ASSESSMENT OF NUTRITIVE VALUE OF *COMMIPHORA SWYNNERTONII* AND ITS EFFECTS ON CHOLESTEROL LEVELS IN *RATTUS RATTUS*

BY

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ABSTRACT

Experimental studies to determine the chemical composition of the *Commiphora swynnertonii* as well as its effect in plasma cholesterol levels and weight changes in *Rattus rattus.* A total of 24 rats were used in the dose and time dependent experiments of resin, *C. swynnertonii* at (0 mg/kg, 50 mg/kg, 100 mg/kg and 200 mg/kg) on daily basis for 21 days. Six samples of root and stem bark were used in the proximate, selected minerals and heavy metals analysis by using AOAC (1995). Weekly weight measurement and plasma cholesterol levels were evaluated for each *R. rattus.* The results showed that the roots and stem bark of *C. swynnertonii* had significant difference in chemical composition ($p < 0.05$). Among the minerals analyzed Magnesium was the most abundant (996.30-1810.01 mg/100g). This plant was found to contain high fibre and carbohydrates. Results shows higher concentration of lead in stem bark (0.25 ppm) than in the root (<0.01 ppm) and the concentration of cadmium in the root and stem bark 0.01 ppm and 0.001 ppm respectively and for mercury the concentration was < 0.01 ppm for both roots and stem bark. There were significant differerence ($p < 0.05$) on cholestrol level and weight between the treated and the control groups. *Commiphora swynnertonii* resin lowered cholesterol level by 54%, 76% and 79% and weight changes by 18%, 31% and 23% for the exposed rats at concentrations of 50 mg/kg, 100 mg/kg and 200 mg/kg respectively and at the higher doses showed side effect including diarrhoea and death. Based on the results, *C. swynnertonii* has shown potential important medicinal plant as it contain some anti-cholesterol properties reduces weight and induces diarrhoea even at low doses.

DECLARATION

I, Sikitu Simon**,** do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

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The above declaration is confirmed

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May the Almighty God bless all abundantly.

DEDICATION

I would like to dedicate this work to the Almighty God for grating me life, the ability to study from the beginning up to this level and to my beloved parents Mzee Simon Clemnet Kihinga and Gaudensia Petro Sengerema who laid the foundation of my education.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA:	Analysis of Variance
AT:	Atherosclerosis
CAD:	Coronary Artery Diseases
DM:	Dry Matters
HDL	High-density lipoprotein cholesterol
LDL:	Low-density lipoprotein
SEAMIC:	Southern Eastern African Mineral Centre
VLDL:	Very Low-Density lipoprotein
WHO:	World Health Organization
xg	g-force

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Medicinal plants contain substances that could be used directly for therapeutic purposes or which can serve as precursors for synthesis of useful drugs. Most people, especially in rural areas, use medicinal plants for different purpose including nutrient supplements (Abolaji *et al.*, 2007). Despite some claimed side effects, utilization of medicinal herbs to treat human ailments is positively accepted globally. The interest toward elucidation of chemical composition of medicinal herb products is also growing as their commercialization increases (Rosli *et al.*, 2008). Herbal drugs have been reported to constitute a major part in all of the traditional systems of medicine. Plants above all other agents, have been used for medicine from time immemorial because they are easily accessible and inexpensive (Sanobar *et al.*, 2010). Medicinal plants have been mentioned in Ayurveda, which literally means 'the science of life' i.e. *Ayur* (life) and *Veda (*knowledge).

Medicinal plants play a significant role in providing medicines for primary health care to rural people and are used by about 80% of the marginal communities worldwide **(**Kochhar *et al.*, 2006). Each medicinal plant species has its own nutrient composition which is essential for the physiological functions of human body (Adnan *et al.*, 2010). Medicinal plants and herbs are of great importance to the health of individuals and communities as they have shown positive role in the prevention or control of some metabolic disorders, such as diabetes, obesity and certain types of cancers **(**Kochhar *et al*., 2006). Cholesterol is a tetra cyclic steroid

with a 3α-hydroxy group, a 5, 6-double bond and an eight-carbon side chain. Cholesterol is a product of *de novo* synthesis in animals, including humans, and is an important component of cell membranes and precursor of steroidal hormones and bile acids. Unlike animals, plants cannot produce significant amounts of cholesterol (Hussain *et al.*, 2009).

Commiphora swynnertonii (Plate 1) that belongs to the family Burseraceae, a group of plant called Myrrh, found in dry areas in East African region is one of medicinal plants used in different parts of Tanzania. It is a small tree, usually not more than 5 metres high. It can be recognized from a distance by spherical top and a short trunk with low branches (Hanus *et al*., 2005). *C. swynnertonii* is a small tree with many of the branchlets ending in spines. The bark is grey-green, sometimes shiny, peeling in membranous scales; slash red, pleasantly scented, exuding a clear gum. The generic name '*Commiphora*' is based on the Greek words 'kommi' (gum) and 'phero' (to bear) (Goji *et al.*, 2009). This tree is believed to treat a number of ailments such as typhoid fever, wounds and elevated cholesterol levels. In India it is also used as food supplement **(**Kochhar *et al.*, 2006). The antilipidaemic activity of other related plants of *Commiphora* spp. has been established. They exert effective lipid-lowering activity by reducing total cholesterol levels (Adebayo *et al.*, 2006). It has been found to contain guggulsterone which lowers cholesterol by acting as an antagonist of the FXR bile acid receptor which is important in cholesterol metabolism (Urizar *et al.*, 2002).

Plate 1: Picture of *C. swynnertonii* plant taken in Simanjiro district in Tanzania

Commiphora spp. lowers serum triglycerides and cholesterol as well as low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) (the “bad” cholesterols). At the same time, it raises levels of High-density lipoprotein (HDL) (the “good” cholesterol). As an antioxidants, it keeps low-density lipoprotein (LDL) cholesterol from oxidizing, an action which protects against atherosclerosis (Anderson *et al*., 2000). It has also been shown to reduce the stickiness of platelets. This effect lowers the risk of coronary artery disease. Also, it acts as a fat burner (thermogenic) and controls obesity. Guggul is a gum like substance in plant extract which helps to produce white blood cells and acts as a disinfectant (Pati, 2010).

1.2 Problem Statement and Justification

Tanzania is endowed with indigenous trees and shrub species with important medicinal values to human and animals. Apparently, the information about these plants in terms of nutritive value is still lacking, although their uses increase worldwide. When conventional medicine fails to treat chronic diseases and conditions such as obesity and without adverse events, many people seek non-conventional therapies including herbal medicine (Kochhar *et al.*, 2006). Among herbal plants, *Commiphora Spp.* are used to prevent heart attacks and have an ability to lower both cholesterol and triglyceride level (Goji *et al.*, 2009). However, no attempts have been made to study the nutritive value and the effectiveness of *C. swynnertonii* in lowering the cholesterol level in both human and animals in East Africa.

Obesity is a major public health and economic problem of global significance. Its prevalence is increasing in all parts of the world, both in affluent Western countries and in poor nations. Men, women and children are affected (Antipatis and Gill, 2001). Indeed, overweight, obesity and health problems associated with them are now so common that they are replacing the more traditional public health concerns such as under-nutrition and infectious diseases such as diarrhoea and cholera (WHO, 1998).

In Tanzania, obesity problem is increasing; resulting in an association with major health problems such as type 2 diabetes, ischemic heart disease, stroke, and cancer. It is necessary to treat obese individuals by both lifestyle interventions and/or pharmacological therapy. However, few attempts have been made to understand the

effectiveness of some medicinal plants in that regard (Anderson et al., 2000). Studies on the effects of *C. swynnertonii* on lipid reduction is lacking and the search for suitable medicinal plants among the *Commiphora* species against the incidence of cardiovascular diseases becomes very imperative (Adebayo *et al*., 2006). Thus there is a need to assess the nutritive value and determine the effectiveness of resin extracts of *C. swynnertonii* on cholesterol levels and weigh using animal model. The study will contribute towards ethno medical uses of *C. swynnertonii* in Tanzania.

1.3 Objectives of the Study

1.3.1 General objective

To evaluate the nutritive and therapeutic value of *C. swynnertonii* in rats in view to improve the ethno medical uses of this plants in Tanzania.

1.3.2 Specific objectives

i. To determine the nutritive value of *C. swynnertonii* by proximate composition analysis.
ii. To establish the effect of *C. swynnertonii* on cholesterol levels and weight in rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin of Medicinal Plants

Since ancient times, plants have been recognized as indispensable sources of both preventive and curative traditional medicine preparations for human beings and livestock. Historical accounts of traditionally used medicinal plants depict that different medicinal plants were in use as early as 5000 to 4000 BC in China and 1600 BC by Syrians, Babylonians, Hebrews and Egyptians (Yirga, 2010**)**. Recent link between nutrition and medicine focuses on the molecular mechanisms and how nutrients behave as pharmaceuticals. The Institute for Medicine's Food and Nutrition Board defined functional foods as "any food or food ingredient that may provide a health benefit beyond the traditional nutrients. In many traditional systems, the medicine and nutrition aspects were not considered as separate fields of study (Adnan *et al.*, 2010). This is supported by Hippocrates, the famous Greek physician, who said, "Let your food be your medicine and your medicine be your food (Ellahi *et al.*, 2007).

World health Organization (2002) emphasizes on the need and importance of determining nutritive content of medicinal plants by proximate analysis. In 1987, guggulipid (*Commiphora mukul*) was officially recognized in India as a lipid lowering remedy and now is widely used for this condition. The uses of complementary and alternative therapies have gained popularity in the Western world. In several studies, both in humans and animals usefulness of guggulipid to reduce low-density lipoprotein (LDL-C), cholesterol and triglycerides has been

demonstrated (Nohr *et al.*, 2009). It is presumed that, the broad-spectrum effectiveness of these species may provide a suitable basis for new nutritional therapies (Newman *et al.*, 2000). In Ayurveda, the Indian traditional system of medicine, the gum resin from the tree *Commiphora* spp. has been used for thousands of years in the treatment of arthritis, inflammation, obesity, and disorders of lipid metabolism (Chander *et al*., 1996).

2.2 Medicinal Use and Health Benefits of the Natural Products

Natural products have been found to have antibacterial, antiviral, and antifungal activities. These actions however, are less clear in humans. Some of the natural products are also claimed to prevent heart diseases (including atherosclerosis, high cholesterol, and high blood pressure) and cancer. Animal studies, and some early investigations in humans, have suggested possible cardiovascular benefits from use of natural products including garlic. A Czech study (Jonathan *et al.*, 1998) found that garlic supplementation reduced accumulation of cholesterol in the vascular walls of animals. Another study by Choudhary (2008) showed similar results, whereby garlic supplementation significantly reduced aortic plaque deposits from cholesterol in rabbits. Another study by Yu-Yan and Lijuan (2001) showed that, supplementation by garlic extracts inhibited vascular calcification in human patients with high blood cholesterol (Wikipedia, 2009). There is need to conduct scientific investigation on traditional herbal remedies for metabolic disorders as may provide valuable information for development of alternative drug and therapeutic remedies (Kochhar *et al.*, 2006). Search for new drugs with better and cheaper substitutes from natural plant is a reasonable option. The medicinal value of *C. sywnnertonii* lies in some

chemical substances that produce a definite physiological action on the human body (Iniaghel *et al.*, 2009).

2.3 Cholesterol

Cholesterol is probably the best known steroid, as it is associated with atherosclerosis. Although it is extensively distributed to all body cells, cholesterol is found mostly in the nerve tissues. It is one of the principal building blocks of the plasma membrane and plasma lipoprotein. It is present in animal fats. The fundamental risk factor for the development of coronary artery diseases or atherosclerosis is an increase of the plasma cholesterol level. Coronary artery diseases are among the major cause of mortality all around the world. There is solid evidence that, administration of antioxidant vitamin cocktails together with lipid-reducing treatment depress the level of HDL cholesterol (Çakılcıoğlu *et al.*, 2007). Despite substantial medical progress in the past two decades, coronary heart disease remains the major health problem in most developed countries. Elevated serum total and LDL-cholesterol concentrations are powerful risk factors for CHD. Each 1% increase in the serum cholesterol concentration results in a 2–3% increase in CHD risk. Furthermore, in primary and secondary prevention trials, a reduction in total and LDL-cholesterol concentrations improved the function of the coronary endothelium and decreased the risk for CHD (Anderson *et al.*, 2000). Although various people have reported hypolipidemic effect of various traditional medicinal plants such as *Commiphora* spp., there is limited information on the nutritional composition of this plant (Kochhar *et al*., 2006).

2.4 Potential of *Commiphora spp.* in Reducing Cholesterol

Commiphora spp. has a potential for reducing cholesterol and triglyceride (Urizar *et al.*, 2002). Some of the *Commiphora spp.* stimulate increase of body's lipid metabolic rate in which the liver is stimulated to metabolize LDL-cholesterol, thereby lowering the amount of cholesterol in the bloodstream (Nancy *et al.*, 2003). Plants have great importance due to their nutritive value and continue to be a major source of medicines. As they have been found throughout human history, 30 to 40% of today’s conventional drugs used in the medicine and curative purpose for various ailments are obtained from herbal supplements, botanicals, nutraceuticals and drugs (Hoareau *et al*., 1999). For instance, 1,500 mg of gugulipid standardized to 2-5% gugulsterones is regarded as the most effective and is safe for lowering cholesterol (Rose *et al.*, 1999).

Throughout the world, great efforts are focused on reducing the risk of coronary heart disease through dietary interventions and by using medicinal plants as nutritional supplements. One of the major risk factors of cardiovascular diseases that can be modulated by dietary intervention is blood cholesterol. A number of dietary agents, including soluble fibres and plant sterols/stanols, have the ability to interfere with cholesterol absorption and to lower its levels in serum (Eldin and Moazzam, 2009). Plant sterols and stanols, also called phytosterols and phytostanols respectively, have chemical structures resembling that of cholesterol but are only available to humans through plant foods such as vegetable oils, nuts, seeds, cereals, legumes, fruits, and vegetables or industrial supplements from plant origin (Piironen *et al.*, 2000). Inclusion of plant sterols/stanols in the diet was known to lower serum cholesterol in man since 1953 and the effects of plant sterols and stanols on cholesterol and bile acid metabolism and their efficacy and safety as serum

cholesterol-lowering agents have been reviewed in several studies (Homma *et al*., 2000).

Cholesterol lowering effect of plant sterols and stanols in normal and hypercholesterolemia has been reported in both males and females. Extracts of *Commiphora spp.* have been employed for a long time in Ayurvedic system of medicine for the treatment of obesity and other weight related problems, rheumatoid arthritis and lipid disorders (Hanus *et al*., 2005). Based on these descriptions, a number of clinical trials to test the effectiveness of this herb in lowering cholesterol and disorders of lipid metabolism as well as lowering body weight have been conducted. The results of the various studies confirmed *Commiphora* spp. to have natural cholesterol lowering substance which are safe and effective (Mahmood *et al*., 2010).

Studies in both animal models and humans have shown that, *Commiphora spp.* can decrease elevated lipid levels. Guggul (*Commiphora spp.*) was approved by the government of India in 1987 for the treatment of hyperlipidemia, hypercholesterolemia, and hypertriglyceridemia. Several well-designed clinical trials using various extracts of guggul have shown significant effect in lowering the levels of triglycerides, total cholesterol, total serum lipids, low density lipoprotein-C, and also causing significant increase in high density lipoprotein-C. No serious adverse reactions have been reported from gugulipid uses. Gugulipid (*Commiphora spp.*) has demonstrated equivalent efficacy to clofibrate in clinical trials for the treatment of hyperlipidemia (Hanus *et al*., 2005). There has been a gradual revival of interest in the use of medicinal plants namely *Commiphora spp.* in developing countries

because herbal medicines have been reported to be safe and without any adverse side effect especially when compared with synthetic drugs (Iniaghe *et al.*, 2009).

2.5 Nutritional Value of Medicinal Plants

Medicinal plants have been used to combat malnutrition. Three non-governmental organizations namely; Trees for Life, Church World Service and Educational Concerns for Hunger Organization have advocated medicinal plants as "natural nutrition in the tropics." Leaves, roots, and bark can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and without loss of nutritional value (Fahey, 2005). Many medicinal plants are used by marginal communities to cure various diseases. Various medicinal plant species are used either in the form of extract or concoction by the local people in different regions. Some of medicinal plants such as *C. swynnertonii* serve as both food and medicine (James *et al.*, 2010). Scientific investigation of traditional herbal remedies for metabolic disorders may provide valuable clue for the development of alternative drugs and therapeutic strategies (Kochhar *et al.*, 2006).

The use of medicinal plants in therapeutics or as dietary supplements goes back beyond recorded history, but has increased substantially in the last decades (Khan *et al.*, 2001). However, the safety of their use has recently been questioned due to the reports of illness, fatalities and poisoning associated with the presence of toxic metals in medicinal plants (Caldas and Machad, 2004). Plants can contain heavy metals from their presence in the soil (including contamination of the plant material with soil), water or air (Shah *et al.*, 2009).

Dietary fibre has major protective effects against atherosclerotic cardiovascular disease. Epidemiologic data suggest that, intake of complex carbohydrates and dietary fibre is inversely related to coronary artery disease (Khan *et al.*, 2001). The current daily intake of plants containing sterols from conventional foods is estimated to be in the range of 160-400 mg/day. This level of natural plant sterol can reduce dietary cholesterol absorption by about 40% compared to sterol-free medicinal plants (Eldin and Moazzam, 2009). Thus, naturally present dietary plant sterols may contribute to reduction in cholesterol absorption especially when combined with other cholesterol-lowering plant foods.

Human body comprises chemical compounds such as water, proteins, fatty acids, nucleic acids and carbohydrates. These in turn consist of elements such as carbon, hydrogen, oxygen, nitrogen and phosphorus and may or may not contain minerals such as calcium, iron, magnesium and zinc. The nutritive value of plant plays a great role in plant and human beings, (Shivraj *et al.*, 2009). Plants have the ability to synthesize primary metabolites (proteins, fats, nucleic acids and carbohydrates) by simple substances including water, carbon dioxide, nitrogen and a number of inorganic salts in small amounts. These primary metabolites are transformed into secondary metabolites like (alkaloids, steroids, terpenoids, saponins, flavonoids etc.,) that can be used as drugs (Manikandan and Victor, 2010). Medicinal plants contain numerous biologically active compounds such as nutrients and phytochemicals which have physiological actions on the human body. The inherent active ingredients are used to cure diseases or relieve pain (Ojokuku *et al.*, 2010).

Although medicinal plants including *C. swynnertonii* are important in the pharmaceutical industry, they are underutilized in medicine despite being used in ethno-medicinal preparations (Belewu *et al.*, 2009). The utilization of medicinal plants in traditional medicine is found to be effective, cheap and practical, various parts of the medicinal plants can be used for various treatments including the leaves, barks, tubers, root, herbs and the plant extracts.

2.6 The Natural Cholesterol-Lowering

High cholesterol and/or triglyceride problems are very common in modern society and are known to increase the risk for heart attack, stroke and other cardiovascular diseases. It has been advised that individuals should strive to achieve a fasting blood cholesterol level below 3.9 mmol/L (150 mg/d) and a fasting triglyceride level below 1.13 mmol/L (100 mg/dL) to maximize their protection against heart attack and stroke (Wang *et al*., 2004). The anti-lipidaemic, properties of a number of *Commiphora* spp. have been extensively studied (Newall *et al*., 1996). The ethanol leaf extract of *Commiphora africana* has been demonstrated to possess an antilipidaemic property (Adebayo *et al*., 2006). *Commiphora* has been shown to exhibit hypolipidaemic activity (Ezekiel *et al*., 2010). The antilipidaemic activity of *Commiphora mukul* (guggulipid) has been established. It exerts effective lipid-lowering activity by reducing total cholesterol (Wang *et al*., 2004).

Extracts of the resin of the guggul tree (*Commiphora mukul*) lowered LDL (low-density lipoprotein) cholesterol levels in humans (Hanus *et al.*, 2005). The plant sterol guggulsterone [4, 17(20)-pregnadiene-3, 16-dione] is the active agent in this extract. It has been shown that, guggulsterone is a highly efficacious antagonist of

the foresaid X receptor (FXR), a nuclear hormone receptor that is activated by bile acids. Guggulsterone treatment decreases hepatic cholesterol in wild-type mice fed a high-cholesterol diet but is not effective in FXR-null mice. Thus, it has been proposed that, inhibition of FXR activation is the basis for the cholesterol-lowering activity of guggulsterone. Other natural products with specific biologic effects may modulate the activity of FXR or other relatively promiscuous nuclear hormone receptors (Urizar *et al*., 2002).

The resin of the *Commiphora mukul* tree has been used in Ayurvedic medicine for more than 2000 years to treat a variety of ailments. Studies in both animal models and humans have shown that this resin, termed gum guggul, can decrease elevated lipid levels. The stereoisomers E- and Z-guggulsterone have been identified as the active agents in this resin. Recent studies have shown that, these compounds are antagonist ligands for the bile acid receptor farnesoid X receptor (FXR), which is an important regulator of cholesterol homeostasis. It is likely that, this effect accounts for the hypolipidemic activity of these phytosteroids (Nancy *et al.*, 2003). Findings of various studies suggested that, the incorporation of certain soluble fibres into the diets of humans and animals can lower blood cholesterol, particularly LDL cholesterol. Clinical studies have shown that, fibre supplements can reduce cholesterol by about 5-9% below the levels achieved with a prudent diet (Bahram *et al.*, 1997). Studies in both humans and animals suggest that the primary mechanism by which dietary soluble fibre lowers cholesterol is through enhanced synthesis of bile acids and their faecal excretion. The enhanced elimination of bile acids also results in increased hepatic cholesterol synthesis (Anderson *et al*., 2000).

2.7 Heavy Metals in Medicinal Plants

The term “heavy metals” refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration (Duruibe *et al*., 2007). The safety information on medicinal plants is required for clinical trials and to support the registration and commercialization of the medicinal plant product. The presence of heavy metals such as lead (Pb), cadminium (Cd) and mercury (Hg) in herbal products at high levels can cause serious risk to public health (Shafii *et al.*, 2011). The determination of heavy metals in medicinal plants is therefore of greater importance in order to protect people from hazards of these metals in which lead and cadmium have no known body beneficial properties (Khan *et al*., 2008). The presence of heavy metals beyond the recommended limits can cause metabolic disturbances in the body. Effect of toxic metals (Cd, Pb and Hg) on human health and the interaction with other trace elements may cause serious problems (Hussain *et al.*, 2006). Plants accumulate metals in both the roots and up to rooted tissues, which then transfer heavy metals from the soil into the food chain. Continuous intake of heavy toxic elements can cause damage to human and other animals due to bioaccumulation. Since there is no effective mechanism for the elimination, effects become apparently after several years of exposure (Khan *et al*., 2008). Heavy metals including (Pb), cadmium (Cd) and mercury (Hg) when consumed in considerable amounts may result in reduced mental and central nervous function (Shafii *et al*., 2011). They also induce various toxic effects in humans at low doses. The symptoms of lead poisoning are colic, anaemia, headache, convulsions and chronic nephritis of the kidneys, brain damage and central nervous system disorders. Cadmium when accumulated in human body and damages mainly the kidneys and liver (Chishti *et al*., 2011). Cadmium is toxic to human even at low concentrations. It is reported to cause osteomalacia, badly affects the cardio vascular system and kidney functioning (Kirmani *et al*., 2011).

CHAPTER THREE

3.0 MATERIALS AND METHODS

This chapter presents materials and methods used in the analysis of nutrient content of *C. swynnertonii* and cholesterol levels in rats. The chapter is divided into two major sections. The first section elucidates the materials used that are *C. swynnertonii* and the animals used, and the second section summarizes the methods and procedures used in the material preparation, animals feeding, determination of the nutrient content, and measurement of plasma cholesterol.

3.1 Materials

3.1.1 Plant materials

C. swynnertonii plant materials were collected from Simanjiro district and were transferred to Sokoine University for preparation and analysis. The stem barks and root parts from *C. swynnertonii* plants were dried in an open air for 24 h as shown in Plate 2. They were grounded into fine powder and stored in air tight container at 4°C until used. Resin was harvested from the plant materials as shown in Plate 3. The concentration of the resin was based on the plant materials One hundred grams (100 g) of the resin was brewed in 750 ml distilled water and thereafter allowed to stand for 30 min. The mixture was filtered using filter paper and stored in a clean bottle before administered to rats. Twenty ml aliquots of the decoction were evaporated to dryness using an electric heater. The residue left was used to determine the concentrations in the *C. swynnertonii* extracts which was administered to different groups of experimental animals. The other dried samples were used for proximate analysis (Edem, *et al*., 2009).

Plate 2: The drying of stem bark and root parts from the *C. swynnertonii*

Plate 3: *C. swynnertonii* resin harvesting process

3.1.2 Sample size determination for experiment animals

The sample size for the experimental animals was determined according to Kirkwood and Sterne, (2003). Absolute sampling error of 10% and confidence interval of 90% was used to obtain the sample size of 24 rats and 6 samples for plants.

$$n > \frac{[u\sqrt{\pi(1-\pi)} + v\sqrt{\pi_{null}(1-\pi_{null})}]^2}{(\pi - \pi_{null})^2} \quad \text{.....(1)}$$

Where:

n = the required minimum sample size,

π = the proportion of interest (0.8),

π_{null} = the null hypothesis proportion (0.2),

u = the one sided percentage point of the normal distribution corresponding to 100%

(With 90% power, u = 1.28),

ν = percentage of the normal distribution corresponding to the required (two sided) significance level (with 5% level of significance, ν = 1.96).

3.1.3 Care of the lost samples

Care of the lost samples was taken by applying Intent-to-treat Analysis of Randomized Clinical Trials. All randomized rats were included in the groups to which they were randomly assigned, regardless of their adherence with the entry criteria, treatment they received, subsequent withdrawal from treatment or deviation from the protocol and anything that happens after randomization as was explained previously in other studies (John, 2000). The rats were used for the following reasons; the body mechanism of the rats is almost the same as that of human being

and other animals and also as the experiment required draw of blood on weekly basis so it was easily for the rats. Lastly at the end of experiment the animals were required to be sacrifices for other study purpose.

3.2 Methods

3.2.1 Experimental animals and treatment

Twenty four rats aged 7 months old of both sexes weighing 125.7-180 grams were used in this study. The rats were obtained from the small animal unit at Sokoine University of Agriculture Faculty of Veterinary Medicine. The animals were randomly assigned into four groups of six rats (n=6) each. All rats were housed in well-ventilated cages (room temperature 24±1°C) and were provided with the normal rat foods (rat feeds) and drinking water during the experimental period (Edem *et al.*, 2009). Group 1 was the control group that received distilled water. Animals in the test groups (II, III and IV) were fed orally 0.5 ml once daily with 50 mg/kg, 100 mg/kg and 200 mg/kg of *C. swynnertonii* resin extract that was prepared on daily basis. The administration of the extract was carried out daily for 21 days as recommended by Iridam *et al*. (2006).

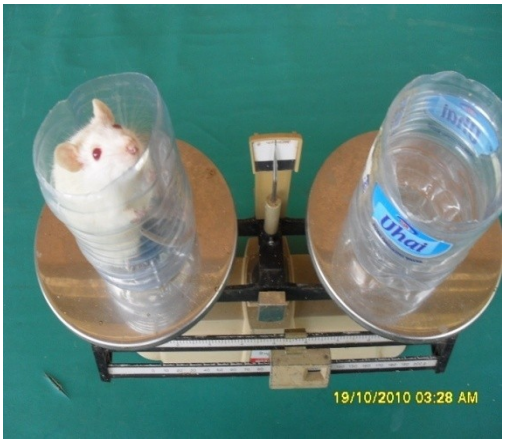

Plate 4: Procedures for measuring weight and drawing blood sample from the rat tail

3.2.2 Preparation of plasma and analysis

At baseline, the body weights of the rats were recorded. Blood samples for plasma preparation were collected from the tail artery of the rats using sterile syringes as shown in Plate 4. Blood samples were stored in EDTA sterile vacutainer tubes as shown in Plate 5. Cholesterol level was determined according to Erba Mannheim protocol (Erba, 2010). The blood sample was thereafter centrifuged at 1308 xg for 5 min using a bench top centrifuge model to obtain the plasma. The plasma was stored in a refrigerator for analysis of biochemical parameters. All analyses on plasma were completed within 24 hours after blood collection as recommended (Goji *et al.*, 2009).

Plate 5: EDTA vacutainer tubes and the centrifuge used to separate the plasma from the blood

The procedure for measuring cholesterol was done using Cholesterol Liquid Stable Reagent kit (CHO-PAP, End Point). This reagent was intended for *in vitro* determination of cholesterol in serum/plasma. Unhaemolysed perished plasma was used in this study. The reagent was mixed well with the serum and incubated for 10 minutes at 20-25^{o}C. Then the absorbance of the Test and Calibrator against reagent

blank was read and recorded (Erba, 2010). Thereafter the calculation was done using the following equation (2):

$$Cholesterol\ (mg/dl) = \frac{Absorbance\ of\ Test \times Concentration\ of\ Calibrator.\ (mg/dl)}{Absorbance\ of\ Calibrator} \quad .....(2)$$

The same procedure was repeated at an interval of 7, 14 and 21 days. The cholesterol level was compared with the four groups (Edem *et al*., 2009). Each sample was analyzed in duplicate.

3.2.3 Chemical analysis of the *C. swynnertonii*

Six samples of *C. swynnertonii* were taken from the stem bark and root of the plants. The samples of *C swynnertonii* were randomly collected from the selected areas of Simanjiro district. The samples were then analyzed for proximate composition and mineral contents. The results obtained were compared regarding their nutritive values.

Plate 6: Some samples of *C. swynnertonii* used for proximate composition and mineral contents

3.2.3.1 Protein content

The protein content of *C. swynnertonii* was determined by Kjedldah (AOAC, 2004; method 925. One gram of *C. swynnertonii* sample was weighted and transferred into a clean digestion tube. Two mercury catalyst tablets were added into the digestion tube and 10 ml of concentrated H_2SO_4 were added and the tube was heated gently in a hot plate in an inclined position. When the initial frothing ceased, strong heat was applied to boil the contents at a moderate rate. The tubes were shaken from time to time and heating continued for one hour until the contents become clear. Digestion was done at 420^{o}C for 3 hours. After cooling, 75 ml of distilled water was added.

The digestion tube with digestion sample solution was connected directly to the rubber joint of the distilling unit (Teccater Kjltec system, model). The tank was filled with alkali and pulled down the handle for dispensing the alkali into the digestion tube. In the receiving flask 25 ml of boric acid solution (4%) and 10 ml Bromocresol green and 7 ml of methyl red were added. The distillation apparatus was connected with the delivery tube dipping below the boric acid solution. The ammonia was distilled into boric acid solution and then titrated with 0.1 N HCl on a titration unit. The end point was detected by development of very faint purple colour. A blank was prepared by digesting the chemicals only, followed by distillation and titration. The blank value was subtracted from the sample values. Percentage crude protein (%CP) was calculated from the percentage nitrogen using the conversation factor of 6.25 to get the percentage of crude protein.

$$\%\ Nitrogen = \left(\frac{(a-b) \times Normality\ of\ acid \times 14.008}{weight\ of\ sample\ (g)}\right) \times 100 \quad \text{.....(3)}$$

Where:

a = ml of titration acid for the sample

b = ml the blank value.

% Protein = % N × Protein factor

3.2.3.2 Fat content

The fat content of *C. swynnertonii* was determined by Soxhlet Ether extraction method (AOAC, 1995; method 920.85). A 2.0 g portion of the sample was weighted into extraction thimble and covered with defatted cotton wool. The thimble was placed in the extraction chamber which was suspended above a flask containing petroleum ether and a condenser. Extraction proceeded for 16 hours at a rate of 2 -3 drops per second by heating the solvent in the boiling flask. The flask containing extracted fat was dried in an oven at 100°C for 3minutes, cooled in desiccators and weighed. The percentage fat was then calculated using the equation (4, 5):

$$\%\ fat\ (V/W\ or\ W/W) = \frac{Weight\ gain\ of\ flask\ (With\ Extracted\ fat) \times 100}{Weight\ of\ sample} \quad \text{.....(4)}$$

$$= \frac{(c-a)\ x\ 100}{b} \quad \text{.....(5)}$$

Where:

a = Weight of empty round flask

b = Weight of sample

c = Weight of flask + residue (fat)

3.2.3.3 Ash content

The ash content was determined using AOAC (2004) method 938.08. Porcelain crucibles and lids were washed in warm soapy water and rinsed in tap water. The crucibles and lids were thereafter soaked in 7.5M HNO_3 overnight in fume hood. The crucibles were thoroughly rinsed with deionised water. The crucible with lids were thereafter dried in an oven set at 110ºC conditioned in muffle furnace up to 600ºC for about 4 hours, and then allowed to cool in desiccators.

The empty crucibles with lid were weighted on an analytical balance. Three grams of homogenized *C. swynnertonii* whole root and stem bark were weighed into the crucible (included lid in weighing). Crucible containing the sample was gently heated on a hot plate on Bunsen burner in a cupboard until smoking ceased then the crucible and lid was transferred in a cool muffle furnace. Then the power was turned off, and temperature was set at 600ºC and the samples were left to heat for 4 hours and until the ash was turned to white colour. After ashing, the power was turned off and the crucibles were left to cool to about 150ºC then stored in desiccators until were completely cool. Finally crucible and lid were weighed to determine the weight of ash. Percentage ash was calculated from the equation (6):

$$\%\ Ash = \frac{Weight\ residue\ in\ crucible\ after\ ashing \times 100}{Weight\ of\ sample}$$

.....*(6)*

3.2.3.4 Fibre content

The percentage fibre content of *C. swynnertonii* was determined using AOAC (1995) method 920.86. One gram of the *C. swynnertonii* sample was digested using the 220 ANKOM Fibre- Tech set at 550ºC for 2 hours. Fibre content was calculated and expressed in percentage using the equation (7):

$$\%\ Fibre = \frac{Weight\ of\ residue\ after\ digestion \times 100}{Weight\ of\ original\ sample}$$

.....*(7)*

3.2.3.5 Carbohydrate content

Carbohydrate content of the *C. swynnertonii* was calculated as difference (AOAC, 1995) using the equation (8):

$$CHO = 100 - (CP + CFat + Ash + CFibre)$$

.....*(8)*

Where:

CHO = Carbohydrate, CP = Crude protein,

Cfat =Crude fat, Cfibre =Crude fibre

3.2.3.6 Moisture content determination

The sample was dried to a constant weight in the oven. The loss in weight during drying was equal to the moisture content of the sample. Oven was pre-set at 100°C. Five grams of the dry sample was weighed into pre- weighed petri dishes. The total weight of the sample and Petri-dishes with sample was placed in the oven pre-set at 100°C. The petri dish was left to dry in the oven for four hours. Thereafter, the petri dishes were removed from the oven and placed in a desiccator to cool. When they cooled to room temperature, the petri dishes with samples were weighed and the petridishes were placed back into the oven and allowed to dry overnight.

Calculation of the moisture contents was done by using the following equation (9):

$$W = \frac{\textit{Weight of dish with sample before drying - Weight of dish and sample after drying}}{\textit{Weight of sample taken}} \times 100 \quad \textit{.....(9)}$$

Where:

W= moisture content in percent

3.2.3.7 Energy content

Energy content of C. *swynnertonii* was determined according to AOAC (1995) procedure. Energy was obtained by multiplying the fat, protein and carbohydrate value by the Atwater conversion factors of 9, 4, and 4 for fat, protein and carbohydrate, respectively.

Energy (kcal) = (CFat x 9) + (CP x 4) + (CHO × 4) .....(10)

Where:

CHO = Carbohydrate; CP = Crude protein; Cfat =Crude fat;

3.2.3.8 Determination of mineral content

The obtained *C. swynnertonii* ash was dissolved in 2 ml concentrated hydrochloric acid. The dissolved ash was then diluted with distilled water (mineral free) and filtered with No. 1 Whatman ash-less filter papers. The filtrate was diluted to 25 ml distilled water and analyzed for Fe, Mg, Cu, P, Zn, and Mn using Atomic Absorption Spectrophotometer (UNICAM 919, Cambridge, U.K.). Each mineral element was determined using a single mineral hollow lamp. The phosphorous was determined by flame photometric method (AOAC, 1995). Each sample was analyzed in triplicate. Quantification was accomplished by comparison with standard curve drawn using standard solution of known concentrations at 0.5, 1.0, 1.5, and 2.5 ppm. The results were determined using the following equation (11):

$$mineral\ conc.\ \left(\frac{mg}{100}g\right) = \frac{GR}{1000ml} x \frac{100ml}{SW}\ x\ DF\ x100 \qquad .....(11)$$

Where:

GR = Reading value (in ppm); SW = dry sample weight; DF = Dilution factor

3.2.3.9 Determination of lead and cadmium

The dried whole root and stem bark powder samples of *C. swynnertonii* were submitted to SEAMIC (Southern and Eastern African Mineral Centre) Dar es Salaam for determination of heavy metals concentration using AOAC (1995) method. The roots and stem bark of *C. swynnertonii* were subjected to an oven's

temperature which was set between 40°C and 3000°C, with a maximum ramp of 2000°C per second. Actual temperature control was achieved through sensors that indicated the resistance to the electrode heads and the temperature of the cooling water. The gas flow used for the flame analysis was programmed at 3 L per min. The standard solutions used for the calibration lines, useful for the determination of each heavy metal (1000 ppm concentration), was as follows Cadmium and lead standard solutions were diluted in HNO_3 (Cd $(NO3)_2 4H_2O$ and $Pb(NO_3)_2$. The wavelengths used to determine the absorbance of the metals were: lead for 217.0 nm, and for cadmium 228.8 nm.

3.2.3 Determination of mercury

The dried and grounded samples of roots and stem bark of *C. swynnertonii* were packed and transferred to, SEAMIC in Dar es Salaam. Mercury was analyzed using atomic absorption spectroscopy (CV– AAS) method. The dried samples of *C. swynnertonii* was further dried in an oven at 40°C for 48 h, and pulverised in an agate mortar. Sub-samples (0.2 to0.3 g) of dried and powdered samples of *C. swynnertonii* were wet digested with 6 ml of concentrated nitric acid in closed PTFE vessels in a microwave oven (Automatic Digestion System, MLS 1200). The digestion was further diluted to 10 ml using double-distilled water. With every set of up to 50 *C. swynnertonii* samples digested, two blank samples were run. Finally the quantification of total mercury content of *C. swynnertonii* samples was performed by cold-vapour atomic absorption spectroscopy (CV– AAS), using a fully automated mercury monitor.

3.2.4 Statistical analysis

The data obtained were compiled, coded and analysed using Microsoft Excel statistical package (2007) and SAS (Statistical Analysis System) program (Version 8.3) for Window^{R}. Results from experimental animals and proximate analysis were expressed as means ± SEM. Data were assessed by analysis of variance (ANOVA) and t-test (Gomez and Gomez, 1984). Tests for differences between the means were done and compared by Duncan's Multiple Range Test (DMRTS) at ($p < 0.05$).

CHAPTER FOUR

4.0 RESULTS AND DISCUSION

4.1 Overview

This chapter presents the results of this study. It is divided into two major sections. Section one highlights the general observation of the rats and cholesterol changes in rats which were fed with the *C. swynnertonii*. The second section delineates the proximate composition and mineral composition of the *C. swynnertonii* while the third section describes the side effect of this medicinal plant to the rats through clinical observation

4.2 General Observation During the Study

Animals used in the study in the control group were apparently in good health condition, as they remained alert, consumed food and water freely and exhibited normal weight increase over time. Animals in the treated groups had diarrhoea and were less active during the daytime. Observation from previous studies by Ruitang (2007) who conducted clinical trials using *C. mukul*, some adverse reactions were observed, in which gastrointestinal discomfort was the predominant side effect reported. Other side effect included loose faeces, mild nausea, and hiccup.

There were three deaths out of twenty four rats treated /exposed rats one at 100 mg/kg and two 200 mg/kg. Other clinical signs observed were diarrhoea in all the treated groups which did not happen in the control group. Results of the effects of *C. swynnertonii* resin extract on the cholesterol levels after 0, 7, 14 and 21 days of treatment are presented in Table 1.

Table 1: Cholesterol level (mmol/L) in the experimental animals

Time	0mg/kg	50mg/kg/Bwt	100mg/kg/Bwt	200mg/kg/Bwt
Baseline	5.65±0.46[a]	5.29±0.46[a]	4.30±0.46[a]	5.49±0.46[a]
1 week	5.71±0.37[b]	3.94±0.37[a]	3.19±0.37[a]	3.89±0.37[a]
2 week	5.50±0.42[d]	3.25±0.42[bc]	1.77±0.48[b]	2.20±0.54[ab]
3 week	5.78±0.41[c]	2.42±0.41[b]	1.03±0.47[b]	1.13±0.54[ab]
Percentage (%) change	+2.3	-54	-76	-79

[abc] Means in row with different superscript are significance different at $p < 0.05$, *Percentage change refer to positive (+) increase and negative (-) decrease in cholesterol levels.

4.3 Cholesterol Changes in Rats

The plasma lipid level of rats before and after administration of *C. swynnertonii* extract at three different levels is shown in Table 1. It was found that, the mean initial value of total cholesterol, before feeding the rats with the *C. swynnertonii* was 5.65±0.46 mmol/L, 4.30±0.46 mmol/L, 5.49±0.46 mmol/L, and 5.49±0.46 mmol/L for 0, 50, 100 and 200 mg/kg respectively. There were decrease in plasma cholesterol levels in all the treated animals and appeared to be dose and time dependent. For the rats receiving 50 mg of *C. swynnertonii* per Kg body weight the total cholesterol levels decreased from 5.67 mmol/L to 3.94 mmol/L after one week, 3.25 mmol/L after two weeks and 2.42 mmol/L after three weeks. For animals receiving 100 mg of the extract per body weight, cholesterol levels decreased from 4.30±0.46 mmol/L (baseline) to 3.19 mmol/L (week 1), 1.77 mmol/L (week 2) and 1.17 mmol/L (week 3). For animals receiving 200 mg of the *C. swynnertonii* extract per body weight, cholesterol levels decreased from 5.49 mmol/L (baseline) to 3.89 mmol/L (week 1), 2.20 mmol/L (week 2) and 1.13 mmol/L (week 3) (Fig. 1).

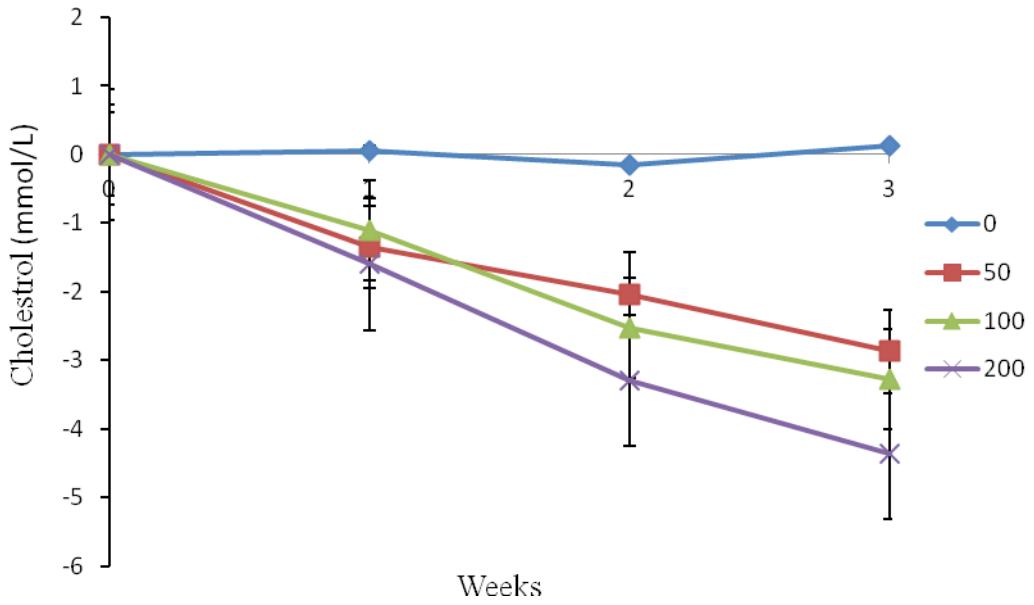

Figure 1: Cholesterol changes in the rats during the experiment

These findings are in line with those of MMed *et al.*, (2007) who reported that chitosan had cholesterol lowering effect. After feeding chitosan to rats for 20 days, there was significant reduction in plasma cholesterol by 25 to 30% without influencing food intake and growth. Findings in this study revealed that, the cholesterol level in the control group did not decrease compared to the treated groups in which the decrease was proportionate to the concentration of *C. swynnertonii* extract given. A study conducted by Fukushima *et al.*, (2001) to examine the effect of mushroom fibres on plasma cholesterol, it was observed that serum total cholesterol concentrations deceased by 11 to 25% compared with the control group. Similar effects were also observed when Fenugreek seeds gum and saponin fractions fed to the rats. Saponin and fenugreek gum compete with cholesterol at binding sites or interfere with cholesterol biosynthesis in liver. Soluble fibres such as gums, pectin and mucilage may block cholesterol absorption in the intestine (Kochhar *et al.*, 2007).

Cholesterol is an important constituent of cell membrane and is the precursor of steroids hormone and bile acid. A high cholesterol level in the blood is, however, the major cause of cardiovascular disorders that is atherosclerosis, coronary heart diseases and myocardial infarction (Adebayo *et al*., 2006). The reduction in plasma cholesterol levels in the doses 50, 100, and 200 mg per kg body weight administered for 21 consecutive days corroborates the findings by Hoareau and DaSliva (1999) suggesting that the plants extract may contain hypolipidaemic and hypocholestrolaemic agents that might prove valuable for the management of cardiovascular diseases. The significant decrease in cholesterol levels further strengthens the fact that the plant extract may also be used to reduce the risk factors for cardiovascular diseases, for it is established that high density lipoproteins (HDLs) may exert a protective effect against atherosclerosis and may promote the mobilization and metabolism of cholesterol, thereby reducing its deposition in the vessels walls (Tiwari, 2008; Sawidis *et al*., 2010). The presence of dimethyl-terpenes (Hanus *et al*., 2005) phenols and tannins (Aliyu *et al*., 2002) in *Commiphora mukul*. may be responsible for its antilipidaemic activity. Some studies have also shown remarkable reduction in serum cholesterol with the administration of phenolic tea (Tandon, 2005).

The lipid lowering activity of resin extracts of *C. swynnertonii* may be attributed to the phyto-constituents present, such as triterpenoids, flavonoids, tannins, glycosides, and saponins (Pengelly, 2004). Saponin derived from *Medicago sativa* were reported to reduce blood cholesterol by competing with cholesterol at binding sites or interfering with cholesterol biosynthesis in the liver (Khadabadi and Bhajipale, 2010). A ketosteroid has been identified as an active principle for the

hypocholesterolaemic and hypolipidaemic activities of *C. mukul* (guggulipid) and African and Indian bdellium, *Commiphora* species (Martinetz, 1993). Guggulipids has shown to prevent endogenous hypercholesterolemia via the stimulation of the thyroid gland and has also been found to reverse the decrease in catecholamine and dopamine –p- decarboxylase activity which is associated with hyper cholestrolaemic (Wang *et al.*, 2004). It seems that a similar mechanism may be involved with *C. swynnertonii* in lowering cholesterol level.

4.4 Percentage Change of Cholesterol

At baseline, the cholesterol levels for all animal groups were similar ($p > 0.05$). After treatment with 50, 100 and 200 mg of *C. swynnertonii* extract per kg body weight, there was significant decline ($p<0.05$) in cholesterol concentration for all the treatment groups. For the group receiving 50 mg of *C. swynnertonii* per kg body weight, cholesterol levels decreased by 54% while for those receiving 100 and 200 mg of *C. swynnertonii* per kg body weight the cholesterol levels decreased by 76 and 79 percent, respectively. This suggested that the higher the dose of *C. swynnertonii* extract per kg body weight the more decrease in the cholesterol concentration (Fig. 2).

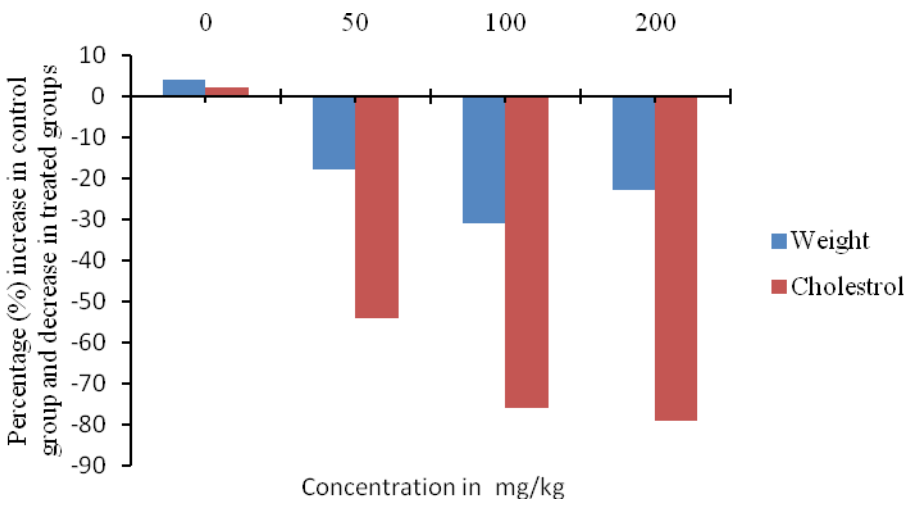

Figure 2: Percentage change in weight and cholestrol in the rats during the experiment

4.5 Weight Changes in Rats

Regarding weights, the animals receiving the *C. swynnertonii* extract lost weight, and this could be attributed to poor digestion and diarrhoea that was observed during the study (Table 2). For animals receiving 50 mg of *C. swynnertonii* per kg body weight, they lost about 16.5% of their average body weight during the first week, 15.7% during the 2nd week and 18.2% during the 3rd week (Fig. 3). For animals receiving 100 mg of *C. swynnertonii* extract weight losses were 24.0% (week 1), 30.0% (week 2) and 31.0% (week 3). Likewise, for animals receiving 200 mg of *C. swynnertonii* extract, weight losses were 23.0% (week 1), 21.5% (week 2) and 23.0% (week 3). Overall, animals receiving the *C. swynnertonii* extract lost weight in the range 18- 31% (Fig. 2).

Despite C. *swynnertonii* resin extract were able to reduce cholesterol, but were adversely affecting growth and this is not desirable. The aqueous resin extract of C. *swynnertonii* although used by Masai for lowering cholesterol in the body in man

Table 2: Weight (g) in the experimental animals

Time	0mg/kg	50mg/kg/Bwt	100mg/kg/Bwt	200mg/kg/Bwt
Baseline	159.53±6.76[b]	137.13±6.76[b]	169.75±6.76[b]	152.23±6.76[ab]
1 week	163.63±7.50[b]	114.50±7.50[a]	129.07±7.50[a]	117.70±7.50[a]
2 week	164.67±6.27[b]	115.65±6.27[a]	118.63±7.14[a]	119.54±8.17[a]
3 week	165.88±6.36[b]	112.18±6.36[a]	117.08±7.24[a]	117.70±8.29[a]
Percentage (%) change	+4	-18	-31	-23

[a] Means ± SEM based on Weight, [abc] Means in rowwise with different superscript are significance different at p< 0.05, *Percentage change refer to positive (+) increase and negative (-) decrease in cholesterol levels

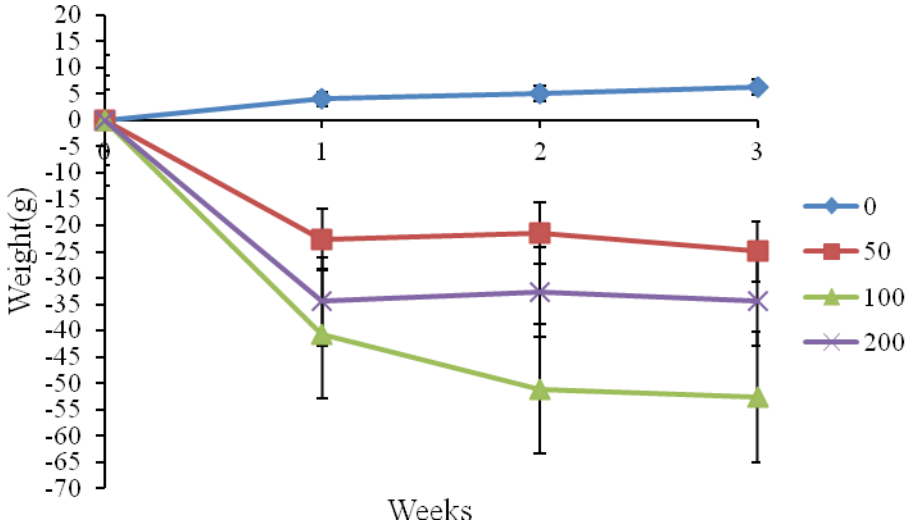

Figure 3: Weight changes in the rats during the experiment

was observed in rats under the conditions of this study to have side effect on the body weight change. Therefore, caution must be taken in its usage especially in high doses. Weight gain was decreased with increasing extract dose, while overall weight gain in the control rats was much higher than those of the extract treated animal. This decrease in weight gain in extract treated rats may be due to decrease in feed consumption since the animals were depressed, inactive and with lost appetite.

4.6 Correlation Between Weight and Cholesterol in Experimental Animals

From Fig. 4, it is observed that there was a significant positive correlation between weight and cholesterol at different concentrations. The control (Fig.4a) group of the rats had ($r = 0.432$ and $p=0.035$) this implies that at 0 mg/kg concentration the correlation is the strongest. However (Fig.4b) at 50 mg/kg ($r = 0.432$ and $p=0.009$) and (Fig.4d) 200 mg/kg ($r = 0.487$ and $p =0.282$) the correlation (Fig.4c) was found to stronger and at 100 mg/kg ($r = 0.712$ and $p=0.000$) the correlation was found to be the weakest. This implies that as the increase in weight tends to increase in cholesterol levels and decrease in weight also decreases cholesterol levels in rats ($p<0.05$). A study by Ibeziako *et al.* (1982) also showed a linear correlation between total cholesterol and phospholipids in the fatal plasma.

4.7 Proximate Composition of *C. swynnertonii*

The proximate analysis was reported on moisture content, ash, fat, protein fibre, carbohydrate and energy contents (Table 3).

4.7.1 Protein content of *C. swynnertonii*

The proximate composition (g/100 g dry weight) of the roots, and stem bark are summarized in Table 3. The protein content ranged from 3.32 g/100g in stem bark to 3.84 g/100g in roots. Most of the medicinal plants contain 5.53-8.59 g/100g crude protein on dried weight basis (Adnan *et al*., 2010).

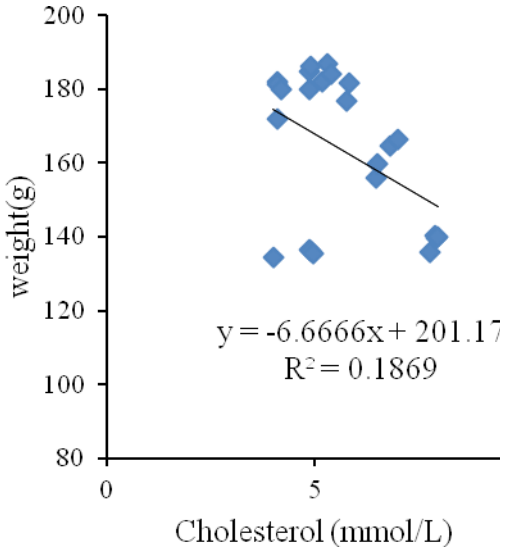

Figure4a: Weight vs cholesterol at 0 mg/kg concentration

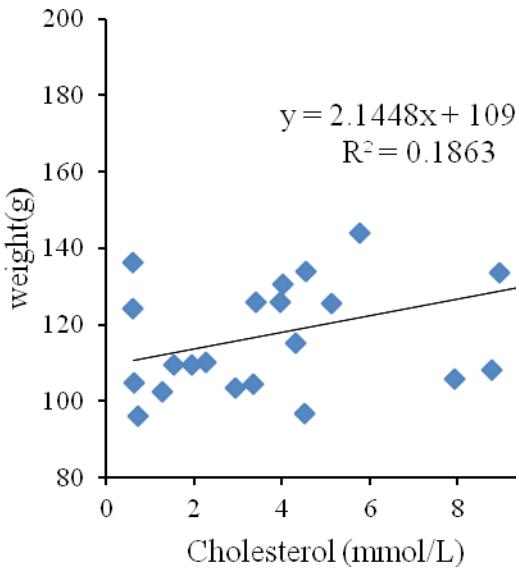

Figure4b: Weight vs cholesterol at 50 mg/kg concentration

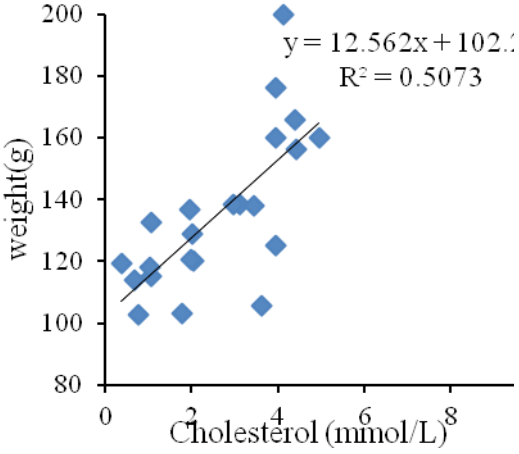

Fig.4c: Weight vs cholesterol at 100 mg/kg concentration

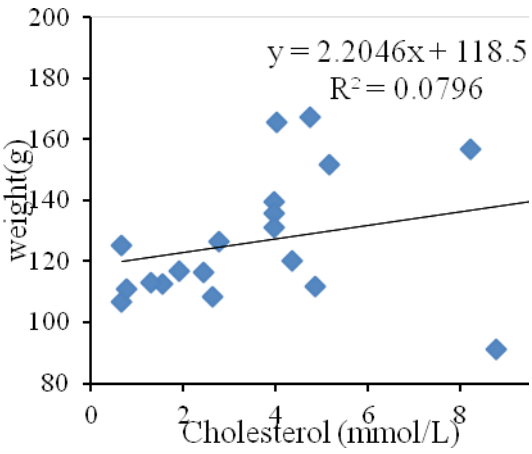

Fig.4d: Weight vs cholesterol at 200 mg/kg concentration

Figure 4: Relationship between total weight and cholesterol levels in rats at different concentrations

Table 3: Proximate composition (g/100g) and energy content (kcal/100g) of *C. swynnertonii* from different parts of the plants.

Proximate composition	Root	Stem bark
Moisture	6.03 ± 0.55	4.98 ± 0.25
Ash	11.59 ± 0.78	10.14 ± 1.41
Fat	3.84 ± 0.10	4.35 ± 0.23
Protein	3.84 ± 0.10	3.32 ± 0.380
Fibre	38.28 ± 4.94	32.60 ± 5.96
Carbohydrate	35.77 ± 4.95	44.61 ± 6.85
Energy	198.85 ± 22.87	230.87 ± 28.60

Means ± SEM based on duplicate analyzes of the same sample

The protein content of *C. swynnertonii* in stem bark was lower than the protein content in the roots. For example, a variety of proteins have been isolated in medicinal plants and found to be bioactive against certain ailments (Ojokuku *et al*., 2010). These demonstrate that *C. swynnertonii* has low protein content than in other medicinal plant plants.

4.7.2 Carbohydrates content

Carbohydrate content of *C. swynnertonii* ranged from 35.77 g/100g (Roots) to 44.61 g/100g (Stem bark) on dry weight basis. Results in Table 3 indicate no significant difference ($p>0.05$) in carbohydrate content between the plant parts. The presence of high carbohydrate content in this plant suggested that it may serve as a source of energy. It may also aid digestion and assimilation of other nutrients (Ojokuku *et al*., 2010). This also can be compared by a study done by Indrayan *et al.*, (2005) in which *Nelumbo nucifera* plant was found to have good nutritive value, particularly high carbohydrates and recommended to be suitable for younger people. This can be supported by (National Academy of Science, 2000) carbohydrates provides energy

to cells in the body, particularly the brain, which is a carbohydrate-dependent organ. The Recommended Dietary Allowance (RDA) for carbohydrate is 130 g/d for adults

4.7.3 Crude fibre constituent

Crude fibre content of *C. swynnertonii* was 38.28 g/100g between the roots and 32.60 g/100g of stem bark (Table 3). Fibre has benefits of enhancing bowel movement and reduces food constipation. Also fibre has some disadvantages that, it impairs digestibility of protein and absorption of trace elements and binds some micro-nutrients such as Fe, Zn and makes them unavailable for absorption. This may probably explain the value and reason for use of the plant to treat constipation (Olowokudejo *et al*., 2008). The concentration varies from 32.60 g/100g in the roots to 38.60 g/100g in the stem bark. There was no significant difference ($p>0.05$) in fibre content between the roots and the stem bark. Fibre is an important dietary component in the diet for preventing overweight, constipation, cardiovascular diseases, diabetes and colon cancer (Whitney *et al*., 1990). An Adequate Intake (AI) for total Fibre is set at 38 and 25 g/d for men and women at the ages 19 to 50, respectively (National Academy of Science, 2000).

4.7.4 Ash constituent

Ash content, which was an indication of the mineral content, was higher in root 11.59 g/100g than in the stem bark 10.14 g/100g (Table 3), but the difference was insignificant ($p> 0.05$). The high ash content is a reflection of mineral content in the sample materials. The ash of *C. swynnertonii* sample was the inorganic residue remaining after the organic matter was burnt away. The importance of the ash content is that it gives an idea of the amount of mineral elements present in the

sample while the organic matter gives an estimate of proteins, lipids (fats), carbohydrate and nucleic acid content in the sample. No specific amount of ash intake has been recommended for different age groups; however, ash content is an important nutritional indicator of mineral content and an important quality parameter for contamination, particularly with the foreign matters (National Academy of Science, 2000).

4.7.5 Crude fat content

The concentration of fat ranged from 4.35 g/100g in the stem bark to 4.49 g/100g of the roots in the *C. swynnertonii* (Table 3). Fat have two major functions as a class of nutrient it required for many normal biological functions, and serve as energy sources for body fuel (National Academy of Science, 2000). Although human body requires dietary fats for normal growth and development, USDA (1995) recommends that, people consume not more than 30% calories from crude fats.

4.7.6 Energy content *C. swynnertonii*

Results in Table 3 shows there insignificant difference in energy content among the plant parts examined in the study. It was 198.85 kcal/100g for roots and 230.87 kcal/100g for the stem bark of *C. swynnertonii*. Some researches on the proximate analysis of medicinal plants (Kochhar *et al*., 2006) have reported slightly less energy content in traditional medicines, (National Academy of Science, 2000) explains that humans and other mammals constantly need energy to perform physical work; to maintain body temperature and concentration gradients; and to transport, synthesize, degrade, and replace small and large molecules that make up body tissue. The daily recommended energy requirement is in human 2100 kcal.

4.8 Mineral Composition of *C. swynnertonii*

The results for minerals composition are presented in Table 4. The minerals analysed in the *C. swynnertonii* were phosphorous (P), copper (Cu), iron (Fe), zinc (Zn), manganese (Mn) and magnesium (Mg).

4.8.1 Magnesium content

This plant *C. swynnertonii* contained 1810.01 mg/100g in the stem barks and 996.30 mg/100g in roots (Table 4) of magnesium. Magnesium concentration differed significantly ($p<0.05$) between the roots and stem bark of *C. swynnertonii*. Magnesium concentration was higher in stem bark than in the whole root (Table 4).

This can be supported by DM recommended daily intake (Food and Nutrition Board, 2004). Most of the plant materials contain above 240 mg/100g of Mg. Magnesium plays important role in formation and function of bones, muscles and prevents high blood pressure and depression. Magnesium is also important in the enzyme activity. Magnesium is a vital for the activity of more than 300 enzymes and plays an important role in neurochemical transmission and muscular excitability (Laires *et al.*, 2004).

Table 4: Mineral Composition of *C. swynnertonii* from different parts of the plants (mg/100g) [1, 2]

Minerals	Root	Stem bark
P	276.10 ± 87.23[a]	250.31± 73.68[a]
Cu	8.34 ± 0.93[a]	7.77± 1.59[a]
Fe	334.41 ± 79.89[a]	335.11± 81.58[a]
Zn	11.90 ±1.0[b]	17.10± 2.3[a]
Mn	29.70 ± 3.30[a]	70.30±23.90[a]
Mg	996.30 ± 81.16[b]	1810.01±1.90[a]

[1]Mean± SEM for duplicate analyses

[2]Means with different superscripts are significance different at $p< 0.05$

4.8.2 Iron content

The level of iron observed in *C. swynnertonii* plant was 335.11 mg/100g in the stem bark and 334.41 mg/100g in roots (Table 4). This could justify its use in the management of iron deficiency such as in anaemia cases. This plant could therefore be used as food supplement during pregnancy. Iron is vital for transporting oxygen in the blood. Physiological requirement for iron increases with rapid growth and the expansion of the blood volume and muscle mass. Iron concentration did not differ significantly ($p<0.05$) between the roots and stem bark. However, all plant parts of *C. swynnertonii* that is stem bark and the roots contained enough iron. Thus it could be advised to use the stem bark for the purpose of treatment of anaemia as it is the part which contained iron than the root.

4.8.3 Copper content

Copper is essential in the absorption and utilization of iron during haemoglobin and myoglobin biosynthesis and forms part of several enzyme systems (King, 1993). For

C. swynnertonii, copper concentration did not differ significantly from the two parts of the plant samples ($p < 0.05$). The observed concentration of copper in the present study ranged from 4.42 mg/100g to 10.01 mg/100g in the stem bark samples and 7.32 mg/kg to 9.02 mg/kg in the roots (Table 4). The concentration of copper observed in this study is comparable with previously reported copper analysis values varying from 0.195 to 1.837 µg/g in some selected medicinal plants (Kirmani *et al.*, 2011).

4.8.4 Zinc content

The level of Zinc from *C. swynnertonii* ranged from 11.90 g/100g of the stem bark to17.10 mg/100g in the roots (Table 3). Zinc is a mineral that is of importance for human growth and normal development, bone metabolism, neuropsychiatric and immune functions, and wound healing. Even moderate deficiencies of Zn can have a profound effect on absorption abnormalities, such as cystic fibrosis and inflammatory bowel diseases. It is found in nearly every cell of the body and is necessary for proper function of over 300 enzymes (Rostan *et al*., 2002). Absorption of toxic heavy metals, especially cadmium and lead, is lower in individuals with high compared to those with low zinc status. There is conclusive evidence in many studies that zinc is the most critical micronutrient in the health of the immune system. This complies with Sawidis *et al*., (2010) observation that normal levels of zinc in most crops and pastures are in the range 1.2–73 mg/kg dry weight. Dietary reference values for zinc vary according to the dietary pattern of the country, assumptions on the bioavailability of dietary zinc, age, sex, and physiological status (WHO, 2001).

4.8.5 Manganese content

Table 4 shows manganese content of *C. swynnertonii* examined. The results show no significant difference in manganese content among the roots and stem bark of *C. swynnertonii* under the study at $p < 0.05$. *C. swynnertonii* contains some amounts of Mn at 70.30 mg/kg in the roots and 29.70 mg/kg in the stem bark. Manganese is a constituent of several enzymes and involved in metabolism and important in borne formation (Russel, 2001). Manganese also is essential for haemoglobin formation but excess is harmful Manganese is an important modulator of cells functions and play vital role in the control of diabetes mellitus (Aliyu *et al*., 2008).

4.9 Heavy Metals Composition of *C. swynnertonii*

The results for heavy metals composition are presented in Table 5. The heavy metals analysed in the two parts (Roots and Stem bark) of *C .Swynnertonii* were lead (Pb), Cadmium (Cd) and mercury (Hg).

Table 5: Heavy metals compositions of *C. swynnertonii* from different parts of the plant (ppm)

Heavy metals	Root	Stem bark
Pb	<0.01	0.25
Cd	<0.01	<0.01
Hg	<0.01	<0.01

Results shows higher concentration of lead of *C. swynnertonii* in the stem bark (0.25 ppm) than in the roots (<0.01 ppm) and the concentration of cadmium from roots and stem bark of the plant collected in both sites at the levels below <0. 01 ppm and 0.001 ppm, for mercury the concentration was < 0.01 ppm. Lead and cadmium are non-essential trace elements both in human's body and in plants. They induce

various toxic effects in humans even at low doses (Chishti *et al.*, 2001). Other studies recommended that limits for lead contents in herbal medicine should not be more than 10 ppm and for the cadmium not more than 0.3 ppm and less than 1 ppm and for mercury (Garg *et al*., 2010). For pharmaceutical purpose, the plant should be collected from the areas not contaminated with heavy metals. It can be concluded that this plant under normal circumstances is non toxic

4.10 Side Effects of *C. swynneronii* on Experimental Rats

The dose of water resin extract of *C. swynnertonii* that produced mortality was 100 mg/kg and 200 mg/kg. The symptoms of toxicity observed with extract administration were dose dependent. Three to seven days after administration of extract all rats in the various groups were very weak. Signs observed before death included loss of appetite, diarrhoea, blindness and coma. Mortality was recorded eight days after 200 mg/kg extract of *C. swynnertonii* treatment. The rats treated with 100 and 200 mg/kg of the resin extract of *C. swynnertonii* were depressed and less active compared to the rats in the other groups.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Results of this study revealed that *C. swynnertonii* is nutritionally rich in fibre, carbohydrates and minerals such as phosphorous, zinc, iron, magnesium, copper and Manganese. *Commiphora swynnertonii* was also observed to lower cholesterol levels in experimental animals (rats). In light of the foregoing, it is evident that *C. swynnertonii* resin extract may possess anticholesterolaemic and antilipidaemic. Although the resin extract of *C. swynnertonii* has been claimed to be used by traditionalists for treatment of different ailments by Masai people in Simanjiro district Tanzania, but under these conditions of this study it was observed to have side effect on the high doses (100 mg/kg and 200mg/kg) so some more studies are recommended especially to find the appropriate dose ranges between 50 mg/kg that seems to be safe and 100 mg/kg that demonstrated some toxicity. Therefore, caution must be taken in its usage especially in high doses, although it has been observed to contain high amount of iron which it can be recommended to treat anaemia. Also the interference of *C. swynnertonii* extract in the digestion which led to diarrhoea is an important observation. The effect of *C. swynnertonii* extract in lowering body weight thus suppressing growth for young animals is very critical.

5.2 Recommendations

i. Further studies should be done to extract the active ingredient and the mechanisms action of the resin extract in *C. swynnertonii* which helps to lower the cholesterol levels.

ii. Although this plant namely *C. swynnertonii* has shown to contain some anti-cholesterol properties, it has some side effects such as diarrhoea and loss of weight even at the lower doses. Thus care should be taken to people who use this plant.

iii. There should be further studies on the safety, efficacy and quality of this plant because until this study was conducted, were not known.

iv. If this plant will be used for medicinal and supplementation purpose in further, there is a need to carry out further studies on the effect of different doses.

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