ANTIOXIDANT EFFECT OF *Crassocephalum rubens* (Jacq.S. Moore) Asteraceae IN ISOPRENALINE INDUCED MYOCARDIAL INFRACTED RATS

BY

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A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY COLLEGE OF BASIC AND APPLIED SCIENCES, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF BACHELOR OF SCIENCE IN BIOCHEMISTRY.

SEPTEMBER, 2021

DECLARATION

I hereby declare that this project has been written by me and it 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.

……..……..

EVEREST GLORY CHIMSOM

……

Date

CERTIFICATION

This is to certify that the content of this project entitled '**Antioxidant Effect Of *Crassocephalum rubens* (Jacq .S. Moore) Asteraceae in Isoprenaline Induced Myocardial infracted rats** and submitted by **EVEREST GLORY CHIMSOM** in partial fulfillment of the requirements for the degree of **BACHELOR OF SCIENCE IN BIOCHEMISTRY**. The original research work was carried out by her under my supervision and is hereby accepted**.**

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Dr. (Mrs.) O.O. Ayodele

Supervisor

…… **(Signature and Date)**

Dr. (Mrs) O.T. Kayode

Head of Department

DEDICATION

I, dedicate this work to God Almighty, for His divine strength, wisdom, and knowledge which saw me through the completion of this work, to my parents, Mr. & Mrs. Everest,sibling for their unwavering supports financially, emotionally, and morally and to my colleagues who encouraged me to be strong despite several challenges. These joint efforts helped in the completion of this work.

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To the King of Israel who answers prayers speedily gives life to all, honor be ascribe unto for the journey so far, He start it and ended it in a miraculously ways, Thank you Jesus.

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ABSTRACT

Myocardial Infarction is a prevalent disorder that leads to the heart failure function, affecting male and female. It is a medical condition that arises when a blood clot completely obstructs the coronary artery leading to death of tissue, which causes a lasting injury to the heart muscle. *Crassocephalum rubens* has been reported to possess an array of medicinal properties, with the leaves being used to treat indigestion, headaches, bruises, nosebleeds and sleeping sickness. The reported pharmacological activities of C. rubens include: anticoagulant properties that can be used in the treatment of clotting disorders. Other activities are: antidiabetic, anti-inflammatory and antioxidant. The present study evaluated the antioxidant effect of *Crassocephalum rubens* leaf extracts on myocardial infarction induced by isoprenaline (ISO) in Wistar albino rats with the view to determine its probable use in the management of heart -related diseases.

Fresh plant leaves were collected, identified, extracted, fractionated and the methanol extracts were partitioned with ethyl acetate and hexane. Sixty adult rats were divided into (12) groups of 5 rats each. Groups I, II, and III were the control groups. Rats in group IV, V and VI were pretreated with 100, 150, and 200 mg/kg of the crude methanol extract; VII, VIII, IX were pretreated with 100, 150, and 200 mg/kg of hexane fraction; X, XI and XII, were pretreated with 100,150, and 200 mg /kg of Ethyl acetate fraction for 14 days. Myocardial infarction was then induced in all rats (except those in Group I) with a single intraperitoneal injection of ISO (100 mg /kg). Afterwards, the rats were sacrificed.

Experiment carried out gave results of a significant increase in lipid peroxidation levels in myocardial –induced rats treated with isoprenaline, there was also an increase in the heart tissue of GSH of myocardial infracted induced rats. The study has shown that *C. ruben* may be able to reduce the effect of oxidative stress induced myocardial infarction.

Keywords: Antioxidant, Myocardial infarction, *Crassocephalum rubens*, Isoprenaline, Lipid peroxidation

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Herbal remedies have been in use in almost every society since time immemorial (Ang-Lee et al. 2001; Goldman, 2001). Herbal medicines mostly used in Africa and Asia countries, and many of these traditional medicines have been discovered to be inexpensive sources of complex bioactive compounds in developed countries (Phillipson, 1994). Modern pharmaceuticals are unique synthetic chemicals with potent clinical activity commonly found in oral dosage forms. In contrast, natural compounds from higher plants are also used in pharmaceutical preparations, either as whole plants or as extracts (Gogtay et al. 2002). Natural products remain one of the best reservoirs of novel structural forms, despite several methodologies for drug development (Hostettmann, 1999). As a result, in the quest to continuously improve the effectiveness and ethics of modern medicine, researchers are increasingly turning to traditional medicine as a source of new drugs (Wayne, 1998; Hoareau Dasilva .1999).

Myocardial infarction (MI) is a critical health challenge, associated with coronary artery occlusion which leads to significant morbidity and mortality worldwide ((Zhang et al. 2019). MI has been linked to apoptosis, inflammation, and oxidative stress, both of which contribute to cardiac failure (Geeta et al. 2016). Isoprenaline (L-b-(3,4-dihydroxyphenyl)-2-isopropyl amino ethanol hydrochloride), is a synthetic beta-adrenoceptor agonist that causes cellular damage and infarct-like necrosis of the myocardium in rats when provided subcutaneously (Islam et al. 2020). The Isoprenaline-induced myocardial necrosis in rat model is a well-accepted standardized model for testing cardiac dysfunction, blood coagulation parameters, and studying the effectiveness of natural and synthetic cardioprotective agents (Sumaira et al. 2014). Modern drugs are used to treat the symptoms associated with MI, but they are often accompanied by side effects.

Crassocephalum rubens is known as Okinawa spinach, Redflower ragleaf, Fireweed, ebolo (in Yoruba, South Western, Nigeria), erimi onu (in Igbo, South Eastern, Nigeria) belonging to the Asteraceae family. This vegetable has been reported to possess an array of medicinal properties, with the leaves being used to treat indigestion, headaches, bruises, nosebleeds and sleeping sickness. (Grubben et al. 2018). The reported pharmacological activities of *C..rrubens* include:

anticoagulant properties that can be used in the treatment of clotting disorders. Other activities are: antidiabetic, anti-inflammatory and antioxidant (Ayodele et al .2020, Bahar et al. 2017). The present study aims at investigating the antioxidant effect of hydromethanol extract and fractions of *C. rubens* in isoprenaline induced myocardial infarcted rat model.

1.2 Statement of the Problem

Myocardial Infarction is a prevalent metabolic disorder that leads to the heart failure function, affecting male and female. It is a medical condition that arises when a blood clot completely obstructs the coronary artery leading to death of tissue, which causes a lasting injury to the heart muscle. (Shier et al. 2013). It has been discovered that an adverse effect of myocardial infarction is high blood pressure which is a risk factor. Modern drugs are available for treating and management of the symptoms associated with MI, but they are often accompanied by side effects. This research thus aims at exploring the antioxidant effect of hydromethanol extract and fractions of *C. rubens* in isoprenaline induced myocardial infarcted rats as possible novel natural antioxidant agent in the management of MI complications and symptoms.

1.3 Aim and objectives of the study

This study aims to investigate the antioxidant effect of methanol extract and fractions of *C. rubens* in Isoprenaline induced on myocardial infarcted rats..

The specific objectives are to:

i. Determine the effects of methanol extract and fractions of *C. rubens* on the extent of lipid peroxidation in isoprenaline induced myocardial infarcted rats.
ii. Determine the effects of methanol extract and fractions of *C. rubens* on catalase activity in isoprenaline induced myocardial infarcted rats.
iii. Determine the effects of methanol extract and fractions of *C. rubens* on reduced Glutathione (GSH) levels in the experimental rats.
iv. Characterize the phytochemical components of the ethyl acetate fraction of *C. rubens* using Gas chromatography-mass spectrometry (GC-MS)

1.4 Significance of study.

Various study has established the fact that myocardial infraction could consequently lead to cardiovascular diseases by clumping of plaques on the walls of the atria. This study will further reveal the antioxidant effect of hydromethanol crude extract and fractions of *C. rubens* in isoprenaline induced myocardial infarction in rats. Consequently, this study could prove impactful in the fight against cardiovascular diseases especially in myocardial patients.

1.5 Scope of the Study

The study entails the administration of methanol extract and fractions to Isoprenaline induced myocardial infarcted rats which may reduce the reactive oxygen species, and thus oxidative stress in the experimental rats. The effect of the plant on endogenous antioxidants (catalase and glutathione) in the experimental rats will be determined. Some phytochemical components of the ethyl acetate fraction will be identified using GCMS.

CHAPTER TWO

LITERATURE REVIEW/BASIC THEORY

2.1 Crassocephalum rubens (Asteraceae) Jacq. S. Moore

Crassocephalum rubens (*C. rubens*), is a wild or semi-domesticated aromatic traditional leafy vegetable that is generally eaten in the South West of Nigeria and only moderately in the South East (Grubben et al. 2018). It is a member of the Asteraceae (sunflower family) and is biologically classified as *C. rubens* (Grubben et al.2018). Roots are used to treat chapped lips (Grubben et al.2018).

2.1.1 Growth Habit:

C. rubens is a herbaceous annual plant that grows up to 4 feet tall and is erect (upright). Its seedlings are light green in color and have spatula-shaped leaves with smooth margins. The first true leaves are light green, elliptical (oval-shaped) with toothed margins, growing in a rosette, the veins of the leaves are slightly red in color, stems are round and ribbed with short, thick hairs near the apical portion of the plant on the stem and branches, and the flower heads are mauve in color. *C. rubens* is a seasonal plant that germinates in a variety of pH, salt, and temperature conditions, preferentially when the seed is on or just below the media surface (Hossain,Nakamura,2009).

C. rubens is an annual herb, 17-19cm high, herbaceous with mucilaginous stem with rounded or cross-section, solid, hairy. Leaves are simple, divided, spirally alternate, margin coarsely dentated, acute apex and base, pinnately veined. Flowers are bisexual, sessile, red or red-brown, grouped in terminal capitulums with only tubular flowers (Kadereit.,Vanijajiva. 2009). *C. rubens* is found to be annual plants which produce a large number of seeds , its normally at temperature between 10^0C and 40^0C and annual rainfall of 600-1500 is suitable, the lower limit of germination explain the incidence at high altitudes. It is an annual weed that flowers. The most important life stages which contribute to plant distribution and invasiveness is seed germination, adaptation to temperature and water stress. *C. rubens* produces seeds with silky hairs that can be easily dispersed by wind and/or water (Denton, 2004). It has been established that *C. rubens* seedlings grow fast and have the potential to form dense thickest displacing native vegetation.

2.2 NUTRACEUTICAL POTENTIAL OF *Crassocephalum rubens*

2.2.1 Hepatoprotective activity

C. rubens was tested in animals against several medications and chemically produced chronic liver disorders, and the results were promising. (Devaraju ,2016).*C. rubens* has also been studied for its hepatoprotective properties against carbon tetrachloride liver injury (Salawu et al.2004).The treatment of chronic liver diseases such as fibrosis, cirrhosis, and chronic hepatitis is typically insufficient due to pharmacological and chemical side effects. To address this issue, current research is focusing on drugs derived from medicinal herbs rich in flavonoid and polyphenolic compounds, as well as hepatoprotective properties (Pereira et al.2015). *C. rubens* root has a long history of use for liver function, as well as treating various dermatologic and systemic disorders, on the theory that the herb improves the liver's ability to detoxify. These concepts have received little research attention. A recent study compared the effects of herbal formula containing dandelion (T. officinalis), turmeric (Curcuma longa), artichoke (Cynara scolymus), rosemary (T. officinalis), schisandra (Schisandra chinensis), and milk thistle (Silybum marianum), a healthy diet, and place on hormone levels in 40 premenopausal women (Greenle et al.2007).

2.2.2 Anti-coagulant activity

Blood is a rapid and effective process that forms clots that must be regulated; a disorder of blood coagulation is a hallmark of many illness conditions. *C. rubens* contains bioactive components that have anticoagulant properties and may be used to treat blood coagulation disorders. The effect of *C. rubens* leaf methanol extract and its partitioned solvent fraction on blood coagulation in healthy human volunteers was studied in vitro. The leaf extract and fraction significantly prolonged the clotting time, Prothrombin and activated partial thromboplastin times in volunteer blood. *C. rubens* has bioactive components with anticoagulant qualities that can be used to treat blood coagulation disorders (Ayodele et al. 2020). *C. rubens* has anticoagulant activity, making it a potential nutraceutical source for the management of thrombotic disorders in diabetes and other diseased states. (Ayodele et al. 2020).

2.2.3 Antidiabetic activity

C. rubens was found to have cell protection and anti-diabetic properties in pancreatic cell culture and Wistar albino rats (Bahar et al.2017). The nutritional composition of *C. rubens* was examined using AOAC standard techniques. The results reveal that the veggies' low fat, protein, and carbohydrate contents, but has high calorie energy, qualify them to be optimal for intake by all, but especially by hypertensive, diabetic, and overweight adults (Arawande et al.2013). *C. rubens* also possess bioactive components that have showed a number of anti-diabetic properties, owing to the pharmacological actions of components such as sesquiterpene lactones, triterpenes/phytosterols (taraxasterol), phenols, flavonoids, and phenolic acids (Schutz et al.2006).

2.2.4 Antioxidant activity

According to (Oluwasesan et al.2019), the extract had a good anti-oxidant impact with an IC50 of 45.78g/ml, with ascorbic acid serving as a positive control at 41.56g/ml. This finding raises awareness about the usage of *C. rubens,* as a therapeutic plant that is frequently eaten as a wild vegetable in many countries. A comparison of the anti-inflammatory, antioxidant, and genotoxicity capabilities of the leaf of *C. rubens*. To evaluate the antioxidant property, cold water extract and hot water extract were studied using red blood cell membrane stabilization technique and In-vitro methods utilizing DPPH assay ferric reducing antioxidant power, thiobarbituric acid-reactive compounds (TBARS).

The study discovered that consuming *C. rubens* leaf in cooked form had a higher medical value than others. The antioxidant and antihyperlipidemic properties of *C ..rubens* methanol extract in vitro. The extract dramatically lowered serum HDL levels in high fat diet hyperlipidemic mice, demonstrating considerable antioxidant activity. According to these findings, the extract aids in the reduction of hyperlipidemia, possibly due to its antioxidant action (Bahar et al.2015) *C. rubens'* free radical scavenging and preventive effects against chemically induced hepatotoxicity. *C. rubens* of water extract effectively scavenged superoxide anion, hydroxyl radical, and the stable radical 1,1diphenyl-2-picrylhydrazyl.*C. rubens* is an effective antioxidant that protects the liver against Ga1N plus Ccl4-induced hepatotoxicity (Aniya et al. 2005).

2.2.5 Antitumor activity

The leaves extract of *C. rubens* are promising for the treatment of breast tumors and the strong potential of *C. rubens* extract as anticancer agents and rational use for drug development (Trinh et

al.2016). The anti-tumor activity has been investigated in In-vitro and in vivo methods. The anti-tumor effect of *C. rubens* was evaluated in S-180-cell bearing mice, the cell growth was used using colorimetric assay, nitrate level were measured by calorimetry. The result shows that the extract of *C. rubens* delay tumor growth in s-180 bearing mice, however it did not inhibit s-180 cell growth in vitro (Tomimori et al.2012). Other researcher conducted in vitro anti-tumor activity of 24 locally medicinal plants which include *C. rubens.* The anti tumour activity of methanol extract obtained from 24 samples were evaluated in vitro on five human cancer cell lines. The results obtained indicate 18 plants extract including *C. rubens* exhibit promising cytotoxic activity against human carcinoma cell lines. (Fadeyi et al.2013).

2.2.6 Anti-bacterial activity

The antibacterial activity of hot aqueous extract of *C. rubens* against three bacterial isolates namely *Staphylococcus aureus, Klebsiella, pneumonia and Escherichia coli*. The antibacterial activity was investigated using disc diffusion method while MIC was determined using broth dilution technique. Phytochemical analysis was also conducted to substantiate their antibacterial activities. All the bacteria were sensitive to hot aqueous extract of *C. rubens* except Klebsiella pneumonia was most sensitive. The result confirmed that *C. rubens* has antibacterial activity (Omotayo et al.2015).

2.2.7 Anti-hyperlipidemic activity

The methanol extract of *C. rubens* was assessed for antihyperlipidemic activity, the extract was also evaluated for antihyperlipidemic activity in high fat diet and triton WR-100 induced hyperlipidemic albino rats by evaluating serum total cholesterol, high density lipo protein cholesterol, low density lipoprotein cholesterol, very low density cholesterol and atherogenic index. The extract showed significant reduction in atherogenic index at 300mg/kg/day dose in both high fat induced and triton induced hyperlipidemic albino rats (Bahar et al.2016).

2.2.8 Local anaesthetic properties

Methanol extract was evaluated for local anaesthetic effect in guinea pigs and frogs using the intradermic wheal technique, plexus anaesthesia, 0.9 percent saline water as a control in both models, and 2 percent xylocaine as a reference medication. The results indicated that *C. rubens* had a strong local anaesthetic activity. (Ananya . 2017)

2.2. 9 Diuretic activity

As a traditional medicine, the diuretic action of *C. rubens* aqueous leaves extract in the treatment of high blood pressure, has considerable diuretic action, traditional healers use it to treat high blood pressure (Fidele et al.2019). The fleshy mucilaginous leaves and steps of *C. rubens* are consumed as vegetables, its lotion made from the leaves is used as a mild medication to strengthen and stimulate the stomach. The plant is reported to possess antiseptic chemicals (which prevent bacterial development) as well as anti-inflammatory properties. Nigerian and British researches proved that a high intake of this plant helps to prevent, rectify, or treat health problems including high blood pressure, hemorrhoids, and gallstones (Medd, Would, 2003). Composition of nutrients and phytochemical control of fireweed *C. rubens* was examined for proximate and mineral components before extraction with acetone, chloroform, ethyl acetate, methanol, and water. Phytochemicals were found in the powdered sample and its solvent extract. The sample included flavonoid, phenol, oxalate, tannin, saponin, phytate, and ascorbic acid, but no alkaloid. Water extract contains all phytochemicals, whereas methanol extract contains all phytochemicals but no flavonoid. Phytate was discovered in ethyl acetate extract, and ascorbic acid and phytate were discovered in chloroform extract. It was discovered that the plant powdered has all of the phytochemicals and is highly rich in nutritional proximate composition (moisture, fat, protein, ash, crude fiber, and carbohydrate (Arawande et al.2013). The nutritional content of certain less common edible leafy vegetables in Nigeria, as well as the proximate composition of vegetables.

Table 2.1: Biological classification of *Crassocephalum rubens* (Grubben et al. 2018)

Kingdom	Plantae
Subkingdom	Viridiplantae
Infra kingdom	Streptophyta
Superdivision	Embryophyta
Division	Tracheophyta
Sub division	Spermatophyta
Class	Magnoliopsida
Super order	Asteranae
Order	Asterales
Family	Asteraceae
Genus	Crassocephalum moench
Species	*Crassocephalum crepidiodes(Benth.) S.Moore* *Crassocephalum rubens* *Crassocephalum biafre* *Crassocephalum buchiense* *Crassocephalum aurantiacum*

Figure 2.1: A picture of *Crassocephalum rubens* (Fire weed) leaves (wikimedia.org/wiki/File)

2.3 MYOCARDIAL INFARCTION

Myocardial infraction (MI) ,is a medical condition that arises when a blood clot completely obstructs the coronary artery leading to death of tissue(Shier et al.2013) .Myocardial infraction is a lasting injury to the heart muscle. ‘'Myo" refers to muscle, ‘'cardial" refers to the heart, and “infraction” refers to tissue death caused by a lack of blood flow ((Shier et al.2013). In the year 2012, 31 percent of all global deaths occurred. 7.4 million of these deaths were caused by coronary heart failure, while 6.7 million were caused by heart attack (Sheu et .al.2010).

Non-communicable diseases kill 16 million people under the age of 60, with 85 percent occurring in low and middle-income countries and 40 percent attributed to CVDs (Sheu et al. 2010). There are many forms of cardio vascular disorders, and the most significant CVDs are Atherosclerosis, Myocardial infarction, Ischemia, and Cardiomyopathy, which are dependent on disease occurrence worldwide. (Sheu et al.2010).

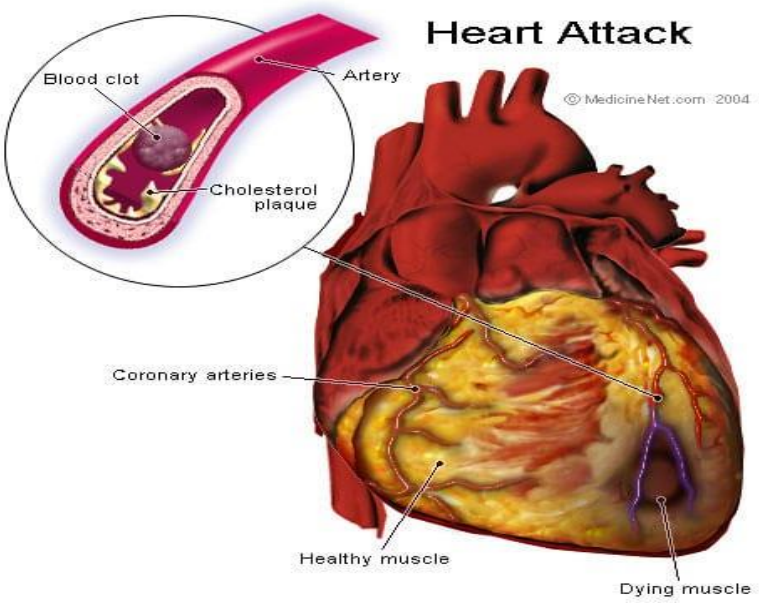

Figure 2.2 A pictorial presentation of myocardial infarction (medicineNet.com)

In (Figure 2.2) above, when a blood clot fully,it obstructs a coronary artery that supplies blood to the heart muscle, The clotted blood triggers the heart attack which develops at the rupture site of an atherosclerotic, cholesterol plaque on the inner wall of a coronary artery. Chest discomfort is the most prevalent sign of a heart attack, heart failure and ventricular fibrillation .Elevated cholesterol levels, high blood pressure, smoking, diabetes, male gender, and a family history of heart attacks at a young age are all risk factors for atherosclerosis and heart attacks. Electrocardiograms and measurements of cardiac enzymes in the blood are used to diagnose heart attacks. The early opening of blocked antiplatelet, anticoagulant, and clot-dissolving medicines, as well as angiotensin-converting enzyme (ACE) inhibitors, beta-blockers, and oxygen, could be used to treat heart attacks. Interventional therapy for heart attacks may involve coronary angiography with percutaneous transluminal coronary angioplasty (PTCA), coronary artery stents, and coronary artery bypass grafting (CABG). Patients who have experience a heart attack are hospitalized for many days to identify cardiac rhythm abnormalities, shortness of breath, and chest discomfort. Aspirin, beta-blockers, angiotensin-converting enzyme (ACE inhibitors), quitting smoking, losing weight, exercising, maintaining good blood pressure and diabetes control, eating a low cholesterol and low saturated fat diet high in omega-3 fatty acids, taking multivitamins with an increased amount of folic acid, lowering LDL cholesterol, and increasing HDL cholesterol are all ways to prevent subsequent heart attacks.

2.4 Risk factors of Myocardial infraction

The risk factors of MI include: Diabetes mellitus, smoking, hypertension, hyperlipidemia, sedentary life style, obesity, stress and depression (Bećkowski ., 2015).

2.5 Symptoms of Myocardial infraction

The symptom of heart attack include: angina, irregular heart beat; pain in the center of the chest; pain that spreads to the arm; nausea and indigestion (Bećkowski., 2015).

2.6 Causes of Myocardial infraction

Atherosclerosis: This is a gradual process in which cholesterol plaques are deposited in the artery walls. Cholesterol plaques promote hardening of the arterial walls and narrowing of the artery's inner channel (lumen). (Daniel,2020). Atherosclerosis of the arteries that provide blood to the brain can result in vascular dementia (mental degeneration caused by the slow death of brain tissue over a long period of time) or stroke (sudden damage and death of brain tissue) (Daniel, 2020).

Atherosclerosis can go undetected in many people for years or decades, providing no symptoms or health consequences. Atherosclerosis can begin as early as adolescence, although symptoms or health concerns do not usually appear until later in life, when the artery narrowing becomes severe. (Daniel, 2020). Cigarette smoking, high blood pressure, raised cholesterol, and diabetes mellitus can all accelerate atherosclerosis and contribute to the onset of symptoms and consequences earlier, especially in persons with a family history of early atherosclerosis. (Daniel, 2020).

Coronary atherosclerosis: It refers to the stiffening and constriction of the coronary arteries caused by atherosclerosis. (Daniel, 2020). Coronary heart illnesses are caused by a decrease in blood flow to the heart muscle as a result of coronary atherosclerosis (Daniel,2020). Coronary heart diseases include heart attacks, abrupt unexpected death, chest pain (angina), irregular heart rhythms, and heart failure caused by heart muscle weakness. (Daniel,2020).

Atherosclerosis and angina pectoris***:*** It is also known as angina is a type of chest pain or pressure that develops when the blood and oxygen flow to the heart muscle is insufficient to meet the muscle's needs. When the coronary arteries are constricted by more than 50 to 70%, the arteries may be unable to increase the supply of blood to the heart muscle during exercise or during periods of high oxygen demand (Daniel,2020). Angina is caused by a lack of oxygen to the heart muscle. Exertional angina is angina that arises during exercise or exertion. In some patients, particularly those with diabetes, the increasing decrease in blood flow to the heart may occur without any pain or discomfort, or it may be accompanied with shortness of breath or unusually early exhaustion(Daniel,2020). Exertional angina typically manifests as a pressure, heaviness, squeezing, or hurting sensation across the chest. This discomfort can spread to the neck, jaw, arms, back, or even the teeth, and it might be accompanied by shortness of breath, nausea, or a cold sweat(Daniel,2020). Exertional angina usually lasts one to fifteen minutes and is eased by rest or by inserting a nitroglycerin pill under the tongue. Both rest and nitroglycerin reduce the demand for oxygen in the heart muscle, so alleviating angina (Daniel,2020). Exertional angina could be the initial symptom of severe coronary artery disease. Chest aches that last only a few seconds are almost never caused by coronary artery disease(Daniel,2020). Angina can also occur while you are at rest. Angina at rest most usually suggests that a coronary artery has constricted to the point where the heart is not receiving adequate oxygen even when at rest. Angina at rest may be caused by a coronary artery spasm (a condition known as Prinzmetal's or variant angina). Unlike a heart attack,

neither exertional nor rest angina causes permanent muscle damage, yet the angina serves as a warning indication that a heart attack is imminent (Daniel,2020).

Saturated fats: It contributes to the building up of plaque in the coronary arteries. Saturated fats are found in meat, dairy products, butter and cheese. These fats lead to arterial blockage by increasing the amount of bad cholesterol in the blood system and reducing the amount of good cholesterol (Luo et al.2018).

2.7 Treatment of myocardial infarction

Myocardial infarction needs urgent medical medical care, which when the arteries supplies blood to the heart would unblocked using a technique known as angioplasty (Musci, 1998). The Coronary artery helps in bypassing graft which could be done in some cases where the patient's veins and arteries have been routed so that blood can circulate through the blockage of the coronary artery vessel. (Luo et al. 2018). These are various drugs, which can be used in the treatment of myocardial infarction, which include: Clopidogre, blood vessels may be widened with nitroglycerin, 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). Other treatment include : Other basic treatments include: exercising regularly, eating healthy, avoid using tobacco products, always controlling your blood pressure, ensure Low LDL cholesterol.

2.8 Oxidative stress

Oxygen, the gas that sustains life, forms reactive oxygen species as a result of oxidation, it can cause necrosis and cell death. Exogenous oxygen species (ROS) such as hydroxyl radical (OH), superoxide radical ($\bullet O_2$), and hydrogen peroxide (H_2O_2) can be produced by oxygen (O_2). (Somogyi et al.2007).

Oxidative stress is defined as any disturbance in the balance of antioxidants and pro-oxidants in favor of pro-oxidants which could be as a result of different factors such as aging, inflammation, drug action and toxicity (Asmat et al.2015). Oxidative stress results in the inefficiency of the functions of some body cells, damage in the structure of cells resulting into different diseased conditions, examples of such structural impairment and diseased conditions caused by oxidative stress are listed in (Table 2.2)

Organs	Diseases
Lungs	Asthma, chronic bronchitis
Kidneys	Glomerulonephritis, chronic renal failure
Joints	Arthritis, rheumatism
Brain	Alzheimer's disease, Parkinson's disease, memory loss, and stroke
Eyes	Cataract, retinal diseases
Fetus	Preeclampsia, IU growth restriction
Heart vessels	Arteriosclerosis, hypertension, ischemia, cardiomyopathy, heart failure
Multiorgans	Cancer, diabetes, inflammation infection, aging
Organs	Diseases
Lungs	Asthma, chronic bronchitis
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Eyes	Cataract, retinal diseases
Fetus	Preeclampsia, IU growth restriction
Heart vessels	Arteriosclerosis, hypertension, ischemia, cardiomyopathy, heart failure
Multiorgans	Cancer, diabetes, inflammation infection, aging

Table 2.2: Organs affected and diseases caused by oxidative stress (Asmat et al.2015).

2.9 Antioxidants

It is a substance capable of inhibiting substrate oxidation is called an antioxidant. Antioxidants can be classified into two categories according to their mechanism or activity (Somogyi et al.2007). These classes include disruptive antioxidants and preventive antioxidants. The main difference between the two is that the preventer reduces the rate of initiation while the breakdown of the antioxidants hinders the spread. Catalase, peroxidase are examples of preventive antioxidants among others that can react with ROOH and metal ion chelators such as ethylenediamine tetraacetate (EDTA), which work by blocking uncontrolled synthesis or production of free radicals and inhibition of their reaction with biomolecules,this is how superoxide dismutase (SOD) scavenges superoxide radicals in vivo. The site of action of these antioxidants depends on their nature with the aquatic environment; For example, SOD is active in the aqueous phase, hydrophilic intracellular antioxidants can be found in the cytosol, and are therefore called cellular antioxidants. They can also be found in mitochondria and other nuclear compartments, while hydrophobic antioxidants are found in membranes where they inhibit lipid peroxidation (Somogyi et al.2007). The lack of enough antioxidants to eliminate ROS often leads to damage that can lead to cumulative damage to cells, macromolecules, and even DNA. This can lead to any disease as shown in Table 2.2, but the presence of antioxidants, even in small amounts, can inhibit the reaction between ROS or free radicals and the organism also biological substances by a process known as redox homeostasis (Somogyi et al.2007).

Some antioxidants are obtained from external sources and are called exogenous antioxidants. They are obtained from the diet, but it is almost impossible to get enough of these exogenous antioxidants from the modern diet, which is why antioxidant supplement therapy is often recommended, for example, these are found naturally in endogenous antioxidant system (glutathione, superoxide dismutase, catalase, etc.) (Asmat et al.2015).

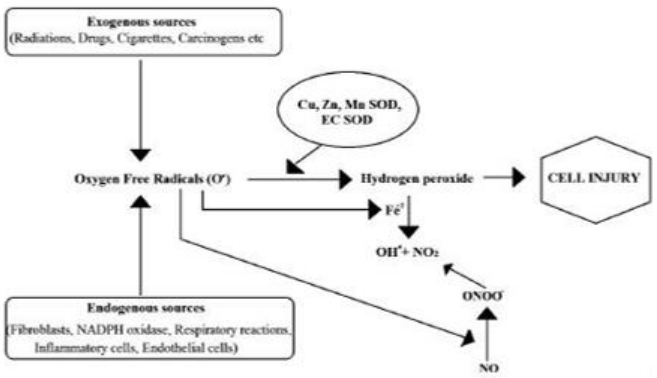

Figure: 2.3 Free radicals induced cell injury (Asmat et al.2015)

2.10 ASPIRIN

It is an analgesic and antipyretic drug used to treat fever and pain, such as in arthritis. It is also been linked to cardiovascular disease. It blocks prostaglandin production by inhibiting cyclooxygenase (prostaglandin H synthase), with a higher selectivity for the COX-1 isoform. The antithrombotic action is related to the suppression of COX-1 in platelets, which prevents thromboxane formation and platelet aggregation. Colorectal and other solid cancers are chemopreventive. Acetylsalicylic acid (aspirin) is a nonsteroidal anti-inflammatory medication. It is generated from salicylic acid and appears as white crystalline powder. Aspirin has been used to stabilize blood during 13,14-dihydro-15-keto-prostaglandin F2 (PGFM) measurement, assess the viability of the BV-2 microglial cell line, in the test for morphogenesis.

2.10.1 MECHANISM OF ACTION

The ability of aspirin to reduce platelet aggregation and thus the possibility of thrombotic vascular events is the most believable clarification for its cardioprotective action. Platelets, platelet products, and thrombosis do have an effect on acute occlusive vascular events such as myocardial infarction (MI) and ischemic stroke. Aspirin has such a strong effect, higher doses which do not appear to provide any additional advantage. Certainly, it has been claimed that much large doses may potentially reverse this trend due to the stimulation of vessel wall enzymes. (Manson et al. 1991).

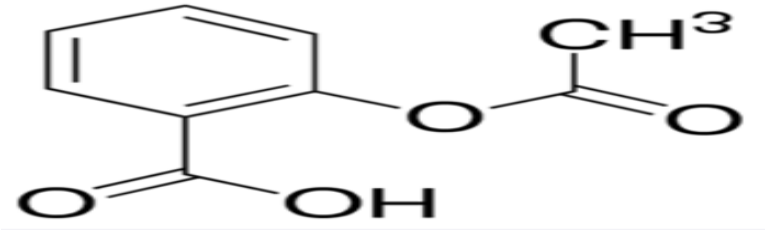

Figure 2.4: molecular structure of Aspirin (dreamstime.com)

CHAPTER THREE

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, Isoprenaline, Aspirin.

Reagent kits for: Lipid peroxidation, Catalase activity, and Reduced Glutathione

3.1.1 Collection and identification of plant materials

Crasssocephalum rubens was obtained locally from farms in Ekiti State in South-Western Nigeria. The plant was authenticated by Dr. Nodza George at the Botany Department, University of Lagos, Nigeria. A voucher specimen 8788 was deposited in the Herbarium of the Departmental herbarium.

3.1.2 Preparation of plant sample

The leaves of *C. rubens* were oven-dried at 40°C, ground into powder with an electric blender, and stored in the refrigerator at 4^{o}C. The ground sample (80g) was soaked for 72 hours at room temperature in 70% methanol at a ratio of 1:8 (w/v), with intermittent shaking. After that, a fine muslin cloth (first) and a filter paper were used to filter the suspension. The filtrate was concentrated under reduced pressure at 45^{o}C in a rotary evaporator, after which it was dried to completion in a hot air oven at 40 °C and stored in an airtight container and refrigerated at 4 °C until further use. A portion (1:5) of the hydromethanol extract was reconstituted with water and subjected to solvent partitioning using Hexane and Ethyl acetate sequentially (Otsuka, 2006).

3.2 Experimental animals

A total number of sixty (60) male Wistar albino rats were obtained and kept in the Animal facility of the Department of Biological Sciences, Mountain Top University. The rats were kept in cages at room temperature in the Animal house, with the provision of a regular food and water (ad libitum). The rats were allowed to adapt to their environment for a period of two weeks. All protocols were done in compliance with the Institutional Animal Ethics Committee's (IAEC) guidelines for the care

and use of laboratory animals, and ethical approval was obtained. A single intraperitoneal injection of isoprenaline (ISO; 100 mg/kg body weight) in phosphate buffer (pH 7.4) was used to induce myocardial infarction in the rats (Syrifah et al. 2018).

The rats were randomly allocated into 12 groups of 5 animals each as thus:

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

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

Group 3: Negative control (ISO without pretreatment).

Group 4: ISO + pretreated with the methanol extract of *C. rubens* (100 mg/kg).

Group 5: ISO + pretreated with the methanol extract of *C. rubens* (150 mg/kg).

Group 6: ISO + pretreated with the methanol extract of *C. rubens* (200 mg/kg).

Group 7: ISO + pretreated with the hexane fraction of *C. rubens* (100 mg/kg).

Group 8: ISO + pretreated with the hexane fraction of *C. rubens* (150 mg/kg).

Group 9: ISO + pretreated with the hexane fraction of *C. rubens* (200 mg/kg).

Group 10: ISO + pretreated with the ethyl acetate fraction of C. *rubens* (100 mg/kg).

Group 11: ISO + pretreated with the ethyl acetate fraction of *C. rubens* (150 mg/kg).

Group 12: ISO + pretreated with the ethyl acetate fraction of *C. rubens* (200 mg/kg).

The animals were kept under observation, and a plant extract was administered orally once daily for 14 days' prior for the induction of myocardial infarction. On day 14, a dosage of 100 mg/kg of Isoprenaline was used for induction. After an overnight fast, the rats were sacrificed and dissected under anaesthetic on day 15. For the preparation of the homogenate, the heart and liver tissues were dissected, rinsed in phosphate buffer. The collected organs were centrifuged at 1200 rpm for 10 minutes. The supernatant was separated from the pellet and stored in a refrigerated at 4^{o}C for assay.

3.3 DETERMINATION OF LIPID PEROXIDATION (LPO)

The level of oxidative damage on the liver and heart was estimated by quantifying the amount of thiobarbituric acid reactive substances (TBARS) present in the sample following the method described by (Varshney and Kale 1990).

Procedure: In a test tube containing 0.4 mL of the homogenate, 1.6 mL of Tris-KCl buffer ,0.5 mL of 30% TCA, 0.5 mL of 0.75% TBA were added and then placed in a water bath for 45 minutes at 800C. The test tube with the content was removed and allowed to cool and centrifuged at 3000 g for 10 minutes. The absorbance of the supernatant was read using a UV spectrophotometer at 532 nm. The level of MDA was determined in units'/mg protein of tissue was computed with a molar extinction coefficient of 1.56 x 105 M-1cm-1.

3.4 DETERMINATION OF CATALASE ACTIVITY

Catalase activity was measured using the method described by (Sinha 1972).

Procedure: In a test tube containing 0.1 mL of homogenate, 1.0 mL of 0.01M phosphate buffer (pH 7.0) and 0.4 mL of 0.02 M H_2O_2 were added to the reaction mixture (1.5 mL). Catalase reagent (2.0mL) was added to halt the reaction. The reaction mixture was read using UV spectrophotometer at 620 nm at one-minute interval against a reagent blank. The catalase activity was calculated using the formula: CAT = A/min TV/SV.

Where:

A = absorbance change

TV = total volume

SV = sample volume

Molar extinction ($\varepsilon = 40M^{-1}cm^{-1}$)

3.5 DETERMINATION OF REDUCED GLUTATHIONE

The reduced glutathione (GSH) levels in the heart and liver homogenates of experimental rats were determined according to the method by Sedllak and Lindsay 1968).

Procedure: To the tissue homogenate (1 mL), 10% TCA was added and centrifuged at 3000 rpm for 10 minutes. Then, 5 mL of the supernatant was measured into a test tube, 0.5mL Ellman's reagents

and 3.0 mL phosphate buffer were added. The sample mixture was read at wavelength of 412 nm. The absorbance was measured against reagent blank. GSH concentration was then calculated using the formula:

Concentration of GSH = $\Delta A \times TV/\varepsilon \times SV$

$\varepsilon = 1.34 \times 10^4\ M^{-1}cm^{-1}$

where;

ΔA = change in absorbance

TV = total volume

SV = sample volume

ε = molar extinction

3.6 Waste disposal

The rat carcasses were buried at the designated burial site, while sample bottles containing unused blood and other biological samples were incinerated

3.7 Statistical analysis

Data obtained from the study was statistically analyzed by one-way Analysis of Variance (ANOVA) followed by Tukey's Multiple comparisons (post-hoc) using GraphPad prism 8.0. Results were expressed as a mean ± standard error of mean (SEM). P values less than 0.05 ($p < 0.05$) were considered statistically significant.

CHAPTER FOUR

RESULTS

4.1 Effect of methanol extracts and fractions of C. *rubens* leaf on the rat body, liver and heart weights.

The results of the weight of both heart and liver (Table 4.1) indicate that in the negative control group was higher compared to the normal control group. There was a significant increase rats given 200 mg/kg of *C. rubens* ethylacetate fraction when compared to the normal control group.

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	176.00±13.66	142.00±7.83	23.94±0.74	0.72±0.08	0.51±0.01	1.43±0.17	0.01±0.02
7	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
8	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
9	162.00± 8.94	157.00±4.01	3.18±1.23	0.73±0.04	0.46±0.99	1.50±0.17	0.96±0.04
10	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
11	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
12	67.00± 3.72	158.00±3.71	57.60±0.27	0.65±0.03	0.41±0.81	1.77±0.20	0.01±0.05

Table 4.1: Effect of methanol extracts and fractions of C. rubens leaf on the rat body, liver and heart weights

Each value represented the mean ±SEM of 5 readings.

Change in body weight (BWT) (%) = (final bwt/finial bwt) X100

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

Groups:

1. Normal control
2. Positive control
3. Negative control
4. Methanol extract (100 mg/kg)
5. Methanol extract (150 mg/kg)
6. Methanol extract (200 mg/kg)
7. Hexane fraction (100 mg/kg)
8. Hexane fraction (150 mg/kg)
9. Hexane fraction (200 mg/kg)
10. Ethyl acetate fraction (100 mg/kg)
11. Ethyl acetate fraction (150 mg/kg)
12. Ethyl acetate fraction (200 mg/kg)

4.2 GC-MS identified Phytochemicals in the Ethyl Acetate fraction of *C. rubens*

Serial Number	R/T (min)	Area (%)	Name of Compound	M/F	Reported biological Activity
1	2.325	0.50	2-Ethoxyethyl isobutyl carbonate Tetraethyleneglycol monomethylethe 2,5,8,11,14,17-Hexaoxanonadecan-19–ol	$C_9H_{18}O_4$	Acetylcholinergic
2	2.519	1.26	Boric acid, trimethyl ester Boric acid, trimethyl ester Boronic acid, ethyl-, dimethyl ester	$C_3H_9BO_3$	Arachidonic acid inhibitor,
3	2.925	0.42	Methanamine, N-hydroxy-N-methyl- Methanamine, N-hydroxy-N-methyl- Cyanogen chloride	C_2H_7NO	Neurostimulant
4	3.519	0.18	3-Amino-2,2-dimethyl-1-propanol 1,4-Dioxaspiro[2.4]heptan-5-one, 7 ,7-dimethyl-3,3-Diethyldiaziridine	$C_5H_{13}NO$	Increase aromatic amino acid carboxylase activity

5	4.239	0.08	O-Butylisourea 2-Butanone, 4-hydroxy-3-methyl- Methanamine, N-hydroxy-N-methyl-	$C_8H_{18}ON_2O$	stimulate osteoblast activity
6	7.097	0.03	Tetradecane, 2,6,10-trimethyl- Decane, 3-methyl- Eicosane	$C_{15}H_{32}$	Methyl donor
7	7.961	0.05	Sulfurous acid, butyl tridecyl ester Heptadecane Nonane, 4,5-dimethyl	$C_{17}H_{36}O_3S$	Urinary acidulant
8	8.592	0.02	Hexadecane Disulfide, di-tert-dodecyl Heptadecane, 9-hexyl-	$C_{16}H_{35}S_2$	Coronary dilator
9	9.118	0.23	1,6-Octadien-3-ol, 3,7-dimethyl-	$C_{11}H_{18}O_2$	Oligosaccharide provider
10	10.419	-0.17	9-Octadecenoic acid (Z)-, methyl ester Hexadecane, 1-(ethenyloxy)- Dodecane, 2,5-dimethyl-	$C_{19}H_{36}O_2$	aromatic amino acid activator
12	10.963	0.26	3,5-Dimethoxytoluene	$C_9H_{12}O_2$	Antioxidant

13	11.282	0.14	(+)-4-Carene1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-Cyclohexene, 4-ethenyl-4-methyl-3-1-methylethenyl)-1-(1-methylethyl)-, (3R-trans)-	$C_{15}H_{24}$	Methyl donor
14	11.370	0.07	Adipic acid, isobutyl 2,3,5,6-tetrachlorophenyl ester Adipic acid, isobutyl 2,3,4,6-tetrachlorophenyl ester 3-Aminooxy-4-chlorobutyric acid, ethyl ester	$C_{16}H_{19}Cl_4O_4$	Acidifier
15	11.451	0.03	2H-Pyran,5,6-dihydro-2-methyl-1,3-Butadiene,2,3-dimethyl-Ethane,1,2-dichloro-1,1,2-trifluoro-	$C_6H_{10}O$	HMG-CoA inhibitor
16	11.658	0.45	.alpha.-Cubebene alfa.-Copaene Copaene	$C_{15}H_{24}$	Alpha-amylase inhibitor

17	11.789	1.62	Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-,[1S-(1.alpha.,2.beta.,4.beta.)] Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-1,5,9-Cyclododecatriene, 1,5,9-tri methyl	$C_{15}H_{24}$	Triglycerigenic
18	12.120	9.44	(E)-.beta.-Famesene cis-.beta.-Farnesene-1,6,10-Dodecatriene,7,11-dimethyl -3-methylene-	$C_{15}H_{24}$	beta-glucronidase inhibitor
19	12.358	0.56	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-,Z,Z,Z- Humulene	$C_{15}H_{24}$	Increases zinc bioavailability
20	12.590	8.74	.beta.-Bisabolene .beta.-Bisabolene .alpha.-Farnesene	$C_{15}H_{24}$	beta-galactosidase inhibitor
21	12.746	2.59	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-,[S-(R*,S*)]-Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-cis-.beta.-Farnesene	$C_{15}H_{24}$	Antimicrobial
22	12.971	4.62	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-1,2-	$C_{12}H_{16}O_3$	anaphylactic

			Dimethoxy-4-(2-methoxy-1-propenyl)benzene Benzenamide, N-cyano-3,4,5-trimethoxy-		
23	13.540	13.41	Apiol 1,3-Benzodioxole, 4,5-dimethoxy-6-(2-propenyl)-	$C_{12}H_{14}O_4$	Anti-proliferative
24	13.959	6.29	1-Butanamine, N-(2-furanylmethylene)-3-methyl-1,2,5-Oxadiazole-3-carboxamide, 4-amino-N-cyclopentyl-Acetic acid, [(Z,Z)-3,7,11-trimeth yl-2,6,10-dodecatrien-1-yl] ester	$C_8H_{19}N$	Increases norepinephrine production
25	14.310	6.77	Phthalic acid, butyl 3-phenylpropyl ester Diethylmalonic acid, di(3-phenylpropyl) ester 2-Oxazolidinone, 4-(hydroxymethyl) 5-phenyl	$C_{17}H_{24}0_4$	Increases superoxide dismutase activity
26	14.447	1.88	1,2-Benzenedicarboxylic acid, bis(4-methylpentyl) ester Bis(tridecyl)phthalate	$C_{16}H_{22}0_4$	Increases aromatic acid decarboxylase activity

			Phthalic acid, 3,3-dimethylbut-2-yl tridecyl ester		
27	14.554	2.72	1,2-Benzenediacetonitril-N'-[(2-Hydroxy-1-naphthyl)methylene]-2-(4-octyloxyphenoxy)acethy drazide 1,3-Benzenediamine, 4-methyl-5-nitro-	$C_{20}H_{30}O_4$	urinary-acidulant
28	14.685	4.19	Ethyl-4-[tetrahydropyran-2-yloxy]-2-butynoate 2-Butenediamide, 2-methyl-, (Z)-22 Isoxazole, 3,5-dimethyl-4-bromo-	$C_{11}H_{16}0_4$	catechol-o-methyl transferase inhibitor
29	14.904	0.94	Propanenitrile, 3-chloro-2,3-dihydroximino-4-Methyl-2,3-hexadien-1-ol 3-Octyn-1-ol	C_3H_4CIN	methyl-guanidine donor
30	14.967	1.25	Furan-2-carboxylic acid,4-diethyl aminomethyl-5-ethyl-Melochinin 1,3,6-Triazahomoadamantane	$C_5H_4O_3$	Anti-inflammatory
			Hexadecanoic acid,	$C_{17}H_{34}O_2$	Hypercholesterole

31	15.104	6.34	methyl ester		mia
32	15.661	12.09	Ethamivan Butyldimethylsilyloxybenzene	$C_{12}H_{20}OSi$	Tocopherols
33	16.487	7.97	9,12-Octadecadienoic acid, methyl ester 7-Octadecenoic acid, methyl ester 9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	Increases zinc bioavailability
34	17.294	1.57	Solanidan-3-one 2-Benzothiazolamine Isosolanidine	$C_{27}H_{43}NO$ $C_7H_6N_2S$	Antibacterial, activity
35	17.525	2.32	Lumazine 5,7-Dihydroxy-4-methylcoumarin 1-(1-(2-Thienyl)cyclohexyl) pyrrolidine	$C_{10}H_8O_4$	Antioxidant activity
36	18.151	0.41	5-Decyne 5-Tridecene 5-Dodecyne	$C_{10}H_{18}$ $C_{13}H_{26}$ $C_{12}H_{22}$	Antibacterial activity(Mohammed; Asif, 2013)
37	18.301	0.52	Pyridine, 4-methyl-, 1-oxide Tungsten, [(2,3-.eta.) bicyclo[2.2.1]hepta-2,5-diene] pentacarbonyl- Pyrazole-3-carboxylic	C_6H_7NO $C_{11}H_8O_4$ $C_4H_3N_3O_4$	Nitric acid synthase inhibitor

			acid, 5-nitro-		
38	18.645	0.14	Thiophene, 2-ethenyl- Mitozolomide 1Cyclopentenylphenylmethane	C_4H_4S $C_5H_8N_4O_3S_2$ $C_{12}H_{14}$	Antimicrobial activity (Maddila; Jonnalagada, 2012)

Table 4.2: GC-MS identified Phytochemicals in the Ethyl Acetate fraction of *C. rubens*

Main activity sources: Duke (2013, 2016).

Ethyl acetate GC-MS analysis of *C. rubens* fraction reveals the existence of compounds together with their relative abundance (area), molecular formulas, and weights. R/T stands for retention time and M/F stands for molecular formula.

The NIST library was used to get the systematic names and molecular formulas of the detected components, as indicated in table 4.2. On the basis of Duke's Phytochemical and Ethnobotanical Databases, the biological actions of substances were substantially predicted (Duke, 2013, 2016).

4.3 Effect of methanol extract and fractions of *C. rubens* on Heart lipid peroxidation in myocardial rats

The result showed significant ($p < 0.05$) increase in heart lipid peroxidation of negative control rats against aspirin treated and normal control only induced rats. There was significant decrease in the heart lipid peroxidation in the groups administered 200 mg/kg of the methanol extract and ethyl fractions 150 mg/kg when compared to negative control (Figure 4.3) . However, there were significant increase recorded in myocardial rats treated with 150 mg/kg, 200 mg /kg hexane fractions and 100 mg/kg ethyl acetate fraction.

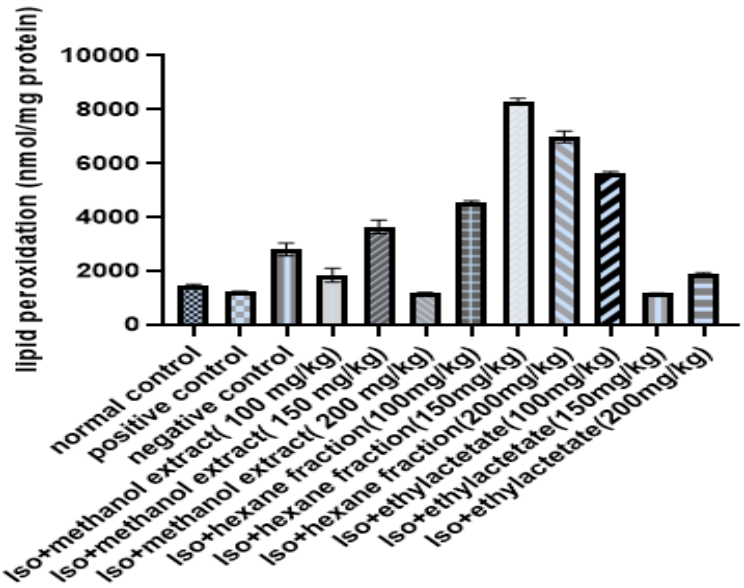

Figure 4.3: Heart Lipid peroxidation of control and myocardial rats pretreated with methanol extract and fraction of *C. rubens*

Values are mean ± SEM; n=4

ISO: Isoprenaline

4.4 Effect of methanol extract and fractions of *C. rubens* on liver Lipid peroxidation in myocardial rats

The result showed no significant ($p > 0.05$) difference in liver lipid peroxidation of normal control and negative control groups. There was significant decrease on liver lipid peroxidation in the groups that were administered 100 mg/kg, 150 and 200 mg/kg of methanol extract when compared to normal control (Figure 4.4) with exception of all tested concentrations of hexane fraction and ethyl acetate100 mg/kg and 200 mg/kg, in which significant increase were recorded.

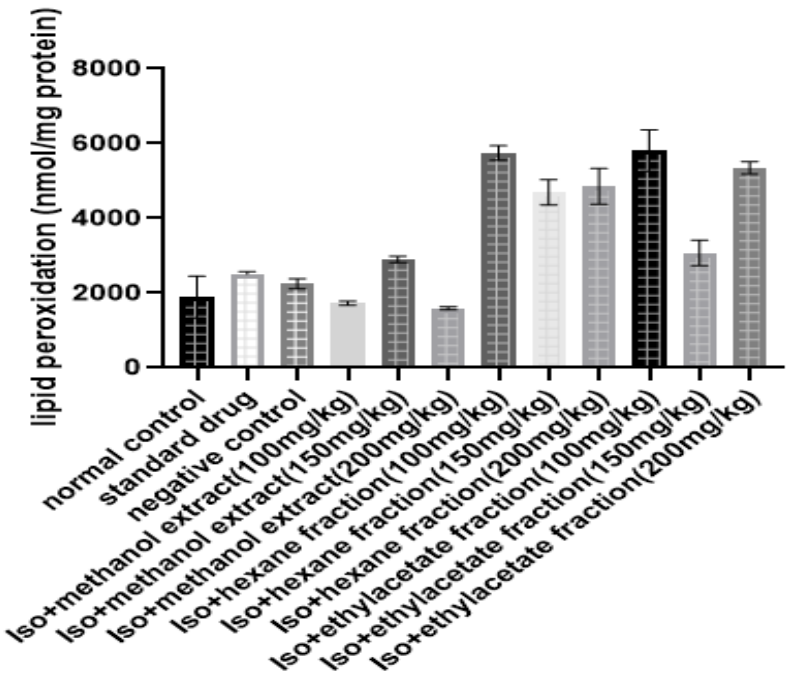

Figure 4.4: Liver Lipid peroxidation of methanol extract and fractions of *C. rubens* in control and myocardial infarcted rats

Values are mean ± SEM; n=4

ISO: Isoprenaline

4.5 Effect of methanol extract and fractions of *C. rubens* on heart catalase activity in myocardial rats

The result showed significant ($p < 0.05$) increase on heart catalase activity of the negative control (isoprenaline only) group against normal control and the treatment groups. There were no significant differences among the treatment groups the normal control group (Figure 4.5).

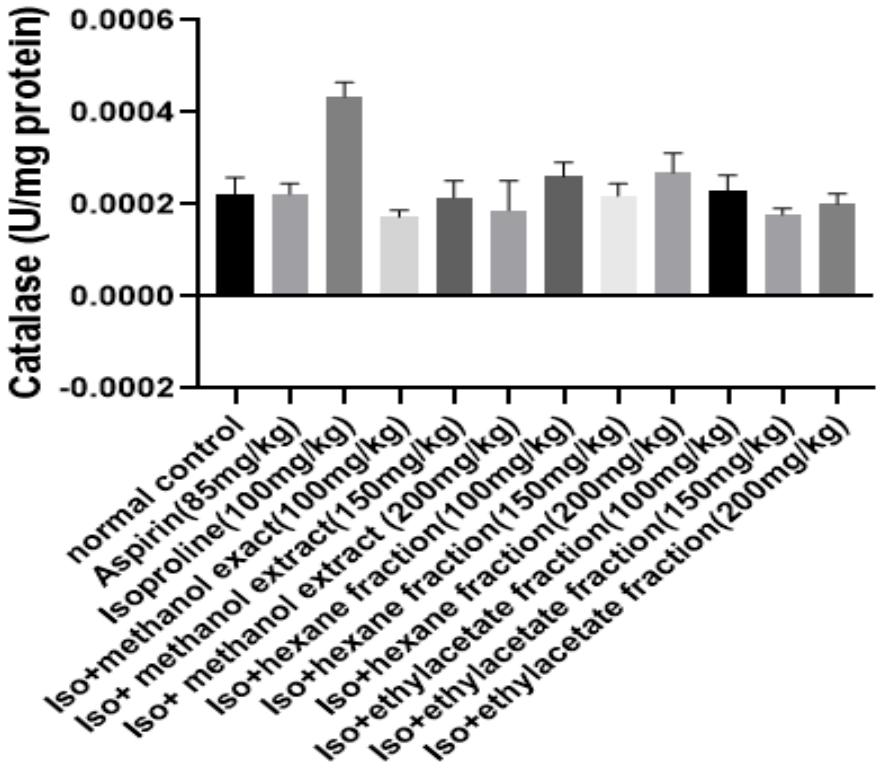

Figure 4.5: Heart catalase activity of control and myocardial rats treated with methanol extract and fraction of *C. rubens*

Values are mean ± SEM; n=4

ISO: Isoprenaline

4.6 Effect of methanol extract and fractions of *C. rubens* on liver catalase activity in myocardial rats

The result showed slight decrease in liver catalase activity of negative control rats against normal control. There was significant ($p < 0.05$) decrease in the liver catalase activity in rats administered with 100 mg/kg, hexane fractions compare with the normal control, while groups treated with 100 and 150 mg/kg methanol extract; and 200mg/kg ethyl acetate fraction as well as Aspirin recorded significantly higher catalase activity compared with the normal and negative control (Figure 4.6).

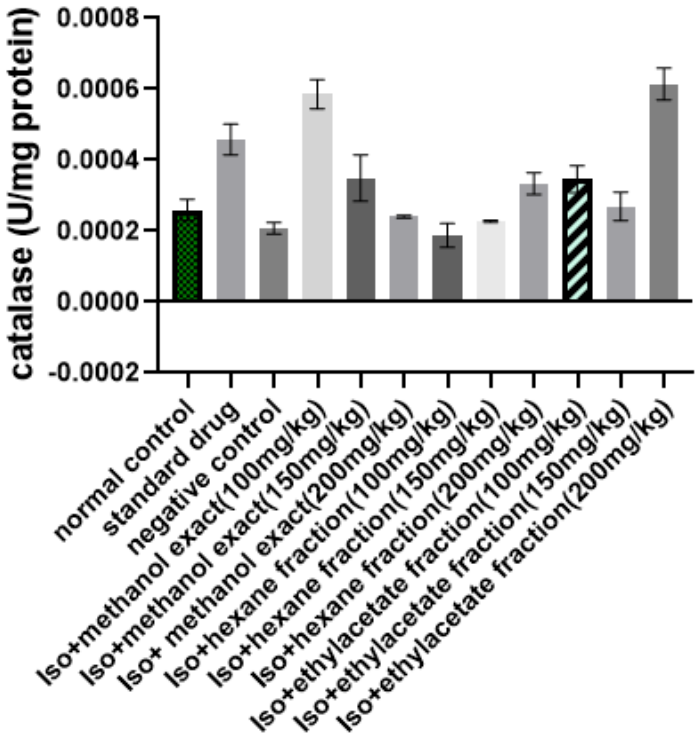

Figure 4.6: Liver catalase activity of control and myocardial rats treated with methanol extract and fractions of *C. rubens*

Values are mean ± SEM; n=4

ISO: Isoprenaline

4.7 Effect of methanol extract and fractions of *C. rubens* on heart reduced glutathione concentration in myocardial rats

The result showed significant ($p < 0.05$) increase in the heart reduced glutathione concentrations in negative control group and aspirin treated group against the normal control. Similar increase were recorded in the groups treated with 100 and 150 mg/kg crude extract; 150 mg/kg hexane; and 150 and 200 mg/kg ethyl acetate. However, significant decrease in heart reduced glutathione concentration were recorded in the groups administered 100 mg/kg, 150 mg/kg, 200 mg/kg of methanol extract; 100 and 200 mg/kg hexane; 100 and 150 mg/kg ethyl acetate fractions compared with the negative control (Figure 4.7).

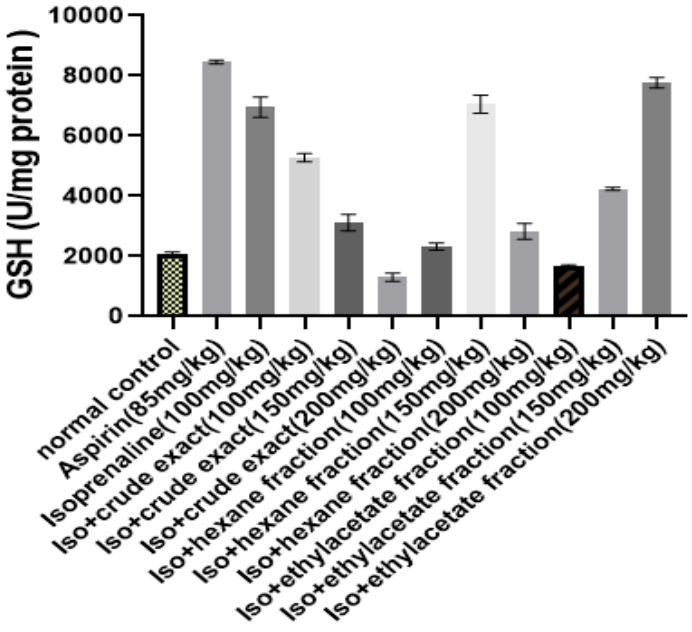

Figure 4.7: Heart reduced glutathione concentration of control and myocardial rats treated with methanol extract and fractions of *C. rubens*

Values are mean ± SEM; n=4

ISO: Isoprenaline

4.8 Effect of methanol extract and fractions of *C .rubens* on liver Reduced Glutathione (GSH) concentration in myocardial infarcted rats

The result showed significant ($p < 0.05$) increase in liver GSH concentration of normal control rats against aspirin treated and isoproterenol only induced groups. There was significant decrease in liver GSH concentration in the groups administered 150 mg/kg, 200 mg/kg of methanol extract and fractions groups when compared to normal control . However, significant increase in liver reduced glutathione concentration were recorded in the group administered 100 mg/kg of methanol extract; compared with the normal control (Figure 4.8).

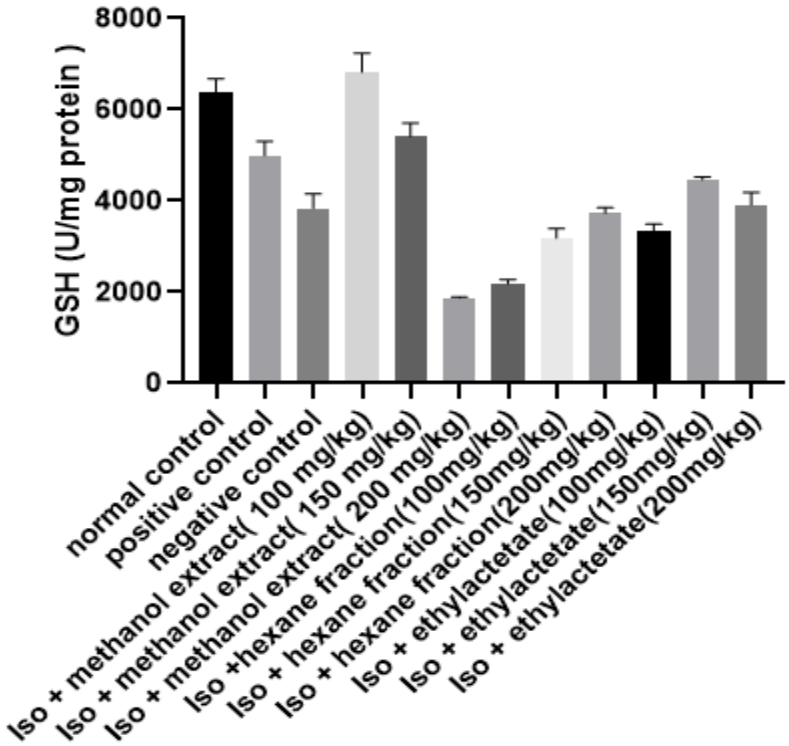

Figure 4.8 Liver reduced glutathione (GSH) concentration methanol extract and fractions in control myocardial infarcted rats of *C. rubens*

Values are mean ± SEM; n=4

ISO: Isoprenaline

4.9 DISCUSSION

Medicinal plants have been reported to have significant levels of active component that elicit the effect of being beneficial to health. The consumption of medicinal plants has been associated with low incidence and low mortality rates caused by heart attack. Plants serve as a major source of dietary antioxidants which also result in the reduction of atherosclerosis related to humans. Dietary components in plants acts as antioxidants which include polyphenols, flavonoids, dietary glutathione, carotenoids.

Myocardial Infarction is a prevalent metabolic disorder that leads to the heart failure function, affecting males and females. It is a medical condition that arises when a blood clot completely obstructs the coronary artery leading to death of tissue, which causes a lasting injury to the heart muscle. (Shier et al. 2013).

Lipid peroxidation is an organic compound with the molecular formula $CH_2(CHO)_2$. It is a colorless liquid, lipid peroxidation is highly reactive compound that occurs as an enol (Nair & O'Neil, 2008) .It occurs naturally and is a marker for oxidative stress. During excessive oxidative, lipid peroxidation increases and total antioxidant capacity (TAC) decreases in body (Farahnaz, 2013). During this study, oral administration of *Crassocephalum rubens* extract increased Catalase and GSH levels, leading to the elimination of ROS which has the potential to restore the oxidative level back to normal. Reduced glutathione is also involved in the removal of H_2O_2.An increase in catalase and GSH in the body than that of lipid peroxidation will bring about low or no oxidative stress occurring.

The crude extract and ethyl acetate fraction of the plant help in sufficient increase in Catalase and GSH activity due to the presences of bioactive constituents such as flavonoid, terpenoids, saponin, tannins, steroid and cardiac glycoside which are antioxidant agents, this agrees with the report of (Makajuola et al. 2016). The presence of these secondary metabolites has contributed to its medicinal value as well as physiological activity. Flavonoids generally have been found to have anti-allergic, anti-inflammatory, anti-microbial, anticancer and anti-diarrheal activities. Tannins have antioxidant, antimicrobial, anti-inflammatory, diuretic properties.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

C. rubens methanol leaf extract and fractions reduced lipid peroxidation in the organs of myocardial infarcted rats. The ethyl acetate fraction increased the activities of some of the endogenous antioxidants (Catalase and reduced glutathione) assayed. The plant is thus a good source of antioxidants that scavenges reactive oxygen species.

5.2 RECOMMENDATION

Further in vivo study to resolve some of the conflicting results are required. Isolation of some bioactive compounds that may be responsible for the antioxidant activity of the plant is advocated.

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APPENDIX

Appendix 1: 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)/	183 U	166	1743 mg in 70 mL PBS (24.9 mg/mL) for 14	1.1
	177 H			1.1
	155 T			0.9

150 mg /kg	166 B		days	1.0
	148 R			0.9
Group 6 (crude extract)/ 200 mg /kg	179 U	176	2464 mg in 70 mL PBS (35.2 mg/mL) for 14 days	1.0
	216 H			1.2
	191 T			1.1
	153 B			0.9
	139 R			0.8
Group 7 (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 8 (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
Group 9 (Hexane extract)/ 200 mg /kg	180 U	162	2268 mg in 70 mL PBS (32.4 mg/mL) for 14 days	1.1
	134 H			0.8
	153 T			0.9
	159 B			1.0
	182 R			1.1
Group 10 (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	107 U		1071 mg in 70 mL	1.1

11 (Ethyl acetate extract)/ 150 mg /kg	87 H	102	PBS (15.3 mg/mL) for 14 days	0.9
	106 T			1.0
	105 B			1.0
	106 R			1.0
Group 12 (Ethyl acetate extract)/ 200 mg /kg	80 U	67	938 mg in 70 mL PBS (13.4 mg/mL) for 14 days	1.2
	69 H			1.0
	63 T			0.9
	59 B			0.8
	62 R			0.9

Appendix 2: 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 3: 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 4: 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 drying (g)	Weight after beaker after drying (g)	Difference in weight (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 5: Showing the calculation of percentage yield

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