG and VLDL-cholesterol in the intoxicated group compared to the normal. CCl_4 produces fatty liver by the following mechanisms: interference with synthesis of apo-protein required to be incorporated in lipoprotein complex, interference with the secretory mechanism itself or interference with conjugation of the lipid moiety with lipoprotein apo-protein (Chatterjea & Shinde, 2007). Pre-treatment with EECD at 500 mg/kg b.w prevented a drastic drop in TG and VLDL-cholesterol. However, the silymarin group gave a better result. Also, TG and VLDL-cholesterol concentrations were significantly ($P \square 0.05$) increased in the alkaloid control group compared to the intoxicated group. This shows synthesis of

lipoproteins, effective formation of lipoprotein complex and subsequent secretion into the peripheral blood.

LDL-cholesterol is a degradation product of VLDL-cholesterol and is therefore rich in cholesterol and cholesterol esters. The intoxicated group showed a significant ($P<0.05$) increase in LDL-cholesterol compared to the normal. LDL-cholesterol is the principal factor in promoting atherosclerosis. During this condition, cholesterol deposited under the endothelial cells, subsequently undergoes oxidation by free radicals released from the endothelial cells. Local elevation of lipid peroxide can lead to thrombosis or occlusion of narrow vascular lumen by superimposed thrombus. The treated group recorded a significant reduction in LDL-cholesterol compared to the positive control. This may be due to increased LDL-cholesterol uptake by the peripheral cells. Protection of LDL – receptors on liver cells are important for LDL uptake and endocytosis. Triiodothyronine enhances LDL-cholesterol mobilization by LDL-receptors. *C. dolichopentalum* leaf extract contains antioxidants such as isorhamnetin that prevent oxidation of LDL-cholesterol in the blood stream.

The results of this study show an increase in HDL-cholesterol concentration in the intoxicated group compared to the normal. However, this increase is not accompanied by a decrease in LDL-cholesterol. In the one week prophylactic study, increased HDL-cholesterol not associated with a decrease in LDL-cholesterol concentration was observed in the positive control group. However an increase was seen in the concentration of HDL-cholesterol in the tannin control group compared to the flavonoid, saponin and alkaloid control groups.

HDL-cholesterol has been demonstrated to influence the binding and uptake of LDL-cholesterol by the peripheral cells. Thus the ratio of LDL-cholesterol and HDL- cholesterol is important in defining cardiovascular risk (Millán *et al.*, 2009; Pereira, 2012). Our results show a very significant ($P<0.05$) increase in the LDL/HDL ratio in positive control rats compared to the

normal control rats. A high value is associated with cardiovascular risk (Pereira, 2012). LDL/HDL ratio of treated groups showed low values compared to the positive control.

Furthermore, our results showed an increase in total cholesterol concentration in the positive group compared to the normal. An elevation of the total cholesterol in plasma is considered a prime risk factor for coronary heart disease (CHD) (Verschuren *et al.*, 1995; Pereira, 2012). The liver plays an active role in the metabolism of cholesterol including its synthesis, esterification, oxidation and excretion. The increase in total cholesterol level may not be due to increased synthesis of cholesterol in the liver but rather due to the presence of altered receptors which prevent normal removal of cholesterol from the blood (entry of cholesterol into peripheral and liver cells occurs by receptor – mediator endocytosis) (Nykjaer & Willnow, 2002; Alberts *et al.*, 2002; Gent & Braakman, 2004).

The reduction in the rate of plasma cholesterol uptake mediated by these receptors thus causes an abnormal elevation of plasma cholesterol. The level of receptors on peripheral and hepatic cells can also be influenced by other factors, such as hormones (especially thyroxine), dietary cholesterol intake, and dietary fiber or non absorbable bile acid-binding resins (given as part of therapy). Overproduction or over release of cholesterol by the liver may also be responsible for hypercholesterolemia. This could result from an altered structure or regulation of hydroxyl–methyl–glutaryl Co-A (HMG-CoA) reductase (so that cholesterol feedback inhibition is less effective), or over production of triglycerides and, apo–protein B. Decreased production and excretion of bile acids and cholesterol through the bile can also lead to hypercholesterolemia (Linder, 1991).

The LDL-cholesterol binding to the liver cells is enhanced by insulin and triiodotyronine (T3) whereas glucocorticoids such as dexamethasone have the opposite effect. These effects are probably mediated through the regulation of apo – B degradation that explains the hypercholesterolemia and increased risk of atherosclerosis (Raju & Madula, 2005).

Renal function

The ability of the kidneys to produce urea and maintain salt and water as well as acid base balance was assessed by estimating the serum concentrations of urea, K^+, Na^+, Cl^- and HCO_3^-.

The measurement of urea concentration is an important investigation in diagnosing kidney damage and its cause. A marked increase in urea as shown in the positive control group is indicative of damaged renal function. Slight increases in urea may occur when there is any condition associated with increased protein breakdown among other reasons or inability of the kidneys to effectively excrete urea. EECD offered protection to the kidney as is evident in the dose dependent reduction of serum urea concentration. Furthermore, pretreatment with alkaloids and flavonoids appeared to stimulate the kidney to effectively excrete urea, resulting in lower concentration of urea in the alkaloid and flavonoid control groups.

Sodium, potassium and chloride are the main electrolytes responsible for fluid osmolarity (osmotic pressure); they also influence ionic strength and thus, the solubility of proteins and other substituents.

Na^+ is the main regulator of the osmotic pressure in the body fluids. Severe salt depletion can occur due to increase in diarrhoea, vomiting and abnormal increase in urine excretion due to reduced aldosterone production. A severe loss of H_2O and salt from the body can lead to a state of shock and low blood pressure, muscular weakness and wrinkled skin. Loss of water and electrolyte is characterized by reduced Na^+, increased urea, raised PCV and haemoglobin and decreased plasma proteins (Chatterjea & Shinde, 2007). In kidney dysfunction, Na^+ is not reabsorbed and is thus excreted in the urine (Chatterjea & Shinde, 2007). A primary loss of NaCl as shown in the positive control usually results in hyponatremia, dehydration and decreased extracellular fluid (ECF) volume. Conditions that can cause hyponatremia owing to loss of NaCl

include diarrhoea and vomiting; or excess water retention will dilute the sodium in the ECF (Raynolds & Seckl, 2005; Loh & Verbalis, 2008).

The flavonoid and alkaloid control groups had sodium ion concentrations very close to that of the normal control. The *C. dolichopentalum* leaf extract contains the alkaloid sparteine which has been shown to be a sodium channel blocker (Körper *et al.*, 1998), and thus posseses a potential to regulate Na^+, this can be to a beneficial effect in reducing blood pressure.

Chloride levels were significantly elevated in the flavonoid, saponin, alkaloid, and tannin control compared to both the intoxicated group and positive control group. Chloride levels are elevated in nephritis, eclampsia and anemia (TCMD, 1993). This indicates that *C. dolichopentalum* leaf extract might be an effective chloride regulator; its individual phytochemicals might not however as shown in the result (Figure 4.55).

The concentration of electrolytes depends on the degree of tubular reabsorption and secretion required to maintain electrostatic equilibrium. The positive control group showed significant ($P<0.05$) increase in plasma volume of K^+. This could be as a result of the inhibition of the activity of the Na^+/K^+ pump. Any substantial increase in the extracellular (EC) concentrations of K^+ will lower the chemical gradients for K^+ and, thus the membrane potential, causing a depolarization of the membrane. If EC $[K^+]$ rises too far, this will interfere with normal heart and nervous function. The tannin extract group showed a better effect as K^+ regulator compared to flavonoid, saponin and alkaloid groups. Sparteine, an alkaloid has also been shown to be an antiarrythymic agent (Körper *et al.*, 1998). Thus the high concentration of K^+ observed in the alkaloid control group shows that the presence of alkaloids other than sparteine could be antagonistic to its action as antiarrythmic agent or K^+ regulator. EECD pretreated rats showed maintenance of electrolyte balance in the face of CCl_4 intoxication.

Maintenance of the pH of blood (7.35–7.45) is one of the prerequisites of life and relatively small changes in pH value of blood can lead to severe metabolic consequences. The primary buffers in blood are heamoglobin in the erythrocytes, and bicarbonate ion (HCO_3^-) in the plasma. Bicarbonate buffer system is the chief buffer of blood and also constitutes the alkali reserve (Chatterjea & Shinde, 2007). Neutralization of strong and non-volatile acids (such as HCl, H_2SO_4, lactic acid) entering the ECF is achieved by the bicarbonate buffers. Strong and non-volatile acids are converted into weak and volatile acids; H_2CO_3 thus formed is diffusible, and eliminated by diffusion of CO_2 through the alveoli of the lungs.

The CCl_4 treated group showed a significant ($P<0.05$) increase in [HCO_3^-] a condition which might lead to alkalosis. The condition is characterized by an absolute or relative increase in [HCO_3^-]. Primary alkali excess or increase in the alkali reserve is the most frequent cause of clinically observed alkalosis. However, the treated group did not show maintenance of acid- base balance, but an increase in alkali reserve in a dose dependent fashion. In other words, the EECD and the silymarin groups might have stimulated a compensatory mechanism that increased pulmonary respiration. This increased ventilation would result in CO_2 loss and reduction in [H_2CO_3] with subsequent increase in [HCO_3^-], increasing NH_3 formation, H^+ excretion compared to K^+ excretion in distal tubule and HCO_3^- reabsorption. The treated groups rather acted like they were exposed to metabolic acidosis (a reduction in plasma level of HCO_3^-).

Intracellular redox active transition metals notably Fe^{2+}/Fe^{3+} and Cu^+/Cu^{2+} have been implicated in catalyzing oxidative stress due to their Fenton activity (Carr & Frei, 1999: Boelsterli, 2007). Thus, iron chelators like desferroxamine (DFO) preclude binding of H_2O_2 to catalytically active iron and thus inhibit the formation of hydroxyl radicals (Sakaida *et al.,* 1995). The results as shown in Figure 4.53 recorded a significant ($P<0.05$) increase in iron concentration of the positive control group compared to the normal. Within the cells, the highest concentration of free, redox-active

iron is found in the lysosomes. If an oxidant damages the lysosomal membrane, then iron leaks out into the cytosol, where it can participate in Fenton reactions and greatly aggravate oxidative damage (Boelsterli, 2007).

Treatment with both concentrations of EECD caused a significant ($P<0.05$) reduction of iron concentrations of the liver in a dose dependent manner. If lysosomal degradation of metalloprotein is inhibited, then oxidant stress induced cell injury can be greatly diminished. Because intracellular iron and other catalytically active transition metals are potentially dangerous to a cell, they are either tightly bound to proteins or low-molecular ligands or sequestered in specific pools (Boelsterli, 2007). It appears that EECD enhanced the sequestering of iron judging from the lower concentration of iron in the treated group compared to the normal.

Zinc is an important constituent of proteins, yet would be highly toxic if the free concentrations of zinc in the cell increased beyond a certain critical level. A significant ($P<0.05$) increase in zinc concentration was recored in the positive control group (Figure 4.57) compared to the normal. This indicates that toxicants could interfere with normal zinc homeostasis. Toxicants indirectly displace zinc from certain key proteins or directly by causing bioavalability of exceedingly high intracellular zinc concentration which induces a stress response. This can either activate or disrupt signalling pathways. If via multiple parallel pathways, xenobiotics can shift the overall equilibrium towards an unfavourable response (Andrew, 2000). Increasing cellular [$zinc^{2+}$] levels activates the metal sensor, MTF-1 (metal-response-element-binding transcription factor) which transmigrates in the nucleus and activates genes involved in the homeostasis of metals, such as those of metallothionein (MT), metal efflux carriers and antioxidant enzymes (enzymes involved in GSH synthesis). Pretreatment with the EECD prevented the disruption of zinc homeostasis significantly ($P<0.05$).

Haematology

The total mass of red blood cells (RBC) in the circulatory system is regulated within a narrow limit, thus adequate red cells are always available to provide sufficient transport of oxygen from the lungs to the tissues. The cells do not become so numerous that they can impede blood flow. Any condition that causes the quality of oxygen transported to the tissues to decrease ordinarily, increases the rate of RBC production as seen in the CCl_4–intoxicated rats of positive group in this study. Destruction of major portions of bone marrow by any means: various diseases of the circulation that cause decreased tissue blood flow might result in hypoxia. Hypoxia causes a marked increase in erythropoietin production and the erythropoietin in turn enhances RBC production until the hypoxia is relieved (Alleyne *et al.*, 2008). Compared to the positive control, the treated group was able to offer little protection against secondary polycythaemic condition. The silymarin group however offered a better protection.

Compared to the normal control, the positive control group showed a significant ($p<0.05$) increase in haemoglobin concentration. Many compounds of diverse structure including insecticides, carcinogens and others, when administered to a mammal can result in marked increase in hepatic δ–ALA synthetase (δ–amino laevulinic acid synthetase; the main rate – limiting enzyme in the synthetic pathway of porphorins). This occurs because most of the drugs are metabolized by cytochrome P450. During metabolism of CCl_4, consumption of haem by cytochrome P450 is greatly increased; this in turn diminishes the cellular concentration of haem, leading to derepression of δ–ALA synthetase with a corresponding increase in ratio of haem synthesis (Chatterjea & Shinde, 2007). Furthermore, the increase in RBC may also result in an increase in the synthesis of haemoglobin to fill the RBC (Guyton & Hall, 2011). Haemoglobin is measured to detect anaemia and its severity. This is because haemoglobin carries oxygen from the lungs to the tissues.

The haematocrit (HCT) or the packed cell volume (PCV) is used to calculate the mean cell haemoglobin concentration (MCHC) and mean cell volume (MCV). These red cell indices are used in the investigation of anaemia when it is not possible to measure haemoglobin, to diagnose polycythaemia vera, and to monitor its treatment. PCV values are increased in all forms of polycythaemia. The PCV values of the intoxicated group are significantly higher than those of the normal control. PCV is that property of the whole blood occupied by red cells. Thus if the positive control group recorded a high RBC and Hb values, it is only rational that the PCV values of the intoxicated group increased. This may not be as a result of healthy condition, but only a response to poor delivery of oxygen from the lungs to the tissues.

There was also a drop in the MCHC, MCV and MCH values in the positive control group compared to the normal. Low MCHC values are found in iron deficiency anaemia and other conditions in which the red cells are microcytic and hypochromic. The MCV values reflect the size of the red blood cell, while MCH and MCHC reflect the haemoglobin content of the blood cells. RBC indices are therefore used to diagnose types of anaemia. Anaemia is defined based on cell size (MCV) and amount of haemoglobin concentration. The values obtained for RBC count in the EECD treated rats indicate that the extract tend to balance the irregularities in the RBC cell count.

The liver harbours a large percentage of resident macrophages (Kupffer cells) and specific resident T cells (YδT cells) that have been implicated in certain forms of immune cell – mediated toxicity induced by xenobiotics. The results of this study showed a drop in the white blood cells (WBC) count. A significant decrease was recorded in the WBC of the positive control group compared to that of the normal. The effects of xenobiotics which can modulate the function of immune system at several levels can have two opposing consequences. If xenobiotics push the normal function of the immune system out of balance, the result can be either an immuno-suppression or an immuno-overestimation.

Either direction portends danger and result in toxicity; immunosuppression can result in a diminished resistance against infections. CCl_4 is bio-activated in the liver and induces centrilobular necrosis (Laskin & Laskin, 2001). Thus CCl_4 could have suppressed the maturation and development of immune cells, and thus caused an immunosuppression. Phagocytes are involved in the scavenger pathway for cholesterol uptake. Phagocytes detect, oxidize, engulf and digest extra circulating LDL-cholesterol especially when the amount of LDL-cholesterol in the bloodstream is excessive (Schaefer, 2002).

Platelet counts are used to investigate abnormal skin and mucosal bleeding, thrombocytopenia and thrombocytosis. This study showed a significant increase in platelet numbers, probably caused by myeloproliferative diseases, such as polycythaemia vera, following tissue injury. The polycaethemic condition agrees with this study since the intoxicated rats (PC) showed increases in RBC, which defines polycethaemia vera. But pretreatment with EECD alleviated this increase. Platelets are tiny cells in the body that help in forming blood cloth. Platelets play important role in blood coagulation, haemostasis and blood thrombus formation. The action of platelets, though quite beneficial in initiating the reaction to injury may actually be harmful in conditions such as coronary occlusion. In that case, platelet function may delay reperfusion and help to cause reocclusion of the vessel.

4.2.5 Histopathology

The result of histological study of the intoxicated group showed massive fatty change and centrilobular necrosis in most areas of the liver compared to the normal control. Also seen were mononuclear cell infiltration mostly macrophages and lymphocytes around the central vein and in the portal areas. Necrotic tissue injury progresses in two phases. The initiating phase where CCl_4 is bioactivated to reactive metabolites ($\cdot CCl_3$, $\cdot OOCCl_3$); interactions of these reactive metabolites

with cellular targets lead to cell injury. The propagatory phase is a phase in which the initial injury is propagated across the neighbouring tissue resulting in large areas of necrosis.

In necrosis, lethal cell injury is first characterised by swollen organelles; pyknotic nuclei (fragmentation, condensation, then lysis), and the formation of small blebs. When the cells finally rupture and the cell undergoes lysis, cytosolic constituents are released into the extracellular space, and highly increased levels of cell-specific markers are found in the peripheral blood e. g aminotransferase, calpains, etc (Boelsterlli, 2007).

The trichloromethyl radical formed, can bind to a liver cell component, the ultimate effect of which is inhibition of lipoprotein secretion. This causes fatty tissue to accumulate in the liver leading to fatty liver or steatosis (Boll *et al.,* 2001). Pathological fatty liver is accompanied by a decrease in plasma lipids (hypolipaemia). Thus fatty liver is caused by agents or conditions which produce either absolute or relative deficiency in certain of the ingredients used by the liver for synthesis for VLDL such as apo-protein itself, cholesterol esters, phospholipids and polyunsaturated fatty acids. Fatty liver is also caused by factors interfering with secretory mechanisms.

Results showed that treatment with EECD at 500 mg/kg b. w and silymarin protected the liver from damage. This shows that EECD might be hepatoprotective in a dose dependent manner. It also confirms the fact that the synthetic drug is hepatoprotective.

Histopathological studies of the kidney showed glomerular and tubular degenerations, interstitial inflammation and oedema congestion of the tubular cell and marked collagen deposition was recorded showing acute tubular necrosis.

Oedema occurs when total protein concentrations fall below 5.0 g % and albumin level fall below 2.5 g % (Chatterjea & Shinde, 2007). In this study, total protein fell by 6.26 g % and albumin fell by 11.12 g %. The osmotic balance between the extracellular compartment and the intracellular

compartment results in water being drawn into the cells producing cellular oedema to restore the balance. Oedema is associated with kidney disorder. Acute failure of the kidney to perform its essential functions: may be due to trauma, any condition that impairs the flow of blood to the kidneys; certain toxic substances such as compounds of mercury, CCI_4, ethylene glycol; bacterial toxins; glomerulonephritis, and acute obstruction of the urinary tract (TCMD, 1993).

Monocytes and macrophages are responsible for phagocytosis of damaged or old cells, cellular debris, foreign substances and pathogens removing them from circulation. They are found in large concentrations in the liver (Kupffer's cells) as well as other tissues.
Interstitial inflammation involves principally the noncellular or supporting elements of an organ such as renal fascia, a sheath of fibrous tissue, which helps to hold the kidney in place.

Following assault by CCl_4 on the liver, an inflammatory process in response to the injury on the liver is initiated by local vasodilation that increases blood flow, followed by increased vascular permeability that enables plasma to move out to the capillaries and into the tissue, producing local oedema. Neutrophils and later monocytes infiltrate the injured tissue. The roles of macrophages in enhancing the signals and aggravating liver injury are manifold. They include the presence of membrane bound NADPH – oxidase which can cause a burst in reactive oxygen production and release into the extracellular space. In addition, nitric oxide is produced, proteases are released and prostanoids and other bioactive lipids are generated. Finally a number of cytokines including Tumour necrosis factor – α (TNFα) and interleukin (IL)–Iβ, are produced. These mediators act as signals and are involved in intercellular cross talk; however, if released in excess quantities, they can also directly participate in tissue destruction (Boelsterli, 2007).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This research has revealed that the leaves of *C. dolichopentalum* are important sources of macronutrients such as carbohydrate, and micronutrients such as sodium, potassium and phosphorus. They are also potential sources of pharmacological active phytochemicals such as tannic acid; flavonoids like apigenin, luteolin, isorhamnetin and kaempferol; alkaloids such as buphanidrine, crinamidine, 6-hydroxyl powelline, undulatine and voacangine, and saponins such as hicogenin, sapogenin, tribuloin and saponine. It has also shown that the leaves are potential source of antioxidant molecules with flavonoid and tannic acid showing greater ability to reduce the pro-oxidant, hydrogen peroxide *in vitro*.

The leaves also showed potential antibactericidal ability against *E. coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Streptococci pneumonia*. Using gas chromatography-mass spectrometry, possible active pharmacological components such as β-lactam, isothiocyanate and cyclohexamine were demonstrated. Apart from the saponin extract which appeared to adversely affect the liver and kidney, the plant's extract did not appear to have toxic effects on animal cells. It dd not also have adverse effects on the cells and/or indices of the functions of the liver, kidney and blood; rather, it enhanced the functions of these organs/tissues and corrected disrupted haemostasis of certain indices of well being. The extract appeared to stimulate the pancreas and small intestine to produce or release bicarbonate, indicating a potential beneficial effect in heartburn.

The extract significantly reduced atherogenic and cardiovascular risk as well as markers of lipid peroxidation; while increasing the activities of the antioxidant enzymes and molecules such as glutathione peroxidase, superoxide dismutase, catalase, vitamin C and vitamin E. These observations suggest that *C. dolichopentalum* extract might be beneficial in the treatment/management of oxidative stress associated diseases and some other disease conditions.

5.2 Recommendations

Considering the above findings, it may be expedient to further state,

I. The exact mechanism of action of the crude alkaloid extract and of the other active agents.

II. The synergistic effect of active agents such as flavonoids, saponins, and tannins of *C. dolichopentalum*.

III. The need to isolate, purify and mass produce these phytochemicals especially the alkaloids with beneficial effects using PCR or microbial agents.

IV. Sequel to the observed effects of the flavonoids and alkaloids, it is plausible to further study their combined effect as anticancer drug due to the presence of apigenin in the flavonoid, and 6-hyroxylpowelline in the alkaloid.

V. The extracts stimulating effect on the placenta and small intestine to produce bicarbonate, its use as an antacid.

VI. Given the high prevalence of oxidative stress implicated diseases and disease conditions and the common availability of *C. dolichopentalum* in all seasons, the use of the plant leaves extract in the treatment and management of diseases can be further investigated and promoted.

VII. *C. dolichopentalum* serving as a source of amino acids and vitamins can also be investigated and promoted.

5.3 Contribution to Knowledge

Combretum dolichopentalum leaves have been shown to contain varying quantities of nutrients and phytochemicals which acts as antioxidants, antimicrobial, antinephrotoxic as well as hepatoprotective agents. The pharmacological properties of the plant can thus help in the management and/or treatment of oxidative stress associated diseases. Also, the knowledge of the identified bioactive components of *C. dolichopentalum* leaves and its medicinal properties will enhance the effective and thorough utilization of the plant.

REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). (2001) Documentation of threshold limits values and biological exposure indices for chemical substances in the workroom air. 7th ed. Supplement. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Adewole, S.O, Ojo, S.K., Adenowo, T.K., Salako, A.A., Naicker, T. & Ojewole J.A.O. (2012). Effects of *Ficus exasperata Vahl*. (Moraceae) leaf aqueous extract on the renal function of streptozotocin-treated rats. *Folia Morphologica, **71,*** 1–9.

Adewole, S.O., Salako, A.A., Doherty, O.W. & Naicker T (2007). Effect of melatonin on carbon tetrachloride-induced kidney injury in Wistar rats. *African Journal of Biomedical Research, **10,*** 153–164.

Aebi, H. (1984). Catalase, In; Bergmeyer, H.L (ed.) In: *Methods in Enzymatic Analysis*, (pp.674-684), Academic press, Cheime, Weinheim, FRG.

Agunu, A., Yusuf, S., Andrew, G.O., Zezi, A.U. & Abdurahman, E.M. (2005). Evaluation of five medicinal plants used in diarrhea treatment in Nigeria. *Journal Ethnopharmacology*, ***101***(1-3), 27-30.

Ahdaya, S.M., Monroe, R.J. & Guthrie, F.E. (1981). Absorption and distribution of intubated insecticides in mice. *Toxicology and Applied Pharmacology, **59,*** 414-423.

Aitken, R. J. & Roman, S. D. (2008). Antioxidant systems and oxidative stress in the testes. *Oxidative Medicine and Cellular Longevity*, ***1***(1), 15–24.

Ajao, A.O., Shonukan, O.O. & Femi-Ondeko, B. (1985). Antibacterial effect of aqueous and alcohol extracts of *Spondias mombin* and *Aknomea cordtfolten*: Two local antimicrobial remedies. *International Journal of Crude Drug Research*, ***23***(2), 67-72.

Albano, E., Bellomo, G., Carini, R., Biasi, F., Poli, G. & Dianzani, M. U. (1985). *FEBS Letters*, ***192***, 184-188

Alberts B., Johnson A., Lewis J., Raff M., Roberts K. & Walter P. (2002). *Transport into the Cell from the Plasma Membrane: Endocytosis*. Molecular Biology of the Cell. 4th edition. Garland Science; New York: Available at: http://www.ncbi.nlm.nih.gov/books/NBK26870/

Alisi, C. S. & Onyeze, G. O. C. (2008). Nitric oxide scavenging ability fraction of methanolic leaf extracts of *Chromolena odorata (Linn)*. *African Journal Biochemisry Research*, ***2***(7), 145-150.

Alisi, C. S., Nwaogu, L. A., Ibegbulem, C. O. & Ujowundu, C. O. (2011). Antimicrobial action of methanol extract of C*hromolaena odorata* – Linn is Logistic and exerted by inhibition of dehydrogenase enymes. *Journal of Research Biology*, ***3,*** 209-216.

Alisi, C.S. & Abanobi, S.E. (2012). Antimicrobial properties of *Euphorbia hyssopifolia* and *Euphobia hirta* against pathogens complicit in wound, typhoid and urinary tract infections. *International Journal of Tropical Disease and Health*, ***2***(2), 72-82.

Alisi, C.S., Nwanyanwa, C.E., Akujobi, C.O. & Ibegbulem, C.O. (2008). Inhibition of dehydrogenase activity in pathogenic bacteria isolates by aqueous extracts of *Musa paradisiacal* (Van sapientum). *African Journal of Biotechnology*, ***7(***12), 1821-1825.

Allain, C.C., Poon, L.S., Chan, C.S., Richmond, W. & Fu, P.C. (1976). Enzymatic determination of total cholesterol. *Clinical Chemistry*, ***20***(4), 470-475.

Alleyne, M., Horne, M.K & Miller, J.L(2008). Individualized treatment for iron-deficiency anaemia in adults. *Annual Journal Medicine*, ***121***, 943.

Amacher, D.E. (1998). Serum transaminase elevations as indicators of hepatic injury following the administration of drugs. *Regulatory Toxicology and Pharmacology*, ***27***, 119-130.

Andrews, G. K. (2000). Regulation of metallothionein gene expression by oxidative stress and metal ions. *Biochemistry and Pharmacology*, ***59***, 95-104.

AOAC (1984). Official Method of Analysis. 13th Ed. Association of Official Analytical Chemists. Virginia.

AOAC (2005). Official Method of Analysis of the AOAC . Horwitz, W. (Ed) Eighteen Edition, AOAC. Washington; D. C.

APHA (American Public Health Association) (1985). *Standard Methods for the Examination of Water and Wastewater*, 18th Edition, APHA, AWWA, WPCF. USA.

APHA (American Public Health Association) (1995). *Vapour Atomic Absorption Spectrometric Method, Standard Methods for the Examination of water and Wastewater*, 20th Edition, APHA, AWWA, WEF. 3112B.

Aruoma, O.I., Hayashi, Y., Marotta, F., Mantello, P., Rachmilewitz, E., & Montagnier L. (2010) Applications and bioefficacy of the functional food supplement fermented *papaya* preparation. *Toxicology*, **278**, 6–16

Arya, V., Demarco, V.G., Issar, M., & Hochhaus, G. (2006). Contrary to adult, neonatal rats shows pronounced brain uptake of corticosteroids, *Drug Metabolism and Disposition*, ***34***, 939-942.

Assman, G., Jabs, H.U., Kornett, U., Nolte, W. & Schriewer, H. (1984). LDL-cholesterol determination in blood serum following precipitation of LDL with polyvinyl sulphate. *Clinica Chimica Acta*, ***140***, 77-83

Asuzu, I. U & Onu, O.U. (1988). Anti-ulcer activity of the Ethanol Extract of *Combretum dolichopentalum* Root. *Journal of Crude Drug Research*, ***25,*** 44 – 48.

Asuzu, I. U. & Onu O.U. (1990). Anti-ulcer activity of the ethanolic extract of *Combretum dolichopentalum*. *International Journal Crude Drug Research*, ***28***(1), 27-32

Atkins, P. (1998) *Diffusion controlled reactions.* In: Physical Chemistry pp. 825–828. (6th ed.), Freeman, New York, NY.

Ayrton, A. & Morgan, P. (2001). Role of transport proteins in drug absorption, distribution and excretion. *Xenobiotica*, ***31***, 469-497.

Azzi, A. (2007). Molecular mechanism of alpha-tocophenol action. *Free Radical Biology and Medicine,* ***43***(1), 16-21.

Babu, B.H., Shylesh, B.S. & Padikkala, J. (2001). Antioxidants and hepatoprotective effect of *Acanthus ilicifolius*. *Fitoterapia*, ***72***, 272-277.

Bahomn, T., Scooratte, M.A, Luximon-Roman, V. & Aruoma, O.I. (2006). Free radicals and antioxidants in cardiovascular health and disease. *Internet Journal of Medical Update*, ***1***, 1-7.

Bardy, G., Vir Solvy, A., Quignard, J.E. Ravier, M.A., Bantraud, G., Daller, S., Cros, G., Magous, R., Richard, S. & Qiry, C. (2013). Quercetin induces insulin secretion by direct activation of L-Type calcium channels in pancreatic beta cells. *British Journal of Pharmacology*, ***169***(5), 1102-1113.

Barja, G. (2004). Ageing in vertebrates and the effect of caloric restriction: a mitochondrial free radical production-DNA damage mechanism? *Biological Reviews, **79,*** 235–251.

Barja, G., Cadenas, S., Rojas, C., & Perez-Campo, M. (1994). A decrease of free readical production near critical targets as a cause of maximum longevity, *Comparative Biochemistry Physiology, **108*B,** 501-512.

Barja, G., Cadenas, S., Rojas, C., Perez-Campo, R. & Lopez-Torres, M. (1994a). Low mitochondrial free radical production per unit O_2 consumption can explain the simultaneous presence of high longevity and high aerobic metabolic rate in birds. *Free Radical Research, **21***, 317-327.

Batra, P. & Sharma A. K. (2013). Anti-cancer potential of flavonoids: Recent trends and future perspectives. *3 Biotechnology, **3***(6), 439–459. http://doi.org/10.1007/s13205-013-0117-5

Baynes, J. W. (2000). From life to death—the struggle between chemistry and biology during ageing: The Maillard reaction as an amplifier of genomic damage. *Biogerontology, **1,*** 235–246.

Beal, M.F. (2002). Oxidatively modified proteins in aging and diastase. *Free Radical Biology and Medicine, **32***, 797-803.

Beecher, G. (2003). Over view of dietary flavonoids: Nomenclature, occurrence and intake: *Journal of Nutrition*, ***133***(10), 1248-3254.

Bellomo, G., Vairetti, M., Stivala, L., Mirabelli, F., Richelmi, P., & Orrenius, S. (1992). Demonstration of nuclear compartmentalization of glutathione in hepatocytes. *Proceeding of National Academy of Sciences USA, **89***, 4412-4416.

Benitez, L. V. (1984). *Amino acid and fatty acid profiles in aquaculture nutrition studies,* In: De Silva S.S (ed.) Fish Nutrition Research in Asia. Proceedings of the Third Asian Fish Nutrition Network Meeting. Asian Fish. Society Special Publication. (pp 23-35).

Berg J.M, Tymoczko J.L. & Stryer L. (2002). Biochemistry. 5th Edition. W.H. Freeman and Company. New York.

Bhutani, K.K. & Gohil, V.M. (2010). Natural products drug discovery research in India: Status and appraisal. *Indian Journal Experimental Biology*, ***48***(3), 199-207.

Biswagner, H. (2008). Enzyme Kinetics: *Principles and Methods*. 2nd Ed. (pp. 62-63). Wiley-VCH Verlag GmbH & Co. KGaA. Weinherm.

Bjorksten, J. (1968). The crosslinkage theory of aging. *Journal of the American Geriatrics Society*, ***16***, 408–427.

Boelsterli, U.A. (2007). Mechanistic Toxicology: *The Molecular Basis of How Chemicals Disrupt Biological Targets* (pp 117-255). 2nd Eds. CRC Press New York.

Boham B.A. & Kocipai-Abyazan R. (1974). Flavonoids and condensed tannins from leaves of *Hawaiian vaccinium vaticulatum* and *V. calycinium*. *Pacific Science, **48**,* 458-463.

Bohm, B.A. & Tod, F.S. (2007). *Flavonoid of the Sun Flower Family (Asteraceae)*. Springer (pp 597).

Boll M., Weber L.W. D., Becker E. & Stampfl A. (2001) Mechanisms of carbon tetrachloride induced hepatotoxicity. Hepatocellular damage by reactive carbon tetrachloride metabolites. *Zeitschrift für Naturforschung C, **56**,* 649-659.

Boll, M., Weber, L.W.D. & Becker, E. (2001) Pathogenesis of carbon tetrachloride-induced hepatocyte injury: bioactivation of CCl_4 by cytochrome P450 and effects on lipid homeostasis. *Zeitschrift för Naturforschung C,* ***56,*** 111–121.

Boritz, W. (1986). Ageing as entropy. *Experimental Gerontology,* ***21,*** 321-328.

Bosetti, C., Spentini, L., Parpinel, M., Gnagnarella, P., Lagiou, P., Negri, E., Franceshi, S., Montolla, M., Peterson, J., Dwyer, J., Giacosa, A. & LaVecchia, C. (2005). Flavonoids and breast cancer risk in Italy cancer epidemiol. *Biomarkers and Prevention*, ***14***, 805-808.

Brown, K.M. & Arthur, J.K. (2001). Seleneproteins and human health, *Public Health Nutrition*, ***4***(213), 593-599.

Butler, M.S. (2008). Natural product to drugs: Natural Product – derived compounds in clinical trials. *Natural Product Reports*, ***28***(3), 475-516.

Byers, T., Nestle, M., McTiernan, A., Doyle, C., Currie-Williams, A., Gansler, T. & Thun, M. (2002). American cancer society guidelines on nutrition and physical activity for cancer prevention: reducing the risk of cancer with healthy food choices and physical activity, *CA Cancer Journal of Clinicians*, ***59***, 92-119.

Calabro, M.L., Tommasini, S., Donato, P., Raneri, D., Stancanelli, R., & Ficarra, P. (2005). Effects on alpha and beta – cyclodextrin complexation on the physiochemical properties and antioxidant activity of some 3-hydroxyflavones. *Journal of Pharmaceutical and Biomedical Analysis*, ***35***(2), 365-371.

Caldenon-Montario, J.M., Burgos-Moron, E., Perez-Guerrero, C. & Lopez-Lazaro, M. (2011). A review of the dietary flavonoid kaempferol. *Mini Reviews Medicinal Chemistry,* ***11***(4), 298-344.

Carr A., McCall, M.R. & Frei B. (2000). Oxidation of LDL by myeloperoxidase and reactive nitrogen species-reaction pathways and antioxidant protection. *Arteriosclerosis, Thrombosis, Vascular, Biology, **20***, 1716- 1723.

Carr, A. & Frei, B. (1999). Does Vitamin C act as a pro-oxidant under physiological conditions *FASEB. Journal, **13*** (9), 1007 – 1024.

Castillo, T., Koop, D.R. & Kamimura, S. (1992). Role of cytochrome P-450 2E1 in ethanol-, carbon tetrachloride- and iron-dependent microsomal lipid peroxidation. *Hepatology, **16***, 992–996.

Chaney. A. & Marbach, E. (1962) Modified reagents for determination of urea and ammonia. *Clinical Chemistry, **8***,130-132.

Chatterjea M.N & Shinde R. (2007). Text Book of Medical Biochemistry. (pp. 93-674). 7th Edition. Jaypee Brothers Medical Publishers. New Delhi.

Christie, G.S. & Judah, J.D. (1954) *Proc. R. Soc. London 142,* 241-244; in Nwachukwu D.C. & Okwuosa C.N. (2013). Investigation of the hepatoprotective activity of leaf extract of *Clerodendrum polycephalum* Indian. *Journal of Novel Drug Delivery 5*(4), 229-233

Clarke, S. (2003).Ageing as a war between chemical and biochemical processes: protein methylation and the recognition of age-damaged proteins for repair. *Ageing Research Reviews, **2***: 263–285.

Committee for Orphan Medicinal Products (2015. April 28). *Phenol, 4- (2-(aminomethyl)-4-thiazolyl)-2, 6-bis (1,1- dimethylethyl) monohydrochloride for the treatment of Huntington's disease.* European Commission-Community Register of Pedicinal products, Designation number: EU/3/15/1481. Public Summary of Scientific Opinion. Retrieved from the website: ec.europa.eu/health/…/html/o1481.htm, on 2015-08-22.

Conklin, K.A. (2004). Chemotherapy-associated oxidative stress: impact on chemotherapeutic effectiveness. *Integrative Cancer Therapy*, ***3***(4), 294-300.

Cordell, G.A. (1981). *Introduction to Alkaloids*. Wiley – Inter science. (pp.1055- 28). New York.

Costa, M.A., Zia, Z.Q., Daria, L.B. & Lawis, N.G. (1999). Toward engineering the metabolic pathways of cancer preventing lignans in cereal-grains and other crops. Phytochemicals in human health protection, nutrition, and plant defense. *Recent advances in photochemistry*, ***33***, 67-87.

Cushnie, T.P., Cushnie, B. & Lamb, A.J. (2014). Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities: *Internal Journal Anti-microbial Agents*, ***44***(5), 377-386.

Dalton, T.P., Chen Y., Schneider S.N., Nebert D.W. & Shertzer H.G. (2004). Genetically altered mice to evaluate glutathione homeostasis in health and disease *Free Radical Biology and Medicine,* ***37,*** 1511 – 1526

De Boer, A.G., Yander Sonidt., & Gaillard, P.J. (2003). The role of drug transporters at the blood brain barriers. *Annual Reviews in Pharmacology and Toxicology*, ***43***, 629-656.

Deapak, V., Gopal, M.D., Hugo, R. & Rosen, M.D. (2000). Abnormal findings on liver function tests: Interpreting result to narrow the diagnosis and establish a prognosis. *Postgraduate Medicine,* ***107***(2), 100-114.

Dewick, P.M. (2002). Medicinal Natural Products. *A Biosynthetic Approach*. (pp. 335). 2nd edition.

Dhanmasiin, J.R., Jayokody, A.C., Galhena, G., Liyanage, S.S.P. & Ratnasooriya, W.D. (2003). Anti-inflammatory and analgesic activities of natural fresh leaves of *Vitex neyundo*. *Journal of Ethnopharmacology*, ***7***, 199-206

Dianzani, M.U. (1993). Lipid peroxidation and cancer. *Critical Reviews Oncology and Hematology*, ***15***, 125-147.

Donnapea, S., Li, j., Yang, X., Ge, A.H., Donkor, P.O., Gao, X.M. & Chang, Y.X. (2014). *Cuscuta chinensislarin*: A systematic review. *Ethnopharmacology*, ***157***, 292-308.

Doumas, B.T., Watson, W.A. & Biggs, H.G. (1971). Albumin standards and the measurement of serum albumin with bromocresol green. *Clinica Chimica acta*, ***31***, 87-97.

Droge, W. (2002). Free radicals in the physiological control of cell function. *Physiological Reviews*, ***82***, 47-95.

Duh, P.D., Tu, Y.Y., & Yen, C.O. (1999). Antioxidant activity of water extract of Harng jyur (*Chrssanthemum morifolium* Ramat). *Lebnesmitteel Wissenschaft und Technologie*, ***32,*** 269-277

Eckl, P.M., (2003). Genotoxicity of HNE. *Molecular Aspects of Medicine*, ***24***, 161–165.

Eitenmiller, R.R. & Landen, W.O. (1999). *Vitamin Analysis for the Health and Food Sciences.*, Fl: CRC Press. Boca Raton

Enechi O.C. & Odonwodo I. (2003). Assessment of the phytochemical and nutrient composition of pulverized root of C*issus quadrangularis*. *Journal of Biological Research and Biotechnology*, *1*, 63-68.

Environmental Protection Agency (2010). *Toxicological Review of Carbon Tetrachloride.* EPA, Washington D.C

Esterbauer, H., Schaur, R.J. & Zollner, H. (1991). Chemistry and biochemistry of 4-hydroxy-nonenal, malondialdehyde and related aldehydes. *Free Radical Biology and Medicine*, ***11***(1), 81-121.

Evans, W.C. (2005). *Trease and Evans Pharmacognosy*. (pp 214-331). Elsevier, New Delhi.

Fanelli, S.L & Castro, J.A. (1995) Covalent binding of carbon tetrachloride reactive metabolites to liver microsomal and nuclear lipid and phospholipid classes from Sprague Dawley and Osborne Mendel male rats. *Teratogenesis Carcinogenesis and Mutagenesis*, ***15*,** 155–166.

Farombi, E.O. (2003). African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. *African Journal of Biotechnology*, ***2***(12), 662-671.

Florence, A.T & Atwood, D. (2005). Physicochemical Principles of Pharmacy. 4th ed.: Pharmaceutical Press, London.

Food & Nutrition Board (FNB) (1998). Institute of Medicine: *Dietary Reference Intakes for Thiamine, Riboflavine, Naicin, Vitamin B6, Folate, Vitamin B12, Pantothenic acid, Biotin and Choline*. National Academy Press. Washington D. C.

Food & Nutrition Board (FNB) (2002). Institute of Medicine: *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenum and Carotenoids*. National Academy Press, Washington DC.

Fowler, J.S.L. (1969) Carbon tetrachloride metabolism in rabbit. *British Journal of Pharmacology*, ***37***, 733–737.

Galelli, M.E. & Castro, J.A. (1998) Effects of trichloromethyl and trichloromethyl peroxyl free radicals on protein sulfhydryl content studies in model and enzymatic carbon tetrachloride

activation systems. *Research Communications in Molecular Pathology and Pharmacology,* ***100***, 227–238.

Gent J. & Braakman I. (2004). Low-density lipoprotein receptor structure and folding. *Cellular and Molecular Life Sciences,* ***61***, 19-20, 2461–70. doi:10.1007/s00018-004-4090-3.

Gibson, E.L., Wardel J. & Watts C.J. (1998). Fruit and vegetable consumption, nutritional knowledge and beliefs in mothers and children. *Appetite*, ***31***, 205-228.

Giner-Chaves, B.I. (1996). Condensed Tannins in Tropical Forages. Ph.D Thesis. (pp 279). Cornell University, New York.

Giovannuci E. (2002). Epidemolic studies of folate and colorectal neoplasia. A review. *Journal of Nutrition*, ***132***, 23505.

Gole, M.K, Dasgupta, S., Sur, R. K. & Ghosal, J. (1997). Hepatoprotective effect of *Amorra rohituka*. *International Journal of Pharmacognosy*, ***35***(5), 318-322.

Gowchi, Y., His-Huai, L. & Hsin-Yi, (2001). Nitric oxide scavenging and antioxidant effects of *Uraria crimitia* root. *Food Chemistry*, ***74***, 471-478.

Green, N.S. (2002). Folic acid supplementation and prevention of birth defects. *Journal of Nutrition*, ***132***, 23565.

Griess, J.P. (1879) *Berichte der Deutschen Chemischen Gesellschaft*, ***12*****(1),** 426-428. 10.1002/cber.187901201117

Grove, T.H (1979). Effect of reagent pH on determination of high-density lipoprotein cholesterol by precipitation with sodium phosphotungstate-magnesium. *Clinical Chemistry*, ***25***, 560-564.

Gulcin I., Oktay M., Kirecci E. & Kufrevioglu O.I. (2003). Screening of anti- oxidant and antimicrobial activities of anise (*Pimpinella anisum* L) seed extracts. *Food Chemistry, **83***, 371 - 382.

Gunawardhana, L., Mobley, S.A., & Sipes, I.G. (1993). Modulation of 1,2-dichiorobenzene hepatotoxicity in the Fischer-344 rat by a scavenger of superoxide anions and an inhibitor of Kupffer ceils. *Toxicology and Applied Pharmacology, **119***, 205-213.

Guo M., Zhang L & Liu Z. (2009). Analysis of saponins from leaves of *Aralia elata* by liquid chromatography and multi-stage tandem mass spectrometry. *Analytical Sciences, **25***(6), 753. DOI: 10.2116/analsci.25.753, JOI: JST.JSTAGE/analsci/25.753

Guyton, A.C. & Hall, J.E (2011). Medical Physiology. *Red Blood Cells Anaemia, and Polycythemia*. (pp. 413-422). 12th Edition. Sanders, Philiadelphia

Hagerman, A.S.E. & Klucher, K.M. (1986). Tannin Protein Interaction. In: *Plant Flavonoids in Biology and Medicine,* (pp 67-76). Cody Vand Meddleton E.J. (ed) Harbon Liss, New York.

Halliwell, B. & Gutteridge, J.M.C. (2007). *Free Radical Biology and Medicine* (4^{th} ed). University Press, Oxford, New York.

Halliwell, B. (1987). Oxidants and human disease: some new concepts. *FASEB Journal, **1**,* 358

Halliwell, B. & Gutteridge, J.M.C. (1999). *Free Radicals in Biology and Medicine.* 3rd ed. University Press, Oxford, New York.

Hallliwell, B. (2007). Biochemistry of oxidative stress. *Biochemical Society Transactions, **35***, 1147-1150.

Hammer K.A., Carson C.F. & Riley T.V. (1999). Antimicrobial activity of essential oils and other plant extracts. *Journal of Applied Microbiology*, ***86***(6), 985.

Harman, D. (1981). The aging process. *Proceedings of the National Academy of Sciences*, ***78***, 712

Hasler C.M. & Blumberg J.B. (1999). Symposium on phytochemicals: biochemistry and physiology. *Journal of Nutrition*, ***129***, 756S-757S.

Henry, R.J. (1974). Clinical Chemistry Principles and Techniques (pp. 42-712). 2nd Edition, Harper and Row Hageistrown.

Herrera, E. & Barbas, C. (2001). Vitamin E: action, metabolisms and perspectives. *Journal of Physiology and Biochemistry*, ***57***(2), 43-56.

Hesse, M. (2002a). *Alkaloids. Natures Curse or blessing?* Wiley-VCH. pp 12-79.

Hesse, M. (2002b). *Alkaloids: Natures Curse or blessing*? Wiley-VCH. pp. 283-309.

Hesse, M. (2002c). *Alkaloids: Natures Curse or blessing*? Wiley-VCH. pp. 283-309.

Hsu, B., Coupar, I.M., & Ng, K. (2006). Antioxidant activity of hot water extract from the fruit of the Doum Palm, *Hyphaene thebaica*. *Food Chemistry*, ***98***, 317-328.

Ibegbulem, C.O. (2008). Inhibition of dehydrogenase activity in pathogenic bacteria isolates by aqueous extracts of *musa paradisiaca* (vicar sapientum). *African Journal of Biotechnology*, ***7***(12), 1821-1825.

Igwe C.U., Nwaogu L.A. & Ujowundu C.O. (2007) Assessment of the hepatic effects, phytochemical and proximate compositions of *Phyllanthus amarus*. *African Journal of Biotechnology*, ***6*** (6), 728-731.

Igwe, C.U., Ojiako A.O., Nwaogu L.A. & Onyeze G.O.C. (2008). Lipid lowering effect of aqueous leaf extract of *Spondias mombin* Linn. *Internet Journal of Pharmacology*, ***6***(1), 1-9.

Igwe, C.U., Onyeze G.O.C, Onwuliri V.A, Osuagwu C.G. & Ojiako A.O. (2010). Evaluation of the chemical compositions of the leaf of S*pondias mombin* linn from Nigeria. *Austrilian Journal of Basic and Applied Sciences*, ***4***(5), 706-710.

Iweala, E.E.J., Uchegbu, F.O. & Adesenoya, O. A. (2013). Biochemical effects of leaf extracts of *Gongronema latifolium* and selenium supplementation in alloxan-induced diabetes rats. *Journal of Pharmacognosy and phytotherapy*, ***5***(5), 91-97.

Jacobs, M.N. & Lewis, D.F. (2002). Steroid hormones receptors and dietary ligands: a selected review. *Proceedings of the Nutritional Society*, ***61***(9), 105-122.

Josef R., Hugo F., Heinz K. & William C.M. (2005)."*Caprolactam", Ullmann's Encyclopedia of Industrial Chemistry*. Weinheim: Wiley-VCH, doi:10.1002/14356007.a05_031.pub2

Joshipura, K.J., Archerio, A., Monson, J.E., Stampfer, M.J., Rimm, E.B., Speizea, F.E.H., Spiegelman, D. & Willat, W.C. (1999). Fruit and vegetable intake in relation to risk of ischemic Stroke. *Journal of American Medical Association*, ***282***, 1233-1239.

Kadam, S.S. & Chavan, J.K. (2000). Dietary tannins. *Basic Life Sciences*, ***59***, 259-280.

Kamel, H.H., Azza, H., Abd-El-Rahman, Walaa, M.S., Ahmed, I. & Amira, H. *et al* (2011). Protective effect of some antioxidants against CCl_4-induced toxicity in liver cells from brl3a cell line. *Journal of American Science*, ***7***(2), 283-296.

Kanakis, C.D., Tarantilis, P.A., Polission, M.G., Doamantoglon, S., & Tajmir-Riahi, H.A. (2005). DNA interaction with naturally occurring antioxidant flavonoids quercetin, kaempferol and delphinidin. *Journal of Biomolecular Structure and Dynamics*, ***22***(6), 719-724.

Kaplowitz, N., Fermandezcheca, J.C., Kannan, R., Garciaruiz, C., Dokhtens, M. & Yi, J.P. (1996). GSH transporters: molecular characterization and role in GSH homeostasis. *Biological Chemistry Hoppe- Seyler, **377***, 267-273.

Karsten E., Erhard H., Roland R. & Hartmut H. (2005)"*Amines, Aliphatic" in Ullmann's Encyclopedia of Industrial Chemistry*. Wiley-VCH, Weinheim, doi:10.1002/14356007.a02_001.

Katavic, P.L., Lamb, K., Navarro, H. & Prisinzana, T. E. (2007). Flavonoids as opoid receptor ligands identification and preliminary structure – activity relationships. *Journal of Natural Products, **70***(8), 1278-1278.

Kelly D.S. & Nelson R.A. (2014). Methods for characterization and quantification of phytochemicals by gas chromatography. *Journal of the Brazilian Chemical Society, **12***, 2.

Kim, H.J, Bruckner, J.V. & Dallas, C.E. (1990). Effect of dosing vehicles on the pharmacokinetics of orally administered carbon tetrachloride in rats. *Toxicology and Applied Pharmacology, **102***, 50–60.

Kim, S.H. & Choi, K.C. (2003). Anti-cancer effect and underlying mechanism(s) of kaempferol. A phytoestrogen on the regulation of apoptosis in diverse cancer cells models. *Toxicological Research, **29***(4), 229-234.

King, A. & Young, G. (1999). Characteristics and occurrence of phenolic phytochemicals. *Journal of American Dietetic Association, **24***, 213-218.

Kittakoop, P., Mahidol, C. & Ruchiwarat, S. (2014). Alkaloids as important scaffolds in therapeutic drug for treatments of cancer, tuberculosis and smoking cessation. *Current Topics in Medicine and Chemistry, **4***(2), 239-252.

Klatt P. & Lamas S. (2000). Regulation of protein function by *S* glutathiolation in response to oxidative and nitrosative stress. *European Journal of Biochemistry*. ***267***, 4928-4944.

Körper S., Wink M. & Fink R.H.A. (1998). Differential effects of alkaloids on sodium currents of isolated single skeletal muscle fibers. *Fed Eur Biochem Soc (FEBS) Letter*. **436** (2), 251–255

Krishman, R., Chandravadana, M.V., Ramachander, P.R. & Bharathkumar, H. (1983). Inter-relationships between growth and alkaloid production in *Catharanthus roseus. G. Don*. *Herba Hungarica*. ***22***, 47-54.

Kushnareva, Y., Murphy, A.N. & Andreyev, A. (2002). Complex I-mediated reactive oxygen species generation: modulation by cytochrome c and $NAD(P)^+$ oxidation reduction state. *Biochemical Journal, **368***, 545-553.

Lacaille – Dubois, M.A. & Wagner, H. (2000). Bioactive saponin from plants: An update. Studies in Natural Products Chemistry, Atta – Ur – Rahman ed. *Elsevier Science. Amsterdam*. ***21***, 633-687.

Laskin, D.L & Laskin, J.D (2001). Role of macrophages and inflammatory mediators in chemically induced toxicity. *Toxicology, **160,*** 111-118

Levites, Y., Weinreb, O., Maor, G., Youdim, Y.B., & Mandel, S (2001). Green tea polyphenol (-)-epigallocatechin-3-gallate prevents N-methyl-4-phenyl-1,2,3,6,tetrahydropyridine-induced dopaminergic neurodegeneration. *Journal of Neurochemistry, **78,*** 1073-1082.

Lewis, R.J (1997) Hawley's Condensed Chemical Dictionary. pp. 213. 13th edition. John Wiley and Sons, Inc. New York, NY:

Linder, M.C (1991). *Cholesterol Nutrition and the Control of Serum Cholesterol Concentration. Nutritional Biochemistry and Metabolism*. (pp 68-75). 2nd Edition. Elsevier, New York.

Liu, J., Edamatsu, R., Kabuto, H. & Mori, A. (1990). Antioxidant action *of guiligji* in the brain of rats with $Fecl_3$- induced rat epilepsy. *Free Radical Biology and Medicine*. ***9***, 451-454

Loeckic, L.Z., Meerman, J.H.N., Commandeur, J.N.M. & Vermanlan, N.P.E. (1999). Biomarkers of free radicals damage: applications in experimental animals and in human. *Free Radical Biology and Medicine, **26,*** 202-226.

Loh J.A. & Verbalis J.G. (2008). Disorders of water and salt metabolism associated with pituitary disease. *Endocrinology and Metabolism Clinics of North America, **37,*** 213-34.

Luchsimger, J. & Mayoux, R. (2004). Dietary factors and alzeheimer's disease. *Lancet Neurology*. ***3***(10), 579-587.

Mahomoodally, M.F. (2013). Traditional medicine in Africa: An appraisal of ten potent African medicinal plants. *Evidence-Based Complimentary and Alternative Medicine, **13***, 1-14.

Mankani K.L., Krishna V., Manjunatha B. K., Vidya S.M., Jagadeesh Singh S.D. & Manohara Y.N., *et al.* (2005). Avinash KR. Evaluation of hepatoprotective activity of stem bark of *Pterocarpus marsupium* Roxb. *Indian Journal of Pharmacology, **37*** (3), 165-168

Marchand, C., McLean, S. & Plaa, G.L. (1970). The effect of SKF 525A on the distribution of carbon tetrachloride in rats. *Journal of Pharmacology and Experimental Therapy, **174***, 232–238.

Marcocci, I., Marguire, J.J., Droy–lefaiz, M.T. & Packer, L. (1994). The Nitric oxide scavenging properties *Ginkgo biloba* extract. *Biochical and Biophysical Research Communications, **201,*** 748-755.

Marquardt D.W. (1964). An algorithm for least squares estimation of non-linear parameters. *Journal of the Society for Industrial and Applied Mathematics, **2***, 431-441.

Mathai, K. (2000). Nutrition in adult Years In: Krause's Food Nutrition and Diet Therapy. 10th edition. *Lk Mahan and S Escott – stump*. ***271***, 274-275.

Matheny, C.J., Lamb, M.W., Bronwer, K.L.R. & Pollack, G.M. (2001). Pharmacokinetic and pharmacodynamic implications of p-glycoprotein modulation. *Pharmacotherapy*, ***21***, 778-796.

McCay, P.B., Lai, E.K. & Poyer, J.L. (1984). Oxygen- and carbon-centered free radical formation during carbon tetrachloride metabolism. Observations of lipid radicals *in vivo* and *in vitro*. *Journal of Biological Chemistry*, ***259***, 2135–2143.

Meagher, E. & Thompson, C. (1999). *Vitamin and mineral therapy*. In: Medical Nutrition and Disease. (pp 33-58). 2nd edition. Morrison G – and Hark, L. (Ed): Black Well Science Inc. Massachusetti.

Meyers R.A. (2001). *Encyclopedia of Physical Science and Technology* - Alkaloids, 3rd edition. Ramatech limited, Tarzaa, Academic Press. California, USA; ISBN 0-12-227411-3

Middleton, J.E., Kandaswami, C. & Theoharides, T.C. (2000). The effect of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacological Reviews*, ***32***(4), 673-751.

Millán, J., Pintó, X., Muñoz, A., Zúñiga, M., Rubiés-Prat, J., Pallardo, L. F. & Pedro-Botet, J. (2009). Lipoprotein ratios: Physiological significance and clinical usefulness in cardiovascular prevention. *Vascular Health and Risk Management*, ***5***, 757–765.

Millogo-kone M, Lomp F, Kini S, A & Ncoulma N (2009). Evaluation of flavonoid and total phenolic contents of stem bark of Parkia biglobosa (Jacq). Benth (Mimosaceae)-free radical scavenging and antimicrobial activities. *Research Journal of Medical Sciences*, ***3***(2), 70-74.

Milne A. (1993). Inhalational and local anesthetics reduce tactile and thermal responses in *Mimosa pudica* linn, *Masui, 1190,* 3.

Miwa, S. & Brand, M.D (2005). The topology of superoxide production by complex III and glycerol 3-phosphate dehydrogenase in Drosophila mitochondria. *Biochimica et Biophysica Acta, **1709**,* 214-219.

Molyneux, R.J., Nash, R.J. & Asano, N. (1996). *Alkaloids: Chemical and Biological Perspectives,* pp. **11**- 303. Pelletier, SW (ed). Pergamon, Oxford, New York.

Moreira, S., Smith, M.A. & Zhu, X. (2005). Oxidative damage and Alzheimers disease; are antioxidant therapies useful? *Drug News Prospect*, ***18,*** 13-19

Morgan, A., Black, A. & Belcher, D.R. (1970). The excretion in breath of some aliphatic halogenated hydrocarbons following administration by inhalation. *Annals of Occupational Hygiene*. ***13,*** 219–233.

Morris, B.J. (2005). A Forkhead in the road to longevity: the molecular basis of lifespan becomes clearer. *Journal of Hypertension, **23***(7), 1285-1309.

Mukherjee, P.K. (2002). *Quality Control of Herbal Drugs*—An Approach to Evaluation of Botanicals. Business Horizons, pp. 518–598. New Delhi, India,

Muralikhrisnan , D., Binu T. & Bala V.M. (2008). Antiparkinson drug – *Mucuna puriens* shows antioxidant and metal chelating activity. *Phytotherapy Research, **22***, 6-11.

Napetschnig, S. (1981). Reactions of amino acids with 4-hydroxy-2,3-transpentenal and therapy of Ehrlich ascites tumors with the amino acid adducts. Austria Univ. Graz. Thesis. .

Narasniga, R. (2003). Bioactive phytochemicals in Indian foods and their potentials in health promotion and disease prevention. *Asia Pacific Journal of Clinical Nutrition*, ***9***, 9-22

Nelson, D.L & Cox, M.M. (2005). *Amino acids*. In: Lehninger Principles of Biochemistry. (pp 859). (4th ed.). W.H Freeman and company Publication, New York.

Ngounou, F.N., Manfouo, R.N., Tapondjou, L.A., Lontsi, D., Kuete, V., Penlap, V., Etoa, F.X., Dubois M.A.L. & Sondengam B.L. (2005). Antimicrobial diterpenoid alkaloids from E*rythrophleum suaveolens* (guill. & perr.) Brenan. *Bulletin of the Chemical Society of Ethiopia*, ***19***(2), 221-226.

NLM (National Library of Medicine). (2003) *Carbon tetrachloride*. Hazardous Substances Data Bank (HSDB). National Institutes of Health, U.S. Department of Health and Human Services, Bethesda, MD. Available online at http://toxnet.nlm.nih.gov (accessed June 24, 2009).

Noveas, R.D. & Leite, J.P.V. (2011). Ethno-pharmacology as current strategy in the search of novel anti ulcerogenic drugs: Case of a brazilian medicinal plant. *Peptic Ulcer Diseaase*. ***20***, 376-400.

Nwaogu, L.A., Ujowundu C.O. & Mgbemena, A.I. (2006). Studies on the nutritional and phytochemical composition of *Amaranthus hybridus* leaves. *Bio-Research*, ***4***, 28-31.

Nweke, C.O., Alisi, C.S., Okolo, J.C. & Nwanyanwu, C.E. (2007). Toxicity of zinc to heterotrophic bacteria from tropical river sediment. *Applied Ecology and Environmental Research*, ***5***(1), 123-132.

Nweke, C.O., Okolo, J.C., Nwanyanwu, C.E. & Alisi, C.S. (2006). Response of planktonic bacteria of new calabar river to zinc stress. *African Journal of Biotechnology*. ***5***(8), 653-658.

Nykjaer A. & Willnow T.E. (2002). The low-density lipoprotein receptor gene family: a cellular swiss army knife? *Trends in Cell Biology*, ***12***(6), 273–80. doi:10.1016/S0962-8924(02)02282-1

O'Neil, M.J. & Smith, A. (2001) The Merck index: *An Encyclopedia of Chemicals, Drugs, and Biologicals*. pp. 305–306.13th edition. Merck and Co., Inc., Whitehouse Station, NJ:

Oakenfull, D. (1981). Saponins in food: A review. *Food Chemistry*, ***6***, 19-40.

Obadoni P.O, & Ochuko, M.C (2001). Practical methods of determining various components from plant extract. *Advance in Environmental Medicine and Biology*. ***102***, 341-398.

Oboh, G., Ekperigin, M.M, & Kazeem, M.I. (2005). Nutritional and haemolytic properties egg plants. (S*olanum macrocarpon*) leaves. *Journal of Food Composition and Analysis*, ***18***, 153-160.

Ojiako O.A. & Nwanjo, H.U. (2009). Biochemical studies of the effects of the aqueous extract of Nigerian garlic on lipid profile and atherogenic risk predictor indices. *Austrialian Journal of Basic and Applied Sciences, **3***(3), 2861-2865.

Ojiako, O.A., Ogbuji, C.A., Agha, N.C. & Onwuliri, V.A. (2010). Chemical Analysis of the Proximate, Mineral and Toxicant Composition of Four Possible Food Security Crops from South Eastern Nigeria (In Press).

Okhawa H., Oishi N. & Yagi K. (1979). Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Analytical Biochemistry*, ***95***, 351-358.

Okoro, I. (2002). *Manual of Pratical of Histology*. 2nd Ed. Peace Publishers, Owerri. Imo State.

Okwu, D.E. & Okwu, M.E. (2004). Chemical composition of *Spondias mombi*n plant. *Journal of Sustainable Agriculture and the Environment*, ***6***, 140-147.

Omaye, S.T., Turnbull, J.D. & Sauberlich H.E. (1979). Selected methods for the determination of ascorbic acid, in animal cells, tissues, and fluids. *Methods in Enzymology, **62**,* 3

Onwuliri, V.A., Attah, A. & Nwankwo, J.O. (2004). Antinutritional factors, essential and non essential fatty acids composition of Ugba (*Pentaclethra macrophylla*) seeds at different stages of processing and fermentation. *Journal of Biological Sciences, **4***(5), 671-675.

Onwuliri, V.A., Yakubu, B. & Samuel, A.L. (2002). Proximate, mineral and antinutrient composition of *Rumex acetosa abd Hibiscus cannabinus*. *Nigerian Journal of Biochemistry and Molecular Biology, **17***, 97-99.

Osawa, T., Kawakishi, S., Namiki, M., Kuroda, Y., Shankai, D.M. & Waters, M.D. (1990). Antimutagenesis and Antimutagenesis Mechanism 11. *New York Plenum*. *9*, 139-153.

O'Sullivan, R. & Jan K. (2012). The great unravelling: Chromatin as a modulator of the aging process. *Trends in Biochemical Sciences, **37***(11), 466-476

Oyaizu, M. (1986). Studies on products of browning reaction prepared from glucoseamine. *Japanese Journal of Nutrition, **44,*** 307–315.

Packer, L. Weber, S. U. & Rimbach, G. (2001). Molecular aspects of alpha-tocotrienol antioxidant action and cell signaling. *Journal of Nutrition, **131*** (2), 369 – 373.

Paglia, D.E. &. Valentine W.N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *The Journal of Laboratory and Clinical Medicine, **70***,158-169.

Palan, P.R., Mikhail, B.S., Basin J. & Romney S.L. (1973). Plasma levels of antioxidant beta-carotene and tocopherol in uterine cervix dysplasis and cancer. *Nutrition and Cancer, **15***, 13-20.

Pamplona, R. & Barja, G. (2006). Mitochondrial oxidative stess, ageing and caloric restriction: the protein and methionine connection. *Biochimica Biophysica Acta, **1757**,* 496-508

Pavia, D.L., Lampman, G.M. & Kriz, G.S. (1982). Introduction to Organic Laboratory Technique. *Nicotine*. pp. 48-49. 2nd Edition. Saunders College Publishing. USA.

Pekkarinen, S.S., Heimonen, I.M. & Hopia, A.I. (1999). Flavonoids: quercetrin, myricetin, kaemferol and (+) – catechin as antioxidants in methyl linoleate. *Journal of the Science of Food and Agriculture, **79**,* 499-506.

Pearson D (1976). The chemical Analysis of food. pp. 3. Churchill Livingstone Edinburgh.

Pereira, C.L., Santos, P.C.L., Ferreira, N.E., Hueb, W.A., Krieger, J.E., & Pereira, Q.C. (2012). Higher incidence of death in multi-vessel coronary artery disease patients associated with polymorphisms in chromosome 9p21. *BMC Cardiovascular Disorders, **12***, 61. DOI: 10.1186/1471-2261-12-61

Peterson, I., Lagiou, P., Samoli, E., Lagiou, A., Katsouyanni, K., La Vacchia, C., Duyer, J. & Trichopoulos, D. (2003). Flavonoid intake and breast cancer risk: a case – control study in Greece. *British Journal of Cancer, **89***, 1255-1259.

Pham-Huy, L.A., He, H. & Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science, **4***(2), 89-96.

Pohl, L.R., Schulick, R.D. & Highet, R.J. (1984) Reductive-oxygenation mechanism of metabolism of carbon tetrachloride to phosgene by cytochrome P-450. *Molecular Pharmacology, **25,*** 318–321.

Pratt, W. B. (1990). The entry, distribution and elimination of drugs. In: Pratt W.B., and Taylor, P, (eds). *Principles of Drug Action*. 3rd ed. Chuchhill Livingstone. New York.

Preethi K.C. & Kuttan R. (2009). Hepato and reno protective action of *Calendula officinalis L*. flower extract. *Experimental Biology*, ***47***, 163–168

Price, K.R., Johnson, L.I, & Feriwick, H. (1987). The chemical and biological significance of saponins in foods and feeding stuffs. *CRC Critical Reviews in Food Science and Nutrition,* ***266,*** 127-135.

Qui, S., Sun, H., Zhang, A.H., Xu, H.Y., Yan, G.L. & Han, Y. (2014). Natural alkaloids, basic aspects, biological roles and future perspectives. *Chinese Journal of Natural Medicine*, ***12***(6), 401-406.

Quiles, J.L., Ochoa, J.J., Huertas, J.R. & Mataix, J (2004a). Coenzyme Q supplementation protects from age-related DNA double-strand breaks and increases lifespan in rats fed on a PUFA-rich diet. *Experimental Gerontology, **39***, 189–194.

Quiles, J.L., Ochoa, J.J., Ramirez-Tortosa, C., Battino, M., Huertas, J.R., Martín, Y. & Mataix, J (2004b). Dietary fat type (virgin olive vs. sunflower oils) affects age-related changes in DNA double-strand-breaks, antioxidant capacity and blood lipids in rats. *Experimental Gerontology, **39***, 1189–1198.

Raja, S., Ahmed, N., Kumar, V., Mukherjee, K., Bandyopadhyan, A. & Mukherjee, K.P. (2007). Antioxidant effect of *Cytisus scoparius* against carbon tetrachloride treated liver injury in rats. *Journal of Ethnopharmacology, **109***, 41-47.

Raja, S., K.F. Ahamed, V., Kumar, K., Mukherjee, A., Bandyopadhyay P.K. & Mukherjee, P.K. (2007). Antioxidant effect of *Cytisus scoparius* against carbon tetrachloride treated liver injury in rats. *Journal of Ethnopharmacology*, ***109***, 41-47.

Raju, S.M & Madala, B (2005). *Interstitial Uptake and Transport of Lipids*. In: Illustrated medical Biochemistry (pp 293-303). 5th Ed. Jaypee Broyhers Medical Publishers (P) Ltd, New Delhi.

Raffauf, R.F. (1996). *Plant Alkaloids: A Guide to Their Discovery and Distribution*. (pp. 12, 76-79). Hawkworth Press, Incorporated, New York.

Ramirez-Tortosa, M.C., García-Alonso, J., Vidal-Guevara, M.L., Quiles, J.L., Periago, M.J., Linde, J., Mesa, M.D., Ros, G., Abellá, P. & Gil, A. (2004). Oxidative stress status in an institutionalized elderly group after the intake of a phenolic-rich dessert. *British Journal of Nutrition, **91,*** 943–950.

Rao, R.V.K., Ali, N. & Reday, M.N. (1978). Occurrence of both sapogenius and alkaloid lycorine in *curculigoorchiodes*. *Indian Journal of Pharmaceutical Sciences, **40***, 104-105.

Rattan, S.I.S. (2006). "Theories of biological ageing" genes, proteins and free radical. *Free Radical Research, **40***(12), 123–1238.

Reader's Digest (1998). *Antioxidants: Sorting Through Facts and Hype*. In: Foods that Harms, Foods that Protects An A-Z Guide to Safe and Healthy ating. (pp 26-27). The Reader's Digests Association, Inc. Pleasantville, New York. USA.

Reinke, L.A & Janzen, E.G. (1991) Detection of spin adducts in blood after administration of carbon tetrachloride to rats. *Chemico- Biological Interactions, **78***, 155–165.

Reiter, R.J., Tan, D., Mayo, J.C., Sainz, R.M. Leon, J. & Czarnocki, Z. (2003). Melatonin as an antioxidant: biochemical mechanisms and pathophysiological implications in humans. *Acta Biochemica Polonica*, ***50***(4), 1129-1146.

Reitman, S. & Frankel, S. (1957). A Colorimetric method of determination of serum glutamic oxaloacetic and glutamic pyruvic transminase. *American Journal of Pathology*, ***28,*** 56–62.

Reynolds R.M. & Seckl J.R. (2005). Hyponatraemia for the clinical endocrinologist. *Clinical Endocrinology*, **63**, 366-374 [PMID: 16181228 DOI: 10.1111/j.1365-2265.2005.02318.x]

Reynolds, E.S., Treinen, R.J. & Farrish, H.H. (1984) Metabolism of [14C] carbon tetrachloride to exhaled, excreted and bound metabolites. *Biochemical Pharmacology, **33**,* 3363–3374.

Riboli, E. & Norat, T. (2003). Epidemiological evidence of the protective effect of fruit and vegetables on cancer risk. *American Journal of Clinical Nutrition, **78**,* 5595-5695.

Ridnour, L.A., Thomas, D.D., Manchardi, D., Espey, M.G., Miranda, K.M. & Paolocci, N. (2004). The chemistry of nitrosative stress induced by nitric oxide and reactive and nitrogen oxide species. Putting perspectives on stressfull biological situations. *Biological Chemistry*, ***385***, 1-10

Rishton, G.M. (2008). Natural products as a robust source of new drugs and drug 12 leads: Art success and Present day issues. *American Journal of Cardiology, **101***(10), 43-49.

Rosalinda G., José L. A., Manuel P., Oscar P. V., Carmen J. S. & Patricia Y. (2009) *Oxidative Stress Modulation by Rosmarinus officinalis in CCl_4-induced Liver Cirrhosis, Phytotherapy Research*, Published online in Wiley InterScience (www.interscience.wiley.com) **DOI**: 10.1002/ptr.2997.

Rozman, K.K. & Klaasen, C.D. (2001). *Absorption, distribution and excretion of toxicants*. In: Klaassen, C. O. ed. Cassarett and Douls toxicology. The Basic Science of Toxicology. 6th ed. McGraw Hill. New York.

Russell, J.J., Seetula, J.A. & Gutman, D (1990) Kinetics and thermochemistry of the equilibrium $CCl_3 + O_2 \approx CCl_3O_2$. *Journal of Physical Chemistry, **94,*** 3277–3283.

Russo, P., Frustaci, A., Del Bufalo, A., Firu, M. & Cesario, A. (2013). Multitarget drugs of plants origin acting on Alzheimer's disease. *Current Medical Chemistry, **20***(13), 1686-1693.

Saad, A. A., Mokhamer, E. M., Mohsen, M. A. A. & Fadaly, G. A. (2014) Attenuation of carbon tetrachloride-induced hepatic fibrosis by glycine, vitamin E and vitamin C. *Journal of Experimental and Integrative Medicine, 4* (3), 180-186. doi:10.5455/jeim.150514.or.104

Saikada, I., Kayano, K., Wasaki, S., Nagatomi, A., Matsumura, Y. & Okita, E. (1995). Protection of acetaminophen- induced liver injury *in vivo* by an iron chelator, deferoxamine. *Scandinavian Journal of Gastroenterology, **30***, 61-67.

Sarhan, N. A. Z., El-Denshary, E. S., Hassan, N. S., Abu-Salem, F. M., & Abdel-Wahhab, M. A. (2012). Isoflavones-enriched soy protein prevents CCl_4-induced hepatotoxicity in rats. *ISRN Pharmacology*, 347930. http://doi.org/10.5402/2012/347930

Sato, A. & Nakajima, T. (1987). Pharmacokinetics of organic solvent vapors in relation to their toxicity. *Scandinavian Journal of Work, Environment and Health, **13***, 81–93.

Schaefer, E. (2002). Lipoprotein nutrition and heart disease. *American Journal of Clinical Nutrition, **75***, 191.

Schauenstein, E. & Esterbauer, H. (1979). Formation and properties of reactive aldehydes. *Ciba Foundation Symposium, **67***, 225–244.

Schaur, R.J. (2003). Basic aspects of the biochemical reactivity of 4- hydroxynonenal. *Molecular Aspects of Medicine, **24***,149–159.

Shacter, E. (2000a). Protein oxidative damage. *Methods in Enzymology, **319***, 428-436.

Shacter, E. (2000b). Quantification and significance of protein oxidation and 20s proteasome aza dependent proteolysis in mammalian cells. *Cellular and Molecular Life Sciences, **58***, 1442-1450.

Shah, H., Hartman, S.P. & Weinhouse, S. (1979). Formation of carbonyl chloride in carbon tetrachloride metabolism by rat liver *in vitro*. *Cancer Research, **39**,* 3942–3947.

Shahani S. (1999). Evaluation of hepatoprotective efficacy of APCL-A polyherbal formulation *in vivo* in rats. *Indian Drugs, **36**,* 628-631.

Slater, T.F. (1981) Free radicals as reactive intermediates in tissue injury. *Advances in Experimental Medicine and Biology, **136,*** 575–589.

Södergren, E., Cederberg, J., Vessby, B. & Basu, S. (2001). Vitamin E reduces lipid peroxidation in experimental hepatotoxicity in rats. *European Journal of Nutrition, **40*** (1), 10-6.

Sohal, R.S. (2002). Oxidative stress hypothesis of aging. *Free Radical Biology and Medicine, **33***(5), 573–574.

Speakman, J.R. & Selman, C. (2011). The free-radical damage theory: Accumulating evidence against a simple link of oxidative stress to ageing and lifespan. *BioEssays, **33***(4), 255-9.

Stadtman, E.R. & Berlett, B.S. (1998). Reactive oxygen – mediated protein oxidation in ageing and disease. *Drug Metabolism Reviews, **30**,* 225-243.

Stadtman, E.R. & Levine, R.L. (2003). Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. *Amino Acids, **25***(3–4), 207–218.

Stadtman, E.R., Remmen, H.V., Richardson, A., Wehr, N.B. & Levine, R.L. (2005). Methionine oxidation and ageing, *Biochimica and Biophysica Acta, **1703,*** 135–140.

Stewart, R.D., Dodd, H.C. & Erley, D.S. (1965). Diagnosis of solvent poisoning. *Journal of American Medical Association, **193***, 115–118.

TCMD (Taber's Cyclopedic Medical Dictionary) (1993). 17th Ed. Davis Company, Philadelphia.

TCMD (Taber's Cyclopedic Medical Dictionary) (1993). Carbon Tetrachloride. (pp 308). 17th Ed. Davis Company, Philadelphia.

Thrabrew, M.I., Joice, P.D. & Rajatissa, W. (1987). A comparative study of the efficacy of *Pavetta indica* and *Osbeckia octandra* in the treatment of liver dysfunction. *Planta Medica*, ***53***, 239-241.

Tietz, N.W. (1976). *Fundamentals of Clinical Chemistry*. 3rd Ed. W.B. Saunders Company, Philadelphia. London.

Tietz, N.W. (1987). *Fundamentals of Clinical Chemistry*. (pp. 470 – 560). 3rd Ed. W.B. Saunders Company, Philadelphia. London.

Tietz, N.W. (1995). *Clinical guide to Laboratory Test*. (pp. 518-519). 3rd Ed. W.B. Sunders Company, Philadelphia.

Tomasi, A., Albano, E. & Banni, S (1987) Free-radical metabolism of carbon tetrachloride in rat liver mitochondria. A study of the mechanism of action. *Biochemical Journal*, ***246,*** 313–317.

Traber, M.G. & Atkinson, J. (2007). Vitamin E, antioxidant and nothing more. *Free Radical Biology and Medicine*, ***43***(1), 4-15.

Traore, F., Faure, F., Ollivier, E., Gasquet, M., Azas, N., Debrauwer, L., Keita, A., Timon – David, P. & Balansard G. (2000). Structure and anti protozoal activity of triterpenoid and saponins from *Glinus oppositifolius*. *Planta Medica*, ***66,*** 368-371.

Trauchimand, S., Browant, P. & Lacazio, G. (2010) The rutin catabolic pathway with special emphasis or quercetinase. *Biodegradation*, ***21***(6), 833-859.

Trease, G. E. & Evans, M.C. (1989). Textbook of Pharmacognosy, (pp. 200- 201, 304 – 348, 419 – 423, 626 – 630, 765 – 775). 13th ed. Bailiere Tindall.

Tretter, L. & Adam-Vizi, V. (2005). Alpha-ketoglutarate dehydrogenase: a target and generator of oxidative stress. *Philosophical Transactions of Royal Society of London B-Biological Sciences, **360***, 2335-2345.

Tretter, L., Takacs, K., Hegedus, V. & Adam-Vizi, V. (2007). Characteristics of alpha glycerophosphate-evoked H_2O_2 generation in brain mitochondria. *Journal of Neurochemistry, **100***, 650-663.

Tsala D. E., Penlab B.V., Nnanga N., Mendimi N. J. Kouamouo J. & Dimo T. (2010) Protective activity of the stem bark methanol extract of *Alafia multiflora* against carbon tetrachloride-induced hepatotoxicity in rats. *International Journal of Pharmaceutical Sciences Review and Research,* **3** (2), 157-163

Tune, B.M. (1998). In: DeBroe M.E., Pates, G.A. & Bennett, M.W., eds. *Clinical Nephrotoxins: Renal injury from drugs and chemicals*. Dordrecht: Kluwer. Academic Publishers.

U.S. EPA (Environmental Protection Agency). (2001) *Toxicological Review of Chloroform*. Integrated Risk Information System (IRIS), National Center for Environmental Assessment, Washington, DC; EPA/635/R-01/001. Available online at http://www.epa.gov/iris (accessed June 24, 2009).

Uehleke, H., Hellmer, K.H. & Tabarelli, S. (1973) Binding of 14C-carbon tetrachloride to microsomal proteins *in vitro* and formation of $CHC1_3$ by reduced liver microsomes. *Xenobiotica, **3,*** 1–11.

Ujowundu C.O., Igwe C.U., Enemor V.H.A., Nwaogu L.A. & Okafor O.E. (2008) Nutritive and anti-nutritive properties of *Boerhavia diffusa* and C*ommelina nudiflora* leaves. *Pakistan Journal of Nutrition, **7*** (1), 90-92.

Ujowundu C.O., Kalu F.N., Nwaoguikpe R.N., Okechukwu R. I. & Ihejirika C.E. (2012) The antioxidative potentials of G*ongronema latifolium* on diesel petroleum induced hepatotoxicity. *Journal of Applied Pharmaceutical Science,* ***02*** (01), 90-94

Ujowundu C.O., Nwokedinobi N., Kalu F.N., Nwaoguikpe R.N. & Okechukwu R.I. (2011). Chemoprotective potentials of O*cimum gratissimum* in diesel petroleum induced hepatotoxicity in albino wistar rats. *Journal of Applied Pharmaceutical Science,* ***01*** (10), 56-61

Ujowundu C.O., Okoye, H.N., Nwaoguikpe, R.N., Belonwu, D.C., Igwe K.O. & Ujowundu F.N. (2014). Hepatoprotective effects of crude extracts of tomato and onion in rats exposed to locally processed beef. *International Journal Biochemistry Research and Reviews,* ***4*****(2)**, 193-203

Valko, M. Morris, H. & Cronin, M. T. (2005). Metals, toxicity and oxidative stress. *Current Medical Chemistry*. ***12*** (10), 1161 – 1208.

Van Gaal L.F., Mertens I.L. & De Block C.E. (2006) Mechanisms linking obesity with cardiovascular disease. *Nature,* **444**, 875-880 | doi:10.1038/nature05487.

Verlangieri, A.J., Kapeghian, J.C., Dean, S., & Bush, M. (1985). Fruit and vegetable consumption and cardiovascular mortality. *Medical Hypothesis,* ***16***, 7-15.

Vita, J.A. (2005). Polyphenols and cardiovascular disease: effects on endothelial and platelet function. *American Journal of Chinical Nutrition,* ***81***(1), 2925-75.

Wallin, B., Rosengren, B., Shetzer, H.G. & Camejo, G. (1993). Lipoprotein oxidation and measurement of thiobarbituric acid reacting substances (TBARS) formation in a single microlitre plate: its use for evaluation of antioxidants. *Annual Journal of Biochemistry,* ***208***, 10-15.

Wardlaw, G.M., Hampl, J.S., & DiSilvestro, R.A. (2004). Perspective in Nutrition, (pp 330-331). 6th edition.McGraw-Hill. New York.

Waterman, P.G. & Mole, S. (1994). *Analysis of Phenolic Plant Metabolites.* (pp 162-173). Blackwell Scientific Publications, New York.

Wettasinghe, M. & Shahidi, F. (2000). Scavenging of reactive-oxygen species and DPPH free radicals by extracts of borage and evening primrose meals. *Food Chemistry, **70***, 17-26.

Wheeler, M.D., Kono, H., Yin, M., Rusyn, J., Froh, M., Connor, H.D. et al., (2001). Delivery of the Cu/Zn-superoxide dismutase gene with adenovirus reduces early alcohol – induced liver injury in rats. *Gastroenterology, **120***, 1241-1250.

Wilcox, B.J., Curb, J.D. & Rodriguez, B.L. (2008). Antioxidants in cardiovascular health and disease: Key lessons from epidemiologic studies. *American Journal of Cardiology, **101***, 75D-86D.

Wilcox, J.K., Ash, S.L. & Gtignani, G.L. (2004). Antioxidants and prevention of chronic disease. Review. *Critical Reviews in Food Science and Nutrition, **44***, 275-295.

Willaims, R.J., Spencer, J.P. & Rice – Evans, C. (2004). Flavonoids: antioxidants or signalling molecules? *Free Radical Biology and Medicine, **36(***7), 839-849.

Wink, M. (1998). Modes of action of alkaloids. In: Roberts, M.F. & Wink, M., eds. *Alkaloids: Biochemistry, Ecology, and Medicinal Applications*, (pp. 301–326). New York: Plenum Press.

Wong, F.W., Chan, W. & Lee, S.S. (1998). Resistance to carbon tetrachloride-induced hepatotoxicity in mice which lack CYP2E1 expression. *Toxicology and Applied Pharmacolgy, **153***, 109–118.

Xin, Z., Waterman, D.F., Henken, R.M. & Harmon, R.J. (1991). Effects of Copper status on neutrophil function, Superoxide dismutase and Copper distribution in Steers. *Journal of Diary Sciences, **74***, 3078.

Yin, D. & Brunk, U.F. (1995). Carbonyl toxification hypothesis of biological aging. In: Macieira-Coelho, A. (Ed.), *Molecular Basis of Aging*. (pp. 421–436). CRC Press, Inc., New York.

Yin, D. (2003). Aging: a war between life and entropy. *Clinical Journal of Gerontology*, ***23***,555-59.

Young D.S. (1995). *Effect of Drug on Clinical Laboratory Tests*, 4th Ed. AACC Press.

Young, I. & Woodside, J. (2001). Antioxidants in health and disease. *Journal of Clinical Pathology*, ***54***, 176-186.

Yu L., Haley S., Perret J., Harris M., Wilson J. & Qian M. (2002). Free radicals scavenging activities of wheat extracts. *Journal of Agricutural and Food Chemistry*, ***50***, 1619-1624.

Yu, B.P. (1996). Aging and oxidative stress: modulation by dietary restriction. *Free Radical Biology and Medicine, **2***, 651–668.

Zangar, R.C., Benson, J.M. & Burnett, V.L. (2000) Cytochrome P450 2E1 is the primary enzyme responsible for low-dose carbon tetrachloride metabolism in human liver microsomes. *Chemco- Biological Interaction, 125*, 233–243.

Zarkovic, K. (2003). 4-Hydroxynonenal and neurodegenerative diseases. *Molecular Aspects of Medicine, **24***, 293–303

Zbidah, M., Lupesv, A., Jilani, K., Fajol, A., Michael, D., Quadri, S. M. & Lang, F. (2012) Apigenin-induced suicidal erythrocyte death. *Journal of Agriculture and Food Chemistry*, ***60***(1), 533-538. Doi: 10-1021/jf204107f PMID 22132906.

Zhang, J., Lin, X., & Lei, X. (2010). Discovery and synthesis of novel luteolin derivatives, as dat agonist. *Biorganic and Medicinal Chemistry*, ***18***(22), 7842-7848.

Zhao, G., Qin, G.W., Wang, J., Chu, W.J. & Guo, L.H. (2010). Functional activation of monoamine transporters by Iuteolin and apigenin isolated from the fruit of *Perilla frutescence (L)*. *Britt Neurochemistry International,* ***56***(1), 108-176.

Zheng, Y., Haworth, I.S., Zuo, Z., Chow, M.S., & A.H. (2005). Physiochemical and structural characterization of guercetin-beta-cyclodextrin complexes. *Journal of Pharmaceutical Sciences,* ***94***(5), 1079-1089.

Zinggy, J. M. & Azzi, A. (2004). Non-oxidant activities of vitamin E. *Current Medical Chemistry* ***11*** (9), 1113 – 1133.

Zoppi, F.P.A., Felini, D., Marcovina, S. & Ramella, C. (1976). Metodo per la determinazione della bilirubin totale e congluta. Uso di un tensioaltivo cationico come agentte solubilizzante. *Giorn Italian Chimica Clinica*, ***1***, 343-359.

Appendix 4A: Threshold inhibitory concentration scavenging ability of Ethanol of Extract *C. dolichopentalum* on some free radicals reactive intermediates

	Threshold Inhibitory Concentration/Scavenging Power of EECD (mg/ml)					
	IC5	**IC10**	**IC20**	**IC50**	**IC70**	**IC80**
Nitric Oxide Radical (No-) Scavenging	0.27	0.69	1.95	12.04	40.50	98.57
Hydroxyl Radicals (OH-) Scavengers	0.32	1.21	5.40	92.39	1173.16	
Lipid Peroxidation in Rabbit Brain homogenate	19.22	32.74	64.70	491.72		

Appendix 4B: The inhibitory concentration of ethanol extract of *C. dolichopentalum* against the total dehydrogenase activity (DHA) of some wound isolates

	Inhibitory Concentration against wound isolates						
	EECD (mg/ml)						
	IC_5	**IC_{10}**	**IC_{20}**	**IC_{50}**	**IC_{70}**	**IC_{80}**	**IC_{100}**
Escherichia coli	1.80	9.06	42.95	355.78	822.58	1167.79	2167.10
Samonella typhi	5.74	19.34	67.74	413.19	886.14	1234.23	2270.68
Streptococcus pneumonia	2.59	9.24	36.74	349.42	1159.25	2195.66	11523
Staphylococcus aureus	705.38	716.82	741.69	843.80	975.47	1138.30	ND

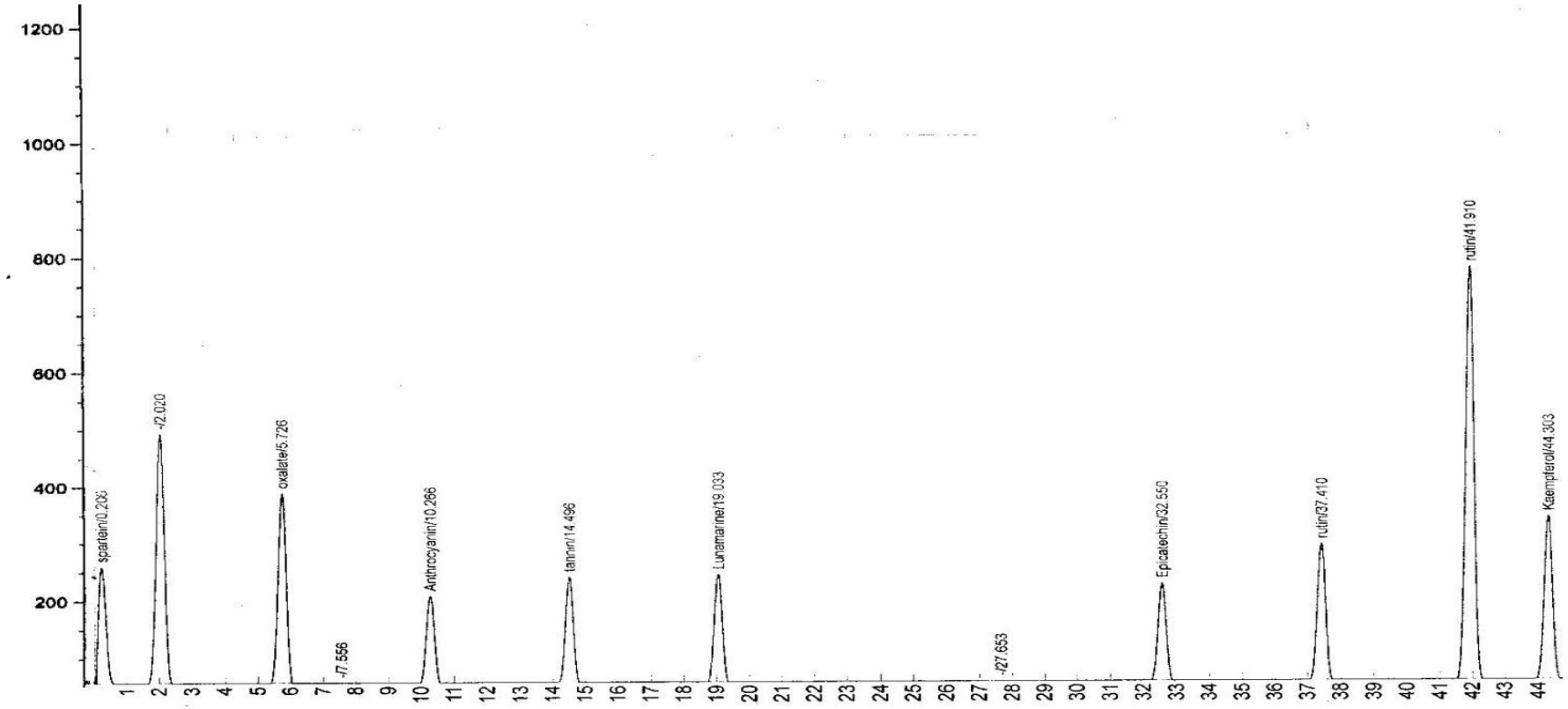
ND = Non-determinable

Appendix 4C: Reducing Power of Ethanol extract of *Combretum dolichopentalum* (EECD) on Iron (iii)

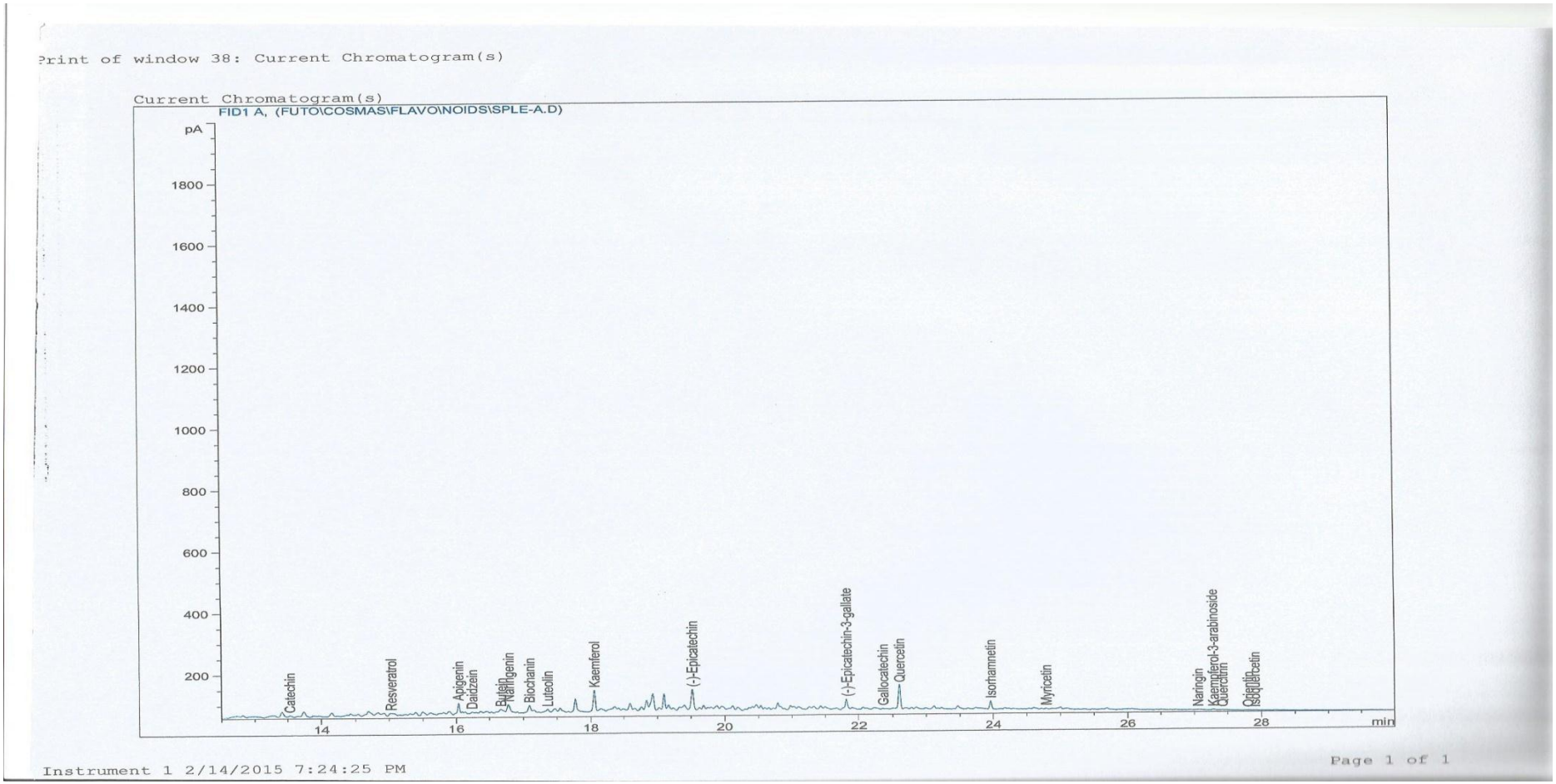
Absorbance Unit concentration/reducing power of EECD (µg/ml)

Reducing Power (RP)	EECD (µg/ml)
0.1	0.61
0.2	16.00
0.3	25.63
0.4	32.97
1.5	39.11

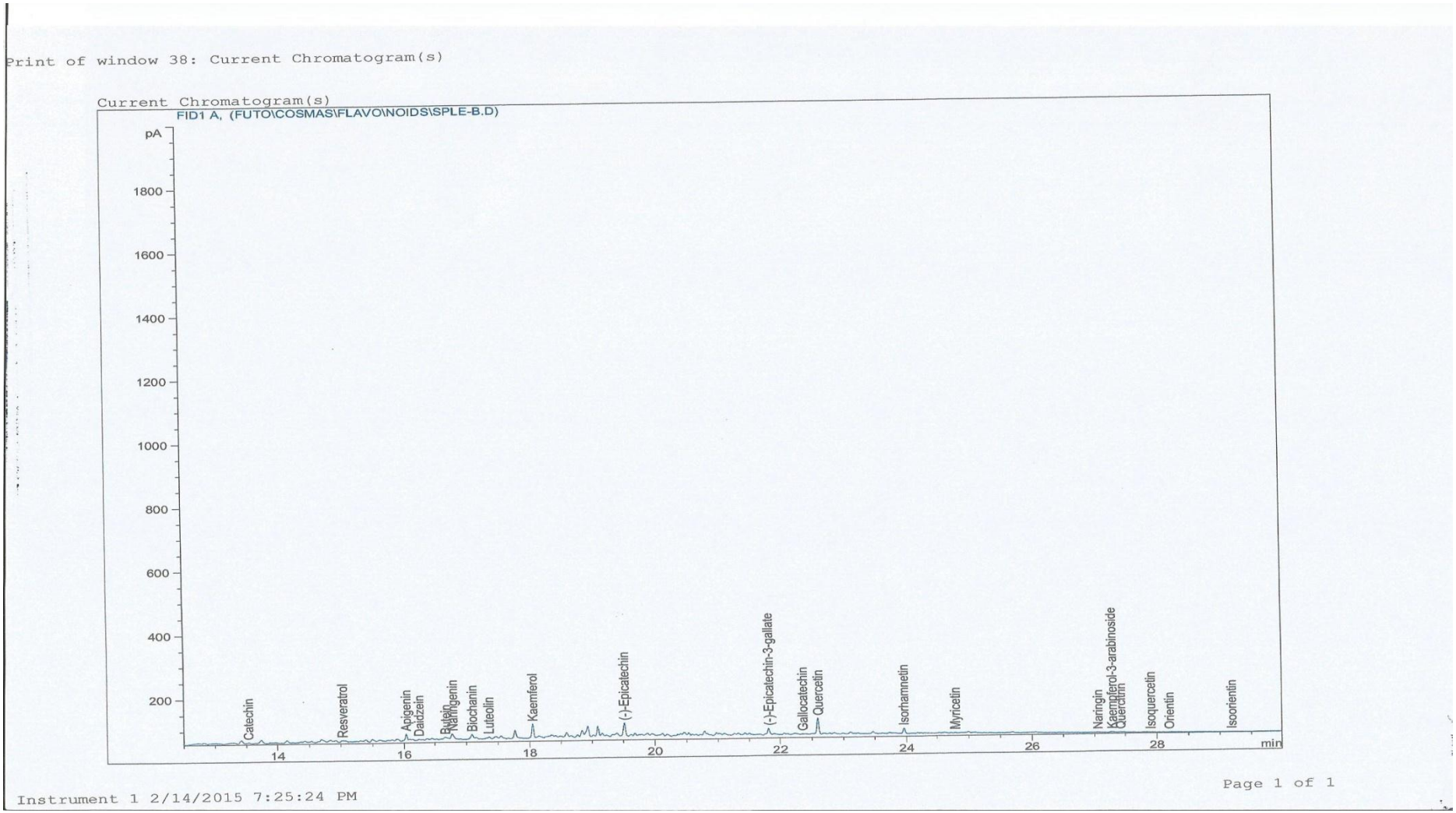
APPENDIX 4D



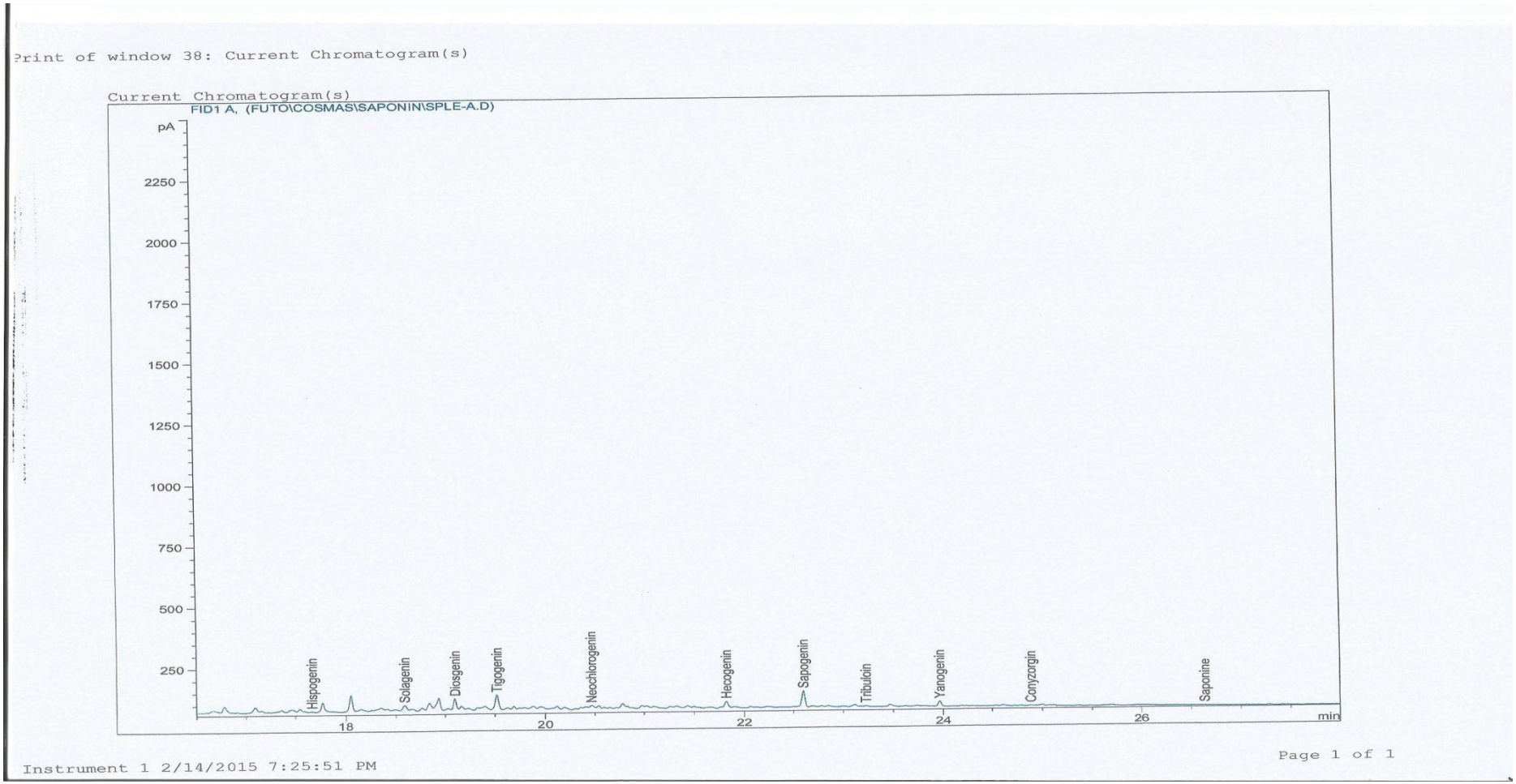
Appendix 4. 1a: Chromatogram of phytochemicals in the leaves of *C. dolichopentalum*



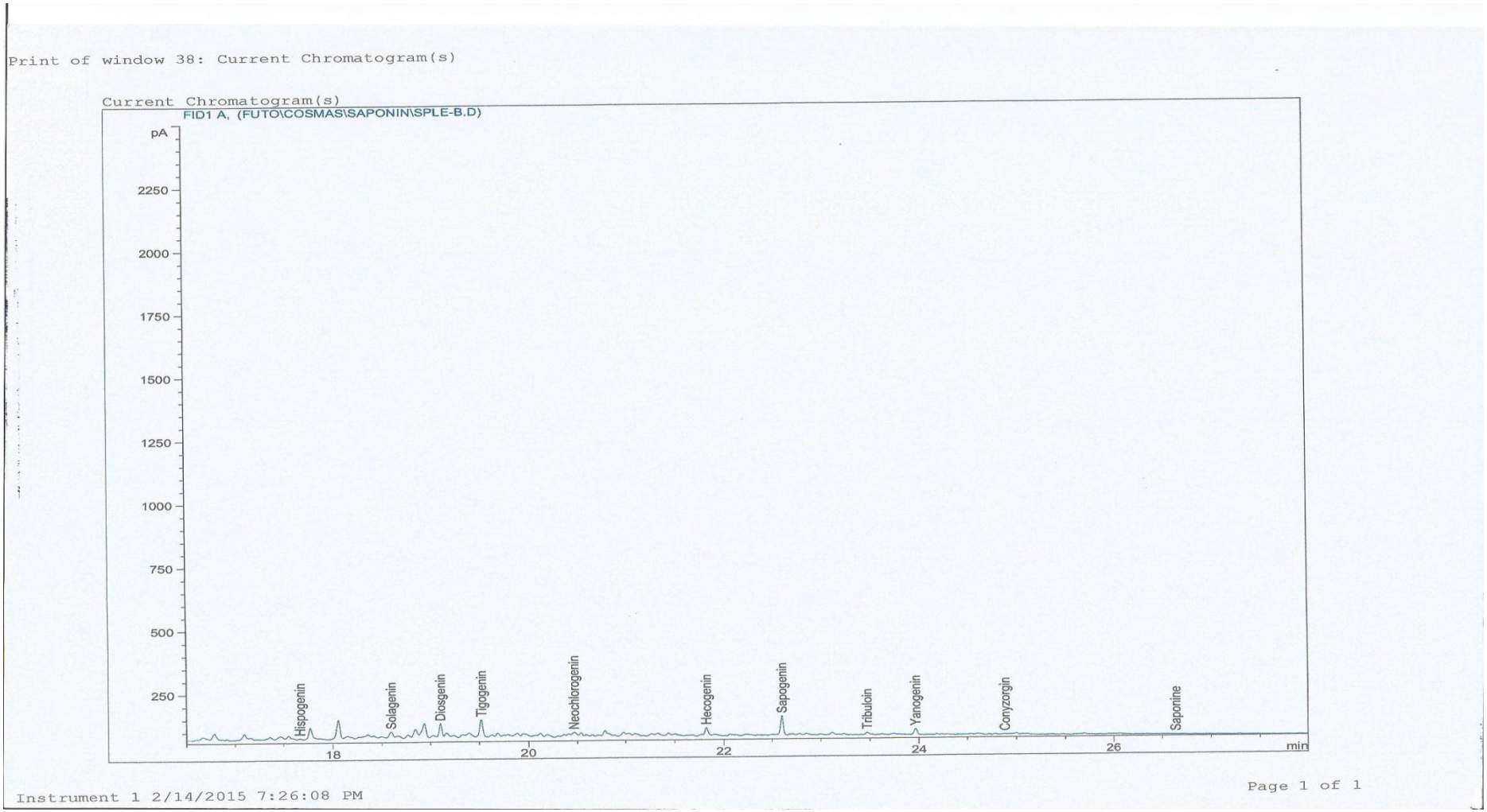
Appendix 4.1b : Chromatogram 1 of flavonoids from *C. Dolichopentalum* leaves



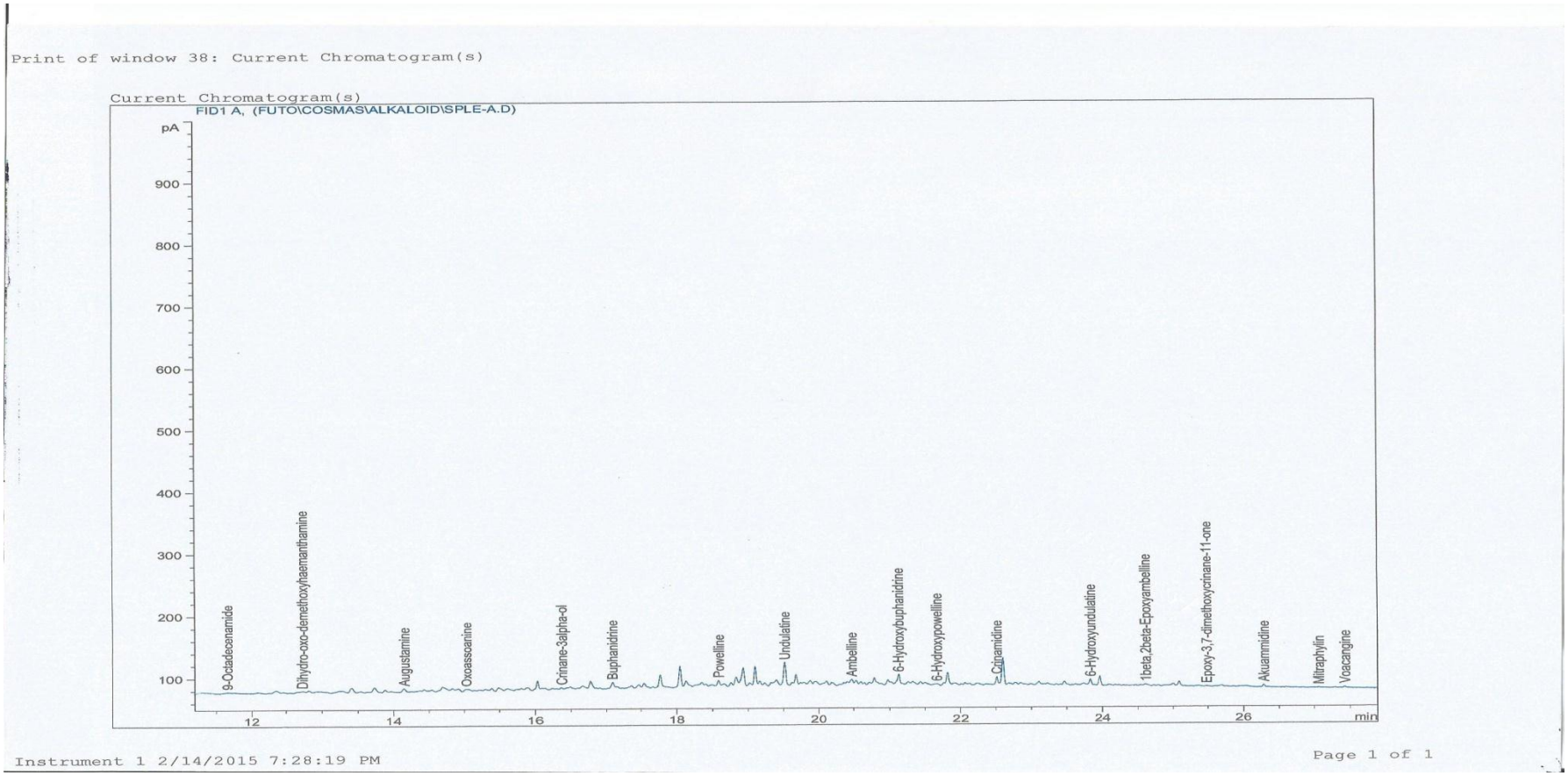
Appendix 4.2 : Chromatogram 2 of flavonoids from *C. Dolichopentalum* leaves



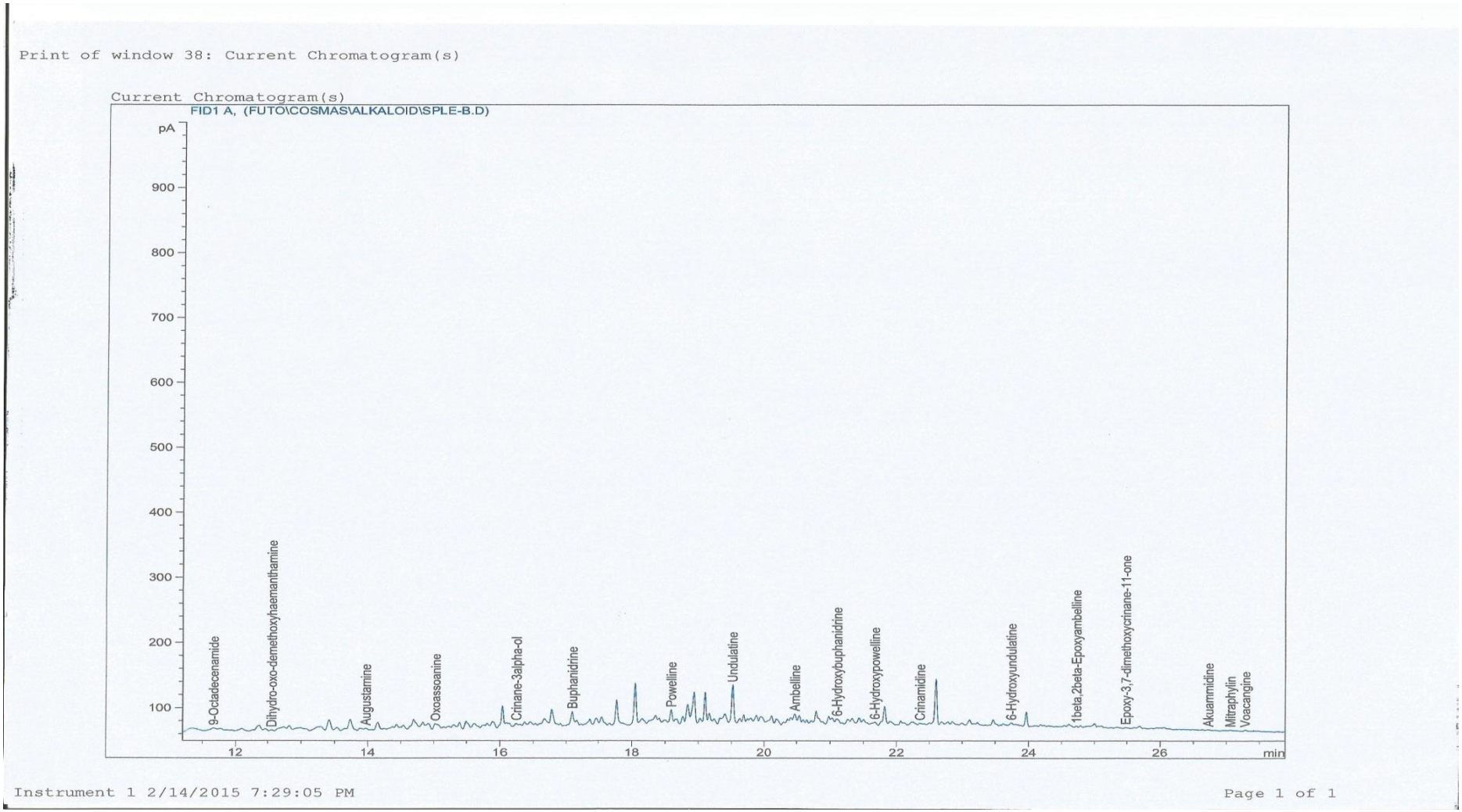
Appendix 4.3 : Chromatogram 1 of Saponins from *C. Dolichopentalum* leaves



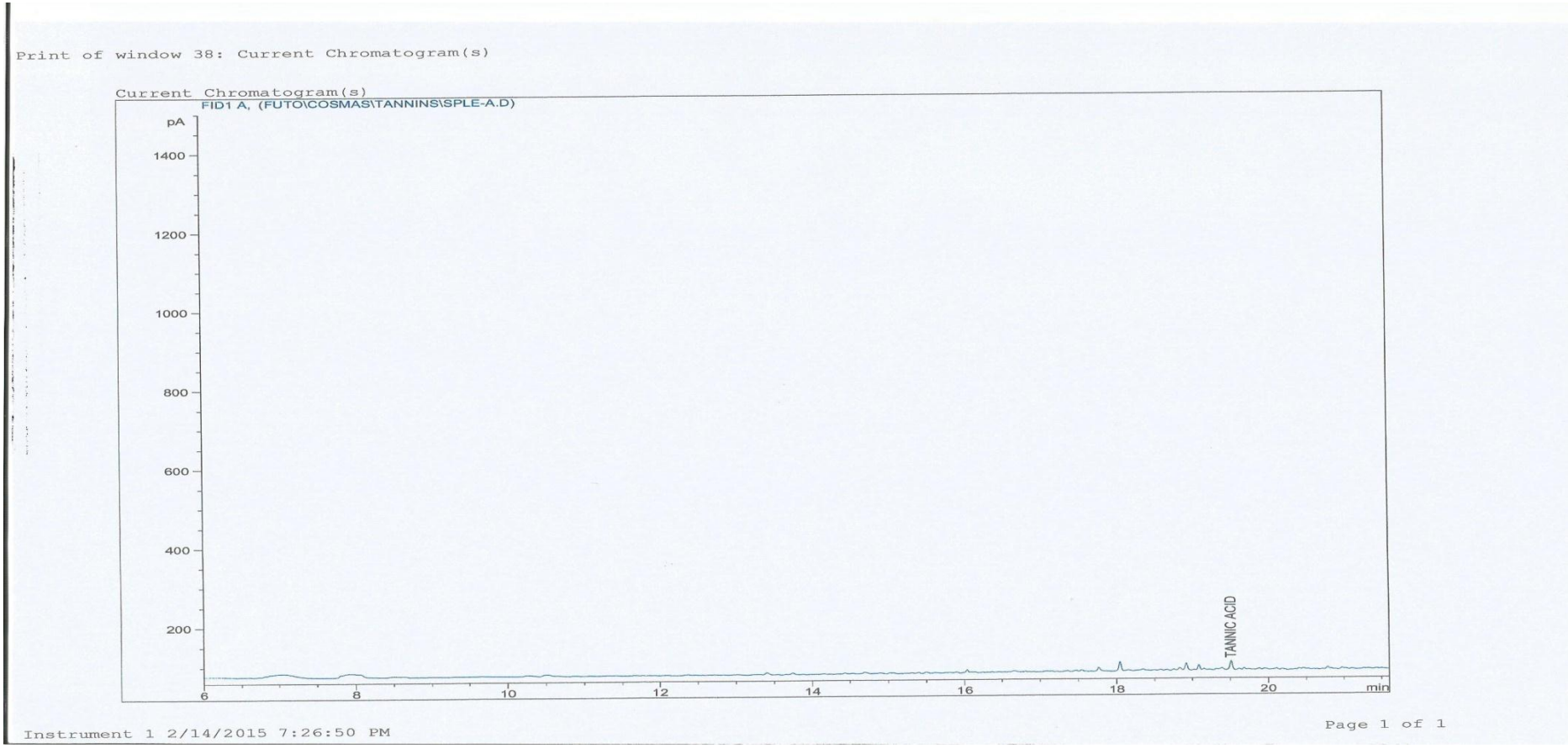
Appendix 4.4 : Chromatogram 2 of Saponins from *C. Dolichopentalum* leaves



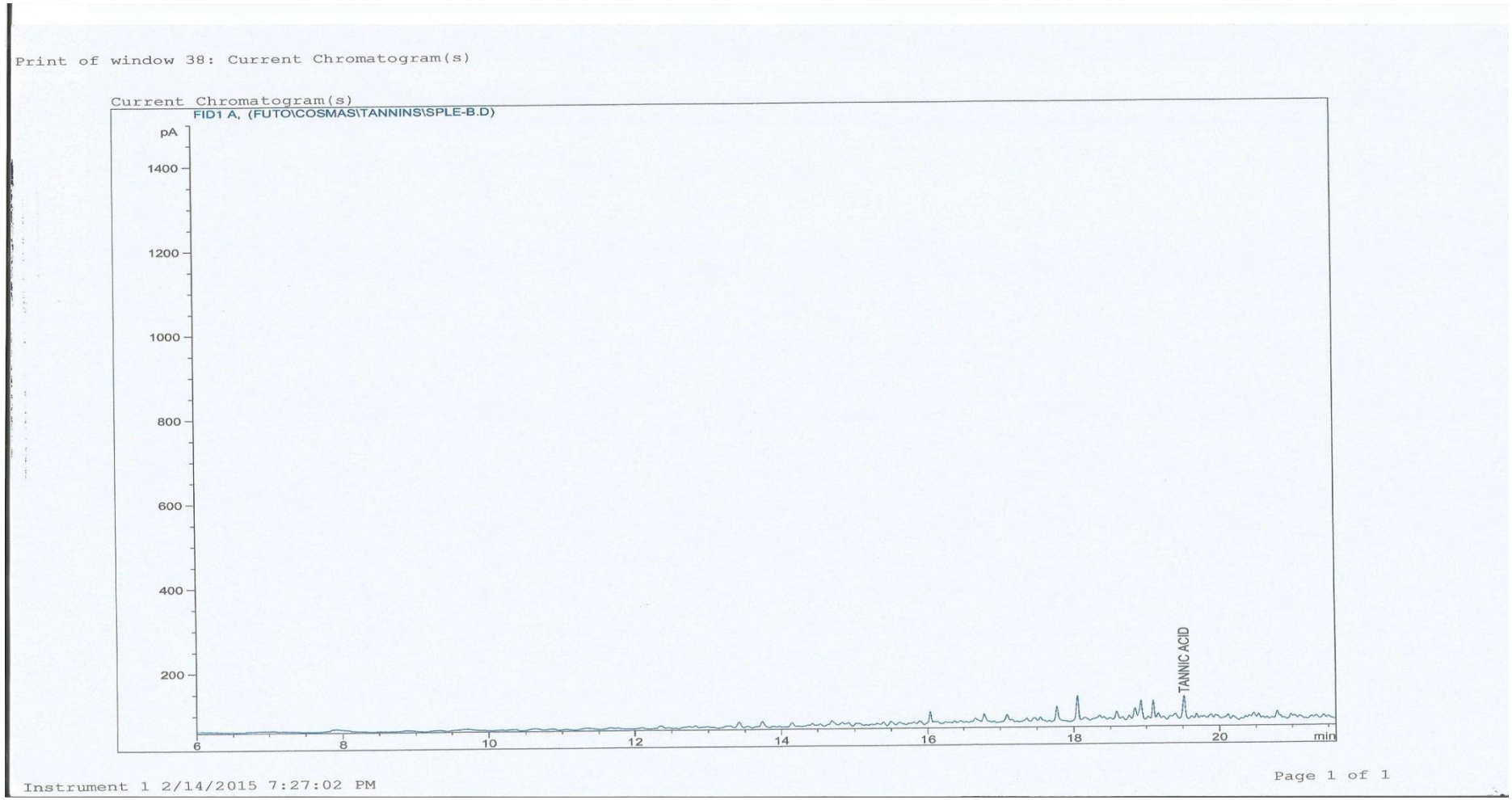
Appendix 4.5 : Chromatogram 1 of alkaloid from *C. dolichopentalum* leaves



Appendix 4.6 : Chromatogram 2 of alkaloid from *C. dolichopentalum* leaves



Appendix 4.7 : Chromatogram 1 of Tannin in leaves of *C. dolichopentalum*



Appendix 4.8 : Chromatogram 2 of Tannin in leaves of *C. dolichopentalum*

Appendix 4.9 : Isolated and Identified Alkaloids 1 in leaves of *C. dolichopentalum* using gas chromatography

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Dilution              :      1.0000

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RetTime  Type     Area      Amt/Area    Amount    Grp   Name
 [min]          [pA*s]                [mg/100g]
-------|------|----------|----------|----------|--|------------------
 11.662 VV T     41.51289 7.35052e-5 3.05141e-3  1 9-Octadecenamide
 12.731 VV T     26.31631 1.41227e-4 3.71657e-3  1 Dihydro-oxo-demethoxyhaemanthamine
 14.150 VV T     44.16800 1.79840e-5 7.94319e-4  1 Augustamine
 15.037 VV T     41.24184 1.55827e-4 6.42660e-3  1 Oxoassoanine
 16.369 VV T     78.30517 1.73406e-4 1.35786e-2  1 Crinane-3alpha-ol
 17.091 VV T    144.86543 2.00984e-4 2.91156e-2  1 Buphanidrine
 18.590 VV T    131.35484 4.62620e-5 6.07674e-3  1 Powelline
 19.519 VV T    164.04178 3.23721e-5 5.31038e-3  1 Undulatine
 20.473 VV T    328.06464 2.01613e-5 6.61421e-3  1 Ambelline
 21.130 VV T    132.26013 2.97526e-5 3.93509e-3  1 6-Hydroxybuphanidrine
 21.666 VV T     78.01532 1.00806e-4 7.86445e-3  1 6-Hydroxypowelline
 22.518 VV T     81.06863 3.89384e-4 3.15668e-2  1 Crinamidine
 23.834 VV T     78.47336 1.20192e-4 9.43189e-3  1 6-Hydroxyundulatine
 24.616 VV T     80.90900 1.14679e-4 9.27855e-3  1 1beta,2beta-Epoxyambelline
 25.482 VV T     31.17142 2.97619e-4 9.27721e-3  1 Epoxy-3,7-dimethoxycrinane-11-one
 26.287 VV T     87.85038 4.82625e-4 4.23988e-2  1 Akuammidine
 27.062 VV T     32.37471 1.38515e-4 4.48438e-3  1 Mitraphylin
 27.431 VB T     25.52666 9.38150e-4 2.39478e-2  1 Voacangine

Totals :                             2.16870e-1

 Results obtained with enhanced integrator!
```

Appendix 4.10 : Isolated and Identified Alkaloids 1 in leaves of *C. dolichopentalum* using gas chromatography

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External Standard Report

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Signal 1: FID1 A,

RetTime [min]	Type	Area [pA*s]	Amt/Area	Amount [mg/100g]	Grp	Name
11.668	VV	66.90569	7.35052e-5	4.91792e-3	1	9-Octadecenamide
12.557	VV	15.39761	1.41227e-4	2.17456e-3	1	Dihydro-oxo-demethoxyhaemanthamine
13.972	VV	45.13803	1.79840e-5	8.11764e-4	1	Augustamine
15.033	VV	92.17308	8.05324e-5	7.42292e-3	1	Oxoassoanine
16.248	VV	96.90446	1.73406e-4	1.68039e-2	1	Crinane-3alpha-ol
17.094	VV	280.31674	2.00984e-4	5.63392e-2	1	Buphanidrine
18.593	VV	225.11903	4.62620e-5	1.04145e-2	1	Powelline
19.522	VV	245.89775	3.23721e-5	7.96023e-3	1	Undulatine
20.471	VV	470.21445	2.01613e-5	9.48013e-3	1	Ambelline
21.103	VV	137.43530	2.97526e-5	4.08906e-3	1	6-Hydroxybuphanidrine
21.672	VV	103.86302	1.00806e-4	1.04701e-2	1	6-Hydroxypowelline
22.361	VV	100.04323	3.89384e-4	3.89552e-2	1	Crinamidine
23.741	VV	151.26994	1.20192e-4	1.81815e-2	1	6-Hydroxyundulatine
24.725	VV	32.92716	1.14679e-4	3.77605e-3	1	1beta,2beta-Epoxyambelline
25.480	VV	40.09403	2.97619e-4	1.19327e-2	1	Epoxy-3,7-dimethoxycrinane-11-one
26.731	VV	16.85006	2.40385e-3	4.05050e-2	1	Akuammidine
27.061	VV	33.46004	1.38515e-4	4.63471e-3	1	Mitraphylin
27.289	VB	19.82763	9.38150e-4	1.86013e-2	1	Voacangine
Totals :				2.67471e-1		

Results obtained with enhanced integrator!

Appendix 4. 11 : Isolated and Identified Flavonoids 1 from *C. Dolichopentalum* leaves using gas chromatography

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External Standard Report

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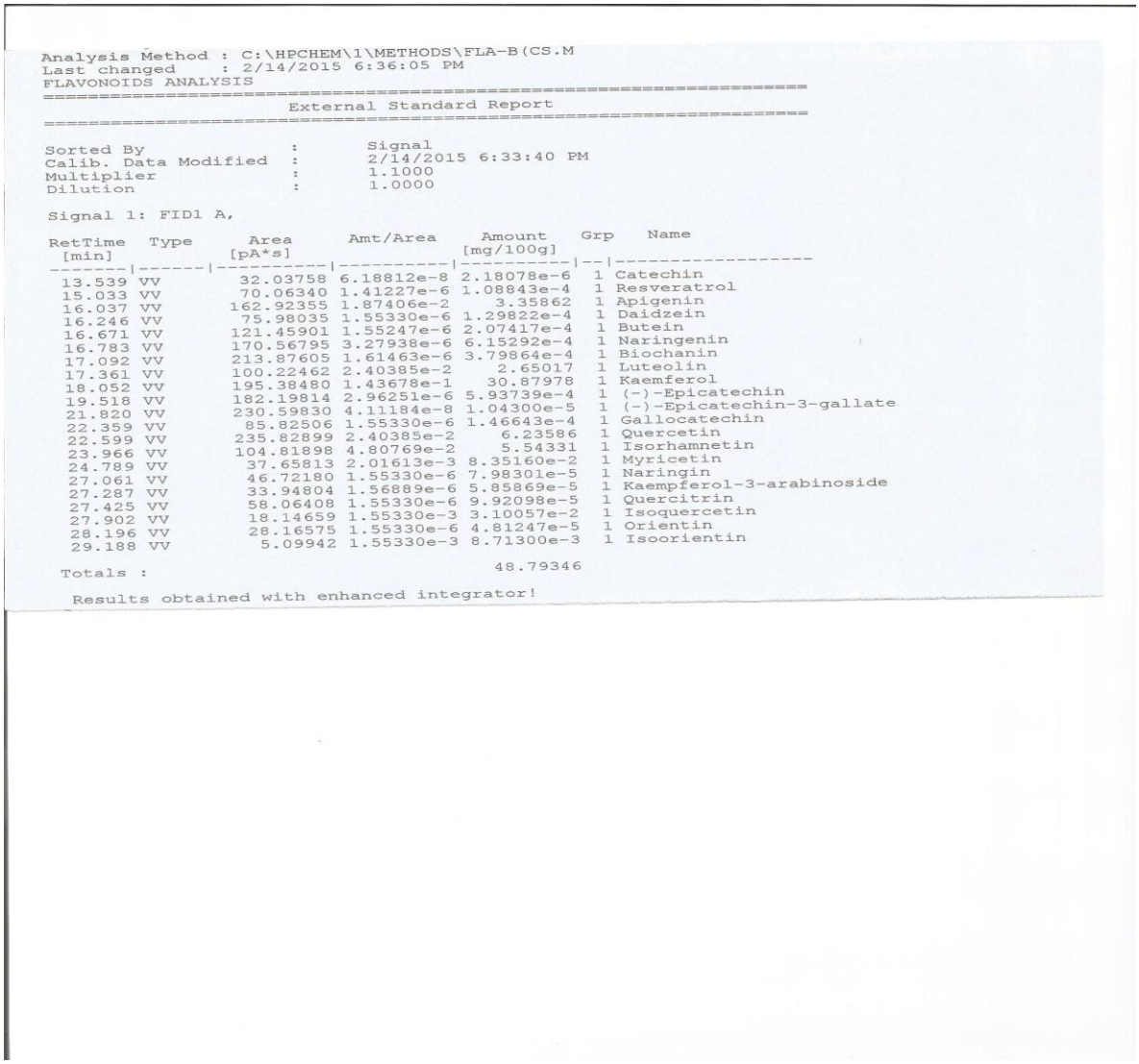
RetTime [min]	Type	Area [pA*s]	Amt/Area	Amount [mg/100g]	Grp	Name
13.538	VV	52.53550	6.18812e-8	3.57605e-6	1	Catechin
15.032	VV	115.66050	1.41227e-6	1.79678e-4	1	Resveratrol
16.039	VV	269.31097	1.14995e-2	3.40665	1	Apigenin
16.247	VV	123.67722	1.55330e-6	2.11318e-4	1	Daidzein
16.673	VV	196.80545	1.55247e-6	3.36087e-4	1	Butein
16.784	VV	277.16980	3.27938e-6	9.99839e-4	1	Naringenin
17.094	VV	351.50620	1.61463e-6	6.24309e-4	1	Biochanin
17.363	VV	164.21095	1.22549e-2	2.21363	1	Luteolin
18.055	VV	321.11240	8.01282e-2	28.30318	1	Kaemferol
19.523	VV	297.45193	2.96251e-6	9.69324e-4	1	(-)-Epicatechin
21.824	VV	237.15164	4.11184e-8	1.07264e-5	1	(-)-Epicatechin-3-gallate
22.361	VV	140.40271	1.55330e-6	2.39896e-4	1	Gallocatechin
22.605	VV	383.00055	1.21832e-2	5.13280	1	Quercetin
23.968	VV	169.88760	2.35849e-2	4.40746	1	Isorhamnetin
24.791	VV	59.92791	2.01613e-6	1.32905e-4	1	Myricetin
27.061	VV	70.50127	1.55330e-6	1.20460e-4	1	Naringin
27.289	VV	53.48841	1.56889e-6	9.23094e-5	1	Kaempferol-3-arabinoside
27.643	VV	26.72414	1.55330e-6	4.56616e-5	1	Quercitrin
27.899	VV	28.61787	1.55330e-3	4.88972e-2	1	Isoquercetin
28.196	VV	42.31996	1.55330e-6	7.23090e-5	1	Orientin
28.839	VV	25.29373	1.55330e-3	4.32175e-2	1	Isoorientin
Totals :				43.55987		

Results obtained with enhanced integrator!

Appendix 4.12: Isolated and Identified Flavonoids 2 from *C. Dolichopentalum* leaves using gas chromatography



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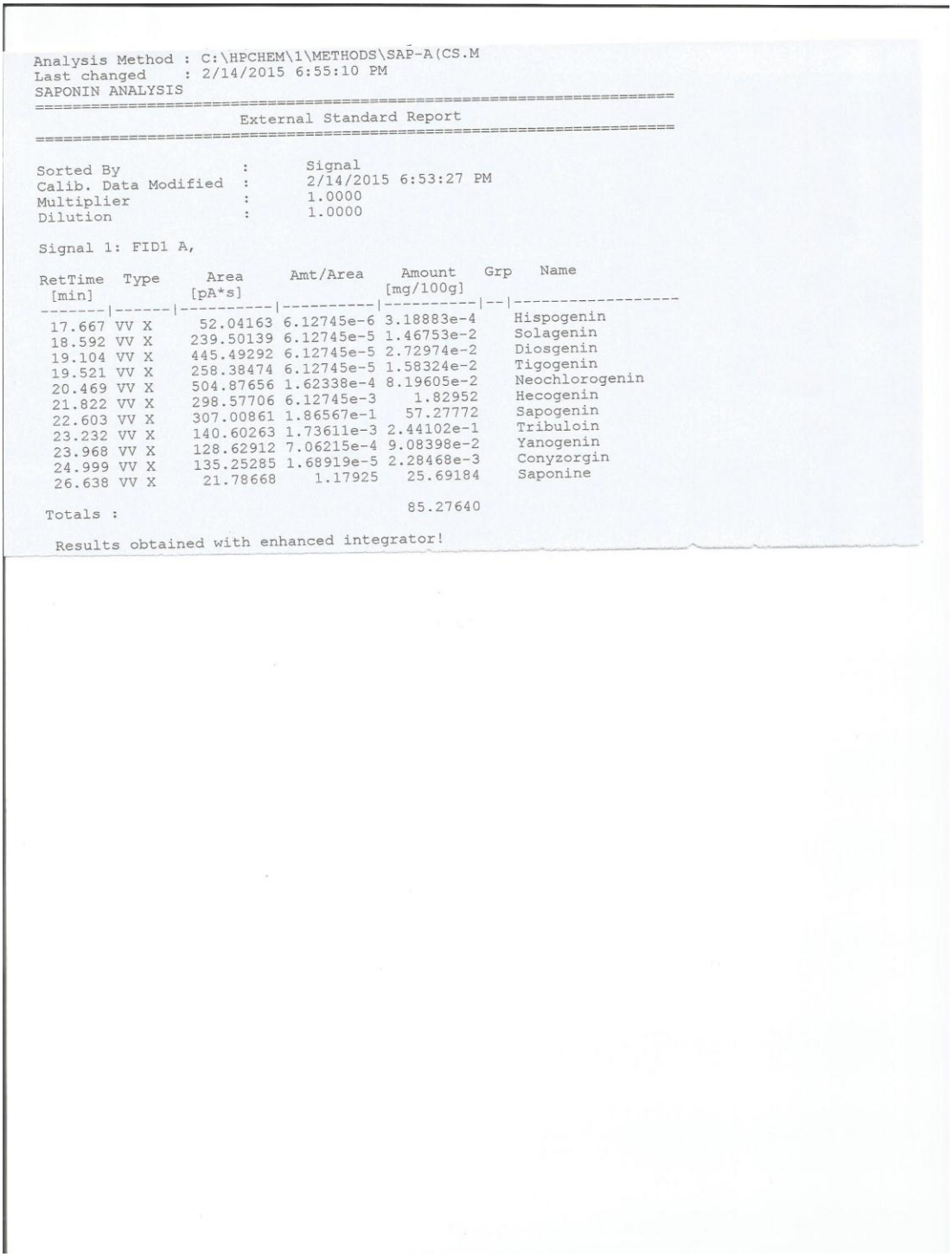
13.539	VV	32.03758	6.18812e-8	2.18078e-6	1	Catechin
15.033	VV	70.06340	1.41227e-6	1.08843e-4	1	Resveratrol
16.037	VV	162.92355	1.87406e-2	3.35862	1	Apigenin
16.246	VV	75.98035	1.55330e-6	1.29822e-4	1	Daidzein
16.671	VV	121.45901	1.55247e-6	2.07417e-4	1	Butein
16.783	VV	170.56795	3.27938e-6	6.15292e-4	1	Naringenin
17.092	VV	213.87605	1.61463e-6	3.79864e-4	1	Biochanin
17.361	VV	100.22462	2.40385e-2	2.65017	1	Luteolin
18.052	VV	195.38480	1.43678e-1	30.87978	1	Kaemferol
19.518	VV	182.19814	2.96251e-6	5.93739e-4	1	(-)-Epicatechin
21.820	VV	230.59830	4.11184e-8	1.04300e-5	1	(-)-Epicatechin-3-gallate
22.359	VV	85.82506	1.55330e-6	1.46643e-4	1	Gallocatechin
22.599	VV	235.82899	2.40385e-2	6.23586	1	Quercetin
23.966	VV	104.81898	4.80769e-2	5.54331	1	Isorhamnetin
24.789	VV	37.65813	2.01613e-3	8.35160e-2	1	Myricetin
27.061	VV	46.72180	1.55330e-6	7.98301e-5	1	Naringin
27.287	VV	33.94804	1.56889e-6	5.85869e-5	1	Kaempferol-3-arabinoside
27.425	VV	58.06408	1.55330e-6	9.92098e-5	1	Quercitrin
27.902	VV	18.14659	1.55330e-3	3.10057e-2	1	Isoquercetin
28.196	VV	28.16575	1.55330e-6	4.81247e-5	1	Orientin
29.188	VV	5.09942	1.55330e-3	8.71300e-3	1	Isoorientin
Totals :				48.79346		

Results obtained with enhanced integrator!

Appendix 4.13 : Isolated and Identified Saponin 2 in leaves of *C. dolichopentalum* using gas chromatography



```
Analysis Method : C:\HPCHEM\1\METHODS\SAP-A(CS.M
Last changed    : 2/14/2015 6:55:10 PM
SAPONIN ANALYSIS
================================================================================
                        External Standard Report
================================================================================

Sorted By             :      Signal
Calib. Data Modified  :      2/14/2015 6:53:27 PM
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: FID1 A,

RetTime  Type      Area      Amt/Area    Amount    Grp   Name
 [min]           [pA*s]                [mg/100g]
-------|------|----------|----------|----------|--|------------------
 17.667 VV X     52.04163 6.12745e-6 3.18883e-4    Hispogenin
 18.592 VV X    239.50139 6.12745e-5 1.46753e-2    Solagenin
 19.104 VV X    445.49292 6.12745e-5 2.72974e-2    Diosgenin
 19.521 VV X    258.38474 6.12745e-5 1.58324e-2    Tigogenin
 20.469 VV X    504.87656 1.62338e-4 8.19605e-2    Neochlorogenin
 21.822 VV X    298.57706 6.12745e-3    1.82952    Hecogenin
 22.603 VV X    307.00861 1.86567e-1   57.27772    Sapogenin
 23.232 VV X    140.60263 1.73611e-3 2.44102e-1    Tribuloin
 23.968 VV X    128.62912 7.06215e-4 9.08398e-2    Yanogenin
 24.999 VV X    135.25285 1.68919e-5 2.28468e-3    Conyzorgin
 26.638 VV X     21.78668    1.17925   25.69184    Saponine

Totals :                                85.27640

 Results obtained with enhanced integrator!
```

Appendix 4.14 : Isolated and Identified Saponin 2 in leaves of *C. dolichopentalum* using gas chromatography

Analysis Method : C:\HPCHEM\1\METHODS\SAP-B(CS.M
Last changed : 2/14/2015 6:55:10 PM
SAPONIN ANALYSIS

External Standard Report

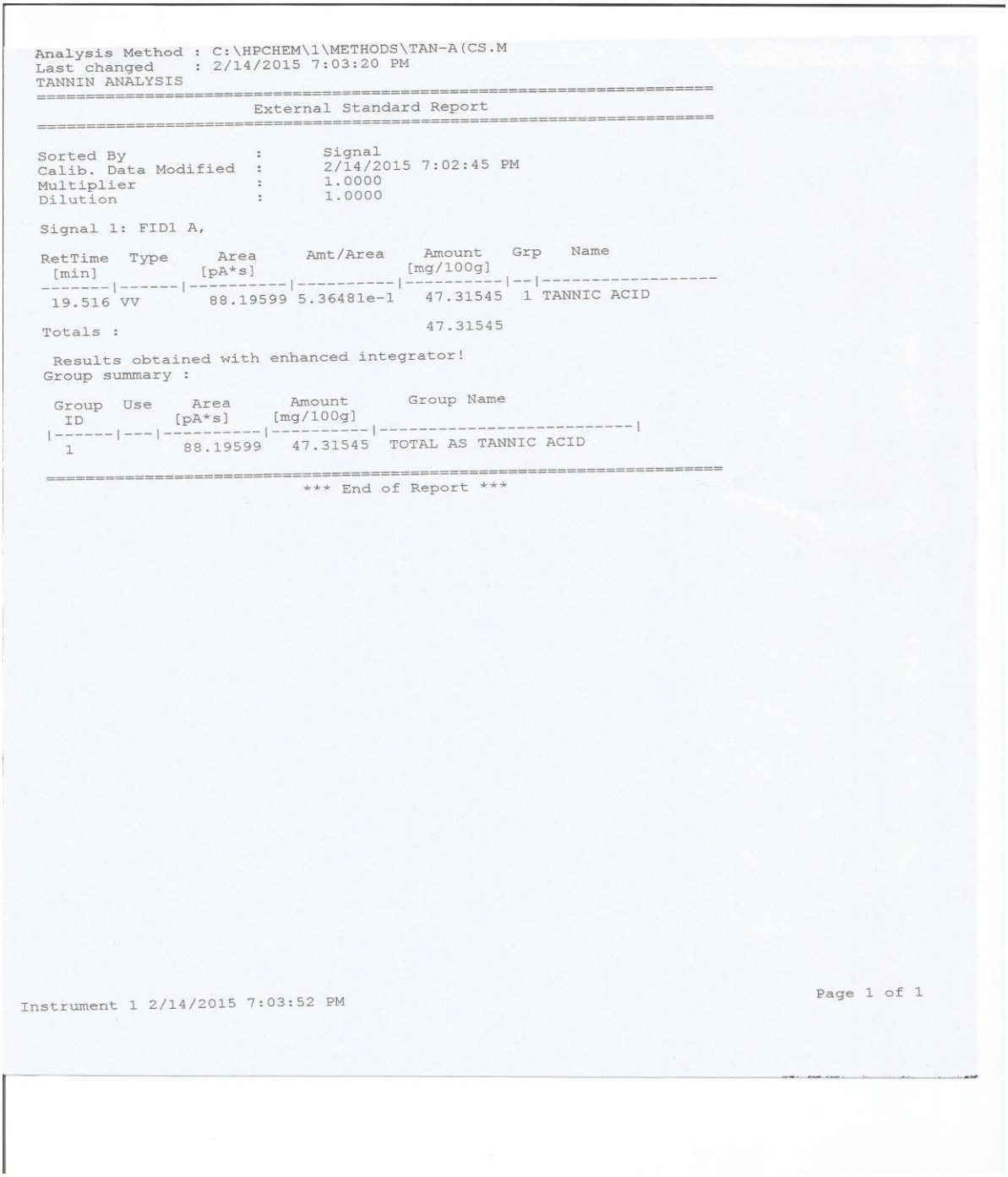
Sorted By : Signal
Calib. Data Modified : 2/14/2015 6:53:27 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: FID1 A,

RetTime [min]	Type	Area [pA*s]	Amt/Area	Amount [mg/100g]	Grp	Name
17.669	VV X	60.75070	6.12745e-6	3.72247e-4		Hispogenin
18.593	VV X	276.24326	6.12745e-5	1.69267e-2		Solagenin
19.105	VV X	516.47308	6.12745e-5	3.16466e-2		Diosgenin
19.522	VV X	294.79272	6.12745e-5	1.80633e-2		Tigogenin
20.471	VV X	585.01593	1.62338e-4	9.49701e-2		Neochlorogenin
21.823	VV X	222.26736	6.12745e-3	1.36193		Hecogenin
22.604	VV X	352.67484	1.86567e-1	65.79754		Sapogenin
23.472	VV X	167.32430	1.73611e-3	2.90494e-1		Tribuloin
23.967	VV X	150.36612	7.06215e-4	1.06191e-1		Yanogenin
24.886	VV X	64.19862	1.68919e-5	1.08444e-3		Conyzorgin
26.638	VV X	25.16888	1.17925	29.68028		Saponine
Totals :				97.39951		

Results obtained with enhanced integrator!

Appendix 4.15: Isolated and Identified Tannin 1 in leaves of from *C. dolichopentalum* leaves using GC



Analysis Method : C:\HPCHEM\1\METHODS\TAN-A(CS.M
Last changed : 2/14/2015 7:03:20 PM
TANNIN ANALYSIS

External Standard Report

Sorted By : Signal
Calib. Data Modified : 2/14/2015 7:02:45 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: FID1 A,

RetTime [min]	Type	Area [pA*s]	Amt/Area	Amount [mg/100g]	Grp	Name
19.516	VV	88.19599	5.36481e-1	47.31545	1	TANNIC ACID

Totals : 47.31545

Results obtained with enhanced integrator!
Group summary :

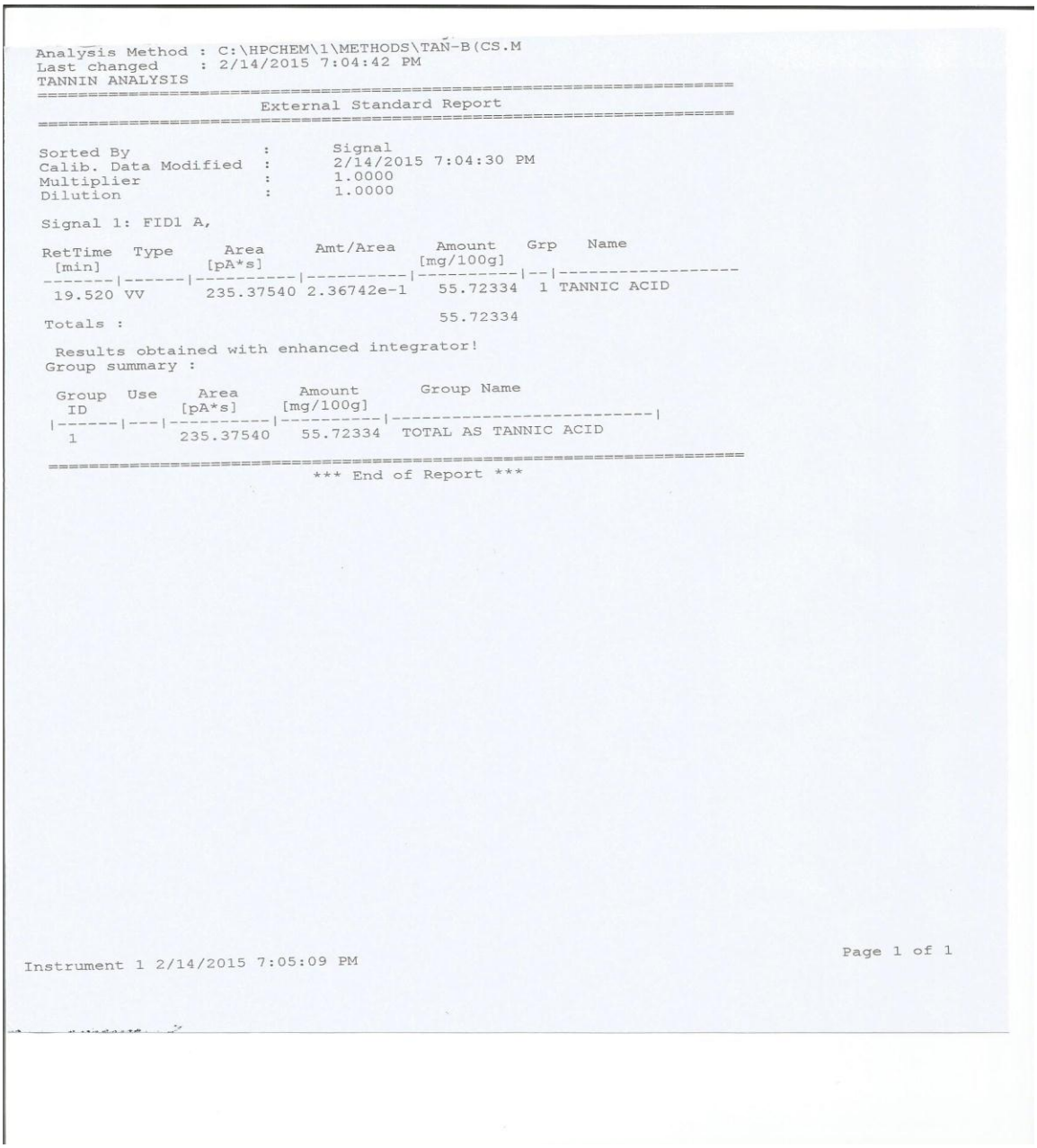
Group ID	Use	Area [pA*s]	Amount [mg/100g]	Group Name
1		88.19599	47.31545	TOTAL AS TANNIC ACID

*** End of Report ***

Instrument 1 2/14/2015 7:03:52 PM

Page 1 of 1

Appendix 4.16: Isolated and Identified Tannin 2 in leaves of from *C. dolichopentalum* leaves using GC



Analysis Method : C:\HPCHEM\1\METHODS\TAN-B(CS.M
Last changed : 2/14/2015 7:04:42 PM
TANNIN ANALYSIS

External Standard Report

Sorted By : Signal
Calib. Data Modified : 2/14/2015 7:04:30 PM
Multiplier : 1.0000
Dilution : 1.0000

Signal 1: FID1 A,

RetTime [min]	Type	Area [pA*s]	Amt/Area	Amount [mg/100g]	Grp	Name
19.520	VV	235.37540	2.36742e-1	55.72334	1	TANNIC ACID
Totals :				55.72334		

Results obtained with enhanced integrator!
Group summary :

Group ID	Use	Area [pA*s]	Amount [mg/100g]	Group Name
1		235.37540	55.72334	TOTAL AS TANNIC ACID

*** End of Report ***

Instrument 1 2/14/2015 7:05:09 PM

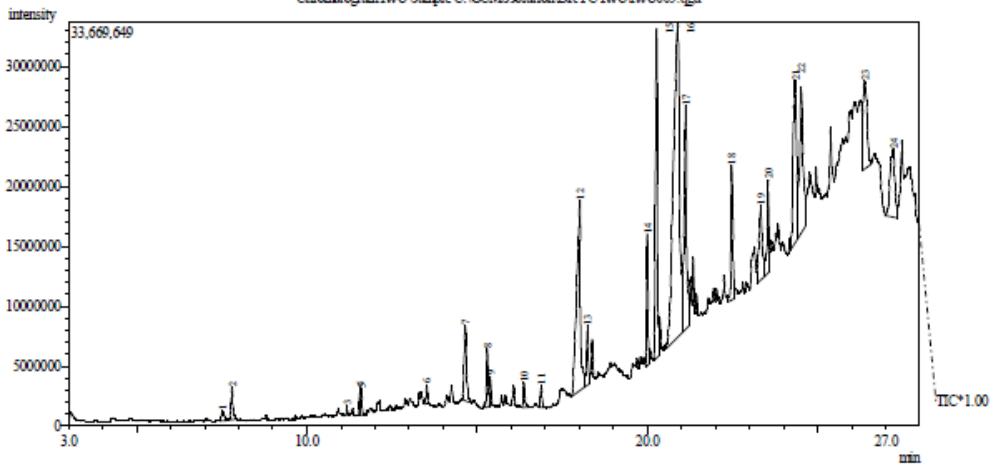
Page 1 of 1

GCMS-QP2010 PLUS
SHIMADZU,JAPAN

NARICT, ZARIA
GCMS ANALYSIS

DR I C IWU [SAMPLE-CRUDE EXT]

Chromatogram
Chromatogram IWU Sample C:\GCMSolution\DR I C IWU\IWU009.qgd



Method

[Comment]

═══ Analytical Line 1 ═══

[AOC-20i]

# of Rinses with Presolvent	:4
# of Rinses with Solvent(post)	:4
# of Rinses with Sample	:3
Plunger Speed(Suction)	:High
Viscosity Comp. Time	:0.2 sec
Plunger Speed(Injection)	:High
Syringe Insertion Speed	:High
Injection Mode	:Normal
Pumping Times	:5
Inj. Port Dwell Time	:0.3 sec
Terminal Air Gap	:No
Plunger Washing Speed	:High
Washing Volume	:8uL
Syringe Suction Position	:0.0 mm
Syringe Injection Position	:0.0 mm
Use 3 Solvent Vial	:1 vial

[GC-2010]

Column Oven Temp.	:80.0 °C
Injection Temp.	:250.00 °C
Injection Mode	:Split
Flow Control Mode	:Linear Velocity
Pressure	:108.0 kPa
Total Flow	:6.2 mL/min
Column Flow	:1.58 mL/min
Linear Velocity	:46.3 cm/sec
Purge Flow	:3.0 mL/min
Split Ratio	:1.0
High Pressure Injection	:OFF
Carrier Gas Saver	:OFF
Splitter Hold	:OFF

Oven Temp. Program

Rate	Temperature(°C)	Hold Time(min)
-	80.0	1.00
10.00	200.0	4.00
10.00	280.0	5.00

< Ready Check Heat Unit >
Column Oven : Yes
SPL2 : Yes
MS : Yes
< Ready Check Detector(FID) >
< Ready Check Baseline Drift >
< Ready Check Injection Flow >
SPL2 Carrier : Yes
SPL2 Purge : Yes
< Ready Check APC Flow >
< Ready Check Detector APC Flow >
External Wait :No
Equilibrium Time :3.0 min

[GC Program]

[GCMS-QP2010 Plus]
IonSourceTemp :230.00 °C
Interface Temp. :250.00 °C
Solvent Cut Time :2.50 min
Detector Gain Mode :Relative
Detector Gain :0.00 kV
Threshold :1000

[MS Table]
--Group 1 - Event 1--
Start Time :3.00min
End Time :28.00min
ACQ Mode :Scan
Event Time :0.50sec
Scan Speed :1250
Start m/z :40.00
End m/z :600.00

Sample Inlet Unit :GC

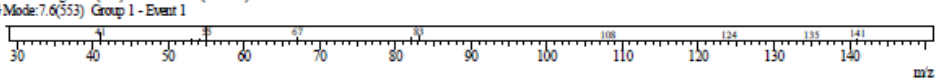
[MS Program]
Use MS Program :OFF

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	7.526	7.442	7.625	4743924	0.38	803241	0.41	5.91		
2	7.808	7.625	7.933	11554295	0.94	2748209	1.40	4.20	V	
3	11.183	11.142	11.242	1561498	0.13	761626	0.39	2.05		
4	11.562	11.508	11.583	5682981	0.46	2348676	1.19	2.42		
5	11.609	11.583	11.667	4924505	0.40	2253546	1.15	2.19	V	
6	13.524	13.442	13.583	4327887	0.35	1587662	0.81	2.73	V	
7	14.652	14.558	14.775	30741491	2.49	6265664	3.19	4.91		
8	15.299	15.250	15.350	15205930	1.23	4904585	2.49	3.10	V	
9	15.391	15.350	15.458	7599414	0.62	2499292	1.27	3.04	V	
10	16.381	16.317	16.508	7484666	0.61	2062372	1.05	3.63		
11	16.887	16.825	16.983	6640834	0.54	1874595	0.95	3.54		
12	18.019	17.758	18.175	129121825	10.46	15901434	8.08	8.12		
13	18.244	18.175	18.308	19008423	1.54	4955409	2.52	3.84	V	
14	20.000	19.942	20.050	31615708	2.56	10998719	5.59	2.87	V	
15	20.271	20.183	20.333	103017169	8.35	27520106	13.99	3.74		
16	20.897	20.558	21.033	331796599	26.88	26237203	13.34	12.65	V	
17	21.131	21.033	21.225	96745034	7.84	18680268	9.50	5.18	V	
18	22.478	22.375	22.567	47854178	3.88	11275575	5.73	4.24	V	
19	23.329	23.217	23.450	43447709	3.52	6271102	3.19	6.93	V	
20	23.549	23.450	23.600	28702067	2.33	7793588	3.96	3.68	V	
21	24.337	24.208	24.417	95467903	7.73	13585649	6.91	7.03	V	
22	24.515	24.417	24.650	92163462	7.47	12225291	6.21	7.54	V	
23	26.378	26.317	26.575	58215328	4.72	7356799	3.74	7.91	V	
24	27.226	26.992	27.350	56725654	4.60	5800473	2.95	9.78		
				1234348484	100.00	196711084	100.00			

Spectrum

Line#:1 R.Time:7.5(Scan#:544)
MassPeaks:75
RawMode:Single 7.5(544) BasePeak:55(141482)
BG Mode:7.6(553) Group 1 - Event 1



Line#:2 R.Time:7.8(Scan#:578)
MassPeaks:55
RawMode:Single 7.8(578) BasePeak:55(272874)
BG Mode:7.8(581) Group 1 - Event 1