INVESTIGATION OF THE EFFECTS OF *Crassocephalum rubens* METHANOL EXTRACT AND FRACTIONS ON LIPID PROFILE OF ISOPROTERENOL INDUCED MYOCARDIAL INFARCTED RATS

By

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A PROJECT SUBMITTEDTOTHE DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF BASIC AND APPLIED SCIENCES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF BACHELOR OF SCIENCE IN BIOCHEMISTRY

SEPTEMBER, 2021.

DECLARATION

I hereby declare that the project has been written by me and is a record of my own research work. It has not been presented in any previous application for a higher degree of this or any other University. All citations and sources of information are clearly acknowledged by means of reference.

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BANIGO, TAMUNOSAIBIBAM CHARLES

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Date

CERTIFICATION

This is to certify that the content of this project entitled **`Investigation of The Effects of Crassocephalum Rubens Methanol Extract and Fractions on Lipid Profile of Isoproterenol - Induced Myocardial Infarcted Rats'** was prepared and submitted by BANIGO TAMUNOSAIBIBAM CHARLESin partial fulfilment of the requirement for the degree of BACHELOR OF SCIENCE IN BIOCHEMISTRY.The original research work was carried out by him under my supervision and is hereby accepted.

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DEDICATION

I dedicate this project to God Almighty my creator, my strong pillar, my source of inspiration, wisdom, knowledge and understanding. He has been the source of my strength throughout this program and on His wings only have I soared. I also dedicate this work to my Family who has encouraged me all the way and whose encouragement has made sure that I give it all it takes to finish that which I have started.

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ABSTRACT

Globally, cardiovascular diseases (CVDs) are becoming the major cause of death. *C. rubens* is oneof endogenous plant which treats a wide range of disease conditions including heart diseases. However, there is limited information on safety and efficacy of the plant, especially hypolipidemic and cardioprotective activities. This study investigated the effects of leaf methanol extract and fractions of *C. rubens* on lipid profile and liver marker enzymes (AST, ALT) of Isoprenaline induced myocardial infracted rats. Forty-five male Wistar rats were randomly divided into nine groups and administered different doses of the extract for 14 days while normal control and myocardial infarcted control received phosphate buffer saline. The lipid profile in the blood plasma and liver of experimental animals were determined using Randox Kits. *C. rubens* leaf methanol extract caused significant ($p< 0.05$) reduction in plasma and liver total cholesterol, triglycerides, VLDL-C, and LDL-Cholesterol, of the myocardial infarcted rats while plasma HDL-C was significantly elevated. Crude extract and solvent fractions of *C. rubens*significantly prevented the deleterious effect of isoproterenol on lipid profiles including triglycerides ($p<0.05$) and total cholesterol. This study showed that the methanol extract of *C. rubens* exerted a hypolipidemic effect which improves the lipid profile in myocardial infarcted rats.

Keywords: Crassocephalum rubens, Myocardial infarction, Isoproterenol, Lipid Profile, Alanine Transaminase, Aspartate Aminotransferase

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Cardiovascular Diseases (CVDs) are conditions that affect the heart and blood vessels (veins and arteries). These diseases are the world's leading cause of death (Lozano et al. 2010). Cardiovascular disorders claim the lives of more people each year than any other cause. Cardiovascular disorders are estimated to be responsible for 17.5 million deaths worldwide, accounting for 31% of overall mortality (Lozano et al.2010). Obesity, heart rhythm disorders, aneurysm, hypertension (high blood pressure), myocardial infarction, and other pathologies are all examples of cardiovascular diseases (Rastogi, et al. 2016).

Coronary heart disease (CHD), cerebrovascular disease (stroke), high blood pressure (hypertension), and myocardial infarction are all heart and blood vessel disorders that lead to cardiovascular diseases. The shortage of oxygen to the brain and heart causes coronary heart disease and stroke, which account for 80% of CVD patients' deaths (Roth, et al. 2017). The accumulation of fatty deposits inside blood vessels narrows the direction of cerebral and coronary arteries, causing them to become blocked. CHD is responsible for nearly 75% of all deaths worldwide, and it occurs in both low- and middle-income countries due to a variety of factors including socioeconomic differences and risk factors resulting from lifestyle changes (Gaziano, et al. 2010).

One of the clinical manifestations of coronary artery disease is myocardial infarction. Other types include silent ischemia, stable and unstable angina, and sudden cardiac death (Goodman,et al. 2006). Myocardial infarction, on the other hand, is the most common type and necessitates prompt diagnosis and treatment. There have been significant advancements in both the diagnostic and therapeutic stages of myocardial infarction in recent years. Just aspirin and nitrates were used in medicines a few decades ago. As a preventative measure, a wide range of prescription and interventional medication (antiaggregants, anticoagulants, beta blockers, angiotensin transforming enzyme inhibitors, and angiotensin receptor blockers) is available today (Patrono, et al. 2017).

Treatment of myocardial infarction is not limited to drugs and medications only.The majority of treatments depend on extracts and active compounds from medicinal plants. Due to the proven effectiveness of medicinal plants in curing certain diseases, there is currently an increase in medicinal plant consumption around the world.Ethno-botanical information indicates that more than 800 plants are used as traditional remedies for the treatment of myocardial infarction(Farnsworth, et al. 1985)

Crassocephalum rubens [Juss. ex Jacq.] S. Moore [Asteraceae]is a traditional leafy vegetable that is used to treat sickness symptoms in several parts of Africa. C. *rubens* is a perennial shrub in the Asteraceae family that is predominantly used as a vegetable in salads and sauces in various parts of Africa. It's been shown to be effective in the treatment and management of disorders like diabetes. C. *rubens* is used in the Benin Republic to cure disorders like diabetes, hypertension, liver issues, breast cancer, and sexually transmitted diseases (Dansi,et al.2012). Several bioactive compounds have been discovered in the plant *C. rubens*as a result of numerous studies on different components of the *C. rubens*family. The leaves of *C. rubens*have long been used to treat stomach upset, headaches, and indigestion (Adjatin, et al. 2012). They have antihyperlipidemic and antioxidant properties, according to research studies (Aniya, et al.2005). Abdominal discomfort, pain, malaria, fresh wounds, and epilepsy are among the diseases that have been reported to be controlled by *Crubens* traditionally in Nigeria (Adjatin,et al. 2012).

In light of these findings, it is essential to further investigate the effect of *C.rubens* leaves on myocardial infarcted rats.

1.2 Statement of problem

Myocardial infarction is a pathologic diagnosis characterized by the loss of normal cardiac myocyte function (Robbins, 2005). The prevalence of cardiovascular diseases is rapidly increasing, reaching pandemic levels, especially in developing countries (Benjamin, et al. 2018). Over the years, a number of antihyperlipidemic and hypolipidemic agents have been produced, but they are not without side effects. Traditional and complementary medicine has grown in popularity in recent years for the treatment of various diseases, owing to the low toxicity of herbal medicines. It is therefore important to investigate and confirm *C. rubens* leaves as a potential source of drugs in this context.

1.3 Aim and Objectives of the study

The study aims at investigating the effects of leaf methanol extract and fractions of *C. rubens* on lipid profile of Isoprenaline induced myocardial infracted rats by achieving the following specific objectives:

- To determine the effects of *C. rubens* leaf methanol extract and fractions on the plasma and liver lipid profile [Total cholesterol, triglycerides, very low-density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL- C] in isoprenaline induced myocardial infarcted rats.
- To determine the effects of the *C. rubens* leaf extract on the atherogenic index of the experimental rats.
- To determine effects of *C. rubens* leaf methanol extract and fractions on the liver concentrations of Aspartate aminotransferase (AST) and Alanine aminotransferase of isoprenaline-induced myocardial infarcted rats.
- To determine the phytochemical components of the ethyl acetate fraction of *C. rubens* leaves using Gas chromatography, mass spectroscopy (GC-MS).

1.4 Scope of the study

This study will investigate and determine the effects of *C. rubens* leaves on the plasma and liver lipid profile, liver marker enzymes (AST and ALT), and atherogenic index ininduced myocardial infarction in rat models. The volatile phytochemical components in the plants will be identified by GC-MS.

1.5 Significance of study

There are so many medicinal plants in the market today; the hydromethanolic extract of *C.rubens* have been reported to improve human health and decrease the risk of blood lipid level and the risk of cardiovascular disorders such as atherosclerosis, hyperlipidemia, thrombosis, and hypertension (Aditya et al.2018). However, it can serve as a novel medication for myocardial infarction. Therefore, it is essential to investigate the effects of *C. rubens* leaf methanol extract and fractions on some biochemical markers and lipid profile parameters of isoprenaline induced

myocardial infarcted rats. This study will provide information on the effects of the *C. rubens* on blood lipid profile parameters and biochemical markers in MI rat model.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Myocardial Infarction

Reduced or complete cessation of blood flow to a portion of the myocardium causes myocardial infarction, also known as "heart attack." Myocardial infarction can be "silent" and go unnoticed, or it can be a life-threatening occurrence that results in hemodynamic deterioration and death (Lauer,et al. 1999). Most incidence of myocardial infarctions are caused by coronary artery disease. The myocardium is deprived of oxygen when a coronary artery is blocked. Myocardial cell death and necrosis will occur when the myocardium is deprived of oxygen for an extended period of time (Goodman, et al. 2006). Patients may complain of chest pain or pressure that spreads to the throat, jaw, shoulder, or arm. Myocardial infarction is a severe medical emergency that requires immediate treatment and is one of the leading causes of death and disability around the world (Lauer,et al. 1999).

A myocardial infarction occurs when myocardial cells die as a result of prolonged ischemia. Myocardial cell death occurs 6 hours after the start of myocardial ischemia, not instantly. Myocardial infarction is almost often caused by atherosclerosis. (Gimbrone, 2000) Hyperlipidemia, diabetes, smoking, hypertension, gender, and age are all significant risk factors for atherosclerosis (Alamir, 2018).The initiation of atherosclerotic plaque formation is aided by endothelial dysfunction and inflammation. The development of an atherosclerotic plaque, which consists of a central lipid core surrounded by foamy macrophages and smooth muscle cells and protected by a fibrous cap, is characterized by lipid accumulation in vessel walls (Watanabe,et al. 1989).The lipid content of the plaque communicates with the blood flowing through the arterial lumen as the fibrous cap ruptures (Virmani, et al. 2005). The platelets are activated by the tissue factor expressed by macrophages, resulting in the development of an intraluminal thrombus. Finally, the thrombus occlusion decreases the blood flow to the myocardial tissues, resulting in ischemia and necrosis, ultimately leading to myocardial infarction (Jennings, 2013).

Myocardial infarction alongside other cardiovascular diseases have been reported to be associated with lipid metabolism disorders (Galloway, 1987). In a study carried out to determine the effect of C. *rubens* on an induced hyperlipidemia model, it was discovered that C. *rubens*

decreases the synthesis of cholesterol and its metabolism thereby decreasing the risk factors for cardiovascular diseases.

2.2 Etiology of Myocardial infarction

Coronary artery disease and myocardial infarction have a similar relationship. According to a review by an international case-control multi-center (INTERHEART), the following were established as modifiable risk factors for coronary artery disease:

- Smoking
- Abnormal lipid profile/blood apolipoprotein (raised ApoB/ApoA1)
- Hypertension
- Diabetes mellitus
- Abdominal obesity (waist/hip ratio) (greater than 0.90 for males and greater than 0.85 for females)
- Psychosocial factors (such as depression, loss of locus of control, global stress, financial stress, and life events such as marital separation, job loss, and family conflicts)
- Lack of daily intake of fruits or vegetables (Yusuf, et al.2004).

Many of the above risk factors were found to be significantly associated with acute myocardial infarction in the INTERHEART analysis, with the exception of alcohol intake, which had a weaker correlation (Yusuf, 2004) Smoking and irregular apolipoprotein ratio was discovered to be the strongest link between acute myocardial infarction. Women were found to have a higher risk of diabetes and hypertension, as well as a higher protective effect of exercise and alcohol (Anand, et al. 2008). A relatively high level of plasma homocysteine, which is an independent risk factor for MI, is another risk factor (Stampfer,et al. 1992). Plasma homocysteine levels that are too high can be managed with folic acid and vitamin B6 and B12.

2.3 Pathophysiology

When one or more epicardial coronary arteries are occluded for more than 20 to 40 minutes, an acute myocardial infarction might ensue (Frangogiannis, 2015). When a plaque in the coronary arteries ruptures, the occlusion is generally thrombotic. Sarcolemmal disturbance and myofibril relaxation occur when there is a lack of oxygen in the myocardium. The initial ultrastructural changes in the MI process are these alterations, which are followed by mitochondrial abnormalities. As a result of the prolonged ischemia, cardiac tissue becomes liquefactive necrosis (Reimer KA et al. 1983). The necrosis extends from the sub-endocardium to the sub-epicardium. It is considered that the sub epicardium improves collateral circulation, which prevents mortality. Depending on the location of the heart injured by the infarction, cardiac function is harmed (Reimer KA et al. 1983). Due to the myocardium's poor regeneration capabilities, the infarcted region recovers through scar formation, and the heart is frequently rebuilt with dilation, segmental hypertrophy of residual viable tissue, and cardiac dysfunction (Kellar, 2006).

2.4 Treatment of myocardial infarction

A heart attack necessitates urgent medical attention. In which the arteries supplying blood to the heart are unblocked using a technique known as angioplasty (Musci, 1998). Coronary artery bypass graft may be done in certain cases where the patient's veins and arteries have been rerouted so that blood can circulate through the blockage of the coronary artery vessel. (Luo et al. 2018).

A variety of drugs can be used to treat a heart attack:

- Aspirin and other blood thinners are often used to break up blood clots and increase blood flow into narrowed arteries.
- Blood clots are often treated with thrombolytics.
- Clopidogrel, an antiplatelet medication, can be used to prevent new clots from forming and established clots from developing.
- Blood vessels may be widened with nitroglcerin.

- Beta-blockers are medications that reduce blood pressure and relax the heart muscle.ACE inhibitors can also be used to lower blood pressure and decrease stress on the heart (Luo et al. 2018).

2.5 Aspirin

Aspirin, commonly known as acetylsalicylic acid (ASA), is a pain reliever, fever reducer, and inflammation reducer (ASHP, 2016). When taken soon after a heart attack, aspirin lowers the risk of death (ASHP, 2016). In those at high risk for heart attacks, ischemic strokes, and blood clots, aspirin is also taken long-term to help prevent additional heart attacks, ischaemic strokes, and blood clots (ASHP, 2016).

2.5.1 MECHANISM OF ACTION

Aspirin's capacity to minimise platelet aggregation and thus the likelihood of thrombotic vascular events is the most plausible reason for its cardioprotective action. Acute occlusive vascular events, such as myocardial infarction (MI) and ischemic stroke, are influenced by platelets, platelet products, and thrombosis. Platelet- and fibrin-rich atherosclerotic plaque disruption can lead to aggressive platelet deposition and, eventually, the formation of a thrombus, which can lead to an acute occlusive event.Small quantities of aspirin permanently acetylate the active site of cyclooxygenase, which is essential for the formation of thromboxane A2, a potent aggregation promoter, in platelets, according to Nobel Prize-winning basic research (Moncada and Vane 1979). Aspirin has such a strong effect that greater doses do not appear to provide any extra benefit. Indeed, due to the stimulation of vessel wall enzymes, it has been claimed that much greater doses may potentially reverse this trend (Manson, et al. 1991).

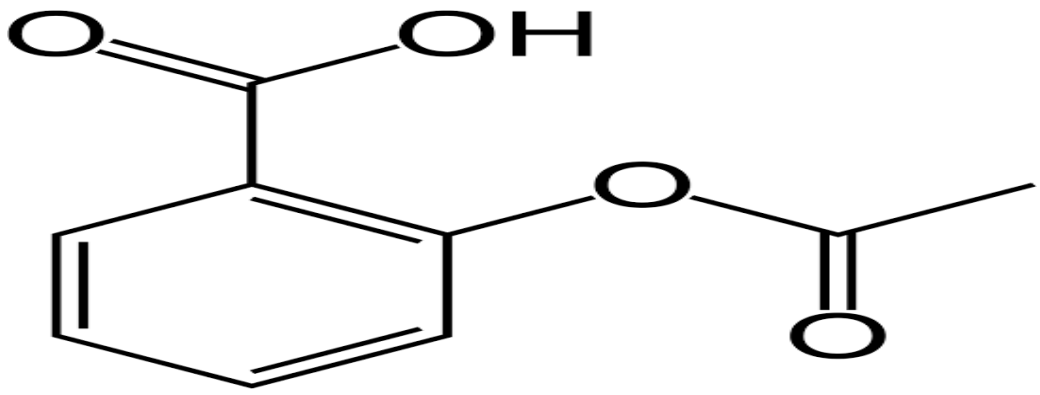

Figure 2.1 : Molecular structure of Aspirin (Tohgi et al., 1992)

2.6 Blood coagulation and Myocardial infarction

Coagulation and thrombotic occlusion of coronary vessels is associated with acute myocardial infarction (AMI).As a result of accumulation of cholesterol and fats in the artery walls, coronary thrombosis (the forming of a blood clot within a blood vessel of the heart) is most frequently induced(Li X, 2014).The smaller diameter of the vessel allows less blood to circulate and makes progression to a myocardial infarction faster.High LDL cholesterol, smoking, sedentary lifestyles, and hypertension are the leading risk factors for coronary thrombosis. (Klatt et al. 2019).

2.7 Isoproterenol

Isoproterenol is a beta-1 and beta-2 adrenergic receptor agonist used to treat bradydysrhythmias in the bronchial, gastrointestinal, and uterine systems, which cause an increase in heart rate, heart contractility, and smooth muscle relaxation (Desmine et al., 2018). It is an isopropylamine derivative of epinephrine that is a non-selective -adrenoreceptor agonist. Its chemical formula is C11H17N03 and it has a molecular weight of 211.258 g/mol, a half-life of two (2) minutes, and a molecular weight of 211.258 g/mol (Szymanski et al., 2021).

2.7.1 Isoproterenol Side Effects

- Nervousness
- Headache
- Nausea
- Visual fuzziness
- Hypertension,
- Hypotension, pulmonary edoema, angina, and other conditions (Szymanski et al., 2021).

2.7.2 Mechanism of action

The G-alpha stimulatory second messenger system mediates the effects of beta-1 and beta-2 adrenergic receptors (Desmine et al., 2018). G-protein coupled receptors are made up of a seven-transmembrane-spanning protein. The extracellular domain contains the ligand-binding site. The intracellular domain interacts to a G-alpha stimulatory protein that is linked to an inactive GDP molecule (Desmine et al., 2018). When a ligand attaches to the extracellular domain of a beta-1

receptor, the alpha subunit switches from GDP to GTP and becomes active. The intracellular

domain binds to a G-alpha stimulatory protein that is linked to an inactive GDP molecule (Desmine et al., 2018). When a ligand attaches to the extracellular domain of a beta-1 receptor, the alpha subunit switches from GDP to GTP and becomes active. Adenylate cyclase is activated when the intracellular domain of the G-alpha protein dissociates. After then, intracellular ATP is converted to cAMP by active adenylate cyclase (Desmine et al., 2018). Protein kinase A is activated by cAMP, the pathway's principal second messenger (PKA). Activated PKA phosphorylates L-type calcium channels in cardiac myocytes, resulting in an increase in intracellular calcium.PKA also allows sarcoplasmic reticulum ryanodine receptors to release more calcium (Desmine et al., 2018).

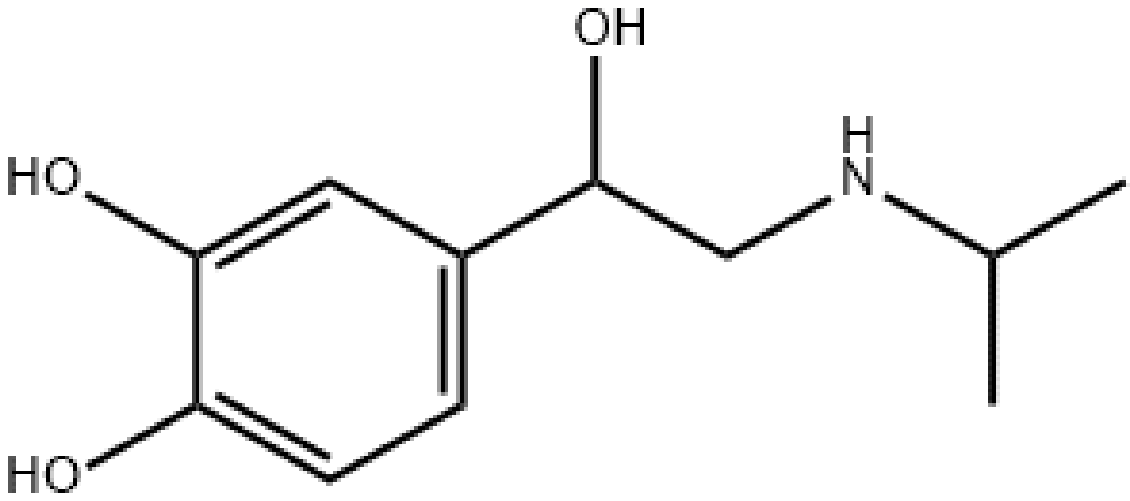

Figure 2.2: Molecular structure of Isoproterenol (Hoff and Koh 2018)

2.8 Cardiac markers

Cardiac markers are intracellular macromolecules (proteins) released from a weakened heart muscle after a myocardial infarction (MI).

Cardiac markers are biomarkers that are used to assess the health of the heart. They are usually contained in the blood and are present at all times. They are, however, substantially higher when the heart muscle is damaged. Aspartate aminotransferase (AST or GOT), troponin I & T (TnI, TnT), creatine kinase MB (CK-MB), myoglobin (Mb), lactate dehydrogenase (LDH), B-type natriuretic peptide (BNP), C-reactive protein (CRP), myeloperoxidase (MPO), and ischemic modified albumin(IMA) are some of the cardiac markers(Azzazy, 2002).These cardiac markers are biomarkers that are used to assess the health of the heart andthey help with disease prediction and diagnosis in the early stages(Rao, et al. 2019).

2.8.1 Aspartate aminotransferase

Glutamic Oxaloacetic Transaminase is another name for Aspartate aminotransferase. It is present in a variety of tissues, with the highest concentrations in the liver, heart, skeletal muscles, and red blood cells. It is an essential enzyme in amino acid metabolism since it catalyzes the reversible transition of a -amino group from aspartate to glutamate (it provides a source of oxaloacetate for Krebs cycle) (Lewandrowskiet al. 2002).

2.8.2 Troponin

Cardiac troponin is a highly sensitive and specific biomarker for detecting cell necrosis. Troponin is composed of 3 proteins – Troponin C, Cardiac troponin I and Cardiac troponin T. The calcium-binding component is troponin C, the tropomyosin-binding component is troponin T, and the inhibitory component is troponin I.Troponin C is not very specific for myocardial injury since its isoforms are similar in skeletal and cardiac muscle. Troponin T and troponin I isoforms vary in skeletal and cardiac muscle, making them highly specific for cardiac tissue necrosis (Apple FS, 1999).Troponin T is mainly found bound to the contractile elements of myocardial cells, but it can also be found free in the cytoplasm. Troponin T has a dual release mechanism, with the cytoplasmic portion released first and the bound component released later. Troponin I is a cardiac muscle-specific protein that has yet to be isolated from skeletal muscle. Cardiac troponin I and cardiac troponin T are rarely detected in healthy people's blood, hence the cut off value for elevated cardiac troponin I and cardiac troponin T levels should be slightly

above the upper limit of the assay's performance characteristics for a typical healthy population (Katus,1991). Myoglobin, creatine kinase MB mass, creatine kinase isoform ratios, and cardiac troponins I and T have similar early sensitivities for acute myocardial infarction.

2.8.3 Creatinine kinase (CK)

In 1979, CK was first identified as a cardiac biomarker. The enzyme CK is mainly present in cardiac muscle and skeletal muscle. This enzyme has 3 isoenzymes: MM, MB and BB (Schlattner, 2006). The skeletal muscle fraction is CK-MM, the cardiac muscle fraction is CK-MB, and the brain fraction is CK-BB (Blomberg,et al. 1975). The complete CK was previously used to check for myocardial infarction. However, since total CK accounts for 95% of the CK-MM fraction, recent concepts have suggested the following use of the relative index score (RI): CK – MB RI =[CK-MB(ng/ml)/Total CK (U/1)] × 100.The CK-MB level in the blood increases 4–9 hours after the onset of chest pain, peaks at 24 hours, and then returns to normal at 48–72 hours.Creatine kinase is expressed in heart muscle in the CK-MB isoform(Wu A.H., et al.1992). It resides in the cytosol and facilitates movement of high energy phosphates into and out of mitochondria. Since it has a limited period, it cannot be used for late diagnosis of acute myocardial infarction but can be used to recommend infarct extension if levels rise again.

2.8.4 Myoglobin (MYO)

Myoglobin is a cytoplasmic oxygen-binding protein that can be present in both skeletal and cardiac muscle (Ordway and Garry 2004). It is released more quickly than CK-MB and Troponin from infarcted myocardium. It is released into the bloodstream 1 hour after the onset of myocardial infarction, peaks at 4–12 hours, and then returns to baseline values almost instantly (Mair J, et al. 1992). The lack of specificity of cardiac tissue caused by the presence of large quantities of MYO in skeletal muscle is MYO's main drawback. MYO levels can also be used in combination with troponins or CK-MB rather than as a single diagnostic marker. MYO levels in the blood can therefore be used to rule out rather than diagnose myocardial infarction (Mair J, et al.1995). The clinical utility of serial myoglobin determinations for the diagnosis of MI is limited, however, by the short period of elevation of less than 24 hours. Serial measurement of cardiac markers in patients with chest pain has limited diagnostic utility, and adding myoglobin to troponin I for excluding myocardial infarction has limited value (Gibler,et al.1987).

2.8.5 Lactate dehydrogenase

Lactate dehydrogenase is an enzyme present in almost all body tissues, but only a trace amount can be detected in the blood. It is normally found inside the cells of the tissues. Cells, on the other hand, release LDH into the bloodstream when they are damaged or killed, causing blood levels to rise. Lactate dehydrogenase catalyzes the interconversion of pyruvate and lactate, as well as NADH and NAD+, at the same time. When oxygen is unavailable or in short supply, it transforms pyruvate, the final product of glycolysis, to lactate.

2.8.6 Ischemia Modified Albumin

The amount of Ischemia Modified Albumin in the blood rises dramatically in ischemic settings, assisting in the detection of acute ischemia prior to the onset of myocardial necrosis (Hjortshøj, et al.2009). The cobalt binding to the weakened N-terminus of the albumin allows for the calculation of Ischemia Modified Albumin (Mastella, et al.2009). The rise in Ischemia Modified Albumin levels occurs soon after the start of ischemia and returns to baseline within 6–12 hours, allowing for early detection of ischemia.

2.8.7 Pro-brain natriuretic peptide

The neurohormone B-type natriuretic peptide is generated by cardiac cells. BNP elevation has been shown in studies to be a predictor of death and heart failure. They are, however, ineffective in the diagnosis of AMI (Morrow, et al. 2003).

2.8.8 Glycogen phosphorylase isoenzyme BB

This enzyme's isoform can be found in cardiac (heart) and brain tissue. Because of the blood–brain barrier, GP-BB can be thought of as a heart muscle-specific protein. GP-BB is a "new cardiac marker" that is thought to help with early diagnosis of acute coronary syndrome. GP-BB is converted to a soluble form and released into the bloodstream during the ischemia process (McLean, et al.2012). Myocardial infarction and unstable angina cause a sudden increase in blood levels. After the ischemia phase, GP-BB levels rise for 1–3 hours.

2.9 Lipid profile

2.9.1 Triglycerides

Triglyceride is a three-fatty-acid ester derived from glycerol. Triglycerides are the most abundant fats in the human body. They can't move freely through the body's cell membranes, so lipoprotein lipase, a rare enzyme found on blood vessel walls, must break down triglycerides into three fatty acids and glycerol. High triglyceride levels trigger atherosclerosis (Mustafa, et al. 2015).

2.9.2 Total cholesterol (TC)

It is a lipid composition measurement that includes LDL-C, HDL-C, and other lipid components.

2.9.3 High density lipoprotein (HDL)

High density lipoprotein also referred to as "good cholesterol." It's one of five lipoprotein families responsible for removing fat molecules from cells. The accumulation of atherosclerosis within the artery wall is strongly linked to increasing HDL concentration. HDL is primarily responsible for transporting cholesterol to the liver, where it is then excreted into the bile and intestines (Mustafa, et al.2015).

2.9.4 Low density lipoprotein (LDL)

They are referred to as "bad cholesterol." They aid in the transport of fat molecules in extracellular water in the body (CDC, 2017). The lipid profile does not quantify LDL particles, but it can be calculated using the (Friedewald, et al. 1972) equation of Total cholesterol – VLDL-C – HDL-C.

2.9.5 Very low-density lipoprotein (VLDL)

The liver produces very low-density lipoprotein, which is then released into the bloodstream. Triglycerides are carried by them. VLDL cholesterol is identical to LDL cholesterol, but LDL mostly transports cholesterol to tissues rather than triglycerides (Mustafa, et al. 2015).

2.9.6 Atherogenic Index (AIP)

Atherogenic index of plasma (AIP) is a novel index composed of triglycerides and high-density lipoprotein cholesterol (Dobiasova and Frohlich, 2001). It has been used to quantify blood lipid levels and commonly used as optimal indicator of dyslipidemia and associated diseases (e.g., cardiovascular diseases) (Bora, et al. 2017). The atherogenic index of plasma is a key parameter that can be used to estimate cardiac risk as a stand-alone index (Khazaal, 2013). Individuals who

have changes in their lipid profiles are more likely to have atherosclerosis problems (Kanthe, et

al.2012). It is defined as the logarithm [log] of the ratio of TG to HDL-C plasma concentrations and is significantly linked to CVD risks. It can be used to supplement an individual's lipid profile. AIP is a better predictor of fractionated esterification rate of HDL-C than standard lipid measures (Dobiásová, et al. 2005). When the other atherogenic risk markers appear to be normal, it can be utilised as a diagnostic signal (Nwagha,et al. 2005). The "zone of atherogenic risk" is estimated using the AIP calculation (Babu, et al. 2015). The atherogenic index of plasma (AIP) has emerged as a plasma atherogenicity predictor (Dobiásová and Frohlich, 2001). It is linked to the particle sizes of HDL, LDL, and VLDL and predicts cardiovascular disease risk (Dobiásová, et al. 2005).

2.10 General Overview of *Crassocephalum rubens*

One of the essential medicinal plants used as medicinal and nutritious herbs in different parts of the world especially Africa is Crassocephalum rubens [Juss. ex Jacq.] S. Moore [Asteraceae]. The plant is also commonly referred to as "EfoEbòlò," (Yoruba), Babohoh (Hausa),Red flower rag leaf or Fireweed (English). It is a perennial herbaceous plant which belongs to the family Asteraceae. It is usually found in abandoned, organically rich farmlands, waste places, wetlands and backyard gardens. C. *rubens* was originally from Tropical Africa, but it is now widely cultivated as a medicinal and nutritional herb in Asia and other parts of the world (Ali et al. 1989). One of the main reasons that the plant is neglected is because of the unpleasant smell and this might be because of the certain phytochemical constituent of the plant (Denton et al. 2018).

C. *rubens* is a plant that has high nutraceutical properties and is used by a large part of the world in folk medicine. It is also nutrient-rich (in antioxidants and phytochemicals, hepatoprotective capacity, anti-diuretic action, antimicrobial, anticancer, antihyperlipidemic, local anesthetic, anti-inflammatory, anticoagulant and other nutraceutical potentials) and readily available. Based on the nutraceutical properties C. *rubens* appears to be safe and beneficial to human being.

Figure 2.3: C. *rubens* plant

2.10.1 Taxonomy hierarchy of C.*rubens*

The C.*rubens* plant belongs to the Plantae kingdom. The order and families of this plant are Asterales and Asteraceae, respectively. The genus is considered Crassocephalum and the plant species is rubens

Other species of Crasssocephalumrubens are:

- Crassocephalumcrepidiodes,
- Crassocephalum aurantiacum,
- Crassocephalum buchiense. (Grubben et al. 2018)

Classification

Domain - Eukaryota

Kingdom - Plantae

Phylum - Spermatophyta

Subphylum - Angiospermae

Class - Dicotyledonae

Order - Sterales

Family - Asteraceae

Genus - Crassocephalum

Species - Crassocephalum rubens

2.10.2 Morphology and environmental condition of C. *rubens*

In tropical or subtropical areas, C. *rubens* are common, but they are mainly available in tropical Africa. Its leaves are slightly succulent and have an irregular edge in form of oval, spiral or elliptical leaves and these mucilaginous leaves and stems are taken as vegetables, C. *rubens* is a fleshy and erect annual plant that can grow up to 180 cm in height and can be branched often (Adjatin et al.2013).

For treatment purposes, parts of the plants are often used. The most attractive aspect of this plant is the flowers. They are coated with green bracts at the top and are red or maroon. They are bisexual, spread similarly, have a tubular corolla and are around 9 to 11 mm in length. The color of the flowers ranges from yellow to orange and the color stays brown-reddish at the tip. Its incidence at high altitudes is explained by the lower limit of germination of rubens germinated at temperatures between 10 and 40ºC and an annual precipitation of 600-1500. C. *rubens* produces a large number of silky-haired seeds that can be easily dispersed by wind and/or water (Denton, 2004. Intra and inter species variability is the genetic diversity and existing relationship of C. *rubens.*

2.10.3 Reported phytochemical contents of *C. rubens*

Phytochemicals are bioactives compounds found naturally in plants, they account for unique color, flavor, and aroma of plant. These functions protect plants against invasion, diseases and infection (Adnan,et. al. 2010).

C. *rubens* has been found to possess various phytochemicals that are associated to their leaves. The plant has been identified for antibacterial, hypoglycemic, antioxidant, anti-inflammatory, antitumor, antimutagenic and antidiabetic properties. (Tomimori, et al. 2012). It contains bioactive compounds such as flavonoids, phenol, oxalate, tannin, saponin, phytate, and ascorbic acid, according to the phytochemical screening of the powdered sample of fireweed (Anandhi and Revathi, 2013).

Flavonoids have antioxidant and detoxification activities and many health promoting effects (Akroum, 2011). Coumarins raise the blood flow and reduces capillary flow in the veins.Tannins, flavonoids and coumarins have antioxidant properties that allow them to protect against free radicals responsible for a lot of human diseases, including cardiovascular diseases. (Adjatin et al. (2012) and Dansi et al. (2013). The cardiac glycosides which are found in the leaves of the plants exerts a positive effect on the heart during cardiac failures by increasing the capacity of the heart muscles to pump blood (Aldred, 2009). Flavonoids are associated with the prevention of diseases that has to do with oxidative stress (Huang et al. 2010).

2.10.4 Pharmacological activities of *C. rubens*

Wound healing activity

C.*rubens* leaves contain the essential oils -cubebene, -farnesene, and -caryophyllene. C. *rubens* leaves have also been used to treat wounds in Vietnam, China, India, a nd some African countries (Loi, et al. 2004).

Antihyperlipidemic activity

The methanol extract of C. *rubens* decreases atherogenic index, which is one of the most significant risk factors of atherosclerotic plaques. Flavonoids have been shown to inhibit hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase activity.

Antidiabetic activity

Diabetes is a metabolic disorder of carbohydrate, fat and protein. (Serirama,. et al. 2010). The amylase and glucosidase enzymes, break carbohydrate down into simple absorbable monosaccharides and raise blood glucose levels. Amylase and glucosidase inhibitors have been shown to be effective as oral hypoglycemic drugs in the treatment of hyperglycemia, especially in patients with type 2 diabetes (Kwon, et al.2006). Inhibition of amylase slows carbohydrate digestion and lengthens digestion time, resulting in a slower rate of glucose absorption and lower blood glucose levels (Chipiti, et al.2015). In both the starch-iodine test and the 5-dinitrosalicylic acid (DNSA) assay, the C. *rubens*extractshowed significant percent inhibition of amylase when compared to the standard an tidiabetic drug, acarbose, which could be attributed to the presence of phenolic and flavonoids co mponents because polyphenols have the ability to bind with proteins and inhibit carbohydrate hy drolyzing enzymes (Lhoret, et al. 2004).

Hepatoprotective activity of C. *rubens*

C.*rubens* has been used in the treatment of acute hepatitis and fever among people (Tomoyuki et al. 2005). It has also been tested against various drugs and chemical induced chronic liver diseases in experimental animals and came out with positive result (Devaraj et al. 2016). According to Salawuet al. 2004, studies of C. *rubens* plant provide hepatoprotective effects

against liver damage as opposed to the side effects caused by different medications and chemicals. As a result of numerous side effects, Current research has concentrated on drugs extracted from medicinal herbs rich in flavonoid and polyphenolic compounds and hepatoprotective properties to resolve this issue (Pereira et al. 2015). C. *rubens* helps liver function and treatment of various dermatological and systemic disorders.

2.10.5 Nutritional composition of C. *rubens*

The important sources of protective foods are vegetables that are extremely helpful for the maintenance of one`s wellbeing and prevention of illnesses (Dansi et al. 2013). Phytochemicals are bioactive compounds that account for unique colour, flavor and aroma of plant. They protect against infection, diseases and invasion. (Adnan et al. 2010).

2.10.6 Mineral Composition of *Crassocephalum rubens*

Using the recommended AOAC method of analysis, the nutritional potential of the species of ebolo (C. rubens) was assessed through proximate composition, mineral, and vitamin C profile (Arawande et al. 2013). For C. rubens, the analysis includes moisture, raw protein, total lipids, ash, fibre, and carbohydrates. Sodium, potassium (K), phosphorus (P), magnesium (Mg), calcium (Ca), iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) are all minerals found in C. rubens. Flavonoid, phenol, oxalate, tannin, saponin, phytate, alkaloid, and ascorbic acid are all found in the powder sample of C. rubens (Arawande et al. 2013).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Materials and Reagents

Volumetric flask, weighing balance, Filter paper, Funnel, Dropper, Test tubes, Test tube racks, Beaker, Measuring cylinder, Rotary evaporator, Spatula, Water bath, Nose mask, Hand gloves, Distilled water, Chloroform, Sodium citrate, Sodium chloride, n-Hexane, Ethyl acetate, Methanol, Isoproterenol, Aspirin.

Reagent kits for triglyceride (TRIG), cholesterol (CHOL), high density lipoprotein (HDL), aspartate transferase (AST) and alanine transaminase (ALT) activity were purchased from Randox Laboratories Ltd, UK

3.1.1 Collection and preparation of plant materials

In April 2021, C.*rubens* was harvested directly from trees at local farms in Ekiti state, South Western, Nigeria. Taxonomic identification of this plant was made by Dr. Nodza George, Department of Botany, University of Lagos, Nigeria. For preservation, a voucher specimen (8788) was deposited at the herbarium of the University of Lagos's Department of Botany. The leaves were dried in a hot air oven at 40 degrees Celsius. The leaves were pulverised in a laboratory blender after drying to a coarse powder. The powder was stored in a clear glass container and kept refrigerated at 4°C until it was used.

3.1.2 Plant extraction and filtration

519.13g of pulverized *C. rubens* aerial part was soaked in4,160mL of 70% methanol by (1:8w/v) in a jar, which was then securely closed and shaken intermittently over the course of three days to allow the plant and the solvent to marcerate.

The methanol and plant mixture were filtered using a sterile muslin cloth. The filtrate was collected in one beaker, while the residues were collected in another. The filtrate was allowed to settle after filtration, then mixed and filtered twice more to guarantee that it was finely filtrated.

3.1.3 Concentration

The filtrate was then concentrated at 45°C in a rotary evaporator. The solvent was successfully separated from the crude extract by the rotary evaporator at the end of the concentration. The

crude extract was then collected in a beaker and evaporated to dryness in a hot air oven at 40^{o}C, after which the percentage yield was determined.

3.1.4 Fractionation of Solvents

For the solvent-solvent extraction method, a separating funnel was used. The crude methanol extract was reconstituted with water and subjected to solvent partitioning using Hexane and Ethyl acetate sequentially. After which, the fractions were concentrated in the rotary evaporator at 40°C. The concentrated fractions were kept each in an airtight container and the refrigerated at 4°C until needed.

3.2 STUDY DESIGN: The study was an *in vivo* animal study.

The rats were randomly allocated into 9groups of 5 animals each:

Group 1: Normal control (given 1ml phosphate buffered saline (PBS).

Group 2: Positive control; ISO + Aspirin (75 mg/kg)

Group 3: Negative control (ISO without pretreatment).

Group 4: ISO + pretreatment with crude hydromethanolextract of *C. rubens* (100 mg/kg).

Group 5: ISO + pretreatment with crude hydromethanol extract of *C. rubens* (150 mg/kg).

Group 6: ISO + pretreatment withhexane fraction of *C. rubens* (100 mg/kg).

Group 7: ISO + pretreatment withhexane fraction of *C. rubens* (150 mg/kg).

Group 8: ISO + pretreatment withethyl acetate fraction of *C. rubens* (100 mg/kg).

Group 9: ISO + pretreatment withethyl acetate fraction of *C. rubens* (150 mg/kg).

3.3 Sample collection and Preparation of Plasma and Tissues

The rats were anaesthetized, blood was obtained through cardiac puncture of the animals into heparinized tubes on the final day of treatment. The liver tissues were excised, washed free of blood in normal saline and blotted with tissue paper for the preparation of homogenate. They

were homogenated using mechanical method. The tissue homogenate was centrifuged at 10 minutes at 12000 rpm. Using a bench centrifuge, blood samples were centrifuged for 15 minutes at 2500 rpm to produce plasma.

3.4 Assay Methods

3.4.1 Determination of Liver and Plasma Cholesterol concentration

Procedure

Using a Randox kit, the concentration of cholesterol in the plasma and liver of experimental rats were determined following the instructions in the manufacturer's kit insert. Tubes were labelled with the identification and group assigned to the experimental animals, as well as a blank and standard tube. 10 µLof standard reagent was pipetted into a clean test tube, followed by 1000 µL of working reagent. 10 µLsamples were pipetted into corresponding test tubes, followed by 1000 µL of working reagent. After a 10-minute incubation period at 37°C, the absorbances were measured at 500nm against a blank containing only the working reagent. The cholesterol concentration was calculated as thus:

Absorbance of Sample/Absorbance of Standard X Standard Concentration

3.4.2 Determination of Plasma Triglyceride concentration

Procedure

Using a Randox kit, the concentration of triglyceride in the plasma of experimental rats were determined following the instructions in the manufacturer's kit insert. Tubes were labelled with the identification and group assigned to the experimental animals, as well as a blank and standard tube. 10 µL of standard reagent was pipetted into a clean test tube, followed by 1000 µL of working reagent. 10 µL samples were pipetted into corresponding test tubes, followed by 1000 µL of working reagent. After a 10-minute incubation period at 37°C, the absorbances were measured at 500nm against a blank containing only the working reagent. The triglyceride concentration was calculated as thus:

Absorbance of Sample/Absorbance of Standard X Standard Concentration

3.4.3 Determination of Plasma and Liver HDL concentration

Procedure

Using a Randox kit, the concentration of HDL in the plasma and liver of experimental rats were determined following the instructions in the manufacturer's kit insert. Tubes were labelled with the identification and group assigned to the experimental animals, as well as a blank and standard tube. 20 µL of standard reagent was pipetted into a clean test tube, followed by 500 µL of working reagent. 20 µL samples were pipetted into corresponding test tubes, followed by 500 µL of working reagent. After a 10-minute incubation period at 37°C, the samples were centrifuged and the supernatants obtained. 10 µL of the supernatant was pipetted into a test tube and cholesterol procedure was carried out. The absorbances were measured at 500nm against a blank containing only the working reagent. The HDL concentration was calculated as thus:

Absorbance of Sample/Absorbance of Standard X Standard Concentration

3.4.4 Estimation of LDL-cholesterolMost of the circulating cholesterol is found in threemajor lipoprotein fractions: very low-density lipoproteins (VLDL), LDL and HDL.

[Total chol] = [VLDL-chol] + [LDL-chol] + [HDL-chol]

LDL-cholesterol is calculated from measured values of total cholesterol, triglycerides and HDL-cholesterol according to the relationship:

[LDL-chol] = [total chol] - [HDL-chol] - [TG]/5

Where [TG]/5 is an estimate of VLDL-cholesterol and all values are expressed in mg/dL.

3.4.5 Estimation of VLDL-cholesterol

Most of the circulating cholesterol is found in three major lipoprotein fractions: very low-density lipoproteins (VLDL), LDL and HDL.

[Total chol] = [VLDL-chol] + [LDL-chol] + [HDL-chol]

VLDL-cholesterol is calculated from measured values of total cholesterol, triglycerides and HDL-cholesterol according to the relationship:

[VLDL-chol] = TG/5

All values are expressed in mg/dL.

3.4.6 Determination of Atherogenic index of the plasma (AIP)

The AIP was calculated with the formula according toNwagha, et al., 2010 as thus:

$$\log_{10}(\text{TG/HDL - cholesterol})$$

3.4.7 Determination of Liver AST concentration

Procedure

Using a Randox kit, the concentration of AST in the liver of experimental rats were determined following the instructions in the manufacturer's kit insert. Tubes were labelled with the identification and group assigned to the experimental animals, as well as a blank and standard tube. 100 µL of standard reagent was pipetted into a clean test tube, followed 500 µL of working reagent. 100 µL samples were pipetted into corresponding test tubes, followed 500 µL of working reagent. After a 30-minute incubation period at 37°C, the procedure repeated with the second set of reagents and incubated for 20 – minutes. 5000 µL of NaOH was added to the mixture and incubated for 5 minutes. The absorbances were measured at 546nm against a blank containing only the working reagent. The AST concentration was calculated as thus:

Absorbance of Sample/Absorbance of Standard X Standard Concentration

3.4.8 Determination of Liver ALT concentration

Procedure

Using a Randox kit, the concentration of ALT in the liver of experimental rats were determined following the instructions in the manufacturer's kit insert. Tubes were labelled with the identification and group assigned to the experimental animals, as well as a blank and standard tube. 100 µL of standard reagent was pipetted into a clean test tube, followed 500 µL of working reagent. 100 µL samples were pipetted into corresponding test tubes, followed 500 µL of working reagent. After a 30-minute incubation period at 37°C, the procedure repeated with the second set of reagents and incubated for 20 – minutes. 5000 µL of NaOH was added to the mixture and incubated for 5 minutes. The absorbances were measured at 546 nm against a blank containing only the working reagent. The ALT concentration was calculated as thus:

Absorbance of Sample/Absorbance of Standard X Standard Concentration

3.5 Phytochemical Characterization

The phytochemical characterization of the ethyl acetate fraction of *C. rubens* was carried out using Gas Chromatography-Mass Spectrometry (GC-MS).

Procedure: a Hewlett Packard Gas Chromatograph (Model 6890 series) with a flame ionization detector and a Hewlett Packard 7683 series 250 °C MS transfer line temperature injector was used in the GC-MS experiment. An HP-5MS fused silica capillary column (30 x 0.25 mm) with a 1.0 m film thickness was installed in the GC. Using helium gas (99.999 percent) as a carrier gas at a steady flow rate of 22 cm / s, the oven temperature was held at 50 ° C for 5 minutes and then increased at a rate of 2 ° C / min from 50 to 250 ° C. At a 1:30 split ratio, a 1.0micron extract (1 mg diluted in 1 ml absolute alcohol) was injected. Agilent Science Network Mass Spectrometer (Model 5973 series) coupled with Hewlett Packard Gas Chromatograph (Model 6890 series) with NIST08 Library software database was studied by MS. The mass spectra were collected at 70 eV/200 °C with a scanning rate of 1 scan/s. The NIST08 Library database was used to perform compound recognition. Each unknown compound's mass spectrum was compared to the known compounds in the library software database.

3.6 Waste Disposal

Experimental wastes were cremated, and the rat carcasses were buried in a specific spot.

3.7 Analytical Statistics

The mean and standard deviation are used to present all of the data. Graph Pad Prism Software was used to assess variation within a collection of data using one-way analysis of variance (ANOVA) (GPPS 9.2). Statistical significance was defined as a value of p0.05.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

TABLE4.1: Percentage yield of crude extract and fractions of *C. rubens*

Percentage yield for Crude (Methanol) Extract	Percentage yield for Ethyl Acetate fraction	Percentage yield for Hexane fraction
13.85%	7.44%	16.42%

% Yield= (Weight of extracts/fractions obtained)/ (Weight of powder used for extraction) ×100

4.1 Effects of hydromethanol extract and fractions of *C. rubens* on liver and plasma cholesterol levels in myocardiac rats

From Figure 4.1, there was a significant decrease ($p< 0.05$) upon administration of Aspirin 75mg/kg on liver cholesterol levels when compared to the normal control. Also, upon administration of all plant extract there were significant decrease in liver cholesterol levels when compared to the normal and negative control groups.

There was a significant decrease ($p< 0.05$) upon administration of Aspirin 75mg/kg on plasma cholesterol levels when compared to the normal control. Similarly, upon administration of all plant extract there was also a significant decrease in plasma cholesterol levels when compared to the normal and negative control groups (Figure 4.2).

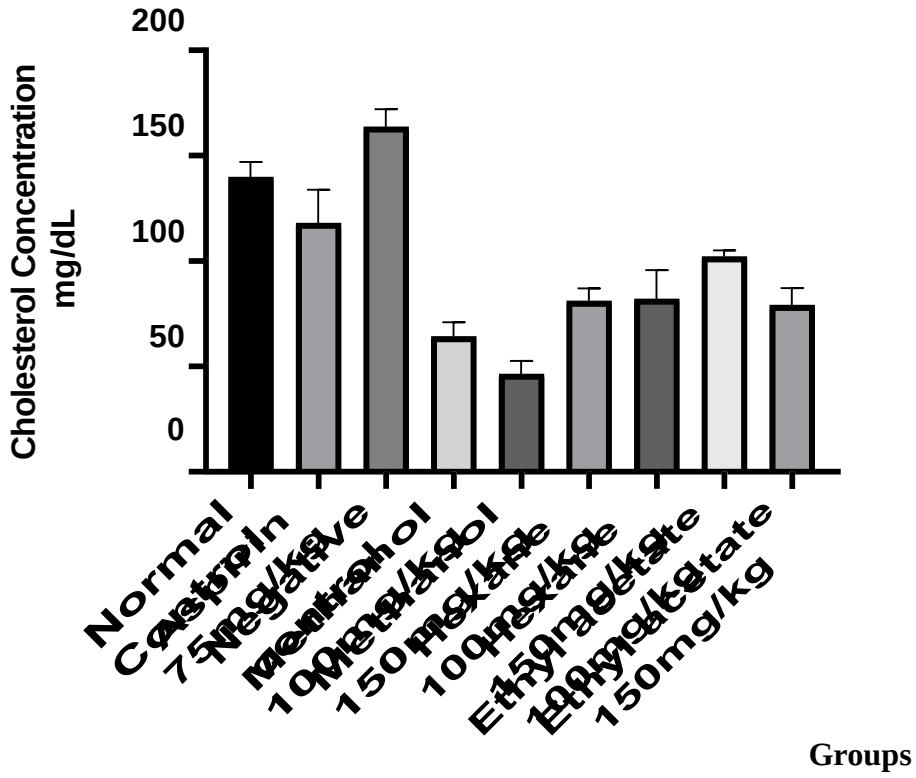

FIGURE 4.1: Liver cholesterol levels of control and myocardiac rats pretreated with hydromethanol extract and fractions of *C. rubens*

Values are mean ± SD; n=5

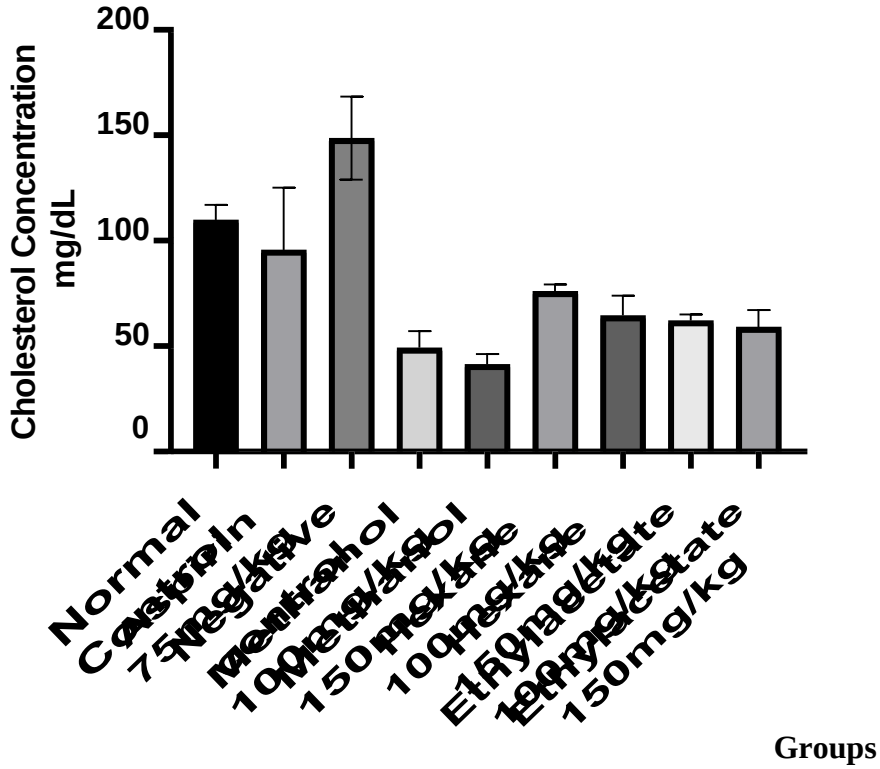

FIGURE 4.2: Plasmacholesterol levels of control and myocardiac rats pretreated with hydromethanol extract and fractions of *C. rubens*

Values are mean ± SD; n=5

4.2 Effect of hydromethanol extract and fractions of *C. rubens* on plasma triglyceride levelsin myocardiac rats

From Figure 4.2, there was a decrease of plasma triglyceride levels in rats administered with Aspirin when compared to the normal control, while the negative control recorded a slightly higher triglycerides level compared with the normal control. However, upon administration of all plant extract, there were significant decreases ($p< 0.05$) in plasma triglyceride levels when compared to the negative control group.

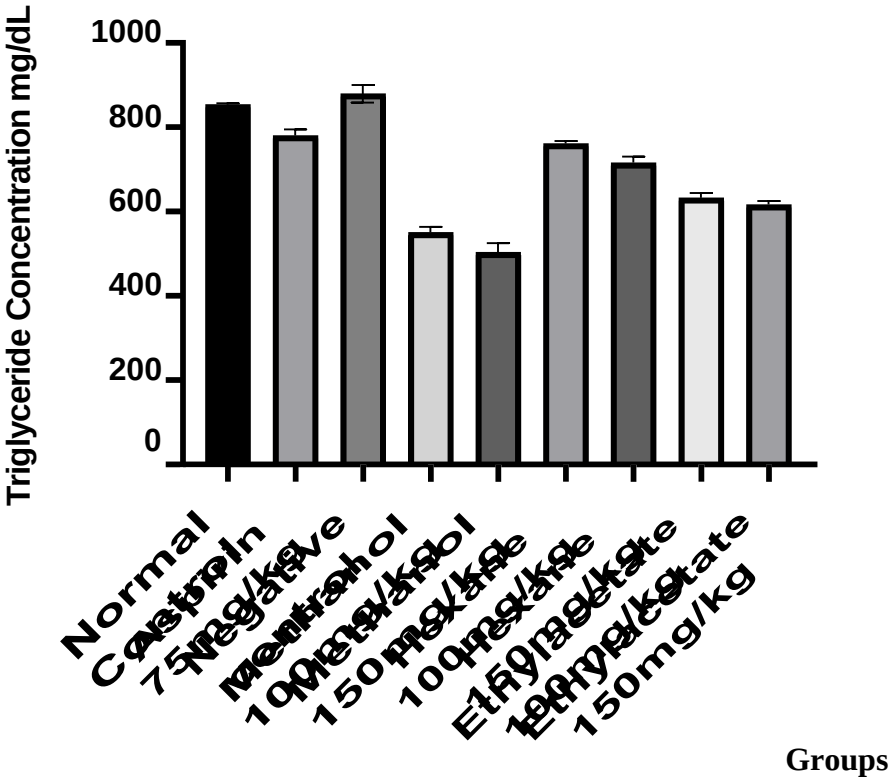

FIGURE 4.3: Plasma triglyceride levels of control and myocardiac rats pretreated with hydromethanol extract and fractions of *C. rubens*

Values are mean ± SD; n=5

4.3 Effect of hydromethanol extract and fractions of *C. rubens* on plasma and liver HDL levels in myocardiac rats

From Figure 4.3, there was a significant increase (p<0.05) upon administration of Aspirin 75mg/kg on plasma HDL levels when compared to the normal control. Similarly, upon administration of all plant extract there was also a significant increase in plasma HDL levels when compared to the positive control (Aspirin 75 mg/kg).

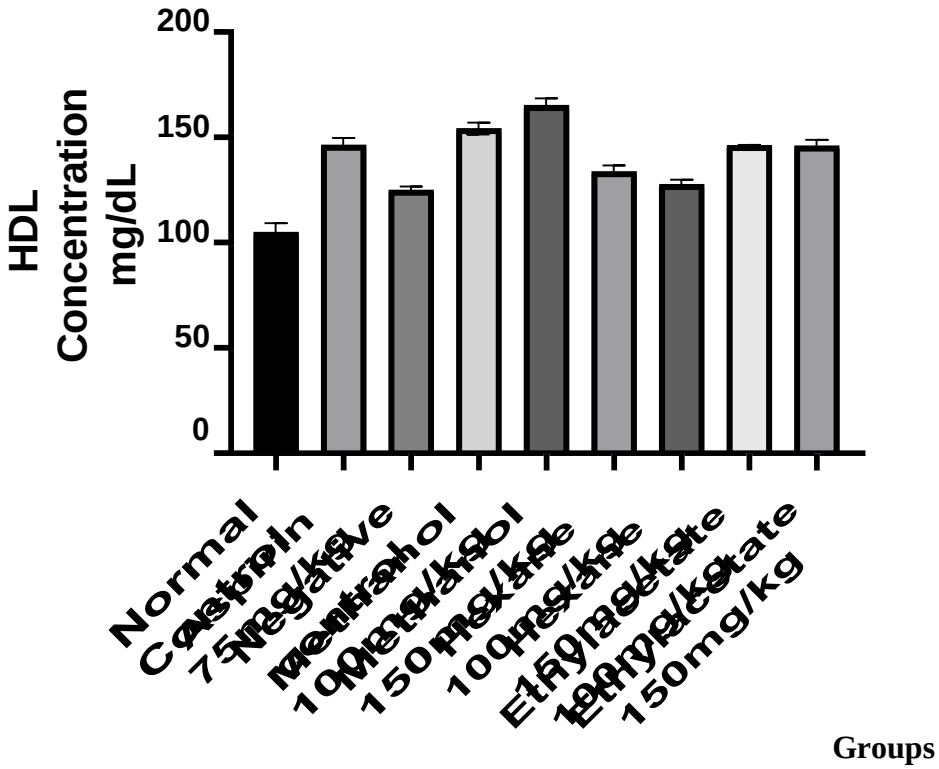

FIGURE 4.4: Plasma HDL levels of control and myocardiac rats pretreated with hydromethanol extract and fractions of *C. rubens*

Values are mean ± SD; n=5

Figure 4.4, there was a significant increase ($p<0.05$) upon administration of Aspirin 75mg/kg on liver HDL levels when compared to the normal control. However, upon administration of all plant extract there was also a significant increase in liver HDL levels when compared to the positive control (Aspirin 75 mg/kg).

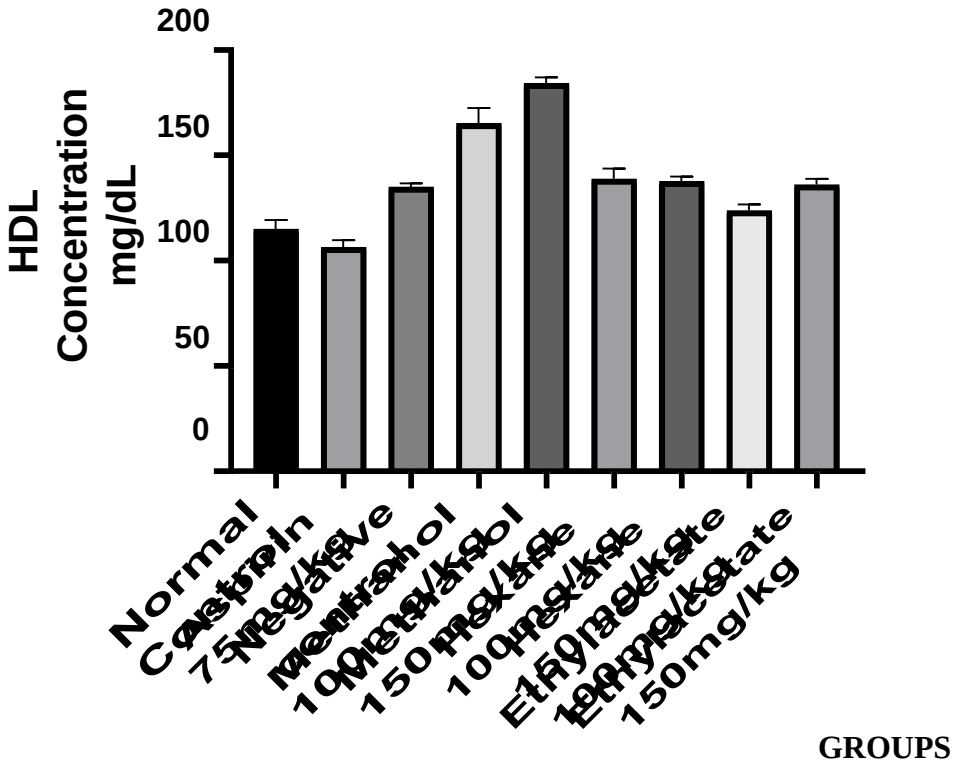

FIGURE 4.5: Liver HDL levels of control, and myocardiac rats pretreated with hydromethanol extract and fractions of *C. rubens*

Values are mean ± SD; n=5

4.4 Effect of hydromethanol extract and fractions of *C. rubens* on plasma LDL levels in myocardiac rats

From Figure 4.5, Upon administration of various fractions of plant extracts, there were no significant difference ($p > 0.05$) between the standard drug treated group (Aspirin 75mg/kg) and other plant fractions with exception of the group treated with Methanol (100mg/kg and 150mg/kg) where there was a significant ($p< 0.05$ decrease in plasma LDL levels when compared to the normal and negative control groups.

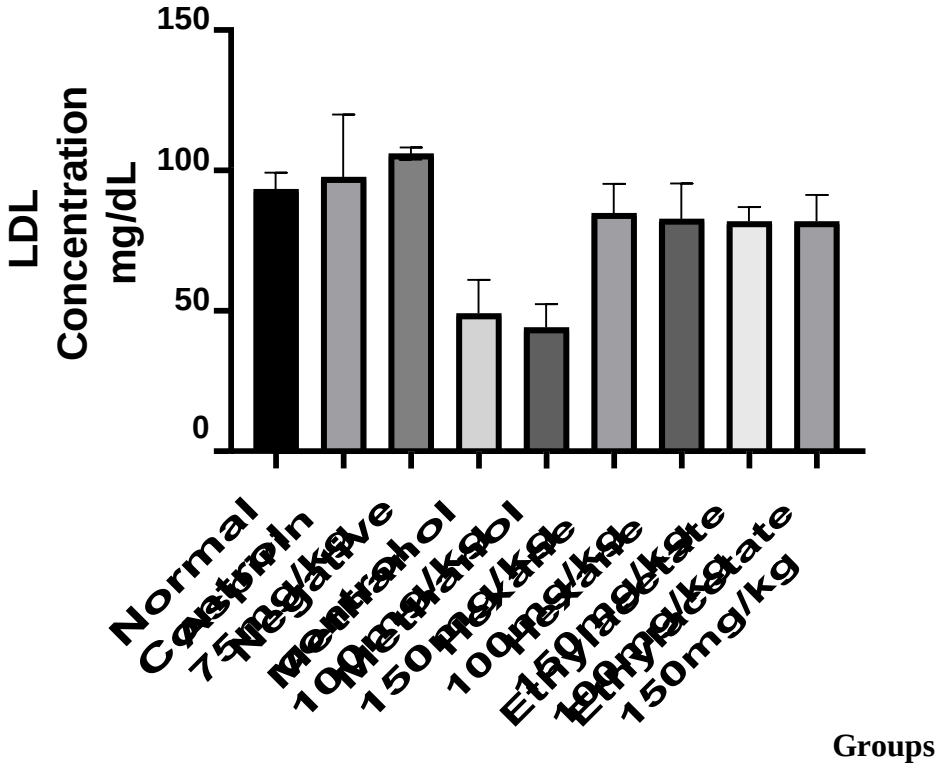

FIGURE 4.6: Plasma LDL levels of control, and myocardiac rats pretreated with hydromethanol extract and fractions of *C. rubens*

Values are mean ± SD; n=5

4.5 Effect of hydromethanol extract and fractions of *C. rubens* on plasma VLDL levels in myocardiac rats

From Figure 4.6, Upon administration of various fractions of plant extracts, there was also no significant difference between the drug treated group (Aspirin 75mg/kg) and other plant fractions with exception of the group treated with Methanol (100mg/kg and 150mg/kg) where there was a significant decrease in plasma VLDL levels when compared to the control groups. Furthermore, all groups treated with plant extract recorded significant decrease ($p< 0.05$) in VLDL levels compared with the negative control group.

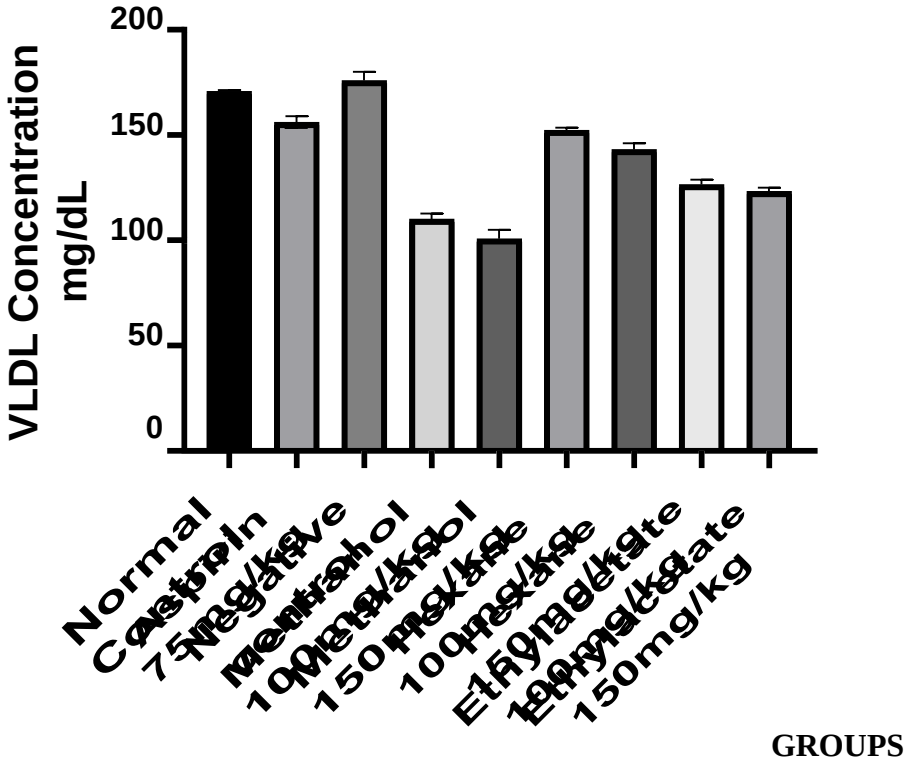

FIGURE 4.7: Plasma VLDL levels of control, and myocardiac rats pretreated with hydromethanol extract and fractions of *C. rubens*

Values are mean ± SD; n=5

4.6 Effect of hydromethanol extract and fractions of C. *rubens*on Atherogenic Index

The effect of Atherogenic Index of Plasma is shown in Table 4.2 below

TABLE 4.2: Atherogenic Index of plasma levels of control, and myocardiac rats pretreated with hydromethanol extract and fractions of *C. rubens*

GROUPS	AIP
Normal control	0.85 ± 0.01
Aspirin (75mg/kg)	0.73 ± 0.01
Negative control	0.91± 0.02
Methanol (100mg/kg)	0.55 ± 0.01
Methanol (150mg/kg)	0.48 ± 0.01
Hexane (100mg/kg)	0.76± 0.01
Hexane (150mg/kg)	0.75 ± 0.01
Ethyl acetate (100mg/kg)	0.64 ± 0.01
Ethyl acetate (150mg/kg)	0.63 ± 0.01

Values are mean ± SD; n=5. AIP -Atherogenic Index of Plasma

4.7 Effect of hydromethanol extract and fractions of *C. rubens* on Liver AST levels in myocardiac rats

From Figure 4.7 Upon administration of various fractions of plant extracts, there was also no significant difference between the drug treated group (Aspirin 75mg/kg) and other plant fractions.

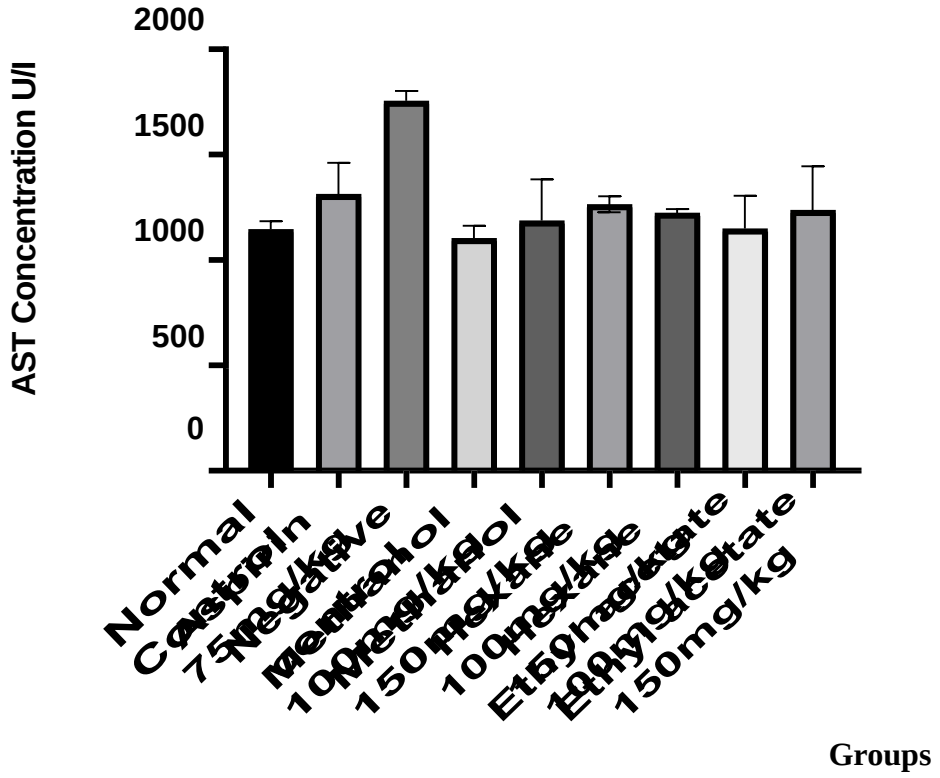

FIGURE 4.8: AST levels of control, and myocardiac rats pretreated with hydromethanol extract and fractions of *C. rubens*

Values are mean ± SD; n=5

4.8 Effect of hydromethanol extract and fractions of *C. rubens* on Liver ALT levels in myocardiac rats

From Figure 4.8, Upon administration of various fractions of plant extracts, there was also no significant differencebetween the normal control and other plant fractions, with the exception of group treated with ethyl acetate (100 mg/kg) which recorded a slight increase.

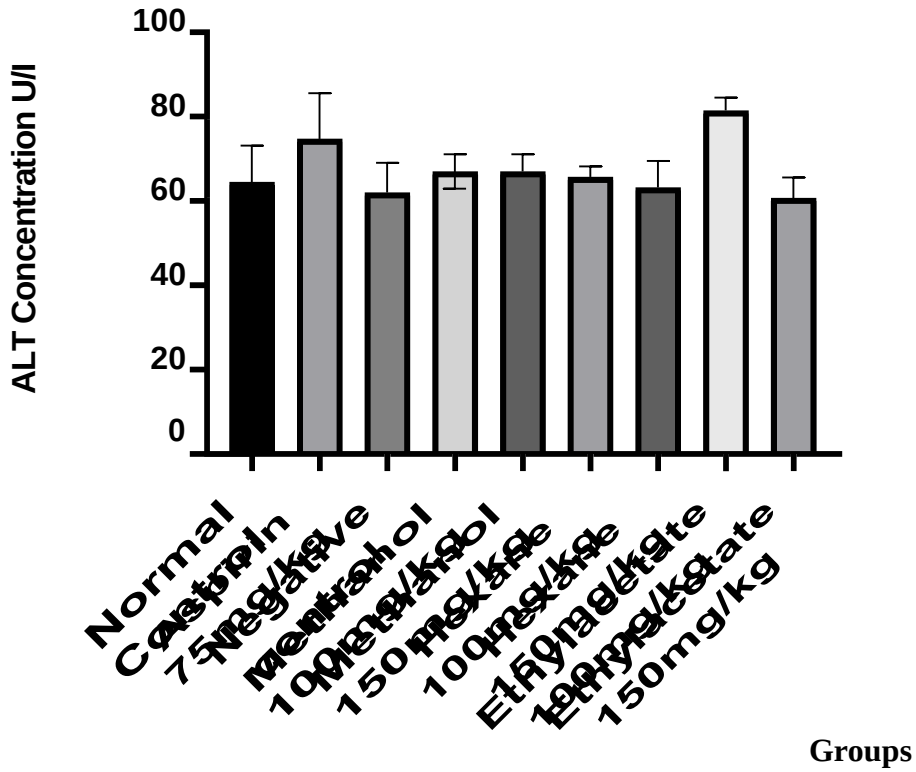

FIGURE 4.9: ALT levels of control, and myocardiac rats pretreated with hydromethanol extract and fractions of *C. rubens*

Values are mean ± SD; n=5

TABLE 4.3: GCMS Identified Phytochemical components of C.*rubens*

Peak no	Retention Time (min)	Area (%)	Name of Compound	Molecular Formula
1	2.325	0.50	2-Ethoxyethyl isobutyl carbonate	$C_9H_{18}O_4$
2	2.519	1.26	Boric acid, trimethyl ester	$C_3H_9BO_3$
3	2.925	0.42	Methanamine, N-hydroxy-N-methyl-	C_2H_7NO
4	3.519	0.18	3-Amino-2,2-dimethyl-1-propanol	$C_5H_{13}NO$
5	4.239	0.08	O-Butylisourea 2-Butanone,	$C_8H_{18}ON_2O$

6	7.097	0.03	Tetradecane, 2,6,10-trimethyl- Decane,	$C_{15}H_{32}$
7	7.961	0.05	Sulfurous acid,	$C_{17}H_{36}O_3S$
8	8.592	0.02	Hexadecane Disulfide,	$C_{16}H_{35}S_2$
9	9.118	0.23	1,6-Octadien-3-ol,	$C_{11}H_{18}O_2$
10	10.419	-0.17	9-Octadecenoic acid (Z)-,	$C_{19}H_{36}O_2$
12	10.963	0.26	3,5-Dimethoxytoluene	$C_9H_{12}O_2$
13	11.282	0.14	(+)-4-Carene1,3-Cyclohexadiene	$C_{15}H_{24}$
14	11.370	0.07	Adipic acid,	$C_{16}H_{19}Cl_4O_4$
15	11.451	0.03	2H-Pyran,5,6-dihydro-2-methyl-	$C_6H_{10}O$

16	11.658	0.45	.alpha.-Cubebene	$C_{15}H_{24}$
17	11.789	1.62	Cyclohexane,1-ethenyl-1-methyl-l	$C_{15}H_{24}$
18	12.120	9.44	(E)-.beta.-Famesene	$C_{15}H_{24}$
19	12.358	0.56	1,5,9,9-tetramethyl-,Z,Z,Z-Humulene	$C_{15}H_{24}$
20	12.590	8.74	.beta.-Bisabolene	$C_{15}H_{24}$
21	12.746	2.59	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-	$C_{15}H_{24}$
22	12.971	4.62	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-1,2-Dimethoxy-4-(2-methoxy-1-propenyl)benzene	$C_{12}H_{16}O_3$

			Benzenamide,	
23	13.540	13.41	Apiol 1,3-Benzodioxole, 4,5-dimethoxy-6- (2-propenyl)-	$C_{12}H_{14}O_4$
24	13.959	6.29	1-Butanamine, N-(2-furanylmethylen e)-3-methyl-1,2,5-	$C_8H_{19}N$
25	14.310	6.77	Phthalic acid,	$C_{17}H_{24}0_4$
26	14.447	1.88	1,2-Benzenedicarboxylic acid	$C_{16}H_{22}0_4$
27	14.554	2.72	1,2-Benzenediacetonitril-N'-[(2-Hydroxy-1-naphthyl)methylene]	$C_{20}H_{30}O_4$
28	14.685	4.19	Ethyl-4-[tetrahydropyran-2-yloxy]-2-butynoate 2-Butenediamide, 2-methyl-, (Z)- 22	$C_{11}H_{16}0_4$

			Isoxazole, 3,5-dimethyl-4-bromo-	
29	14.904	0.94	Propanenitrile	C_3H_4CIN
30	14.967	1.25	Furan-2-carboxylic acid	$C_5H_4O_3$
31	15.104	6.34	Hexadecanoic acid	$C_{17}H_{34}O_2$
32	15.661	12.09	Ethamivan Butyldimethylsilylo xybenzene	$C_{12}H_{20}OSi$
33	16.487	7.97	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$
34	17.294	1.57	Solanidan-3-one 2-Benzothiazolamine	$C_{27}H_{43}NO$
35	17.525	2.32	Lumazine 5,7-Dihydroxy-4-methylcoumarin	$C_{10}H_8O_4$
36	18.151	0.41	5-Decyne	$C_{10}H_{18}$
			Pyridine,	C_6H_7NO

37	18.301	0.52		
38	18.645	0.14	Thiophene, 	C_4H_4S $C_5H_8N_4O_3S_2$ $C_{12}H_{14}$

4. 9 Discussion of Results

This study was designed to look into the cardioprotective effects of *C. rubens* leaf extracts in an experimental rat model of Isoproterenol-induced myocardial infarction. The level of cardiac enzymes (AST, ALT) and lipid profiles, including triglycerides and total cholesterol, were elevated in the Isoproterenol-induced groups, whereas the crude and solvent fractions of *C. rubens*, particularly the crude fractions groups mitigated the elevations. One of the most basic criteria for detecting heart damage is the AST and ALT values. During myocardial infarction, several cardiac biomarkers were increased. The explanation for this is that these enzymes were increased in the heart and were released into the bloodstream as a result of membrane breakdown and cardiac muscle cell rupture. As a result, a rise in cardiac enzymes indicates cellular leakage and a loss of functional integrity of the heart's cell membrane (Sabeena et al.,2004).

AST and ALT are known to be secreted in reaction to liver injury, in addition to cardiac impairment. AST, on the other hand, is more sensitive to heart injury, and ALT is more sensitive to liver disease. As a result, calculating the AST/ALT ratio is critical in determining which organ is the more relevant source. The AST level in the rats' given isoproterenol was considerably higher than in the normal control group in the current investigation. After treatment with the crude extract and solvent fractions of *C. rubens*, the elevated levels of cardiac enzymes in the plasma were shown to recover to a virtually normal profile. By lowering the increased levels of these enzymes, pretreatment of the rats with crude extract and solvent fractions of *C. rubens* leaves stopped the harmful effect of isoproterenol on the heart. As a result, the suppression of elevated plasma levels of AST, and ALT by the crude extract and solvent fractions of *C. rubens,* particularly the crude extract towards the respective normal value is an indication of plasma membrane stabilization as well as repair of isoproterenol-induced cardiac tissue damage.

The increased levels of total cholesterol and triglycerides in the isoproterenol-induced group suggest that isoproterenol is interfering with lipid production or metabolism. Isoproterenol inhibited cardiac lipoprotein lipase (LPL) production, resulting in an increase in total cholesterol and triglyceride levels in lipid indicators. Lipoprotein lipase is a triglyceride-degrading enzyme that converts triglycerides to fatty acids. Treatment with *C. rubens* crude and solvent fractions resulted in a dose-dependent reduction in plasma lipid profile levels.

The hexane fraction exhibited just a little reduction in total cholesterol. This could indicate that the plant's active ingredients are polar molecules that the hexane solvent could not extract. This suggests that pretreatment of the rats with plant extracts and ethyl acetate may help to reduce isoproterenol -induced cardiotoxicity and hyperlipidemia.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

According to the findings of this investigation, *C. rubens* crude extract and solvent fractions exhibited significant hypolipidemic and cardioprotective efficacy in a dose-dependent way. The crude extract, followed by ethyl acetate, exhibits the most substantial protective effect against the adverse effects of isoproterenol in lipid profiles, and cardiac biomarkers. The fraction containing hexane was the least active. *C. rubens* could therefore be novel source of hypolipidemic and cardioprotective agent in pharmaceutical drug development.

5.2 RECOMMENDATION

Isolation of the components is recommended to determine the exact phytoconstituents responsible for *C. rubens* crude extract and fractions' cardioprotective effects. However, one of the study's drawbacks was that the MI-inducing medication isoproterenol could harm organs other than the heart.

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Appendix I

Showing the data obtained from Animal study.

Selected dose	Animal body weight (g)	Average weight (g)	Stock solution (conc.)	Daily dose in Ml
Group 1 (normal control)	162 U	154	1 mL of PBS daily; 70 mL for 14 days	1.0
	151 H			1.0
	187 T			1.0
	159 B			1.0
	112 R			1.0
Group 2 (positive control) / 75mg/kg (Aspirin	169 U	174	913.5 mg in 70 mL PBS (13.05 mg/mL) for 14 days	1.0
	204 H			1.2
	133 T			0.8
	162 B			0.9
	202 R			1.2
Group 3 (negative control)	234 U	217	1 mL of PBS daily; 70 mL for 14 days	1.0
	246 H			1.0
	233 T			1.0
	195 B			1.0
	177 R			1.0
Group 4 (crude extract)/ 100 mg /kg	179 U	172	1204 mg in 70 mL PBS (17.2 mg/mL) for 14 days	1.0
	185 H			1.1
	175 T			1.0
	128 B			0.7
	193 R			1.1
Group 5 (crude extract)/ 150 mg /kg	183 U	166	1743 mg in 70 mL PBS (24.9 mg/mL) for 14 days	1.1
	177 H			1.1
	155 T			0.9
	166 B			1.0
	148 R			0.9

	216 H			1.2
	191 T			1.1
	153 B			0.9
	139 R			0.8
Group 6 (Hexane extract)/ 100 mg /kg	182 U	169	1183 mg in 70 mL PBS (16.9mg/mL) for 14 days	1.1
	213 H			1.3
	133 T			0.8
	179 B			1.1
	138 R			0.8
Group 7 (Hexane extract)/ 150 mg /kg	183 U	180	1890 mg in 70 mL PBS (27 mg/mL) for 14 days	1.0
	129 H			0.7
	202 T			1.1
	162 B			0.9
	225 R			1.3
	134 H			0.8
	153 T			0.9
	159 B			1.0
	182 R			1.1
Group 8 (Ethyl acetate extract)/ 100 mg /kg	117 U	114	798 mg in 70 mL PBS (11.4 mg/mL) for 14 days	1.0
	112 H			1.0
	116 T			1.0
	109 B			0.9
	121 R			1.1
Group 9 (Ethyl acetate extract)/ 150 mg /kg	107 U	102	1071 mg in 70 mL PBS (15.3 mg/mL) for 14 days	1.1
	87 H			0.9
	106 T			1.0
	105 B			1.0
	106 R			1.0
	69 H			1.0
	63 T			0.9

	59 B			0.8
	62 R			0.9

Appendix II

Showing the values for the weight of the petri dishes before and after drying of the crude extract using the hot air oven

Weight of petri dishes before drying (g)	Weight of petri dishes after drying (g)	Difference in weight (g)
A (7.41)	8.33	1.42
B (7.40)	8.74	1.34
C (7.40)	9.58	2.18
D (7.42)	10.98	3.56
E (6.60)	8.62	2.02
F (7.40)	10.66	3.26
G (6.60)	11.00	4.40
H (7.40)	11.69	5.07
I (6.67)	8.03	1.36
J (6.60)	7.97	1.37
K (5.99)	6.77	0.78
L (6.01)	6.96	0.95
M (6.44)	7.11	0.67
N (6.41)	6.74	0.33
O (5.99)	6.72	0.73
P (6.02)	6.90	0.88
Q (6.00)	6.77	0.77
R (6.58)	7.37	0.79
A1 (31.50)	36.94	5.44

B1 (34.80)	39.21	4.41
C1 (31.56)	35.87	4.31
D1 (35.38)	38.14	2.76
E1 (35.32)	39.79	4.47
F1 (39.19)	42.28	3.09
G1 (37.88)	41.63	3.75
Total		71.91

Appendix III

Showing the values for the weight of the petri dishes before and after drying of the ethyl acetate fraction using the hot air oven

Weight of petri dishes before drying (g)	Weight of petri dishes after drying (g)	Difference in weight (g)
A (28.38)	28.70	0.32
B (30.26)	30.89	0.63
C (30.90)	31.41	0.51
D (30.94)	31.47	0.53
E (29.29)	30.25	1.06
F (31.73)	31.97	0.24
G (32.44)	33.45	1.01
Total		4.3

Appendix IV

Showing the values for the weight of the beakers before and after drying of the hexane fraction using hot air oven

Weight of beaker before	Weight after beaker after	Difference in weight (g)

drying (g)	drying (g)	
A (99.11)	100.83	1.72
B (104.15)	105.70	1.55
C (98.33)	104.54	6.21
Total		9.48

Appendix V

Showing effect of methanol extracts and fractions of Crassocephalum rubens leaf on the rat body,liver and heart weights.

Groups	Initial body weight(g)	Final body weight(g)	Change in body weight (%)	Weight of heart(g)	Ratio of body to heart (%)	Weight of liver(g)	Ratio of body to liver (%)
1	154.00±12.15	142.00±12.31	8.45±1.30	0.52±0.03	0.37±0.24	1.14±0.21	0.80±0.02
2	174.00±13.29	184.00±18.32	5.43±0.27	0.75±0.07	0.41±0.38	1.81±0.19	0.98±0.01
3	217.00±13.17	170.00±11.88	27.65±10.86	0.70±0.04	0.41±0.34	2.12±0.26	0.01±0.02
4	172.00±11.41	149.00±12.83	15.44±11.07	0.63±0.04	0.42±0.31	1.77±0.19	0.01±0.14
5	166.00± 6.54	135.00±1.11	18.67±4.89	0.65±0.04	0.50±0.04	1.73±0.36	0.01±0.32
6	169.00±14.94	155.00±12.88	9.03±0.16	0.76±0.04	0.50±0.32	1.13±0.09	0.73±0.69
7	180.00±16.50	155.00±9.57	16.13±0.72	0.75±0.08	0.48±0.84	1.00±0.15	0.65±0.02
8	115.00± 2.07	156.00±12.37	26.28±0.83	0.73±0.06	0.47±0.49	2.31±0.29	0.01±0.02
9	102.00± 3.81	146.00±7.36	30.14±0.48	0.70±0.04	0.48±0.54	1.64±0.28	0.01±0.04

Each value represented the mean ±SEM of 5 readings.

Change in body weight (BWT)(%) =(final bwt/finial bwt)X 100

Ratio of body to heart (%) =weight of heart /final body weight) X100

Group:

1. Normal control
2. Positive control
3. Negative control
4. Methanol extract (100mg/kg)
5. Methanol extract (150mg/kg)
6. Hexane fraction (100mg/kg)
7. Hexane fraction (150mg/kg)
8. Ethyl acetate fraction (100mg/kg)
9. Ethyl acetate fraction (150mg/kg)

Appendix VI

Percentage Yield of Crude Extract and Solvent Fractions of C. *rubens*

The Percentage Yield of the Crude Extract

% Yield= (Weight of extracts obtained)/ (Weight of powder used for extraction) ×100.

Total gram of sample after pulverizing = 519.13g

519.13g of pulverized *C. rubens* was soaked in 4,160mls of solvent (70% methanol) and dried.

Total weight after drying = 71.91g

Total weight before drying = 519.13g

Percentage yield for crude (methanolic) extract = $\frac{71.91}{}$ x 100% = 13.85%

519.13

14.17g was kept for phytochemical characterization (Gas chromatography mass spectrometry)

71.91 – 14.17 = 57.74g

The Percentage Yield of the Fractions

% Yield= (Weight of fractions obtained)/ (Weight of crude extracts used) ×100.

Percentage yield for ethyl acetate fraction

$= \frac{4.30}{57.74} \times 100\% = 7.44\%$

Percentage yield for hexane fraction

$= \frac{9.48}{57.74} \times 100\% = 16.42\%$

Appendix VII

Result for Assay of cholesterol activity in the liver

Normal Control	Aspirin 75mg/kg	Negative control	Methanol 100mg/kg	Methanol 150mg/kg	Hexane 100mg/kg	Hexane 150mg/kg	Ethyl acetate 100mg/kg	Ethyl acetate 150mg/kg
148.76	138.19	154.22	63.45	38.56	82.34	89.48	102.15	77.4
131.26	99.94	168.92	70.92	47.28	74.33	93.67	102.39	89.8
139.64	116.33	159.22	55.3	53.51	88.5	63.14	98.45	70.3
139.89	118.15	172.34	67.44	46.45	79.2	82.11	105.61	79.3

Appendix VIII

Result for Assay of cholesterol activity in the plasma

Normal Control	Aspirin 75mg/kg	Negative control	Methanol 100mg/kg	Methanol 150mg/kg	Hexane 100mg/kg	Hexane 150mg/kg	Ethylacetate 100mg/kg	Ethylacetate 150mg/kg

118.76	138.19	124.22	43.45	38.56	72.34	69.48	62.15	57.4
101.26	69.94	158.92	60.92	47.28	74.33	73.67	62.39	69.8
109.64	86.33	169.22	45.3	43.51	78.5	63.14	58.45	50.3
109.89	88.15	142.34	47.44	36.45	79.2	52.11	65.61	59.3

Appendix IX

Result for Assay of triglyceride activity in the plasma

Normal Control	Aspirin 75mg/kg	Negative control	Methanol 100mg/kg	Methanol 150mg/kg	Hexane 100mg/kg	Hexane 150mg/kg	Ethylacetate 100mg/kg	Ethylacetate 150mg/kg
850.34	775.8	877.24	545.86	497.59	756.21	706.21	628.62	624.48
857.24	787.96	851.72	570.34	536.21	768.1	719.66	635.15	623.1
854.48	764.14	890.34	546.89	491.38	757.93	703.79	646.89	613.7
854.02	796.3	899.77	541.03	491.73	765.08	735.22	620.22	607.43

Appendix X

Result for Assay of VLDL activity in the plasma

Normal Control	Aspirin 75mg/kg	Negative control	Methanol 100mg/kg	Methanol 150mg/kg	Hexane 100mg/kg	Hexane 150mg/kg	Ethylacetate 100mg/kg	Ethylacetate 150mg/kg
170.068	155.16	175.448	109.172	99.518	151.242	141.242	125.724	124.896
171.448	157.592	170.344	114.068	107.242	153.62	143.932	127.03	124.62
170.896	152.828	178.068	109.378	98.346	151.586	140.758	129.378	122.74
170.804	159.26	179.954	108.206	98.276	153.016	147.044	124.044	121.486

Appendix XI

Result for Assay of HDL activity in the liver

Normal Control	Aspirin 75mg/kg	Negative control	Methanol 100mg/kg	Methanol 150mg/kg	Hexane 100mg/kg	Hexane 150mg/kg	Ethylacetate 100mg/kg	Ethylacetate 150mg/kg

117.71	102.22	132.63	166.35	182.86	136.44	139.35	125.39	134.85
119.5	108.33	135.89	168.73	184.24	136.25	139.08	121.61	136.25
113.067	105	136.49	171.34	188.22	136.36	134.81	121.49	133.26
110.09	109.95	135	155.38	181.77	146.25	138.08	127.07	139.92

Appendix XII

Result for Assay of HDL activity in the plasma

Normal Control	Aspirin 75mg/kg	Negative control	Methanol 100mg/kg	Methanol 150mg/kg	Hexane 100mg/kg	Hexane 150mg/kg	Ethylacetate 100mg/kg	Ethylacetate 150mg/kg
107.71	142.22	122.63	152.86	166.35	135.39	129.35	146.44	144.85
109.5	148.33	125.89	154.24	168.73	131.61	129.08	146.25	146.25
103.067	145	126.49	158.22	161.34	131.49	124.81	146.36	143.26
100.09	149.95	125	151.77	165.38	137.07	128.08	146.25	149.92

Appendix XIII

Result for Assay of LDL activity in the plasma

Normal Control	Aspirin 75mg/kg	Negative control	Methanol 100mg/kg	Methanol 150mg/kg	Hexane 100mg/kg	Hexane 150mg/kg	Ethylacetate 100mg/kg	Ethylacetate 150mg/kg
87.138	71.402	105.392	57.588	56.402	75.25	85.25	82.866	77.354
101.092	114.466	108.768	58.818	39.312	86.678	80.678	81.61	91.43
94.142	117.642	106.504	47.192	41.811	78.502	67.502	75.432	70.82
91.004	87.386	103.554	33.146	39.176	98.84	97.84	87.816	87.734

Appendix XIV

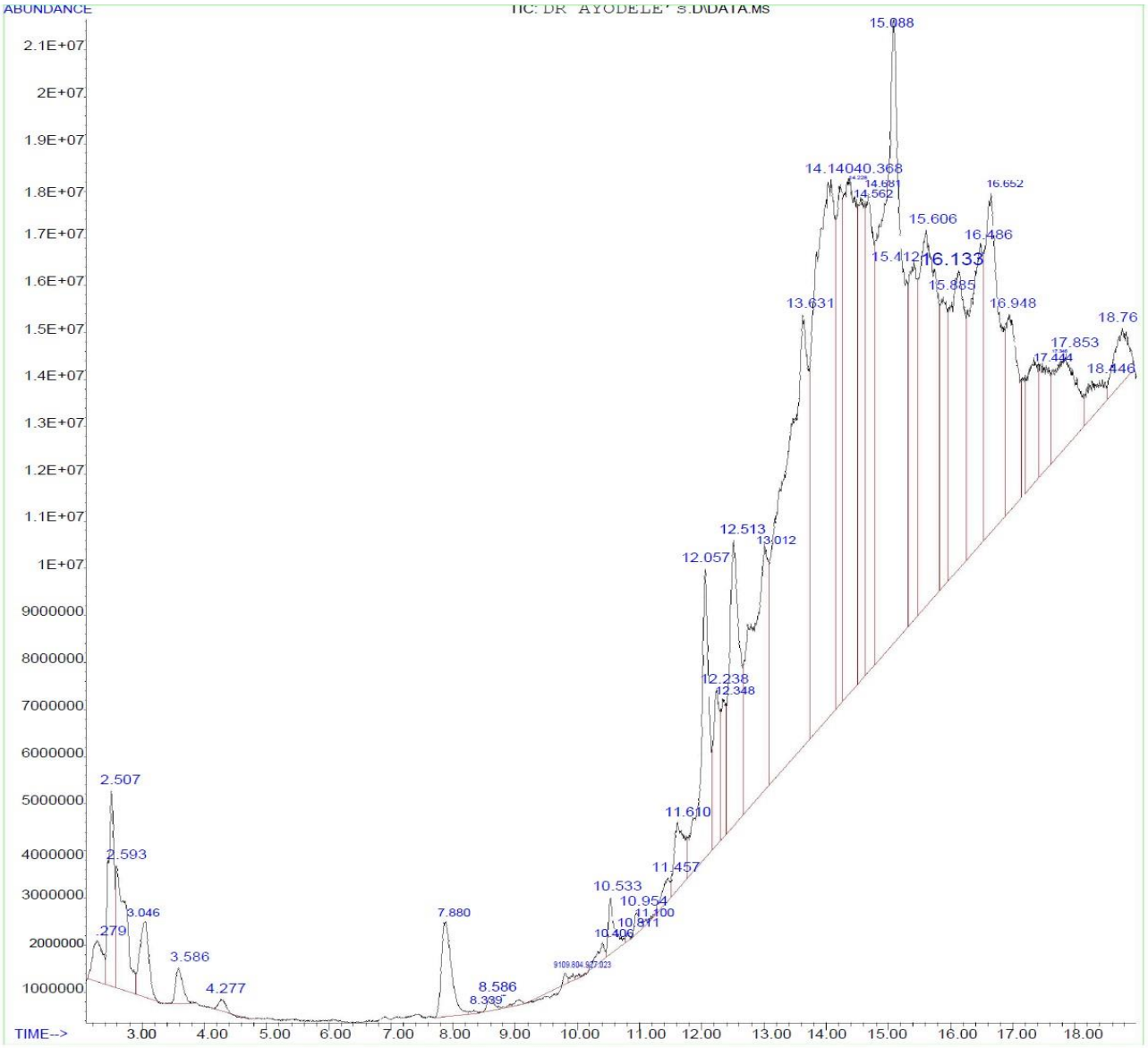
Result for Assay of Alanine transaminase activity in the liver

Normal Control	Aspirin 75mg/kg	Negative control	Methanol 100mg/kg	Methanol 150mg/kg	Hexane 100mg/kg	Hexane 150mg/kg	Ethylacetate 100mg/kg	Ethylacetate 150mg/kg
52	72	57	67	62	67	62	83	57
67	88	72	72	67	67	72	83	57
72	62	57	62	72	62	57	77	67
67	77	62	67	67	67	62	83	62

Appendix XV

Result for Assay of Aspartate amino transferase activity in the liver

Normal Control	Aspirin 75mg/kg	Negative control	Methanol 100mg/kg	Methanol 150mg/kg	Hexane 100mg/kg	Hexane 150mg/kg	Ethyl acetate 100mg/kg	Ethyl acetate 150mg/kg
1135.485	1151.66	1815.505	1025.49	1093.43	1292.475	1220.45	1041.67	1154.04
1106.37	1274.59	1751.09	1112.84	1458.99	1288.67	1211.985	1008.365	1138.15
1197.335	1511.13	1698.38	1171.11	1009.32	1210.46	1214.365	1196.95	1546.33
1146.39	1312.46	1754.99	1103.15	1187.25	1263.87	1248.93	1348.995	1112.84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