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Antimalarial, toxicity and phytochemicals evaluation of lippia kituiensis and cucumis metuliferus species found in Tanzania

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NM-AIST

ANTIMALARIAL, TOXICITY AND PHYTOCHEMICALS EVALUATION OF *Lippia kituiensis* AND *Cucumis metuliferus* SPECIES FOUND IN TANZANIA

Theopista Mzena

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Life Sciences and Bioengineering of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

This study aimed at evaluating the antimalarial, toxicity and phytochemical profile of *Cucumis metuliferus* and *Lippia kituiensis* used for treatment of malaria in Tanzania. Pulverised plant materials were sequentially extracted in chloroform, ethyl acetate and methanol, the solvent was removed using a rotary evaporator. This extracts were evaluated for antimalarial activity using animal model infected with *Plasmodium berghei*. The negative and positive controls were treated with 1% DMSO and chloroquine (CQ) respectively. *Cucumis metuliferus* percentage suppression was 98.55%, 88.89% and 84.39% for chloroform, methanolic, and ethyl acetate extract respectively. For *L. kituiensis* the percentage suppression was 95.19%, 93.88% and 74.83%, for ethyl acetate, chloroform and methanolic extracts respectively at a dose of 1500, 600 and 300 mg/kg respectively. Phytochemical profile of *C. metuliferus* and *L. kituiensis* methanolic, ethyl acetate and chloroform leaf extract were also determined by GC-MS technique. The analysis revealed the presence of 11 major compounds. Moreover, the extracts were evaluated for acute and sub-acute toxicity. In acute toxicity test, the result showed no significant difference was observed in behavior, body weight and hematology parameters.

The LD_{50} of the *C. metuliferus* and *L. kituiensis* extracts in mice was determined to be not greater than 2000 mg/kg body weight. In sub acute toxicity the rats were orally treated with doses of 150 mg/kg, 300 mg/kg and 500 mg/kg body weight. The results revealed a significant change in body weight, organ weight, hematological and biochemical parameters of rats administered with 300 mg/kg and 500 mg/kg body weight. Histopathological examination revealed the distraction of glomerula and bowman's capsule, distraction of tubules and inflammation of kidneys and bile duct hyperplasia, hepatic necrosis and vacuolation of the liver while the lung showed thickened alveolar wall in a dose of 300 mg/kg and 500 mg/kg body weight. These findings suggest that *C. metuliferus* and *L. kituiensis* have demonstrated antimalarial activities, but with toxicity. To reduce their toxicity and improve their pharmacologic properties, the study suggests that isolation, characterization, structural elucidation of different types of bioactive compound with high potency may serve as candidate to reduce their toxicity effect and hence developing a new beneficial drug.

DECLARATION

I, Theopista Mzena do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology 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

MzenaTheopista _____ _____

Name and signature of candidate **Date**

The above declaration is confirmed by

Prof. HuldaSwai_____ _____

Name and signature of supervisor **Date**

Dr. Musa Chacha _____ _____

Name and signature of supervisor **Date**

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for examination of a dissertation entitled "Antimalarial, toxicology and phytochemicals evaluation of *Lippia kituiensis*, and *Cucumis metuliferus* species found in Tanzania" in fulfillment of the requirements for the Degree of Doctor of Philosophy in Life Sciences and Bioengineering (LiSBE) at Nelson Mandela African Institution of Science and Technology (NM-AIST).

Prof. HuldaSwai_____ **_____**

Name and signature of supervisor **Date**

Dr. Musa Chacha_____ **_____**

Name and signature of supervisor **Date**

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DEDICATION

This dissertation is dedicated to my beloved husband Godson Mafita and our lovely kids Merry Godson Mafita, Nuru Godson Mafita and Isaac Godson Mafita.

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LIST OF ABBREVIATIONS AND SYMBOLS

ACTs	Artemisinin-based combination therapies
ALB	Albumin
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AST	Aspartate transaminase
CREA	Creatinine
CQ	Chloroquine
D0	Day zero
D4	Day four
DEET	NN-diethyl toluamide
DMSO	Dimethylsulfoxide
GC-MS	Gas Chromatography-Mass Spectrometry
GLU	Glucose
HCT	Hematocrit
HGB	Hemoglobin
IRS	Indoor Residual Spraying
ITNs	Insecticide-Treated Mosquito Nets
LYM	Lymphocytes
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MON	Monocytes
MST	Mean survival time
MUHAS	Muhimbili University of Health and Allied Sciences
NEUT	Neutrophil granulocytes
NIMR	National Institute of Malaria Research
OECD	Organization for Economic Cooperation and Development
PBS	Phosphate Buffered Saline
PCV	Packed cell volume
RBC	Red Blood Cell
RDW	Red blood cells Distribution Width
SD	Standard Deviation
SUA	Sokoine University of Agriculture
TB	Total bilirubin
TCHO	Total cholesterol
TG	Triglyceride
TP	Total protein
TPRI	Tropical Pesticides Research Institute
UREA	Urea
WBC	White blood cell

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Malaria is a vector-borne disease caused by protozoan plasmodia parasites namely *Plasmodium falciparum, Plasmodium vivax, Plasmodium malaria* and *Plasmodium ovale* and the most severe form of malaria is caused by *P. falciparum* (Kantele *et al*., 2011), that accounts for over 96% of all malarial infections in sub-Saharan Africa (Galshir *et al*., 2001; Marsh *et al*., 1995). Malaria can be transmitted by infective bites from a female Anopheline mosquito or, rarely, through transfusion of infected blood products or in utero from the mother to the newborn through the placenta and during delivery (Malhotra *et al*., 2006). This parasite targets the erythrocytes by shuttling its own proteins into the surface of the erythrocytes, as a result, the erythrocytes stick to the blood vessels (Bhattacharjee *et al*., 2012). The stickiness of the erythrocytes with the veins is the key factor that leads to hemorrhagic problems of malaria (Adm, 2002). However, the management of malaria is complicated because the parasites that cause the disease are resistant to most of the safest and cheapest first line treatments developed so far.

1.1.1 Global burden of Malaria

Malaria is an important disease that has a global distribution and a significant health burden. In 2016, there were an estimated 445 000 deaths from malaria globally, compared to 446 000 estimated deaths in 2015 as reported by World Health Organization (WHO, 2017). The WHO African Region accounted for 91% of all malaria deaths in 2016, followed by the WHO South-East Asia Region (6%). Fifteen countries accounted for 80% of global malaria deaths in 2016, all of these countries are in sub-Saharan Africa, except for India (WHO, 2017). According to the world health report, about 214 million cases of malaria were reported worldwide in 2015, and an estimated 446 000 deaths (WHO, 2015), 92% of all malaria deaths occur in Africa region (WHO, 2016). Most malaria cases in 2016 were in the WHO African Region 90%, followed by the WHO South-East Asia Region 7% and the WHO Eastern Mediterranean Region 2% (WHO, 2017). As shown in Fig. 1, in Sub-saharan Africa, malaria remains a major public health hazard owing to its high morbidity and mortality despite being the focus of significant financial support and research. It is estimated that malaria costs Africa USD 12 billion per year in direct costs and reduces GDP growth by 1.3% annually (WHO, 2014). The

burden is carried mostly by poor, rural families that have less access to current prevention and treatment services. The reduction of malaria occurrence and transmission of parasites from humans to mosquitoes have been achieved largely through the use of insecticide treated mosquito nets (ITNs) and indoor residual spraying (IRS). The burden has been reduced by an estimate of 55% in children by the use of ITN whereas about 116 million people worldwide reported to be protected by IRS in 2014 (WHO, 2015). In addition, Artemisinin-based combination therapies (ACTs) are highly effective against *P. falciparum*, the most prevalent and lethal malaria parasite affecting humans. Globally, the number of Artemisini-based Combination Therapy treatment produced by manufacturers estimated to be 409 million treatment courses of Artemisinin-based Combination Therapy (ACT) in 2016, an increase from 311 million in 2015. Over 69% of these procurements were reported to have been made for the public sector. The African Region accounted for most (99%) manufacturer deliveries of ACTs in 2017. Despite such efforts, malaria still represents a major health burden, particularly in Africa. Therefore, more effort in many Africa countries is needed to address these challenges.

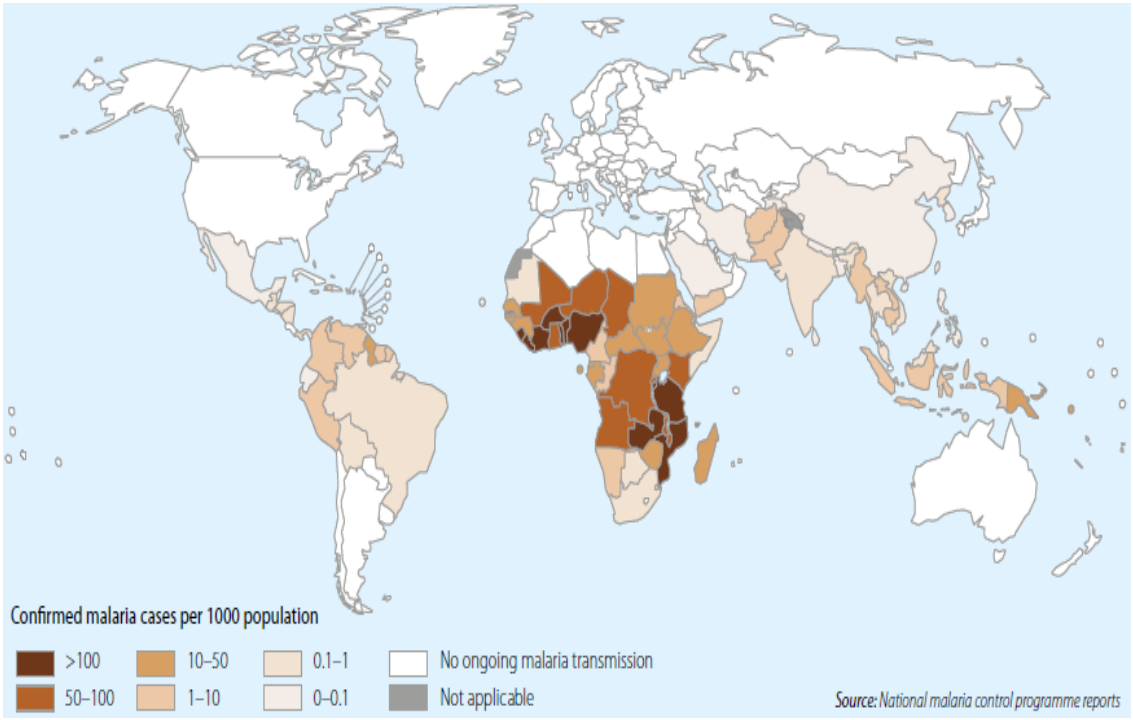


Figure 1: Countries with ongoing malaria transmission, more cases in sub-Saharan countries (WHO, 2014)

1.1.2 Malaria in Tanzania

Malaria remains a major public health problem in sub-Saharan Africa, with approximately 1 million deaths and more than 400 million cases a year. Over 93 % of the Tanzania mainland populations live in areas where malaria is endemic. In Tanzania, there is great variation in the risk of malaria transmission and prevalence ranging from 1–33 %, with an average of about 10 % as reported by The Tanzania Commission for AIDS (TACAIDS, 2012). Malaria risk in Tanzania is diverse with malaria prevalence rates, parasite densities and entomological inoculation rates varying from one area to another. The prevalence rates usually vary by region from <1% in Northern parts to >30% along the Lake Victoria shores. In Tanzania Mainland, it is estimated that more than 90% of the 44 million people are at risk of contracting malaria and about 10-12 million malaria cases cost their lives annually as reported by Ministry of Health and Social Welfare (MOHSW, 2015). *Plasmodium falciparum* accounts for 96% of malaria infection in Tanzania, with the remaining 4% due to *P. malariae* and *P. ovale* as reported by The United States Agency for International Development (USAID, 2013). The principal vectors of malaria in Tanzania are mosquitoes of the Anopheles gambiae complex (USAID, 2013). Contrary to that, morbidity and mortality have been substantially reduced, with a decrease of ≥75% in the numbers of malaria cases, in-patient malaria cases and deaths in recent years (WHO, 2016). The decline of malaria burden in many parts of the country attributed to the deployment of malaria interventions including mass distribution of insecticide treated nets (ITNs), Indoor residue sprays (IRS) and treatment with antimalarials (Jacobson *et al*., 2014). Additionally, changes in malaria treatment policy in 2006 from Sulphadoxine/pyrimethamine to Artemisinin-based Combination Therapy (ACTs) as first line drug for malaria treatment in Tanzania National Malaria Control Programme (NMCP, 2006). Despite such reduction, residual malaria is still observed in many parts which still cause morbidity and mortality both in rural and urban areas (Russell *et al*., 2015). Therefore, efforts are still needed to maintain an effective surveillance system to prevent and to treat malaria properly as the risk of *Plasmodium falciparum* resistance to available drugs and insecticides is still high. In this context, the search for new anti-malarial agents remains a priority.

1.1.3 Malaria and life cycle of plasmodium species

There are numerous transitions and stages, which comprises the life cycle of the malaria parasites and these can be divided into three main stages as shown in Fig. 2.

(i) Mosquito stage: This is also referred to as the sexual stage from gametocytes to sporozoites in the female *Anopheles* mosquito.

(ii) Human liver stage: This is the asexual exo-erythrocytic schizogony (tissue schizogony or the multiple fission of a trophozoite or schizont into merozoites) in the liver cells of the primate host.

(iii) Human blood stage: This is the asexual erythrocytic schizogony in the red blood cells. When an infected female *Anopheles* mosquito bites a person, elongated motile sporozoites in her saliva are injected into the human blood circulation (step 1). The parasites rapidly leave the blood circulation and are taken up into the liver, where they pass through the Kupffer cells and infect the hepatocytes to initiate the human liver stage infection (step 2). This asymptomatic tissue stage of infection lasts for 5 to 16 days, depending on the species of *Plasmodium*. However, in all species of *Plasmodium*, these parasites multiply and develop to form exo-erythrocytic (tissue) schizonts from which several thousand merozoites develop (step 3). In *Plasmodium vivax* and *Plasmodium ovale* only, a proportion of the liver stage parasites remain in the hepatocytes as a dormant form or hypnozoite (Hemmer, 2006).

This stage of the parasite can remain dormant for a few weeks or up to several years and can, therefore, start a life cycle of asexual reproduction without the need for further mosquito bites. Hence, the *P. vivax* infection is also referred to as relapsing malaria. However, in *P. falciparum* and *P. malariae* infections, the tissue schizonts rupture more or less simultaneously leaving no form of the parasites in the liver. The infected hepatocytes rupture and release these merozoites into the bloodstream as aggregates called merosomes that allow the merozoites to evade antibodies and rapidly invade red blood cells (step 4). This initiates the human blood stage or the erythrocytic cycle of infection. The intra-erythrocytic parasites undergo asexual development from young ring forms to trophozoites and finally to mature schizonts. Asexual blood stage parasites produce 8-20 new merozoites every 48 hours (or 72 hours for *P. malariae*), causing the parasite number to rise rapidly to higher levels in the human host. The *P. falciparum* is also distinguished by its ability to bind to end othelium during this blood stage of the infection and sequester in organs, including the brain. Antibodies that blocks (step 5) binding of *P. falciparum*-infected erythrocytes to end othelium might prevent disease and control parasitemi. The asexual stages are pathogenic whereas the sexual stage parasites are non-pathogenic but transmissible to the *Anopheles* vector (Aravind *et al*., 2003). The parasites

replicate synchronously to release more merozoites into the blood stream leading to the classical cycle of fever that is observed as afebrile clinical attack. The released merozoites then invade more erythrocytes to continue the cycle, which proceeds until the death of the host or modulation by drug treatment or acquired immunity (step 6). At a certain stage of malaria, some merozoites infecting red blood cells differentiate into sexual forms known as male and female gametocytes (step 7). In *P. falciparum*, these are formed in the later stages of infection. By contrast, they are formed at the same time as the asexual stages in *P. vivax.* Gametocytes are taken up into the female *Anopheles* mosquito gut during a blood meal to initiate the mosquito stage of infection (step 8). The male gametocytes are activated (ex- flagellation) followed by male gametogenesis to form gametes which fuse with the female gametes (fertilization) in the gut of the insect to form diploid ookinetes. These ookinetes migrate to the midgut of the insect and the resulting zygote develops in the gut wall to form the oocysts (step 9). These eventually give rise to the infective sporozoites by meiotic division and then migrate to the salivary gland of the mosquito (Fidock *et al.*, 2004). The periodicity of parasitaemia and febrile clinical manifestations in tertian or quartan malaria thus depends on the timing of schizogony of a generation of erythrocytic parasites (Goodman & Gilman, 1992).

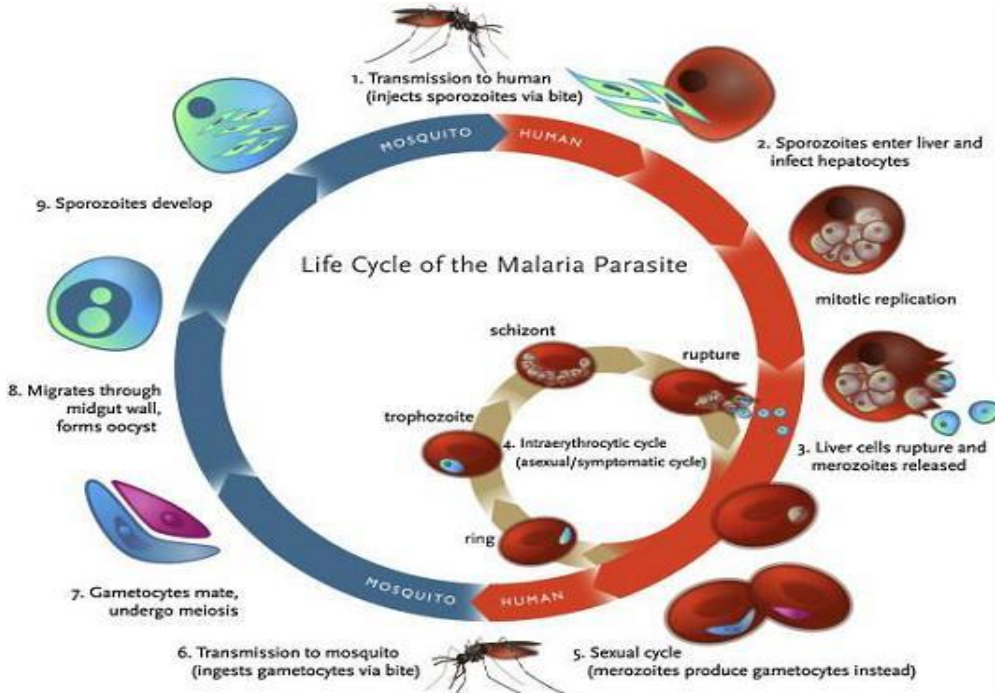


Figure 2: Life Cycle of the malaria parasite (Klein, 2013)

1.1.4 Malaria and natural products drug development

Natural compounds have been the source antimalarial drug known to date. Recent surveys have identified many extracts of various organisms (mostly plants) as having antiplasmodial activity. Huge libraries of fractionated natural compounds have been screened with impressive hit rates. Natural products are the sources of the two most important drugs currently available to treat severe *falciparum* malaria, that is, quinine and artemisinin derivatives. In the case of artemisinin, relatively simple chemical modification of the natural product parent compound has led to a series of highly potent antimalarials that are playing an increasingly important role in the treatment of malaria (Meshnick, 2002). With the current spread of drug resistance and the emergence of artemisinin-resistant parasites, new drugs for the treatment of malaria are urgently needed. However, the challenge facing malaria chemotherapy today is to find safe and selective agents whose potencies are not compromised by malaria parasite resistance and thus promote more efforts to discover lead compounds providing valuable bioactive scaffolds, which could be further adjusted by semi-synthetic approaches to obtain effective antimalarials.

1.1.5 Screening of traditional medicinal plants for antimalarial activities

In sub-Saharan Africa and other parts of the world where malaria is endemic, herbal remedies are commonly used to treat the disease. The understanding is that traditional medicinal plants that are employed for the treatment of malaria represent a potential for discovery of lead molecules for development into potential antimalarial drugs (Rukunga *et al*., 2007). In most African, about 80% of the population rely on medicinal plants for primary health care (WHO, 2008b), and since the discovery of artemisinin as an effective antimalarial isolated from the herb plant *Artemisia annua* a lot of interests have shifted to plant sources as antimalarial agents Klayman *et al.,* (1985). The screening of plants known to cure malaria in ethno medicine is therefore, an important strategy in the treatment and control of malaria. *Cucumis metuliferus* and *Lippia kituiensis* are some of the medicinal plants traditionally used to treat malaria in Tanzania. *Cucumis metuliferus* (Cucurbitaceae) also called 'African horned cucumber' is a monoecious, climbing annual herb, with staminate flowers typically appearing several days before pistillate flowers (Morton *et al.,* 1987). The fruits are ovoid berries of between 8 and 10-centimeter-long, 4 and 5 cm in diameter, reddish orange at maturity, hanging, and covered with strong spiny out-growth (Benzioni *et al.,* 1993). It occurs in tropical Africa, especially Nigeria, Botswana, South Africa, Namibia, Zimbabwe, Malawi and Tanzania (Morton *et al*., 1987). In some areas this plant has been used by traditional healers for the management of

various ailments including peptic ulcer disease, diabetes mellitus, hypertension and HIV/AIDS (Personal communication, 2006). The fruit pulp was found to possess alkaloids, flavonoids, glycosides, which are known to produce biological activity. Information on toxicological studies done on the plant material is not much however, Wannang *et al*. (2007) demonstrated that the extract altered serum enzymes in laboratory animals.

Lippia is a genus of flowering plants in the verbena family, Verbenaceae. The genus contains roughly 200 species of tropical shrubs that are found around the world. Plants are fragrant due to their essential oils, which vary between species but may include estragole, carvacrol, linalool, or limonene. The leaves of certain species, such as *L. graveolens*, can be used as a culinary herb similar to oregano. Lippia species are also used traditionally for the treatment of a variety of ailments such as gastrointestinal, respiratory complaints and for relaxation and sedation (Joubert *et al*., 2011). Other studies also linked the use of *Lippia kituiensis* and *C. metuliferus* multiflora to the treatment of blood pressure, malaria, diarrhea and mild hypertension (Arthur *et al*., 2011).

However, little scientific information about their antimalarial activities against *Plasmodium* species and safety for use are available. It is important, therefore, to investigate the antimalarial activities of these plants to determine their potentials as new antimalarial compounds

1.2 Problem statement

Malaria is a major public health problem that is currently complicated by the increasing resistance of *P. falciparum* against the mainstay drugs (Batista *et al*., 2009; Nguta *et al.*, 2011). Despite extensive efforts to control malaria, the disease remains a major public health threat throughout the tropics, with persistent severe malarial morbidity and increasing resistance to malaria drugs, including new artemisinin-based combination therapy (ACT). There is a compelling need for new and improved treatments for malaria. Discovery of the lead compounds against malaria parasites is a crucial step to ensure a sustainable global pipeline for effective antimalarial drugs.

Considering important roles for natural products in the treatment of malaria, the screening of medicinal plants that are traditionally used to treat malaria is a credible approach to discover new antimalarial leads. In view of the increasing use of medicinal plants, it is necessary to carry out toxicity studies to determine lethality and the safest and effective dose that is appropriate for human consumption with a view to validate and document the safety of these medicinal

plants with antimalarial properties to the society. This study, therefore, focused on the antimalarial activity, toxicity and phytochemical analysis of two medicinal plants namely *Lippia kituiensis* and *Cucumis metuliferus* growing in Tanzania. These plants were selected on the basis of the literature reviews and verbal information collected from local traditional healers in Ugweno ward within Mwanga District, Kilimanjaro Region in Tanzania.

1.3 Rationale of the study

About 80% of the World's people rely on Traditional medicine (UNEP, 2010). Plants have always been considered to be an alternative source of new drugs and some of the antimalarial drugs in use today such as artemisinin were derived directly from medicinal plants or structures modeled on plant derived compounds (Nguta *et al*., 2010). The important advantages for therapeutic uses of medicinal plants in various ailments are their safety, economical, effective and easy availability. Increased side effects of conventional drugs/development of resistance by parasites that cause malaria has become a global concern and thus highlights the need to develop novel antimalarial drugs that are not only active against drug resistant parasites, but more importantly, kill persistent parasites and shorten the length of treatment.

1.4 Objectives

1.4.1 General objectives

The general objective of this study was to evaluate antimalarial activity, toxicity and phytochemical analysis of *L. Kituiensis* and *C. metuliferus* used for management of malaria in Tanzania.

1.4.2 Specific objectives

(i) To evaluate *in-vivo* ant plasmodium activity of *L. kituensis,* and *C.metuliferus* species found in Tanzania.

(ii) To screen for phytochemical compound present in *L. kituiensis* and *C. metuliferus* extract by using Gas Chromatography-Mass Spectrometry (GC-MS).

(iii) To evaluate *in-vivo* acute and sub acute toxicity of *L. kituiensis* and *C. metuliferus* extract in mice and rats' models respectively.

1.5 Research questions

The study sought to answer the following questions

(i) What are the ant-plasmodium potentials of crude extract of leaves from *Lippia kintuensis* and *Cucumis metuliferus* species?

(ii) What are the phytochemical constituents present in *Lippia kintuensis* and *Cucumis metuliferus* of methanol, ethyl acetate and chloroform extracts?

(iii) What are the acute and sub acute toxicity effects in albino rats of crude extracts of *Lippia kintuensis* and *Cucumis metuliferus* species?

1.6 Significance of the study

This study opens up the search for novel antimalarial 'leads' from *L. kituiensis* and *C. metuliferus* the medicinal plants with reported antimalarial efficacy and locally used to treat malaria in Tanzania. This study also illustrates secondary metabolites that can be associated with their antimalarial efficacy. In this study, the compounds associated with antimalarial efficacy of plant extract are highlighted, paving the way for further research to be done to isolate the identified compound(s).

The *in vivo* toxicity profiles from *L. kituiensis* and *C. metuliferus* have been made evidenced in this study. The study has shown that the toxicity of the extracts from the plant exceeded the therapeutic levels hence these plants should be used with care. This research adds to the current knowledge on the effectiveness of the plant and thus validates its ethnobotanical value for local communities. This data can extensively contribute to policy development towards the channeling of safe and efficacious medicinal plants to primary healthcare, particularly in Malaria management. In addition, with the increasing interests in medicinal plants, the present study revealed the possibility of developing plant-based antimalarial agents for the treatment of malarial diseases. Furthermore, the study provided scientific evidence on the toxic effects of these plants in animal models.

1.7 Delineation of the study

This research is concerned with evaluatig antimalarial activity and its compounds of two medicinal plants namely *C. metuliferus* and *L. kituiensis* used traditionally to treat malaria in Tanzania. Furthermore, the study provided scientific evidence of the efficacy of these plants through in *vivo*-acute and Sub-acute toxicity in an animal model, that all plant materials entailed for medical purposes should be thoroughly tested for their efficacy and safety before being approved for human consumption. The antimalarial activity of these two plants was expressed by counting the number of parasites in a given volume of the blood. The study aimed

to use the mice model to test the activity of these plants against *Plasmodium berghei*. This result has established the rationale for the traditional use of the plants in the treatment of malaria.

The study uses GC-MS to reveal unique chemical classes present in the *L. kituiensis* and *C. metuliferus* that might be responsible for the antiplasmodial effect against *Plasmodium berghei.* The study identified unique chemical classes present in the *C. metuliferus and L. kituiensis* that the antimalarial effects observed may be attributed to these compounds observed. The study went far on evaluating adverse effects or safety of a substance that result either from a single and multiple exposures within 14 and 28 day to determine the toxicity of the substance. The study uses a mice model to test toxicity for single exposure and rat's model for everyday doses the plants showed severe toxic to animals. Since malaria is a major public health problem that presently is complicated by the increasing resistance of *P. falciparum* against the current drugs, this has necessitated the search for novel antimalarial agents that have a unique mechanism of action.

CHAPTER TWO

LITERATURE REVIEW

2.1 Historical background

Effective malaria treatment originated in the 17th century with quinine, extracted from the back of the cinchona tree (*Cinchona officinalis*) (PATH, 1990). For more than three centuries quinine and other alkaloids of cinchona trees, such as quinidine, cinchonine and cinchonidine were the only effective drugs available for the treatment of malaria (Brautbar *et al*., 1985). In recent times, however, synthetic antimalarials were introduced as they were found to be superior to quinine and also less toxic (Brautbar *et al*., 1985). These include 8-amino quinolines e.g. primaquine; 4-aminoquinolines e.g. chloroquine and amodiaquine; biguanides e.g proguanil, diamino Pyrimidine e.g. pyrimethamine, sulfones and sulfonamides and antibiotics e.g. tetracycline. For these synthetic antimalarials, chloroquine was found to be excellent both as curative and preventive, with a very wide range of other useful qualities. It is non-toxic safe, reliable, inexpensive and highly effective and a rapid blood schizontocide against all forms of malaria parasites. Therefore, at that time it was adopted as the first line drug of choice (PATH, 1990; Bruce-Chwatt *et al*., 1985). In the 1960's, however, resistant strains of *P. falciparum* began to emerge in South – East Asia and Latin America (PATH, 1990). In Africa, resistant *P. falcipanum* malaria was first reported in Kenya by Jepsen *et al.* (1979) and since then it has spread throughout the continent, with resistance first recorded in Nigeria (Olorunnisola *et al*., 1989; Daniel *et al*., 1989). The widespread resistance to chloroquine and other synthetic antimalarials necessitated the development of alternative second line drugs such as Sulphadoxine/Pyrimethamine, Pyrimethamine/Sulphaline, sadly cases of resistance to these second line drugs became widespread and persistent. It is creditable to note that the emergence of widespread resistance of *P. falciparum* to chloroquine, the drug of choice, led to additional studies that produced new recent and effective antimalarial drugs the artemisinin derivatives (Ozer *et al*., 2007). These artemisinin based compounds which were developed first in China fortunately happened to arrive on the market just at a time when resistance to earlier drugs was becoming a very serious issue (Anderson *et al*., 2007). The World Health Organisation recommended that all countries experiencing resistance to monotherapies such as chloroquine, primaquine should use artemisinin-based combination therapies in order to ensure a high cure rate of *P. falciparum* malaria and to reduce the spread of resistance (WHO, 2006). As a result, the majority of *falciparum* malaria endemic countries have adopted Artemisinin-based Combination Therapy (ACT) as first line treatment and the deployment of ACTS in the public

sector has increased exponentially in recent times (WHO, 2006). In order to delay eminent emergence of resistance, artemisinins are now combined with long-acting drugs, especially with amodiaquine, mefloquine, lumefantrine or sulfadoxine-pyrimethamine (Gotep *et al*., 2009). Currently four artemisinin combination therapies (ACTs) are recommended for treatment of malaria: Artemether-lumefantrine, artesunate-amodiaquine, artesunate-mefloquine and artesunate –sulfadoxine – pyrimethamine (WHO, 2015).

2.2 Resistance to antimalarial drugs and the use of artemisinin combination therapy (ACT)

Resistance is defined as 'the ability of a parasite strain to survive and/ or multiply despite the administration and absorption of a drug in doses equal to or higher than those usually recommended but within limits of tolerance of the subject' (WHO, 1973). The development and spread of *P. falciparum* resistance to most commonly used antimalarial drugs is a major challenge in the control of malaria since it hinders our capacity to roll back malaria (Olliaro *et al*., 2005). Some scientists such as Kochurova *et al*. (2009) pointed out that parasite resistance has caused some of the least expensive traditional antimalarial drugs to be ineffective. Additionally, they said that, because there is a concern that resistance will emerge against the current first-line drugs such as the ACTs, there is currently great interest in discovering the next generation of antimalarial drugs. A strategy that has received much attention recently to combat drug resistance is the use of a combination of antimalarial drugs, such as mefloquine, sulfadoxine/pyrimethamine (SP), or amodiaquine, with an artemisinin derivative (Vasu *et al*., 2006). Currently, four artemisinin combination therapies (ACTs) are recommended for treatment of malaria: Artemether-lumefantrine, artesunate-amodiaquine, artesunate-mefloquine and artesunate - sulfadoxine – pyrimethamine (WHO, 2009). Balogun (2003) seems to have somehow a contrary view of malaria therapy using ACT in Africa. He said that while ACT unquestionably holds wonderful promise as a malaria treatment, the reality of ACT today is somewhat problematic. One problem he noted was availability; that substantial quantities of quality artemisinin drugs are actually hard to come by. The second problem is the cost. Artemisinin combination therapies are far more expensive than currently used treatments and more than most African economies can sustain. Be that as it may, ACTs are being used as first- line drugs for malaria treatment in most African countries (WHO, 2009). However, there is the fear among scientists that the most common and most debilitating malaria parasite in the tropics, *P. falciparum*, might soon develop resistance to artemisinin-based combination drugs. Bantie *et al.* (2007) pointed out that although the artemisinin derivatives retain excellent

efficacy, the selection of resistance to the artemisinin is a matter of time. He observed that since treatment courses are generally short, the artemisinin component is dependent upon the partner drug for adequate clinical efficacy. Furthermore, when resistance to the partner drug reaches critical level, the efficacy of ACTs would fall and would no longer be suitable for malaria treatment.

2.3 Medicinal plants as alternative sources of antimalarial compounds

Alsllami *et al*. (2007) pointed out that the problem created by drug-resistant strains of malaria especially *P. falciparum* in malaria therapy has accelerated antimalarial drug research over the last two decades. While synthetic therapeutic agents continue to dominate research, attention has increasingly been directed to natural products. Similarly, Grover *et al.* (2008) said that drug resistant strain of *P. falciparum* has compromised malaria therapy and has led to the search for new lead compounds in medicinal plants used in folk medicine for the treatment of the diseases. In search of new, safe, affordable and effective antimalarial drugs, phytochemical screening of extracts of plants used in traditional medicine becomes necessary as pointed out by Tchouankeu *et al.* (2007). Medicinal plants have been part of African cultures for centuries, therefore, the search for newer, more effective anti-malaria drugs is a major challenge (Jimam *et al*., 2007) and a very welcome development, and it is believed that traditional plants which have been used to cure malaria are bound to give us effective antimalarial compounds (Anderson *et al*., 2007). Additionally, plants commonly used in traditional medicine are assumed to be safe due to their long usage in the treatment of diseases according to knowledge accumulated over centuries (Ajaiyeoba *et al*., 2006).

2.4 Current status of drug discovery from plant sources

Many medicines used against different diseases including malaria, cancer, diabetes, hypertension, neurodegenerative disorders and infectious diseases have been sourced from a plant or designed based on scaffolds of compounds isolated from plants. The latest of these include artemether (antimalarial), galantamine (for Alzheimer's disease), nitisinone (for tyrosine-associated metabolic disorder) and tiotropium (anticholinergic), which have all recently been introduced in the United States or are currently involved in late-phase clinical trials (Balunas *et al*., 2005). Drug discovery from medicinal plants involves a multi-thronged approach that includes, but is not limited to traditional medicine practitioners, botanists, medicinal chemists, pharmacologists and molecular biologists. Conventionally, plants are selected either randomly or based on their claimed historical medicinal relevance and subjected

to sequential extraction and purification steps. This can be very tedious and time-consuming and more effective methods for identifying new lead molecules from plants have been explored. These include chemoinformatics and bioinformatics as tools for in silico drug discovery (Lagunin *et al.*, 2014), systems/polypharmacology approach which integrates oral bioavailability tests, druggability, blood-brain barrier permeation, target identification and network analysis owing to the complex composition of medicinal plant extracts and their diverse physiological effects (Liu *et al*., 2013). High throughput pharmacological screens and genetic manipulation have also been applied to discover new drug leads from plants, in which plants extracts are screened against an array of receptors with or without gene manipulation and compared to existing drugs (Litteleton *et al*., 2005).

2.5 Approaches in antimalarial drug discovery

Six major approaches to antimalarial drug discovery have been identified and reviewed, including the investigation of natural products (Rosenthal *et al*., 2003). A plant-based approach is particularly useful in resource-poor, malaria-endemic areas where nearly one-fifth of patients rely on herbal remedies to treat malaria and febrile illnesses (Willex *et al*., 2004). The choice of plants for antimalarial drug discovery maybe based on both random and empirical methods to explore biodiversity or through studies guided by the traditional use of the plant in the treatment of fever. The latter ethnopharmacological approach has been recognized to give higher success rates for finding active compounds, as over 50% of extracts from ethno medicinal plants were active *in vivo* and/or *in vitro* (Cawalho *et al*., 1998).

2.6 Ethnopharmacology-based plant selection and extraction

Herbal medicines have played a pivotal role in health and disease management for many centuries. Different ancient civilizations, including Mesopotamian, Indian ayurveda, ancient traditional Chinese medicine and Greek unani medicine, show documented evidence for the use of herbs in the treatment of different ailments. In Africa, knowledge of traditional medicine constitutes part of a holistic system, passed through generations by oral communication and indigenous practices (Romero-Daza *et al*., 2002). The scientific exploitation of herbs used ethno medicinally for pain relief, wound healing and abolishing fevers has resulted in the identification of a wide range of compounds that have been developed as new therapeutics (Harvey *et al*., 2008). The major role of ethnopharmacology is to discover new plant-derived compounds based on the traditional use of medicinal plants. The knowledge on the use of plants for fevers and other symptoms of malaria are used to guide the selection of plants to be

subjected to antimalarial screening and isolation of active constituents. This is a favored and conservative approach in drug discovery as historical use of a plant as medicine increases the possibility that safe and pharmacologically active compounds would be isolated from it.

2.7 In vivo and in vitro antimalarial activities of medicinal plants reputed to treat malaria

The literature on researches conducted in the tropics and sub-tropics on the *in vitro* and *in vivo* antimalarial activities of plants traditionally used in the treatment of malaria and related fevers are vast. Screening of medicinal plants for antimalarial activities is a strategy employed by scientists in the malarious regions of the world to validate or justify or support the use of such plant remedies in malaria therapy. These researches are also efforts to produce the next effective, affordable and safe antimalarial drugs (Anderson *et al*., 2007).

In vitro antiplasmodial evaluation studies involve the use of micro-test well plates for the assessment of the response of fresh isolates of *Plasmodium* species obtained from malaria patients (Berzins *et al*., 1997; WHO, 2001b; Basco & Ringwald, 2007) or from continues cultures of chloroquine sensitive or resistant strains of *P. falciparum* (O'Neil *et al*., 1986; Ngemenya *et al*., 2006; Omar *et al*., 2007). On the other hand, the evaluation of *in vivo* plasmodial activity normally involves the inoculation of experimental animals (mice or rats) with *Plamodium berghei* followed by administration of drug or extract to observe therapeutic effects (Abosi *et al*., 2003; Okokon *et al*., 2006; Bickii *et al*., 2007; Ogbunufagor *et al*., 2008).

Jenett-Siems *et al*. (1999) studied the *in vitro* antiplasmodial activity of Central American medicinal plants. Their result revealed antiplasmodial activity *in vitro* of some of the remedies tested and concluded that selection of plants by ethnobotanical criteria may provide promising sources of potential antimalarial lead compounds. The antimalarial activity of *Swartziamada scariensis*, *Cumbretum glutunosum* and *Tinosporabakis*, Burkina Faso medicinal plants was evaluated by Ouattara *et al*. (2006). They reported that the extracts of *S. madagascariensis, C. glutunosum*and *T. bakis* possess some measure of antimalarial activity. The *in vitro* antiplasmodial activity of *Enicostemmalittorale*, a plant traditionally used for malaria treatment in India, was evaluated by Soni *et al*. (2009). Their report demonstrated the antiplasmodial activity of *E. littorale* against *P. falciparum* and the potential antimalarial action of the plant and its active phytoconstituents. Moreover Ngemenya *et al.* (2006) screened some products of *Turreanthus africanus*, a plant used in traditional medicine to treat malaria in Southwest Cameroon. Their result showed that *T. africanus* has weak antiplasmodial activity, which

probably when combined with other antiplasmodial plants results in enhanced antimalarial effect. Kayembe *et al.* (2010) investigated the *in vitro* antimalarial activity of 20 quinones isolated from 4 plants used by traditional healers in the Democratic Republic of Congo. They reported that quinones isolated from *Cassia alata*, *C. occidentalis, Garcinia kola* and *Ocimum basilicum* have interesting antimalarial activities IC50 < 1 μg/ml for 12 of them. In the same vein, Bero *et al.* (2009) evaluated the *in vitro* antiplasmodial activity of crude extracts of 12 plant species traditionally used in Benin for the treatment of malaria in order to validate their use. Their study justified the traditional use of some of the investigated plants to treat malaria in Benin. They concluded that the dichloromethane extracts of *Acanthospermum hispidumaerial* parts, *Keetialeucantha* (leaves and twigs), *Carpolobia lutea* aerial parts and *Strychnos spinosa* leaves showed promising antiplasmodial activities. Ademowo *et al.* (2007) also investigated the *in vitro* antimalarial activity of methylene blue (MB) against field isolates of *P. falciparum* from children in Southwest Nigeria. Their preliminary study showed that MB has a potential to be used as schizonticidal antimalarial. The *in vivo* antimalarial activity of root bark and leaves of *Vernonia amygdalina* (bitter leaf) was evaluated by Abosi *et al*. (2003). The leaf extract produced 67% suppression of parasitemia while the rootbark produced 53% suppression, justifying the use of the plant parts for malaria treatment in herbal medicine. Ogbunogafor *et al*. (2008) also studied the tolerance and antiplasmodial activity of *Ritchealongipedicellata*in *Plasmodium berghei*. Their result revealed a dose dependent therapeutic activity, which means a higher dose is required to clear the parasites from the blood stream once infection has been established. The result also justifies the use of *R. longipedicellata* as an antimalarial in herbal medicine in Nigeria. Okokon *et al*. (2006) studied the *in vivo* antimalarial activity of stem bark of *Mammea africana*, a plant used traditionally by the Ibibios of the Niger Delta region of Nigeria for the treatment of malaria and related fever. They reported that *M. Africana* possesses antimalarial activity and therefore justifies its folkloric use as an antimalarial. Innocent *et al*. (2009) screened traditionally used plants in Tanzania for *in vivo* antimalarial activity in mice to establish the validity of their claims. Their work revealed that the extracts of six plants used in traditional medicine exhibited *in vivo* antimalarial activity, but three had very weak activity. They concluded that *Caesalpinia bonducella* root and *Cassia abbreviate* leaf ethanol extracts were the most promising.

2.8 Toxicity studies

Toxicity is the degree to which a substance can harm humans or animals. It can be measured by its effects on the target organism, organ, tissue or cells (Kifayatullah *et al*., 2014). The toxic

effects of a substance on animal physiology can range from minor changes such as reduced weight gain, small physiological alteration or change in the levels of circulating hormones, to severe effects in organ functional loss leading to death. Intermediate levels of toxicity may cause pain and suffering (Home office, 2004). During toxicity studies, five major aspects are put into consideration. These include organs affected by the chemical, relevance of quantification effect, concentration of the chemical to be tested, *in vitro* markers of toxicity that is relevant to the chemical and how to use the data from *in vitro* test for risk assessment (Said *et al.,* 2000).

Toxicological studies in the pharmaceutical field have been growing exponentially. These developments have been prompted by the discovery of teratogenic effects of drugs such as thalidomide, exposure of chemicals to the environment and employees and by conduct and assessment of toxicity studies as part of good manufacturing practice (Traina *et al.,* 2006). In the study of herbal remedies, it has been found that toxicity may result from inadvertent substitution of one plant species another. For example, rapidly progressive renal failure resulting in end-stage renal disease has been reported in women who have taken weight-reducing pills containing the Chinese herbs *Stephania tetrandra* and *Magnolia officinalis*. This so called Chinese–herb nephropathy is characterized by a pattern of interstitial fibrosis (Kripa *et al*., 2011). The cause of the disease was later noted to be due to inadvertent inclusion of *Stephania fangchi* containing the nephrotoxic and carcinogenic aristolochic acids instead of *S. tetrandra* that contains weight reducing tetrandrine (Nortier *et al.,* 2000; Rotblatt *et al*., 2002).

Another reported case on herbal toxicity involved patients using a dietary supplement containing herbal constituents yohimbine in addition to norephendrine, sodium usiniate and 3,5 diiodothyronine. All patients developed hepatotoxicity within 3 months, recovering spontaneously on withdrawal of the supplement (Flavreau *et al*., 2002). Another study carried out in Zimbabwe confirmed an increase in incidences of poisoning by herbal remedy since 1971 (Nyanzema *et al.,* 1986). These facts support the need to have toxicity profiling of all herbal remedies. Qualitative toxicity assessment of a chemical substance in laboratory animals or *in vitro* toxicity testing gives information on its potential to cause toxic effects in humans or animals (Descote *et al*., 1996). To succeed in this exercise, an appropriate choice of control group must be selected, a sufficient number of laboratory animals used and a good selection of rigorous experimental protocols. Furthermore, the severity of the effect described on major organs and the relevance of the mechanisms involved including the variations in different

species assist in the extrapolation of toxicological findings from laboratory animals to man (Descote *et al*., 1996).

During the study, the target organ of toxicity in laboratory animals are identified, the mechanism of induced changes is noted and compared to the properties of the target site in man. Determination of the toxic potential of new compounds constitutes a major part in drug development and it involves both *in vivo* and *in vitro* toxicological tests. These tests are very critical in the assessment of the safety of all pharmaceutical products before they are released for general use. Animal models are used in *in vivo* studies as indicators of human toxicity (Magna *et al*., 2007). Toxicity testing on herbal extracts is carried out on the same principles as conventional medicine. *In vitro* toxicity testing employs the use of models such as the brine shrimp lethality test (BST) whereas in *in vivo* methods, animals such as mice or rats are used. The advantage of the brine shrimp in toxicity testing is that the shrimp has a lot of homogeneity in eggs and in newly born nauplii which are highly sensitive to chemicals. The eggs are easily available and hatch with ease within 16-24 hours to the nauplii. The nauplii are utilized within 24-48 hours post hatching. At 24 hours post exposure of nauplii to the test chemical, the concentration causing 50 % lethality (LC_{50}) is determined. The BST method is used to screen for bioactivity, anticancer, cytotoxicity, toxicity, pesticidal activity or gastro protective action among other pharmacological effects of plant extracts. Brine shrimp lethality is a rapid method that is reliable, inexpensive and convenient as an in-house general bio-assay tool (Meyer *et al.*, 1982).

This method is basically used to predict toxicity and the results obtained are compared with oral acute toxicity in rodents (Amenya *et al.,* 2011). *In vivo* toxicity testing mainly employs the use of rats although other rodents may be used. Dogs and monkeys are restricted to advanced stages of testing (Amenya *et al*., 2011). Organization for Economic Cooperation and Development (OECD) guidelines are used during acute and sub-acute oral toxicity testing (Diener *et al*., 1995). It is important to optimize the information obtained by using the smallest number of animals to comply with animal welfare regulations. Further, it is important to avoid excessive pain or tissue damage in the animals, pharmaceuticals with irritant or corrosive characteristics should not be administered in concentrations that produce severe toxicity after administration. During toxicity studies, all the animals must be checked for morbidity, mortality and specific signs of toxicological relevance. For example, neuro functional and neurobehavioral, pharmacological observation, bodyweight and food/water intake. The key

hematological parameters investigated are mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), haemoglobin levels, hematocrit levels, packed cell volume (PCV) total and differential leukocytes, erythrocytes and platelet counts. Clinical biochemistry is crucial to investigate major toxic effects on organs especially the kidney and the liver. Some of the parameters include total protein, albumin, major electrolytes, total cholesterol, alanine aminotransferase, aspartate aminotransferase, creatinine and alkaline phosphatase that aid in hepatocellular evaluation. All long-term studies such as chronic toxicity must include urinalysis (urine output, color, protein and osmolarity). Pathological studies and gross necropsy are done by examining the body, orifices, abdominal cavity, body weight and organ weight changes among others. In addition, histopathological studies are done on adrenals, lung, liver, kidney, testis, ovaries among others (OECD 407, 2008).

These organs are considered to be the most important during toxicity studies in rodents and non-rodents (Michael *et al.*, 2007). Taking the weights of organs is necessary because organ to body weight ratios or organ weight index (OWI) is commonly calculated and are considered more useful when body weights are affected (Michael *et al*., 2007). By carrying out toxicity tests, the effects of an increase in dose on the mortality and other effects of the lethal dose that kills are determined. Estimating different levels of toxicity by use of LD_{50} for instance can help in estimating the probabilities of an outcome for a given individual in a population. The determination of acute, sub-acute, subchronic and chronic toxic effects of the test compounds is therefore crucial (Traina, 2006).

2.8.1 Acute toxicity

Acute toxicity is caused by an agent when it is administered in one or more doses over a period not exceeding 24 hours and involves harmful effects to the organism through a single or short term exposure**.** Acute toxicity studies have also been used during the selection of starting doses for phase-I human and animal studies and provide information relevant to acute overdosing in humans and animals. The testing is based on the route of substance administration to the animal and therefore, it is classified from Class-1 to Class-5 for oral, dermal, gas inhalation, vapor/dust/mist inhalation and injection. Dosing can be repeated during the administration of test material by a variety of routes of exposure, including gaveling which involves stomach intubation or forced feeding, injection, skin, painting and inhalation. The acute toxic class method, a stepwise procedure, involves the use of three animals of a single sex per step. Depending on the mortality and/or moribund status of the animals, on average 2 to 4 steps may

be necessary to allow judgment on the acute toxicity of the substance. The OECD Guideline 423 (2001) provides a reproducible method that uses few animals.

2.8.2 Sub-acute toxicity

The sub-acute toxicity test in the study was based on OECD guideline 407 (2008), repeated dose 28-day oral toxicity study in rodents. In this form of toxicity, adverse effects occur as a result of repeated daily dosing of a chemical or exposure to the chemical, for part of an organism's lifespan usually not exceeding 10 % of the animals' lifespan. With experimental animals, the period of exposure may range from a few days to 6 months. Exposure for 28 days provides a first-hand indicator of potential sub acute toxicity. The test is intended to investigate effects on a very broad variety of potential targets of toxicity. It provides information on the possible health hazards likely to arise from repeated exposure over a relatively limited period of time, including effects on nervous, immune and endocrine systems. The duration of exposure is normally 28 days in rodents where results are used for hazard identification and risk assessment (OECD, 2008). All the knowledge gathered from the studies is used in selecting doses for repeat-dose studies as a source of preliminary identification of target organs of toxicity and may also reveal delayed toxicity. Sub-acute toxicity studies in animals are essential for any pharmaceutical product especially those intended for human use.

2.8.3 Sub-chronic toxicity

This is the ability of a toxic substance to cause effects for more than one year but less than the lifetime of the exposed organism. This form of toxicity is studied for at least 90 days in animal models notably rodents. The test is carried out after getting initial information on toxicity from acute or 28-day sub-acute toxicity studies. It provides information on possible hazards likely to arise from repeated exposures over a prolonged period of time covering post-weaning maturation and growth well into adulthood. The study provides information on the major toxic effects, indicates target organs and the possibility of accumulation. It can also provide an estimate of a non-observed-adverse effects level (NOAEL) of exposure which can be used in selecting dose levels for chronic studies and to establish safety criteria for human studies (OECD 408, 1998).

2.8.4 Chronic toxicity

This is the ability of a substance or mixture of substances to cause harmful effects over an extended period, usually upon repeated or continuous exposure, sometimes lasting for the entire life of the exposed organism. The three main routes for chronic study include oral, dermal and inhalation depending on the characteristic of the test substance and the predominant route of exposure in humans. The objectives of chronic toxicity studies include identification of the hazard properties of a chemical, identification of target organs, characteristic of dose-response relationship and identification of NOAEL or point of departure of Benchmark Dose (BMD). It also helps in the identification of chronic toxic effects in human exposure levels and provision of data to test hypotheses regarding the mode of action (OECD 452, 2008).

2.9 Future perspective of medicinal plants as antimalarial agents

The importance of plants is becoming clear from the fact that more than 80% of the world population fulfills its medical needs from medicinal plants (Jimma *et al*., 2015). In some African countries such as Tanzania, about 60% of the people especially in rural areas rely on traditional medicines for their healthcare needs (Magano *et al*., 2001). Demand for these herbal medicines for therapeutic purposes is increasing and will continue in the future mainly because of less toxicity and side effects of these medicines (Balkema *et al.,* 2007). Since the demand of such plants as antimalarial agents is highly increasing in both developed and developing countries, many of these plant species are at greater risk of being extinct (Martinez *et al.,* 2013). According to the International Union for Conservation of Nature, there are many plant species that are used for medicinal purposes worldwide (IUCN, 2007). Among these species, few of them are threatened with extinction from overharvesting, especially in China, India, Nepal, Tanzania, Kenya and Uganda (Nalawade *et al.,* 2003; Heywood *et al.,* 2003; Zerabruk *et al*., 2012). In order to alleviate this concern and to meet public demands, sustainable use of medicinal plants should be highly considered. Similarly, herbal medicines should be brought under legal control in all countries where they are used for therapeutic purposes and more efforts should be made to raise public awareness about the benefits and risk of extinction of these herbal medicines (Ramanan *et al.,* 2007). Additionally, the development of genetic engineering and advancement in tissue culture can also be a promising alternative for the production of those medicinal plant species that are disappearing at a high speed (Rao *et al*., 2002).

CHAPTER THREE

ANTIMALARIAL ACTIVITY OF *Cucumis metuliferus* AND *Lippiakituiensis* AGAINST *Plasmodium berghei* INFECTION IN MICE[1]

Abstract

The search for new antimalarial drugs has become progressively urgent due to plasmodial resistance to most of the commercially available antimalarial drugs. As part of this effort, the study evaluated the antimalarial activity of *C. metuliferus* and *L. kituiensis,* which are traditionally used in Tanzania for the treatment of malaria. In vivo antimalarial activity was assessed using the 4-day suppressive antimalarial assay. Mice were infected through an intraperitoneal route with 1×10^7 erythrocytes infected with *Plasmodium berghei*. Extracts were administered orally Chloroquine (10 mg/kg/day) and 1% of dimethylsulfoxide (5 mL/kg/day) were used as positive and negative controls, respectively. The level of parasitemia, survival time, packed cell volume and variation in body weight of mice were used to determine the antimalarial activity of the extract. The chloroform, ethyl acetate and methanolic extracts of *C. metuliferus* and *L. kituiensis* significantly ($p < 0.05$) suppressed parasitemia in a dose-dependent manner. The highest suppression was recorded at dose level of 1500 mg/kg. At this dose, *C. metuliferus* had percentage suppression of 98.55%, 88.89% and 84.39% of chloroform, methanolic, and ethyl acetate extracts respectively whereas *L. kituiensis* exhibited suppression of the pathogens of 95.19%, 93.88% and 74.83% ethyl acetate, chloroform and methanolic extracts respectively. The extracts prevented loss of body weight at a dose of 600 mg/kg and 1500 mg/kg in all solvent's extracts. In addition, the extracts prolonged the mean survival time of *P. berghei*-infected mice compared to the non-treated control. The significant reduction of packed cell volume was observed at a dose of 300mg/kg body weight. From this study, it can be concluded that the leaves extract of *C. metuliferus* and *L. kituiensis* showed good chemo suppressive thus supporting further studies of the plant for its active components

Keywords: Malaria, *Cucumis metuliferus, Lippia kituiensis, Plasmodium berghei,* Crude extracts.

[1]*Research and Reports in Tropical Medicine 9: 81-88; 2018.*

3.1 Introduction

Substantial progress has been made in fighting malaria since the year 2000 (WHO, 2016). Estimates between 2000 and 2015 indicated that malaria case incidence has been reduced by 41% and malaria mortality rates by 62% (WHO, 2016). At the beginning of 2016, malaria was considered endemic in 91 countries and territories down from 108 in 2000 (WHO, 2016). This progress is associated with the wide-scale deployment of malaria control interventions. Despite this remarkable progress, malaria continues to have a devastating impact on people's health and livelihoods. It is estimated that 214 million cases occurred globally in 2015, leading to 446 000 deaths, most of which were children aged under 5 years (WHO, 2016). The burden of the disease is heaviest in Africa, where 82 and 90 % of all global cases and deaths occurred, the report further indicates that 90% of all malaria deaths occur in African regions; 78% of all deaths occur in children under five years of age. Increased side effects of conventional drugs and the development of resistance by malaria parasites have become a global concern and thus call for continued efforts to search for antimalarial agents and plants. There fore; medicinal plants have made and continue to make a great contribution to antimalarial chemotherapy as they contain molecules with a great variety of structures and biological activities.

Lippia kituiensis and *C. metuliferus* have been utilized by rural communities in Tanzania for the management of malaria. *L. kituiensis* belongs to the verbena families (Verbenaceae) which are aromatic due to their essential oils (Arthur *et al.,* 2011). Apart from its use for the management of malaria, leaves are used as a culinary herb (Tucker *et al*., 2009). Camphor isolated from *L. kituiensis* essential oil was found to have a strong repellent activity against maize weevil compared with NN-diethyl toluamide (DEET) (Mwangi *et al*., 1992). It also used for the treatment of chronic joint pains, such as osteoarthritis and/or rheumatoid arthritis, but also in different farms, they use *L. kituiensis* for milk treatments in animals (Wambugu *et al*., 2011). *Cucumis metuliferus* (Cucurbitaceae) is a monoecious annual herb with staminate flowers that grows wild (Wannang *et al*., 2007). The family is commonly referred to as African horned cucumber, jelly melon in English. *Cucumis metuliferus* is an annual climbing or rarely trailing herb; vegetative parts are rough with spreading hairs, its fruits occur in two forms - the bitter and non-bitter forms, which occur mostly in the wild state. It has been established that the fruit of *C. metuliferus* possesses antibacterial activity against *S. gallinarum in vitro* (Usman *et al*., 2014). The pulp extract of *C. metuliferus* was shown to have anti-ulcer property (Wannang *et al*., 2009). Glycosides extracted from the fruit pulp of *C. metuliferus* possess anti-hyperglycemic activity against alloxan-induced diabetes mellitus in rats (Jimam *et al*., 2010;

Gotep *et al.*, 2011; Tomar *et al.*, 2014). Despite the fact that *L. kituiensis* and *C. metuliferus* are used in Tanzania for the management of malaria, there is no report on the antimalarial activity of the two plants. This paper reports antimalarial activity of methanolic, ethyl acetate and chloroform extract of *C. metuliferus* and *L. kituiensis* against *Plasmodium berghei* infected in mice.

3.2 Materials and methods

3.2.1 Collection and extraction of the plant materials

Fresh leaves *of L.kituiensis* and *C. metuliferus* were collected from Ugweno ward within Mwanga District, Kilimanjaro region in Tanzania. Ugweno ward is situated at 3° 39' 0" South and 37° 39' 0" East in the Pare Mountains. Herbalists residing in Ugweno ward and a taxonomist (Josephat Mboya) were consulted and involved in the identification and collection of the plant materials. Voucher specimen coded (Voucher no. 160for *C. metuliferus* and 161 *L. kituiensis*) ware deposited at the Nelson Mandela African Institution of Science and Technology.

Extraction of the plants: Extraction of the plants: The collected plant leaves were cleaned with tap water and dried under shade at room temperature for two weeks and coarsely powdered using an electric blender. Total extraction was employed in the extraction process in which 600 g of the pulverized materials were divided into three portions (200 g each). The three portions were soaked with 80% methanol (1600 mL), 100% ethyl acetate (1600 mL) and 100% chloroform (1600 mL) for three consecutive days with occasional stirring in Erlenmeyer flask for 48 h at room temperature.

After 48 h, the extracts were separated from the marc (the residue left after the extraction) using gauze and further filtered by Whatman filter paper No. 1 (Whatman®, England). Solvents (80% methanol, 100% ethyl acetate and 100% chloroform) were removed through the vacuum using a rotary evaporator to obtain 134 g, 136 g and 138 g respectively. The portions of the yield were solubilized in 10% DMSO for confirmation of the antimalarial activity and the remaining was then kept in a refrigerator at -20^{0}C until further use.

3.2.2 Experimental animals

Swiss albino male mice, weighing 25 to 27 g of 6 to 8 weeks old were collected at Sokoine University of Agriculture (SUA) and housed at Muhimbili University of Health and Allied Sciences (MUHAS) in plastic cages with softwood having and chips as beddings. They were exposed to a 12:12 dark-to-light cycle and provided with free access to a pellet diet and clean drinking water. All mice were acclimatized to the working environment for one week before the beginning of the experiment.

3.2.3 Malaria parasites and materials

Chloroquine-sensitive *Plasmodium berghei* was used for the induction of malaria in experimental mice. Blood stage *P. berghei* parasites used in the study were kindly donated by Dr. Lindsay Stewart of the Department of Pathogen Molecular Biology, London School of Hygiene and Tropical Medicine, United Kingdom. Materials and reagents: methanol (Okhla Industrial, India), Giemsa (Sciencelab, USA), trisodium citrate (Deluxe Scientific Surgico, India) and normal saline (Addis Pharmaceuticals Factory, Ethiopia), chloroform (Okhla Industrial, India), ethyl acetate (Okhla Industrial, India), Dimethylsulfoxide (DMSO) were purchased from Techno-Net Scientific (Dar es Salaam, Tanzania) whereas chloroquine diphosphate was purchased from Sigma (Sigma®, Steinheim, Germany)

3.2.4 Preparation of infected red blood cells suspension

In-vivo antiplasmodial activity of the crude extract against early *P.berghei* strain infection was carried out according to the method described by Peters *et al.* (1975). A donor mouse infected by chloroquine sensitive *P. berghei* strain with a rising parasitemia 40-45% was used for infecting mice in the 4^{th}day suppressive test procedures. The donor mice were anesthetized by diethyl ether blood (parasitized erythrocytes) was collected by cardiac puncture into a test tube having 0.5 % trisodium citrate which was added as an anticoagulant. The inoculum was prepared by determining percentage parasitemia of the donor mice. According to Waako *et al*. (2005) appropriate inoculum for infecting mice had to be low to 1% by dilution process. In this dilution Phosphate Buffered Saline (PBS) 9.77 ml of PBS were required to dilute 0.23 ml of blood from the donor mice with 44.40% parasitemia to obtain 10 ml of blood with 1% parasitemia, which was used in infecting 50 experimental mice each receiving 0.2 ml of the diluted blood through intraperitoneal route containing approximately 1.0×10^7 parasitized cells.

3.2.5 Inoculation of parasites and administration of extracts

Each mouse was infected through the intraperitoneal route with 1×10^7 iRBCs in 0.2 mL suspension of 1×10^7 iRBCs per mL. The extracts were solubilized in 10% DMSO and Chloroquine was dissolved in normal saline. Three hours post infection the mice were randomly allocated into groups, for *L. kituiensis* extract forty five (45) mice were grouped into three groups (chloroform, ethyl acetate and methanol of fifteen mice per group. Each group consists of three doses (300, 600 and 1500 mg/kg) having five mice in each dose making a total of forty five (45) mice, two females and three males. A similar procedure was repeated for *C. metuliferus* extract that forty five (45) mice were grouped into three groups (chloroform, ethyl acetate and methanol of fifteen mice per group. Each group consists of three doses (300, 600 and 1500 mg/kg) having five mice in each dose making a total of forty five (45) mice, two females and three males different sex were separated to avoid physiological interaction like mating. Ten mice received a vehicle five as a positive control and five as negative control making a total of ninety five mice. The negative control group received 1% DMSO (5 mL/kg/day), the positive control group received chloroquine 10 mg/kg/day and each mice was observed daily for determination of survival time. Treatment started three hours after mice had been inoculated with the parasite (Trager & Jensenon, 1976) at day 0 and then continued daily for four days from day 0 to day 3. On the 5th day (day 4), thin films were made from the tail blood of each mice and smear on to a microscope slide to make a film (Saidu *et al*., 2000). The blood films were fixed with methanol, stained with 10% Giemsa at pH 7.2 and parasitaemia was examined microscopically to determine parasitemia level as per formula below.

$$\%\ Parasitemia = \frac{Number\ of\ effected\ \ (iRBCs)}{Total\ number\ of\ \ RBCs} X100$$

$$\%\ Suppression = \frac{Parasitemia\ in\ negative\ control - Parasitemia\ study\ group}{\text{Parasitemia in negative control}} X100$$

3.2.6 Determination of packed cell volume, mean survival time and body weight

The cell Packed volume (PCV) of each mouse was measured before infection (D0) and after infection (D4). For this purpose, blood was collected from the tail of each mouse in heparinized microhematocrit capillary tubes. The tubes were sealed by crystal seal and placed in a microhematocrit centrifuge with the sealed ends outwards. The blood was centrifuged at 12 000 rpm for 5 min. The volume of the total blood and the volume of erythrocytes were measured and PCV was calculated according to Mengistie (2012) as given below.

$$PCV = \frac{\text{Volume of erythrocytes in a given volume of blood}}{\text{Total blood volume}} X100$$

3.2.7 Determination of mean survival time and body weight

Mean survival time (MST) was determined according to Alli *et al*. (2011) mortality was monitored daily, the number of the days from the time of infection up to death was recorded for each mouse in the treatment and control groups throughout the follow-up period and the MST were calculated for each group by using the following formula.

$$MST = \frac{\text{Sum of survival time (days) of all mice in a group}}{\text{Total number of mice in that group}} X100$$

The change of body weight of each mouse in all groups was determined before infection (D0) and after treatment (D4) by using sensitive electrical balance (ADP 720 L, Adam Equipment) (Krettli *et al*., 2009). Then, body weight change of extract treated groups was compared with the control groups.

3.2.8 Ethical consideration

Before the experimental work, an ethical clearance with notification number NIMR/HQ/R.8a/Vol. IX/2146 was given by the National Health Research Ethics Sub-Committee (NatHREC) of the National Institute for Medical Research (NIMR) in Tanzania.

3.2.9 Data analysis

The data of the study were expressed as mean ±SD (standard deviation of the mean) for each group of experiments. Data on the levels of parasitemia, variations in body weight and survival times were analyzed using Statistical Analysis System **(**SAS). Values were considered statistically significant at $P < 0.05$ and more significant at $P<0.01$. All results were represented

asMean ± SD (n = 5) Values with different superscripts were significantly different ($P < 0.05$) and ($P<0.01$)**.**

3.4 Results

3.4.1 In vivo evaluation of the antiplasmodial activity of plant extracts

The results of *in vivo* antimalarial activity revealed that *C. metuliferus* and *L. kituiensis* extract exhibited dose dependent suppression of parasite growth. At a dose of 1500, 600 and 300 mg/kg/day, of *C. metuliferus* extract, the highest suppression was obtained from chloroform (98%, 70%, and 48%) followed by methanolic (88%, 58% and 36%) and ethyl acetate (84%, 64% and 30%) respectively as indicated in Table1. In *lippia kituiensis* extract the highest suppression was obtained for ethyl acetate (95%, 70% and 42%) followed by chloroform (93%, 69% and 46 %) and methanolic extracts (74%, 68% and 35%) respectively as shown in Table 2. All extracts of *C. metuliferus* and *L. kituiensis* leaf reduced parasite growth and the reduction were statistically significant ($P < 0.05$) in mean percentage parasitemia as compared to the negative control.

Table 1: Antimalarial activities of methanolic, chloroform and ethyl acetate extracts of leaves of *C. metuliferus* and mean survival time of *P. berghei* infected mice

Extract	***C. metuliferus***	**Antimalarial activity on D4 post-infection**		**Mean survival time (days) ± SD**
	Dose (mg/kg)	**% Parasitemia ± SD**	**% suppression± SD**	
Methanol	300	30.09±0.78*	36.99±1.64	9±1*
	600	19.82±0.93**	58.91±1.68	10±2*
	1500	5.49±0.30**	88.89±0.83	14±0**
Chloroform	300	25.9±0.39**	45.81±1.22	9±1*
	600	14.37±0.95**	70.69±2.86	10±1.52*
	1500	1.10±0.13**	98.55±0.7	14±0**
Ethyl Acetate	300	34.03±0.98*	30.73±2.73	7.66±0.57*
	600	17.08±1.68**	64.23±3.53	10.33±1.52*
	1500	8.95±0.47**	84.39±3.98	14±0**
CQ	10	0	100	14±0
NC		47.15±1.74	-	6.0±1

Key: Values are presented as Mean ± SD; n = 5; DSMO = negative control (1 %); CQ positive control (chloroquine), NC negative control CQ chloroquine (positive control); * = values are significantly different ($p < 0.05$) from that of the negative control);** = values are more significantly different ($p < 0.01$) from that of the negative control.

Table 2: Antimalarial activities of methanolic, chloroform and ethyl acetate extracts of leaves of *L. kintuensis* and mean survival time of *P. berghei* infected mice

Extract	***L. kentuensis***	**Extract antimalarial activity on D4 post-infection**		**Mean survival time (days) ± SD**
	Dose (mg/kg)	**% Parasitemia ± SD**	**% suppression± SD**	
Methanol	300	30.08±3.05*	35.94±5.49	9±1*
	600	14.21±1.95**	68.76±3.34	12±2*
	1500	12.46±0.52**	74.83±2.35	13±0*
Chloroform	300	25.61±2.17**	46.38±4.63	9±1*
	600	14.44±0.20**	69.96±0.50	12±1.52*
	1500	3.75±0.38**	93.88±2.47	14±0**
Ethyl Acetate	300	27.74±3.60*	42.42±8	8±0.57*
	600	14.64±1.57**	70.14±3.85	10±1.52*
	1500	2.53±0.13**	95.19±1.11	14±0**
CQ	10	0	100	14±0**
NC		47.15±1.74	NC	6±1

Key: Values are presented as Mean ± SD; n = 5; DSMO = negative control (1 %); CQ positive control (chloroquine), NC negative control CQ chloroquine (positive control); * = values are significantly different ($p < 0.05$) from that of the negative control); ** = values are more significantly different ($p < 0.01$) from that of the negative control.

3.4.2 Effect of extracts on packed cell volume (PCV)

In the fourth-day suppressive test, packed cell volume (PCV) of *P. berghei* infected mice were measured. The results showed a significant ($p < 0.05$) reduction in packed cell volume (PCV) between day zero (D0-inoculation day) and day four (D4) in mice treated with chloroform, methanolic and ethyl acetate extracts of leaves of *Lippia kituiensis* at dose level of 300 mg/kg. Whereas mice treated with a dose level 600 mg/kg and 1500 mg/kg in chloroform, methanolic and ethyl acetate extracts did not show a significant reduction (Table 3). In *Cucumis metuliferus*, the mice treated with chloroform, methanolic and ethyl acetate extracts exhibited significant reduction ($p < 0.05$) in packed cell volume (PCV) between day zero (D0-inoculation day) and day four (D4) in mice treated at a dose of 300 mg/kg. While the mice treated with chloroform, methanolic and ethyl acetate extracts from the two plants at a dose of 600 mg/kg and 1500 mg/kg did not show significant ($p > 0.05$) PCV reduction (Table 4).

3.4.3 Effect of extract on survival times of mice

In the four-day suppressive test, 300 mg/kg, 600 mg/kg and 1500 mg/kg extract treated groups lived longer than the corresponding negative control. There were significant differences in survival times among extract treated groups of *C. meturiferus* and *L. kituiensis* in all solvent and dose levels as shown in Table 1 and Table 2.

3.4.4 Effect of extract on body weights of mice

In the 4-day suppressive test, all extract treated groups prevented body weight except in dose level 300 mg/kg of *C. metuliferus* and *L. kituiensis* in chloroform, ethyl acetate and methanolic extract between day zero (D0) and day four (D4) as compared to the negative control. However, there were no significant differences of weight in dose level 600 mg/kg and 1500 mg/kg in all extracts when compared with the control as shown in Table 3 and Table 4.

Table 3: Effect of methanolic, chloroform and ethyl acetate extracts of *Lippia kintuensis* on body weight and PCV of *P. berghei* infected mice

Extracts	**Dose (mg/kg)**	**PCV**		**Body weight**	
		D0	D-4	D0	D-4
Methanol	300	51.23±1.21	43.32±5.6*	29.70±3.21	24.65±2.11*
	600	50.12±2.32	48.13±1.3	30.11±1.63	28.24±2.12
	1500	59.31±1.2	57.91±1.2	32.22±1.01	31.90±2.11
Chloroform	300	51.23±1.50	44.21±2.3*	26.54±2.13	21.44±1.33*
	600	51.32±2.3	49.23±1.2	29.33±1.55	27.12±1.5
	1500	50.18±2.1	49.91±1.2	30.45±1.03	29.93±1.02
Ethyl acetate	300	49.23±1.5	43.32±5.3*	32.12±0.23	25.52±3.23*
	600	51.34±3.1	48.23±2.1	28.4±2.11	25.23±2.11
	1500	50.32±1.2	49.32±0.1	28.15±1.23	27.25±2.55
CQ	10	55.41±1.03	54.66±0.52	30.23±0.44	29.92±0.12
NC		55.44±1.25	40.45±1.45*	32.17±1.4	25.13±4.35*

Key: Values are presented as mean ± SD; n = 5; PCV packed cell volume, NC negative control (1% DSMO), CQ chloroquine (positive control); * = the difference between day 0 and day 4 is significant ($P < 0.05$).

Table 4: Effect of methanolic, chloroform and ethyl acetate extracts of *Cucumis metuliferus* on body weight and PCV of *P. berghei* infected mice

Extracts	Dose(mg/kg)	PCV		Body weight	
		D0	D-4	D0	D-4
Methanol	300	48.32±1.61	40.32±5.6*	28.70±3.21	22.65±2.11*
	600	49.32±2.32	47.13±1.3	30.11±1.63	28.24±2.12
	1500	51.31±1.2	49.91±1.2	32.22±1.01	31.90±2.11
Chloroform	300	51.23±1.50	44.21±2.3*	26.54±2.13	20.44±1.33*
	600	51.32±2.3	49.23±1.2	29.33±1.55	27.12±1.5
	1500	53.23±2.1	52.32±1.2	30.45±1.03	29.93±1.02
Ethyl acetate	300	50.23±1.5	40.32±5.3*	32.12±0.23	26.52±3.23*
	600	51.34±3.1	49.23±2.1	27.14±2.11	25.23±2.11
	1500	49.32±1.2	48.32±0.1	28.15±1.23	27.25±2.55
CQ	10	55.41±1.03	54.66±0.52	30.23±0.44	29.92±0.12
NC		55.44±1.25	40.45±1.45*	32.17±1.4	25.13±4.35*

Key: Values are presented as mean ± SD; n = 5; PCV packed cell volume, NC negative control (1% DSMO), CQ chloroquine (positive control); * = the difference between day 0 and day 4 is significant ($P < 0.05$).

3.5 Discussion

The emergence of widespread resistance of *Plasmodium* species to most antimalarial drugs has led to a more vigorous and concerted research in traditional medicinal plants for the treatment of malaria. *Cucumis metuliferus* and *Lippia kituiensis* are two such plants which are traditionally used for the treatment of malaria in Tanzania, but for which there is limited scientific proof of their efficacies. The results of the present study revealed that *Cucumis metuliferus* and *Lippia kituiensis* exhibited potent antimalarial activity against *Plasmodium berghei*. Similarly, Odugbemi *et al.* (2007) reported that the two plants have been traditionally claimed to relieve fever and cure malaria

Parasitemia refers to the level of infection by a particular parasite or the number of parasitized cells circulating in the blood (Waako *et al*., 2005). A decline in parasitemia in a given organism is essential for the recovery of symptomatic malaria (Alli *et al.,* 2011). In this study, the percentage parasitemia in the negative control group on D4 after infection with *Plasmodium berghei* parasites was 47.15% and Chloroquine had the least parasitemia of 0.00% whilst none of the extracts reached this level. For the case of plant extracts, the lowest parasitemia was observed in *Cucumis metuliferus* with 1.1% chloroform extract at dose level 1500 mg/kg and in *Lippia kituiensis* the lowest parasitemia was observed with ethyl acetate 2.53% at a dose level of 1500 mg/kg. This result is in agreement with the antimalarial properties reported by Renata (2015) and Ajaiyeoba (2005) that support the *in vivo* activities of *C. metuliferus* and *L. kituiensis.*

Chemo-suppression is inversely related to parasitemia, the plant extracts, which have been shown to reduce parasitemia to low levels have been shown to have a corresponding high chemo-suppression. The study revealed that the highest suppression in *Cucumis metuliferus* extract was observed from chloroform (98%, 70% and 48%) followed by methanolic (88%, 58% and 36%) and least was ethyl acetate (84%, 64% and 30%) extracts treated mice at the dose levels of 1500 mg/kg, 600 mg/kg and 300 mg/kg, respectively. In the same way, the ethyl acetate, chloroform and methanolic extracts of *lippia kituiensis* extract the highest suppression was observed form ethyl acetate (95%, 70% and 42%) followed by chloroform (93%, 69% and 46 %) and least was methanolic extracts (74%, 68% and 35%) treated mice at the dose levels of 1500 mg/kg, 600 mg/kg and 300 mg/kg, respectively.

According to Ajaiyeobal and Coweker (2005) in vivo antimalarial activity of plant extracts can be categorized as moderate, good and very good if the extract showed 50% or more chemo-suppression. Hence, the current finding in both plants has very good and good 4-day suppressive antimalarial activity in dose level 1500 mg/kg and 600 mg/kg while in the lowest dose both plants have low, 4-day suppression antimalarial activity. However, the highest peak chemo-suppression was recorded in chloroform extracts of *C. meturiferus* with 98.53% ($p<0.05$), followed by ethyl acetate of *L. kituiensis* with 95.19% which was equivalent to that induced by chloroquine. This observation was quite interesting since the extract was only a crude preparation. The chemo-suppression showed by *C. meturiferus* and *L. kituiensis* was in agreement with other studies on medicinal plants used for malaria such as *Artemisia annua* (Aduga *et al*., 2014), *Annona senegalensis* (Aduga *et al*., 2014) and *Adhathodas chimperian* (Petros *et al*., 2012) which showed 90.48%, 96.20%, and 97.80%, respectively. According to Zhengming *et al*. (2009), the antimalarial activity of the plant might be induced by the presence of active bioactive compounds. However the observed low antimalarial activity of plant extracts could be partly explained by the fact that many antimalarial traditional medicinal plants may lack direct antiplasmodial activity to cure the disease but their beneficial role could be in their antipyretic, analgesic and immune stimulatory effect as demonstrated in other studies (Krettli *et al*., 2009; Makonnen *et al*., 2003).

According to Abdu *et al*. (2008) the effectiveness of a given extract can also be influenced by the rate of gastrointestinal uptake and the half-life in plasma metabolism of the active compound. The difference between the antimalarial activities of extracts of both plants in all the three solvents may indicate differences in level and composition of active compounds Muregi *et al*. (2007). In addition to that the difference may also be due to differences between the solvents used in terms of polar and nonpolar solvent. Also the administration of plant extracts on mice was done orally, due to this, some of the mice may not have taken the whole 100 mg/kg dosage and this may have affected the parasitemia in the groups.

Mean survival time is another parameter that evaluates the antimalarial activity of plant extracts as indicated in Table 1 and Table 2. Plant materials that can prolong the survival time of infected experimental animals compared to the negative controls are considered as active agents against malaria (Oliveira *et al*., 2009; Adugn *et al.*, 2014). In this study, the mice treated with 300 mg/kg, 600 mg/kg mg and 1500 mg/kg of extract had significantly lived longer than negative control in the four-day suppressive test. This means that the tested extracts contain the

anti-malarial compound, which reduces the number of parasites and hence prolong the survival time. The current finding was in agreement with other reports on medicinal plants used for malaria such as *Dodonaeaa ngustifolia* seed (Mengistie *et al*., 2012).

Body weight is another parameter that can be used to assess the effect of extract in treated mice (Taherkhani *et al.*, 2013). In this study, the result showed that there were significant ($P < 0.05$) decrease in body weight between day zero (D0) and day four (D4) of mice treated with chloroform, methanolic and ethyl acetate in dose level of 300 mg/kg for both *C. metuliferus* and *L. kituiensis* and as shown in Table3 and Table 4. This may be due to the depressant action on the appetite of the mice and the consequences of disturbed metabolic function and hypoglycemic effect of the parasite (Basir *et al.*, 2012). Similar results were reported by Gituaj *et al*. (2012). The reduction of packed cell volume (PCV) values of *P. berghei* infected mice signifies malaria infection. *Plasmodium berghei* infected mice treated with extracts at 300 mg/kg exhibited a significant PCV reduction whilst insignificant PCV reduction was recorded for infected mice treated with extracts at 600 mg/kg and 1500 mg/Kg. Similar results were obtained in studies reported from the same species of *A. Remota* species used to treat malaria (Gupta *et al*., 2005; Gituaj *et al*., 2012).

3.6 Conclusion

From this study, it can be concluded that the leaf of *C. metuliferus* and *L. kituiensis* showed good antimalarial activities against *Plasmodium berghei* infection in mice. The highest chemosupression was observed from *C. metuliferus* chloroform extract of 98.55% and 95% in *L. kituiensis* ethyl acetate extract in dose level of 1500 mg/kg. Accordingly, with the essence of further studies this plant could serve as the potential source of new and novel antimalarial leads and/or drugs for the treatment and prevention of malaria. However, antimalarial compounds from these plants which could significantly contribute to drug discovery remained unveiled thus need phytochemical investigations.

CHAPTER FOUR

GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF COMPONENTS FROM *Cucumis metuliferus* AND *Lippia kituiensis* PLANTS

Abstract

Objective: From the recommendations given in the previous chapter, phytochemicals evaluation of the leaf extract of *C. metuliferus* and *L. kituiensis* plants was conducted in this chapter to reveal the unique chemical classes present in these plants that might be responsible for the antiplasmodial effect against *P. falciparum*.

Methods: Gas Chromatography-Mass Spectrometry (GC-MS) techniques were used in the identification of compounds with low molecular weight of the leaf extracts of *C. metuliferus* and *L. kituiensis.* The collected samples were dried and extracted with three different polarity (chloroform, ethly acetate and methanolic) solvents.

Result: Preliminary phytochemical analyses of methanol, ethyl acetate and chloroform extracts have revealed the presence of steroids, alkaloids, flavonoids, tannins, glycosides, amino acids, and phenol compounds. The gas chromatography-mass spectroscopy result revealed the presence of 11 major compound namely Cyclotrisiloxanehexamethyl; Z-Z-6, 28-Heptatriactontadien-2-one; D-Manitol, 1, 1, 1'-o-1, 16-hexadecanediylbis; Trans, CIS-1, 8-Dimethylspiro(4, 5)-Decane and L-(+)-Ascorbic acid 2, 6-dihexadecanoate, 3, 7, 11, 15-Tetramethyl-2-Hexadecen-1-ol; Oleyl alcohol tri fluoroacetate; 1-Pentatriacontanol; N-propyl 11-octadecenoate, fucosterol, Artemiseole compound and other usefully compound.

Conclusion: The medicinal properties of *L. kituiensis* and *C. metuliferus* might therefore due to the presence of these groups of phytochemicals. The results of this study offers a base of using *C. metuliferus* and *L. kituiensis* as an herbal alternative for the synthesis of antimalarial agents.

Keywords: *C. metuliferus* and *L. kituiensis*, GC-MS analysis, pharmacological properties and phytochemicals.

4.1 Introduction

The uses of medicinal plants as a source of drugs in primary health care have become popular universally, particularly in developing countries (Michiels *et al*., 2012). Plants have been a source of the bioactive compound, which serves as a drug (Seyyednejad *et al*., 2010), these bioactive compound work with nutrients and fibers to form an integrated part of a defense system against various diseases and stress conditions (Thilagavathi *et al.,* 2015). Several plant products have been shown to exert a protective role against the formation of free radicals and playing a beneficial role in maintaining disease conditions. Therefore, there is a need for screening of bioactive compounds in plant for establishiment of lead compounds which can be further developed into potential herbal products for the treatment of several ailments (Bohlin & Bruhn, 1999). Gas chromatography coupled to mass spectrometry (GC-MS) has commonly been used for the analysis of relatively low molecular weight compounds (Eisenhauer *et al.,* 2009; Prabhadevi *et al.,* 2012). Taking into consideration of the medicinal importance of these compounds, it is essential to thoroughly investigate their composition and hence promote the use of such compounds as potential sources of drug templates (Bohlin & Bruhn, 1999).

Genus *Lippia* (Verbenaceae, Lamiales Magnoliopsida) includes about 200 species of herbs, shrubs and small trees mainly distributed in Central and South America and Africa Tropical (Terblanche *et al*., 1996; Santos *et al*., 2009). *Lippia* species have shown a large number of important usages in folk medicine for various diseases, particularly in the treatment of cough, bronchitis, indigestion, liver, hypertension, dysentery (Pham *et al*., 1988; Pascual *et al*., 2001), and skin diseases (Matos *et al*., 1998). Many*Lippia* species have promising biological activities, including antiviral (Abad *et al*., 1997), antimalarial (Valentin *et al*., 1995), anti-inflammatory, analgesic, antipyretic (Forestier *et al*., 1996: Forestier *et al*., 1996), antimicrobial (Lemons *et al*., 1990), insecticidal (Lima *et al*., 2013), and anticonvulsant (Abena *et al*., 1998) properties. Moreover, *Lippia* species revealed *in vitro* antitumor activity on leukemia (K-562, HL-60, and CEM), colon (HCT-116), breast (MCF-7), glioblastoma (U-251), and prostate (PC -3) cell lines (Costa *et al*., 2001; Gonzalez-Guereca *et al*., 2010).

Cucumis metuliferus belongs to the family Cucurbitaceae, and is a monoecious, climbing, annual vine that can be grown practically anywhere, provided the season is warm (Benzion *et al*., 1993). The fruits are ovoid berries of 8-10 cm long and 4-5 cm in diameter with horn-like spines (hence the name horned cucumber), yellow-orange skin and a lime green jelly-like flesh when ripe (Wanning *et al*., 2008). This plant is used by traditional medical practitioners in

different place to treat diseases such as peptic ulcer, diabetes mellitus, hypertension and HIV/AIDS (Wanning *et al*., 2008). It was also discovered through personal interaction with poultry farmers and other individuals in the Plateau state of Nigeria, that the fruit pulp is used to treat poultry diseases and in the management of Hepatitis B. According to Wanning *et al*. (2007), the fruit pulp is used as a remedy to all diseases hence its local name 'Kanda' which means 'stop it before it comes' or 'a local vaccine. Moreover, the crude extract of *C. metuliferus* has been shown to possess antiviral properties (Noel *et al*., 2009). Moreover, the two plants *L. kituiensis* and *C. metuliferus* have been used in Tanzania for the management of malaria (Mzena *et al.*, 2018). Since the crude extract of *L. kituiensis* and *C. metuliferus* has been shown to possess antimalarial property (Mzena *et al.*, 2018) and it is known that certain constituents of a plant can be largely responsible for its medicinal activities, this study, therefore, evaluates phytochemical compounds of *L. kituiensis* and *C. metuliferus* that are responsible for its antimalarial activity.

4.2 Materials and methods

4.2.1 Collection of plant and extraction of the plant materials

Fresh leaves *of L. kituiensis* and *C. metuliferus* were collected from Ugweno ward within Mwanga District, Kilimanjaro Region in Tanzania. Ugweno ward is situated at 3° 39' 0" South and 37° 39' 0" East in the Pare Mountains. Herbalists residing in Ugweno ward and a taxonomist (Josephat Mboya) were consulted and involved in the identification and collection of plant materials. The voucher specimen coded (Voucher no. 160 for *C. metuliferus* and 161 *L. kituiensis*) was deposited at Nelson Mandela African Institution of Science and Technology.

Extraction of the plants: The collected plant leaves were cleaned with tap water and dried under shade at room temperature for two weeks and coarsely powdered using an electric blender. Then 200 g of the pulverized materials were soaked with 80% methanol (1600 mL), 100% ethyl acetate (1600 mL) and 100% chloroform (1600 mL) until the powder was fully immersed, incubated overnight and filtered through a whatmann No.1 Whatman®, England). The filtrate is then concentrated to 1ml by bubbling nitrogen gas into the solution. The extract obtained was employed in GC-MS for analysis of different compounds

4.2.2 Preliminary phytochemical screening

Preliminary phytochemical screening of chloroform, ethyl acetate and methanol of *L. kituiensis* and *C. metuliferus* leaf extract was tested for alkaloids, terpenoids, flavonoids, tannins,

glycosides, phenol, steroids and saponins. The qualitative results are expressed as (+) for the presence and (−) for the absence of phytochemicals.

(i) Test for alkaloids

A few drops of Dragendroff's reagent were added to a test tube containing 1 ml of plant extract and a colour change was observed. The appearance of an orange colour was an indication of the presence of alkaloids (Firdouse & Alam, 2011).

(ii) Test for terpenoids

In this test, about 5 ml of plant extract was added to 2 ml of chloroform and 3 ml of concentrated sulphuric acid (H_2SO_4). The presence of terpenoids was indicated by reddish brown colour (Edeoga *et al.,* 2005).

(iii) Test for flavonoids

About 2 ml of plant extract was treated with few drops of dilute sodium hydroxide (NaOH), followed by addition of dilute hydrochloric acid (HCl). A yellow solution with NaOH turned colorless with dilute HCl, which indicated the presence of flavonoids (Onwukaeme *et al.,* 2007).

(iv) Test for phenols and tannins

About 2 ml of plant extract was stirred with 2 ml of distilled water and a few drops of ferric chloride ($FeCl_3$) solution were added. Formation of dark blue precipitate was an indication of the presence of tannins or a blue-green or black coloration indicated the presence of phenols and Tannins (Kumar *et al.,* 2007).

(v) Test for saponin

About 5 ml of plant extract was shaken vigorously with 5 ml of distilled water in a test tube. The formation of stable foam was taken as an indication of the presence of saponins (Parekh & Chanda, 2007).

(vi) Test for glycosides

Liebermann's test- crude extract was mixed with each of 2 ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H_2SO_4 was added. A colour change from violet to blue to green indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside.

(vii) Test for steroid

Crude extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2 ml of chloroform. Then 2 ml of each of concentrated H_2SO_4 and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids

4.2.3 GC-MS analysis

The GC-MS analysis was carried out using Agilent 6890N GC connected to the Agilent 5975 MS (Agilent Technologies, USA) with a capillary column (HP-5) of 30 meter length, 0.25 mm diameter and 0.25 µm film thickness. Helium gas (99.99%) was used as carrier gas at a constant flow of 1mL/min and an injection volume of 1 µL was employed. The injector temperature was maintained at 250°C, the ion-source temperature was 280°C, the oven temperature was programmed at 110°C (isothermal for 2 min), with an increase of 10°C/min to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. The mass spectrometer operated in electron ionization mode with an ionizing energy of 70 eV and the ion source temperature was 230°C. The inlet line temperature was 200ºC and the total GC-MS running time was 36 minutes. Interpretation of the mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than sixty two thousand patterns. The mass spectra of the detected compounds from the *C. metuliferus* and *L. kituiensis* chloroform extract were compared with the spectra of the known compounds stored in the NIST library.

Figure 3: A Gas Chromatography connected to the Mass Spectrometer instrument used in the study

4.2.4 Identification of phyto components

Interpretation on mass-spectrum GC-MS was conducted using the database of central electrochemical research institute characterization and measurement laboratory having more than 62 000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the central electrochemical research institute characterization and measurement laboratory. The name, molecular weight and structure of the components of the test materials were ascertained.

4.3 Results

The preliminary phytochemical screening of chloroform, ethyl acetate and methanolic extract of *L. kituiensis* and *C. metuliferus* plants revealed the presence of major groups of phytochemical compounds namely steroids, alkaloids, flavonoids, tannins, glycosides, sapponin, and phenol compounds (Table 5). The study revealed that the leaf extracts of *Lippia kituiensis* showed the presence of alkaloids, flavonoids, phenols, tannins, terpenoids and saponins in all solvent extract. Glycosides are completely absent in methanolic and chloroformic extracts but it is present in ethyl acetate extract. For *Cucumis metuliferus* alkaloids, glycosides, flavonoids, phenols, terpenoids and tannins were present in all solvent extracts Saponins are absent in methanolic extracts, but present in Chloroform ethyl acetate extracts.

Table 5: Preliminary phytochemical screening of *L. kituiensis* and *C. metuliferus* methanol, ethyl acetate and chloroform leaf extract

Plants name	Alkaloids	Terpanoids	Flavonoids	Phenol	Tannis	Sapponin	Glycosides	Steroids
Methanol								
L.kituiensis	+	+	+	+	+	+	---	---
C. metuliferus	+	+	+	+	+	---	+	+
Ethyl acetate								
L.kituiensis	+	+	+	+	+	+	+	+
C. metuliferus	+	+	+	+	+	+	+	+
Chloroform								
L.kituiensis	+	+	+	+	+	+	---	---
C. metuliferus	+	+	+	+	+	+	+	+

Key: + (Presence of compound), --- (Absence of compound).

Furthermore, the volatile phytochemical compounds present in these plants *C. metuliferus* and *L. kituiensis* were identified by GC-MS technique. The retention time, peak areas, molecular formulas, molecular weights and biological activities of these compounds presented in Table 6 and Table 7, and the structures of the identified compounds are presented in Table 8. The result revealed the presence of 11 major compound namely Cyclotrisiloxanehexamethyl; Z-Z-6, 28-heptatriactontadien-2-one; D-Manitol, 1, 1, 1'-o-1, 16-hexadecanediylbis; Trans, cis-1, 8-dimethylspiro(4, 5)-Decane and L-(+)-Ascorbic acid 2, 6-dihexadecanoate, 3, 7, 11, 15-Tetramethyl-2-Hexadecen-1-ol; Oleyl alcohol tri fluoroacetate; 1-Pentatriacontanol; N-propyl 11-octadecenoate, fucosterol, Artemiseole compound. For *C. metuliferus* plants were: Cyclotrisiloxanehexamethyl; Z-Z-6, 28-Heptatriactontadien-2-one; were found in methanolic extract and D-Manitol, 1, 1, 1'-o-1, 16-hexadecanediylbis; Trans, CIS-1, 8-Dimethylspiro (4,5)-Decane and L-(+) -Ascorbic acid 2, 6-Dihexadecanoate were found in ethyl acetate extract table 5 scheme 1.While the constituents found in *L. kituiensis* plants were 3, 7, 11, 15-Tetramethyl-2-Hexadecen-1-ol; Oleyl alcohol trifluoroacetate; in methanolic extract and 1-Pentatriacontanol; N-propyl 11-octadecenoate, fucosterol compound were found, in ethyl acetate extract while in chloroform extract Artemiseole was found as showed in Table 7

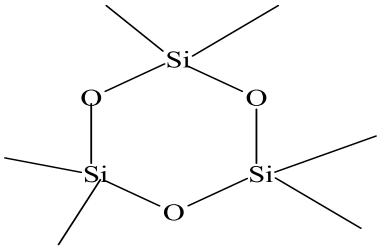
Table 6: The antmalarialcompound present in *Cucumis metuliferus*

RT (min)	PA (%)	Name of Compound *(Cucumis metuliferus)*	M. F	M. W	Solvent used
27.033	0.899	Cyclotrisiloxanehexamethyl	$C_6H_{18}O_3Si_3$	222	Chloroform
16.769	4.989	Z, z-6,28-heptatriactontadien-2-one	$C_{37}H_{70}O$	530	Methanol
16.349	2.790	D-Manitol, 1,1'-O-1,16-Hexadecanediylbis	$C_{28}H_{58}O_{12}$	586	Ethyl acetate
17.044	1.253	Trans, cis-1,8-dimethylspiro [4.5] decane	$C_{12}H_{22}$	166	Ethyl acetate
18.165	24.969	L- (+) -ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	Ethyl acetate

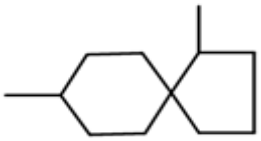
Table 7: The biomolecules present in *Lippia ketuiensis*

RT (min)	PA (%)	Name of Compound *(Lippia ketuiensis)*	M. F	M. W	Solvent used
17.234	1.904	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	Methanol
18.025	3.191	Oleyl alcohol, trifluoro acetate	$C_{20}H_{35}O_2F_3$	364	Methanol
19.495	0.978	1-Pentatriacontanol	$C_{35}H_{72}O$	508	Ethyl acetate
19.845	14.735	N-propyl 11-octadecenoate	$C_{21}H_{40}O_2$	324	Ethyl acetate
29.394	40.593	Fucosterol	$C_{29}H_{48}O$	412	Ethyl acetate
9.3	1.15	Artemiseole	$C_{15}H_{16}O$	152	Chloroform

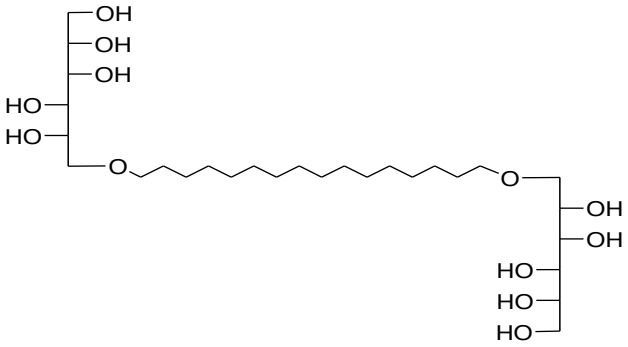
Table 8: Major chemical structures found in *C. metuliferus* and *L. kituiensis* extract



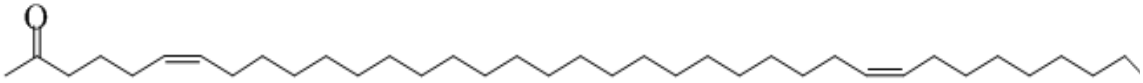
Cyclotriloxane hexamethyl



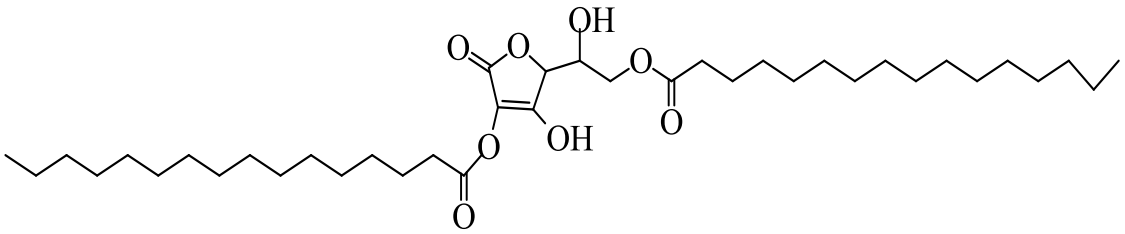
Trans, cis- 1, 8–Dimethylspiro (4, 5) –Decane



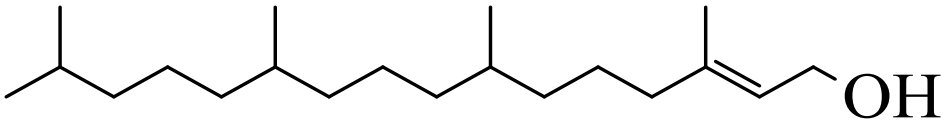
D-Mannitol, 1,1'–O–1,16–Hexadecanediylbis



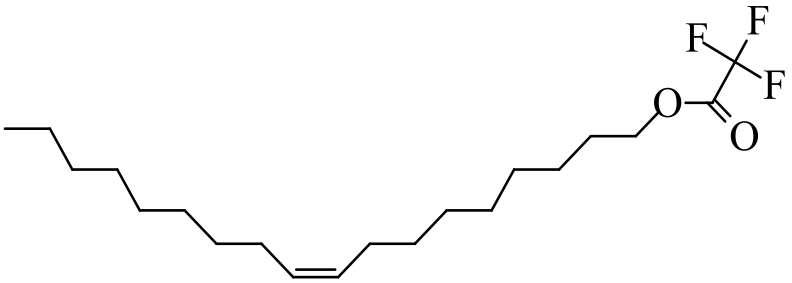
Z–Z–6, 28–Heptatriactontadien–2–one;



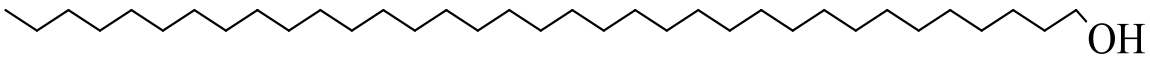
L–(+)–Ascorbic acid 2, 6-Dihexadecanoate



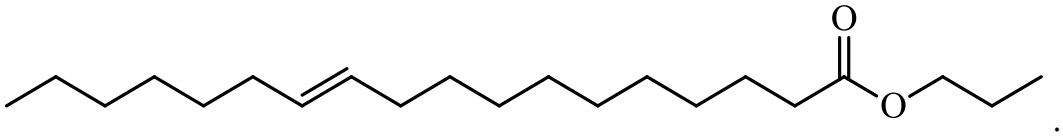
3, 7,11, 15- Tetramethyl-2-Hexadecen-1-ol;



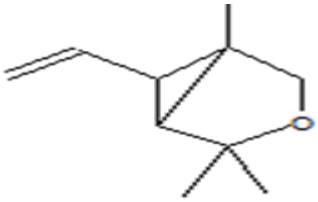
Oleyl alcohol, Trifluoroacetate;



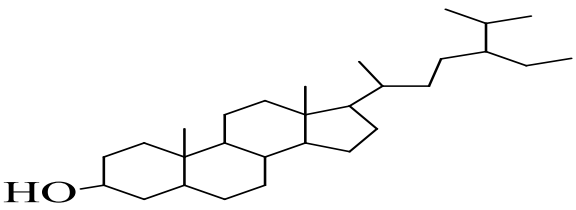
1-Pentatriacontanol



N-propyl 11-octadecenoate



Artemiseole



Fucosterol

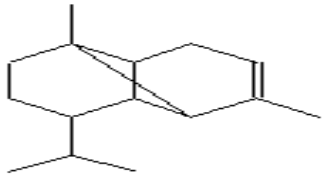
Other usefully compounds present in both plants *C. Metuliferus* and *L. kituiensis* ware, Terpineol cis β, β-Tocopherol, Squalene, Vitamin E, n-hexadecanoic acids, Octadecadienoic acids, Heneicosane, Santolina epoxide, Tetradecanoic acids, Copaene, Tetratriaicontane, Octadecadienoic acid, α-curcumene, α-cubebene, Epizonarene, α-Tocopherol, Stigmastero, Tetratriacontane, Oleic aci (Table 9).

Table 9: The biomolecules present in both *C. metuliferus* and *L. kituiensis*

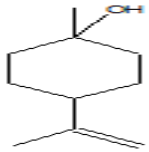
RT (min)	P A (%)	Name of Compound	M. F	M. W	Reported bioactivity
11.43	5.73	Terpineol cis β	$C_{10}H_{18}O$	154	Antibacterial
31.03	5	β-Tocopherol	$C_{28}H_{48}O_2$	416	Antioxidants
10.17	3.19	Squalene	$C_{30}H_{50}$	410	Antioxidants,
24.07	1.88	Vitamin E	$C_{29}H_{50}O_2$	204.35	Antioxidants
16.89	1.78	*n*-hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	Antitumor
18.88	1.65	Octadecadienoic acid	$C_{18}H_{32}O_2$	280.45	Antitumor
14.01	1.49	Heneicosane	$C_{15}H_{44}$	296	Antioxidant
23.29	1.23	Santolina epoxide	$C_{10}H_{16}O$	152.23	Cardiovascular
16.82	1.2	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	Antitumor
33.16	1.89	Tetratriaicontane	$C_{34}H_{70}$	478	Antioxidant
18.88	1.65	Ooctadecadienoic acid	$C_{18}H_{32}O_2$	280.45	Antitumor
8.84	0.61	α-cubebene	$C_{15}H_{24}$	204.35	Antioxidant
14.45	0.56	α-Tocopherol	$C_{28}H_{48}O_2$	416	Antioxidants
21.44	0.52	Stigmastero	$C_{29}H_{48}O$	412	Antimicrobial
9.89	0.32	Tetratriacontane	$C_{34}H_{70}$	478	Antioxidant
8.52	0.15	Oleic Acids	$C_{18}H_{34}O_2$	282	Antioxidant

KEY- RT-Retention time, M.F-Molecular formula, M.W-Molecular weight (g/mol)

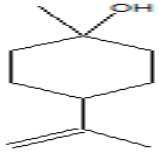
Table 10: chemical structures found in all extract of *C. metuliferus* and *L. kituiensis*



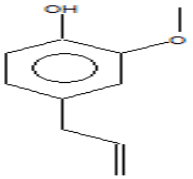
Copaene



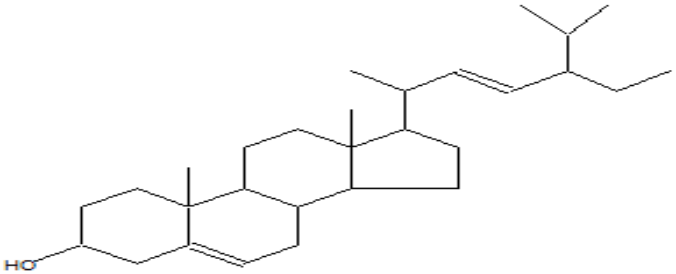
cis-β-Terpineol



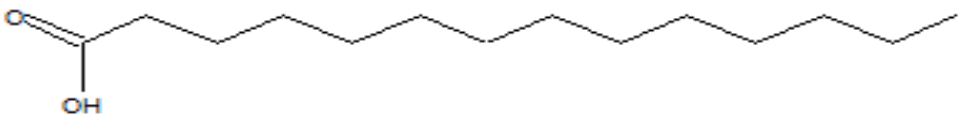
Terpineol, cis-β-



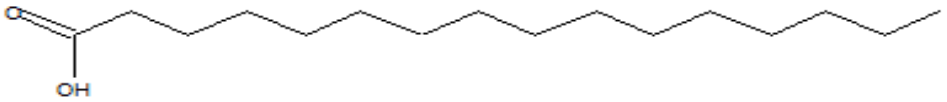
Euginol



Stigmasterol

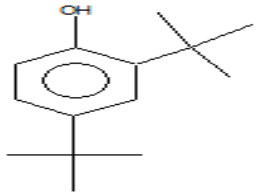


Tetradecanoic acid

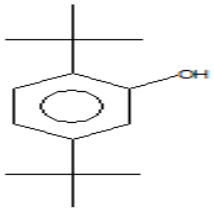


N-hexadecanoic acid

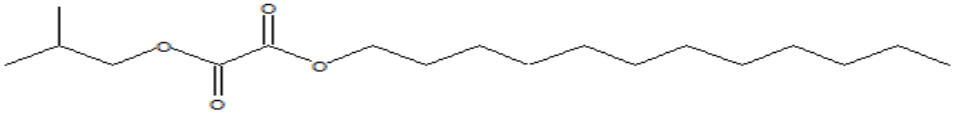
Tridecanoic acid



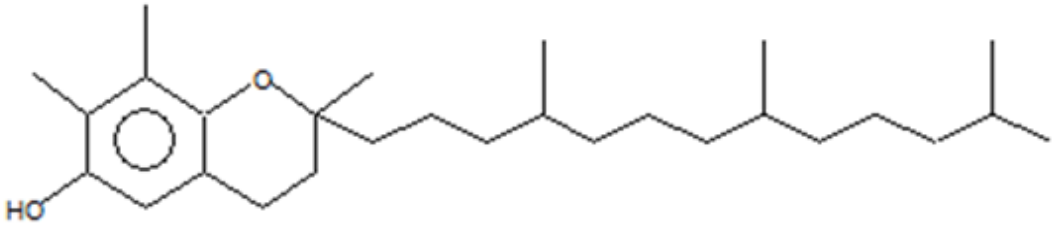
Phenol, 2, 4-bis (1, 1-dimethylethyl)



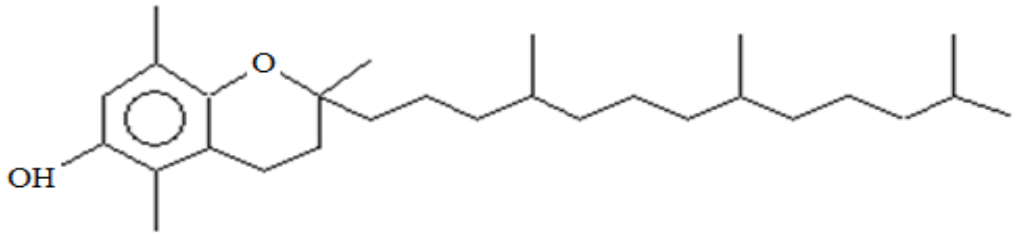
Phenol, 2, 5-bis (1, 1-dimethylethyl)-



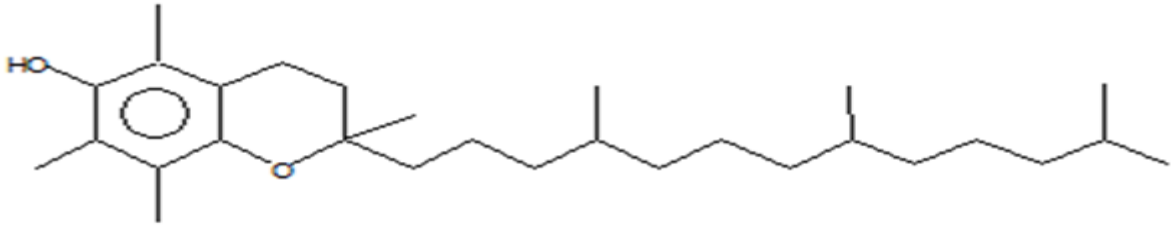
Oxalic acid, dodecyl isobutyl ester



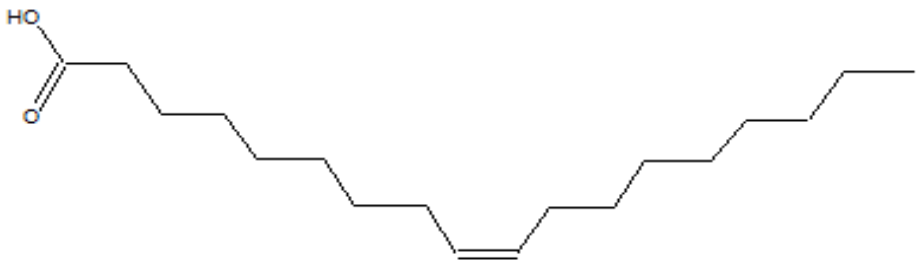
γ-Tocopherol



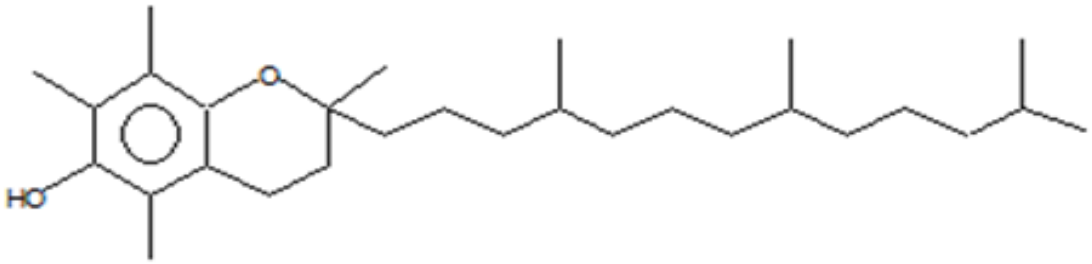
β-Tocopherol



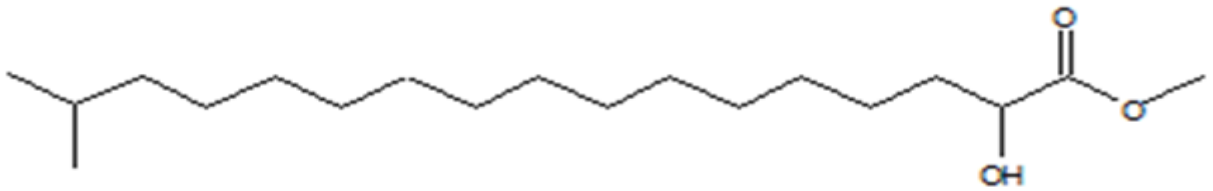
Vitamin E



Oleic Acid



α-Tocopherol



Hexadecanoic acid

4.4 Discussion

The preliminary phytochemical analysis of the crude extract of *C. metuliferus* and *L. kituiensis* showed the presence of phenolics, flavonoids, tannins, steroidal compounds and saponins. Phenolic compounds like flavonoids and tannins are good antioxidant substances that have been reported to have biological activities and prevent or control oxidative stress related disorders (Naskar *et al*., 2010). Previous studies indicated that many phenolic and steroidal compounds possessed anti-plasmodial activities (Laphookhieo *et al*., 2009; Beroa *et al*., 2009). Malarial pathogenesis was reported to be associated with free radicals' formation and decrease of antioxidant levels (Kronenberger *et al.,* 2014; Becker *et al.,* 2003; Mulker *et al*., 2004). This suggests that an increase in the body antioxidant can initiate the decrease in parasite number and severe infection in the long run (Kronenberger *et al.,* 2014; Wannang *et al*., 2010). Oxidative stress has been implicated in the pathophysiology of malaria (Shahidi *et al*., 1992). The efficacy of antioxidants is usually associated with their ability to inhibit oxidative damage by scavenging free radicals. The extract also has phenolic compounds that are antioxidant agents that also act as free radical terminators (Shahidi *et al*., 1992). The phenolic compounds found in the extract may be involved in the antimalarial activity exhibited by the *C. metuliferus* and *L. kituiensis* crude extract. The antimalarial activity of phenolic compounds was reported to be due to elevation of red blood cell oxidation, inhibition of parasite's protein synthesis and also by counteracting the oxidative damage induced by the malaria parasite (Wannang *et al*., 2010). Plants with antioxidant compounds may ameliorate the progression of malarial infection and possibly prevent the development of cerebral complications and other malaria related complications. However, the challenge here is that some antimalarial drugs generate free radicals to produce their effect suggesting that, their activity may be reduced in the presence of antioxidants. The anti-plasmodial activity observed in this study is consistent with the traditional use of the *C. metuliferus* and *L. kituiensis* as herbal medications against malaria in Tanzania.

Moreover, the crude extract tested positive to alkaloids. Alkaloids are one of the major classes of compounds possessing antimalarial activity, and one of the oldest and important antimalarial drugs, quinine belongs to these compounds (Dharani *et al*., 2008). The presence of alkaloids in *C. metuliferus* and *L. kituiensis* extracts might have contributed to antimalarial activities exhibited by the plant extracts. This finding is similar to the findings reported by Ferreira *et al*. (2012) that alkaloids were present in species of *Lippia.*

Additionally the study identified the major 11 compounds using GC-MS analysis namely Cyclotrisiloxanehexamethyl; Z-Z-6, 28-heptatriactontadien-2-one; D-Manitol, 1, 1, 1'-o-1, 16-hexadecanediylbis; Trans, cis-1, 8-Dimethylspiro (4, 5)-Decane and L-(+)-Ascorbic acid 2, 6-dihexadecanoate, 3, 7, 11, 15- Tetramethyl-2-Hexadecen-1-ol; Oleyl alcohol tri fluoroacetate; 1-Pentatriacontanol; N-propyl 11-octadecenoate, fucosterol, Artemiseole compound. According to Sowmiya *et al.* (2015) several of these compounds were screened *in vitro* against both chloroquines (CQ) sensitive and resistant *P. falciparum* isolates and were found to exhibit antiplasmodial activity. In our study, *C. metuliferus* was matched with major compounds of cyclotrisiloxane hexamethyl and the antiplasmodial activity exhibited by this compound was (IC_{50}18.14 μg/ml).

4.5 Conclusion

The different polarity solvent (methanol, ethyl acetate and chloroform) extracts of *C. metuliferus* and *L. kituiensis* were evaluated for phyto chemical compound. The GC-MS results revealed unique chemical classes present in the *L. kituiensis* and *C. metuliferus* that might be responsible for the antiplasmodial effect against *Plasmodium berghei* observed in previous chapter. Further studies are highly warranted for the antiplasmodial activity of identified unique chemical classes present in the *C. metuliferus and L. kituiensis* and also the safety and efficacy through animal model studies. However, a detailed experimental analysis of toxicity present in these plants remains unveils to complete the safety profile of this plant.

CHAPTER FIVE

In vivo EVALUATION OF ACUTE AND SUB-ACUTE TOXICITY OF CHLOROFORM, ETHYLACETATE AND METHANOL EXTRACT OF *Lippia kituiensis* and *Cucumis metuliferus* EXTRACT IN MICE AND RATS MODELS

Abstract

Objective: From the recommendations given in the previous chapter, acute and sub-acute oral toxicity of *L. kituiensis* and *C. metuliferus* leaf extracts in mice and rats was conducted in this chapter to complete the safety profile of this plant extract. **Methods**: Acute oral toxicity study of chloroform, ethyl acetate and methanol extracts of *L. kituiensis* and *C. metuliferus* was carried out by the administration of 300, 600, 1500, 2500 and 5000 mg/kg body weight to mice in the respective groups. The LD_{50} of the *C. metuliferus* and *L. kituiensis* extracts was determined to be not greater than 2000 mg/kg body weight. Sub-acute toxicity study was conducted by oral administration of the extracts at daily doses of 150, 300 and 500 mg/kg body weight in the respective groups of rats for 28 days, and a positive control consisting of 1% DMSO and 5 mL was given to each. **Results**: In the first phase of acute toxicity, all treated groups revealed neither mortality nor significant alteration in behavior, body weight, and hematology parameters. However, the significance difference was observed in organ weight at a dose of 600 mg/kg and 1500 mg/kg of the tested plant extract in both plants. In the second phase, all animals in dose level of 5000 mg/kg died within 24hrs while in dose level of 2500 mg/kg died within 8 days. In sub acute study the result revealed neither mortality nor significant alteration in behavior between treated and control. The significant increase ($P<0.05$) was observed in body weight in all doses in both plants while organ weight and haematological parameters showed significant difference in dose level 300 mg/kg and 500 mg/kg when compared to the control. Moreover, the significant change was observed in biochemical parameters of both sexes in a dose of 300 mg/kg and 500 mg/kg body weight of *C. metuliferus* and *L. kituiensis* extract. A significant histological change was observed in the liver, kidney, lungs and spleen in all extractextract of *C. metuliferus* and *L. kituiensis* in a dose of 300 mg/kg and 500 mg/kg body weight. **Conclusion**: These plant scan causes' severe toxicity to animals.

Key words: Sub acute, toxicity, *Lippia kituiensis*, *Cucumis metuliferus*

5.1 Introduction

The search for new drugs which are plant-derived has been receiving renewed interest among researchers throughout the world in view of discovering new drugs that possess potency to combat the menace of drug resistant agent (About *et al.,* 2000; Pimenta *et al*., 2003). Plants can be useful either in their crude or advanced forms, offering a source of drugs in their pure state (Saint *et al*., 2010; Soejarto *et al.,* 2009). According to the World Health Organization's questionnaire, it is announced that 80% of the population in the world are relying on unconventional medicine, mainly in plant sources, in the primary health protection (WHO, 2015). Recognized for their ability to produce a wealth of secondary metabolites, many of these natural products have been shown to present interesting biological and pharmacological activities, which could serve as the starting point in the development of modern medicines (Abubakar *et al*., 2010)

Many drugs such as vinblastine and vincristine (first cures in human cancer) from *Catharanthus roseus,* Quinine (anti-malarial agent) from *Cinchon species*, Scopolamine (sedative) from *Datura metel* L., and many others that remained in use until the present day were developed from plants (Farnsworth & Soejarto, 2009). Although many plants have valuable properties, some of them are known to carry toxicological properties as well (Magano *et al*., 2011). Recent studies indicate that although numerous plants are used as food sources, some of them may have mutagenic or genotoxic potential (Tülay & Özlem, 2007). Numerous research studies have recently focused on both pharmacology and toxicity of medicinal plants used by humans (Jimma *et al*., 2011). This is of high importance to achieve a safe treatment with plant products (Parra *et al*., 2001). The toxicity of the plants may originate from different contaminants or from plant chemical compounds that are part of the plant (Balkema *et al*., 2003; Martinez *et al*., 2011). Various assays are used for the research of potential toxicity of herbal extracts based on different biological models, such as in vivo assays on laboratory animals. Therefore, this study reports the toxicity activity of *C. metuliferus* and *L. kituiensis* extracts from medicinal plants traditionally used for the treatment of malaria in Tanzania.

5.2 Materials and methods

5.2.1 Plants material and preparation of extract

The plants (*C. metuliferus* and *L. kituiensis*) were collected in September 2016 from Ugweno ward within Mwanga District, Kilimanjaro Region in Tanzania. Herbalists residing in Ugweno ward and a taxonomist (Mr. Josephat Mboya) were consulted and involved in the identification and collection of plant materials. The voucher specimen coded (Voucher no. 160 and 161) was deposited at Nelson Mandela African Institution of Science and Technology.

After the extraction process as described in chapter three above the obtained extracts were, measured and dissolved in 10% DMSO making a ratio of 1:9. For chloroform extract, 918 mg of extract was measured and dissolved in 2.27 ml of DMSO and 28.44 ml of water making a total of 30.71 ml. For ethyl acetate extract, 500 mg of extract was measured and dissolved in 1.01 ml of DMSO and 17.10 ml of water making a total of 18.11 ml. While for methanolic extract 1000 mg of extract was measured and dissolved in 3.07 ml of DMSO and 35.64 ml of water making a total of 38.71 ml stock solution and stored at 4°C.

5.2.2 Experimental animals

Winstar albino rats weighing 62-96 g for male sex and 45-77 g for female sex and swiss albino mice weighing 25–30 g for male sex and 20-27 for female sex from the College of Veterinary Medicine and Medical Sciences of Sokoine University of Agriculture (SUA) were used in these experiments. All animals were kept in a meshed cage covered with sawdust beddings at room temperature 25°C - 30°C. Different sexes were separated to avoid physiological interaction like mating and fighting. All experimental animals were exposed to a 12:12 dark-to-light cycle and provided with free access to a pellet diet and clean drinking water. All rats were acclimatized to the working environment for one week before the beginning of the experiment.

5.2.3 Acute toxicology assessments

The acute oral toxicity test of chloroform, ethyl acetate and methanol extracts of *L. kituiensis* and *C. metuliferus* ware evaluated in swiss albino mice as reported by Muhammad *et al*. (2015) with little modifications that involved two phases. The first phase was conducted as follows. For *L. kituiensis* extract forty five (45) mice were grouped into three groups (chloroform, ethyl acetate and methanol) of fifteen mice per group. Each group consist of three doses (300, 600 and 1500 mg/kg) having five mice in each dose making a total of forty five (45) mice, two

females and three males. A similar procedure was repeated for *C. metuliferus* extract that forty five (45) mice were grouped into three groups (chloroform, ethyl acetate and methanol) of fifteen mice per group. Each group consists of three doses (300 600 and 1500 mg/kg) having five mice in each dose making a total of forty five (45) mice, two females and three males different sex were separated to avoid physiological interaction like mating. Five mice received a vehicle as a positive control making a total of ninety five mice (95).

Group 1: Vehicle (1%DMSO)

Group 2: 300 mg/kg of extract

Group 3: 600 mg/kg of extract

Group 4: 1500 mg/kg of extract

Following an overnight fast, the mice were weighed, and the dose was calculated in reference to their body weight. The first phase received 300, 600 and 1500 mg/kg body weight of the chloroform, ethyl acetate and methanol extracts of *L. kituiensis* and *C. metuliferus*. The positive control received 1% of DMSO. The extract was dissolved in 10% DMSO and given via oral gavage route. The animals were observed keenly for about 30 min for any signs of toxicity or mortality, and further observations were made every 8 h for 24 h after administration of the extracts. The general behavior such as urination, food intake, water intake, respiration, convulsion, and temperature, changes in eye, skin colors and body weight were observed for a period of 14 days post-treatment. After 14 days other parameters such as body, organ weight and hematology were determined.

The absence of death of any animals in this phase necessitated the conduct of the second phase. In the second phase, 24 mice were grouped into six groups of four mice each. The first three groups received 2500 and 5000 mg/kg body weight of chloroform, ethyl acetate and methanol extracts of *L. kituiensis* while the other three groups were administered with of chloroform, ethyl acetate and methanol extracts of *C. metuliferus* at a dose of 2500 and 5000 mg/kg body weight. The mice were observed for any signs of toxicity or mortality within 24 h. In this phase, all animals in dose level 5000 mg/kg died within 24 while the animal in dose level 2500 mg/kg died within 8 days

After the test procedure, the formula employed in the calculation of the LD_{50} is shown below:

$$LD_{50} = \frac{(M_0 + M_1)}{2}$$

Where M_0 = Highest dose of test substance that gave no mortality,

M_1 = Lowest dose of test substance that gave mortality

5.2.4 Sub-acute toxicology assessments

Sub-acute toxicity study (28-day repeated oral toxicity study) was carried out according to Muhammad *et al.* (2015). Both sexes of rats (62-96 g for male sex and 45-77 g for female sex) were divided into groups, each comprising 5 animals. The same number of rats was used as in acute study first phase ninety five (95) rats was used, the 1st group served as the positive control, while 2nd, 3rdand 4thwere considered as tested groups and orally received *C. metuliferus* and *L. kituiensis* chloroform, ethyl acetate and methanolic extract at dose levels of 150, 300, and 500 mg/kg. Before dose administration, the body weight of each animal was determined and the dose was calculated according to the bodyweight of each animals. The group 1 received 1% DMSO vehicle orally at a dose volume of 5 ml/kg body weight and served as a control group. All the groups of rats were observed ones a day for mortality, morbidity, bodyweight and other clinical signs until the completion of the experiment. Blood samples were collected on the 29th day the last day of the experiment from retro orbital plexus of the eye using VITREX NRIS soda lime glass 80IU/ml heparinised microhematocrit tubes into EDTA and plain (with clot activator tubes) vacutainer tubes for hematological and biochemical laboratory analysis, after which the rats were sacrificed and vital organs (liver, lung, kidney and spleen) were collected and fixed in 10% buffered formalin for histopathology.

5.2.5 Hematological and serum biochemical examination

On the 29th day of the study, following an overnight fasting, all animals in various groups were anesthetized with chloroform and blood samples were collected into heparinized bottles for hematological analysis. Red blood cell count (RBC), white blood cell count (WBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), lymphocytes (LYM), monocytes (MON) and red blood cells distribution width (RDW) were analyzed using *NS4s* auto analyser, Made in Germany. Blood samples without anticoagulant were used for serum biochemistry analysis; the samples were placed at room temperature for 1 h and then centrifuged at 1500 × g for 10 min to obtain serum. The serum was used for analyzing the following parameters, Alanine transaminase (ALT), Alkaline phosphatase (ALP), Aspartate transaminase (AST), Albumin (ALB), Total bilirubin (TB), Total protein (TP), Creatinine (CREA), urea (UREA), Triglyceride (TG), Total cholesterol (TCHO) and Glucose (GLU).

5.2.6 Determination of absolute and relative organ weight

Absolute and relative organ weight of vital organs (liver, kidney, heart, lungs and spleen) of a treated animal was determined using an electronic balance and compared with that of a control group. Organ-to-body weight ratio was calculated by dividing the weight (g) of each organ by the weight (g) of mice before sacrifice as per the given formula.

$$\text{ROW} = \frac{\text{Absolute organ weight (g)}}{\text{Rat's body weight}} \times 100$$

5.2.7 Histopathological examination

For histopathological examinations, organs were fixed in buffered formalin 10%, before being embedded in paraffin. After routine processing, five micrometers of paraffin sections were prepared and stained with hematoxylin and eosin before microscopic examination. All the sections were examined under a light microscope under 40x magnifications.

5.2.8 Statistical analysis

Data collected from the biochemical and hematological analyses were expressed as Mean ± SD. One-way ANOVA was used to test the means. Values were considered statistically significant at $P < 0.05$. All results were represented as Mean ± SD ($n = 5$).

5.2.9 Ethical consideration

Prior to the experimental work, an ethical clearance with notification number NIMR/HQ/R.8a/Vol. IX/2146 was given by the National Health Research Ethics Sub-Committee (NatHREC) of the National Institute for Medical Research (NIMR) in Tanzania.

5.3 Results

5.3.1 Acute oral toxicity study

The acute toxicity effect of chloroform, ethyl acetate and methanolic extract was determined as per Muhammad *et al.* (2015), with little modifications that involved two phases. In the first phase no treatment-related toxic symptom or mortality was observed after oral administration of the tested plants (*C. meturiferus* and *L. kituiensis*) extract at a dose of 300 mg/kg, 600 mg/kg and 1500 mg/kg. No change in behavior pattern, body weight, organ weight and hematological parameter of the treated animals observed as compared to the control. All the animals given the extracts survived for 14 days period of observation.

5.3.2 Effect of *C. meturiferus* and *L. kituiensis* extract on absolute and relative organ body weight

The mean absolute and relative organ weight of male and female mice are shown in Table 11, 12, 13, 14, 15 and 16. The absolute and relative organ weight of spleen, heart, lungs, liver and kidneys in mice treated with chloroform, ethyl acetate and methanolic extract in dose level of 300 mg/kg, 600 mg/kg and 1500 mg/kg were determined. A significant difference was observed in some organs when compared to controls.

For chloroform extract theof *C. metulifures* the result showed a significant increase in liver, lungs and kidney organs in dose level of 600 mg/kg and 1500 mg/kg for both males and females when compared to control (Table 11). For ethyl acetate extract the result reveared significant increases in kidney of the male mice treated in dose levels 600 mg/kg and 1500 mg/kg and in lungs of the mice treated in dose level 1500 mg/kg when compared to the control. For female mice, the significant increase of kidney, lungs and spleen in mice treated in dose level 600 mg/kg and 1500 mg/kg was observed when compared to control (Table 12).

For mice treated with methanolic extract of *C. metuliferus*, the result revealed significant increases of liver, kidney and heart at a dose of 600 mg/kg and 1500 mg/kg and lungs at a dose of 1500 mg/kg as compared to control. Female mice the significance increases were observed

in dose level 600 mg/kg and 1500 mg/kg body weight of liver, lung and kidney ($P > 0.05$) when compared to control (Table 13).

For mice treated with *L. kituiensis* chloroform extract revealed significant reduction of kidney and lungs of the male mice treated in a dose of 1500 mg/kg body weight. Female mice, the significant reduction were observed in treatement dose level of 600 mg/kg and 1500 mg/kg of kidney and lungs when compared to control (Table 14).

For the mice treated with ethyl acetate extract the result showed significant reductions of liver and kidney in mice treated in a dose of 1500 mg/kg when compared to control, while female mice, the significant reductions was recorded in tratement dose level 600 mg/kg and 1500 mg/kg of liver, lungs and spleen when compared to control (Table 15). No significant difference was observed at a dose of 300 mg/kg, 600 mg/kg and 1500 mg/kg in both male and female treated with methanolic extract (Table16).

Table 11: Effect of chloroform extract of *C. meturiferus* on absolute (A) and relative organ weight (R %) of mice after 14 days of treatment

Sex		**Organ**	**Dose mg/kg n=5**				**Sex**		**Organ**	**Dose mg/kg n = 5**			
			300mg/kg	600mg/kg	1500mg/kg	0mg/kg				300mg/kg	600mg/kg	1500mg/kg	0mg/kg
F	A(g)	Liver	0.90±0.21	1.50±0.41*	1.89±0.01*	1.30±0.10	M	A(g)	Liver	1.50±0.03	1.71±0.9*	1.99±0.10*	1.68±0.01
		Heart	0.11±0.01	0.12±0.01	0.14±0.01	0.13±0.21			Heart	0.15±0.10	0.18±0.10	0.19±0.10	0.18±0.10
		Kidney	0.41±0.1	0.61±0.01*	0.71±0.01*	0.31±0.01			kidney	0.27±0.11	0.35±0.21	0.54±0.11*	0.28±0.11
		Lungs	0.33±0.10	0.43±0.10	0.62±0.10*	0.30±0.10			lungs	0.40±0.10	0.55±0.11*	0.74±0.12*	0.21±0.12
		Spleen	0.44±0.01	0.62±0.01*	0.71±0.01*	0.18±0.01			Spleen	0.16±0.30	0.18±0.12	0.19±0.14	0.18±0.13
	R (%)	Liver	1.71±0.01	1.60±0.02	2.43±0.15	1.98±0.13		R (%)	Liver	1.84±0.15	1.92±0.11	1.88±0.25	1.98±0.11
		Heart	0.22±0.14	0.24±0.14	0.28±0.04	0.26±0.05			Heart	0.67±0.12	0.26±0.13	0.29±0.13	0.26±0.12
		Kidney	0.89±0.15	0.72±0.25	0.91±0.15	0.61±0.15			Kidney	0.42±0.11	0.25±0.10	0.28±0.15	0.28±0.17
		Lung	0.49±0.01	0.54±0.02	0.72±2.06	0.63±0.03			Lung	0.43±0.20	0.62±0.15	0.69±0.14	0.46±0.18
		Spleen	0.18±0.07	0.18±0.07	0.18±0.07	0.18±0.07			spleen	0.25±0.14	0.24±0.14	0.18±0.14	0.19±0.17

Values are expressed as mean± SD. A: absolute organ weight (g); R: relative organ weight (%). * P< 0.05 compared with the control (0mg/kg).

Table 12: Effect of ethyl acetate extract of *C.meturiferus* on absolute (A) and relative organ weight (R %) of mice after 14 days of treatment

Sex		**Organ**	**Dose mg/kg n =5**				**Sex**		**Organ**	**Dose mg/kg n = 5**			
			300mg/kg	600mg/kg	1500mg/kg	0mg/kg				300mg/kg	600mg/kg	1500mg/kg	0mg/kg
F	A(g)	Liver	1.28±0.1	1.28.±0.2	1.32±0.01	1.30±0.01	M	A(g)	Liver	1.68±0.01	1.69±0.20	1.68±0.33	1.68±0.01
		Heart	0.11±0.10	0.12±0.01	0.14±0.01	0.13±0.21			Heart	0.18±0.13	0.23±0.50	0.35±0.10	0.28±0.10
		Kidney	0.45±0.01	0.61±0.41*	0.70±0.12*	0.31±0.01			Kidney	0.48±0.10	0.68±0.11*	0.96±0.15*	0.28±0.11
		Lungs	0.43±0.03	0.45±0.20*	0.51±0.30*	0.30±0.10			Lung	0.38±0.12	0.55±0.12*	0.68±0.22*	0.21±0.12
		Spleen	0.21±0.01	0.32±0.01*	0.50±0.01*	0.18±0.01			Spleen	0.15±0.13	0.16±0.13	0.17±0.13	0.18±0.13
	R (%)	Liver	1.46±0.04	1.58±0.05	1.48±0.33	1.62±0.03		R (%)	Liver	1.87±0.25	1.88±0.11	1.98±0.16	1.88±0.01
		Heart	0.22±0.03	0.22±0.05	0.43±0.02	0.41±0.14			Heart	0.42±0.13	0.42±0.12	0.44±0.16	0.46±0.10
		Kidney	0.20±0.01	0.62±0.15	0.61±0.15	0.56±0.05			Kidney	0.39±0.15	0.48±0.12	0.45±0.17	0.48±0.11
		Lung	0.42±0.06	0.66±0.02	0.73±0.02	0.68±0.06			Lung	0.39±0.14	0.43±0.22	0.46±0.15	0.21±0.12
		Spleen	0.24±0.06	0.19±0.27	0.19±0.05	0.19±0.07			Spleen	0.17±0.11	0.17±0.13	0.18±0.19	0.19±0.13

Values are expressed as mean± SD. A: absolute organ weight (g); R: relative organ weight (%). * P< 0.05 compared with the control (0mg/kg).

Table 13: Effect of methanolic extract of *C. meturiferus* on absolute (A) and relative average organ weight (R %) of mice after 14 days of treatment

Sex		**Organ**	**Dose mg/kg n = 5**				**Sex**		**Organ**	**Dose mg/kg n = 5**			
			300mg/kg	600mg/kg	1500mg/kg	0mg/kg				300mg/kg	600mg/kg	1500mg/kg	0mg/kg
F	A(g)	Liver	1.40±0.51	1.58±0.01*	1.97±0.01*	1.30±0.01	M	A(g)	Liver	1.56±0.09	1.82±0.17*	1.89±0.01*	1.68±0.01
		Heart	0.13±0.10	0.14±0.31	0.16±0.30	0.13±0.21			Heart	0.33±0.10	0.47±0.12*	0.59±0.10*	0.18±0.10
		Kidney	0.45±0.31	0.55±0.10*	0.62±0.21*	0.31±0.01			Kidney	0.34±0.20	0.47±0.11*	0.68±0.11*	0.28±0.11
		Lungs	0.33±0.10	0.43±0.50	0.52±0.10*	0.30±0.10			Lungs	0.25±0.12	0.27±0.12	0.45±0.12*	0.21±0.12
		Spleen	0.17±0.31	0.18±1.01	0.17±1.10	0.18±0.01			Spleen	0.17±0.23	0.16±0.14	0.16±0.15	0.18±0.13
	R (%)	Liver	1.24±0.06	1.48±0.03	1.76±0.03	1.67±0.03		R (%)	Liver	1.63±0.25	1.78±0.12	1.98±0.13	1.98±0.09
		Heart	0.28±0.14	0.28±0.14	0.25±0.14	0.29±0.04			Heart	0.68±0.11	0.88±0.13	0.79±0.11	0.79±0.10
		Kidney	0.62±0.35	0.62±0.02	0.62±0.15	0.65±0.03			Kidney	0.27±0.17	0.28±0.17	0.39±0.17	0.28±0.11
		Lungs	0.06±0.03	0.06±0.16	0.15±0.04	0.07±0.06			Lung	0.45±0.14	0.44±0.12	0.46±0.18	0.44±0.12
		Spleen	0.19±0.02	0.19±0.03	0.19±0.04	0.18±0.07			Spleen	0.17±0.12	0.18±0.19	0.18±0.19	0.19±0.13

Values are expressed as mean± SD. A: absolute organ weight (g); R: relative organ weight (%). * P< 0.05 compared with the control (0mg/kg).

Table 14: Effect of the chloroform extract of *L. kituiensis* on absolute (A) and relative organ weight (R %) of mice after 14 days of treatment.

Sex		**Organ**	**Dose mg/kg n = 5**				**Sex**		**Dose mg/kg n = 5**			
			300mg/kg	**600mg/kg**	**1500mg/kg**	**0mg/kg**			**300mg/kg**	**600mg/kg**	**1500mg/kg**	**0mg/kg**
F	**A(g)**	Liver	1.10±0.01	0.80±0.01*	0.70±0.01*	1.30±0.10	**M**	**A(g)**	1.53±0.02	1.45±0.10	1.05±0.09*	1.68±0.01
		Heart	0.12±0.01	0.12±0.01	0.13±0.01	0.13±0.21			0.16±0.10	0.15±0.20	0.15±0.30	0.18±0.10
		Kidney	0.33±0.01	0.08±0.01	0.04±0.01*	0.31±0.01			0.16±0.31	0.15±0.21	0.07±0.11*	0.28±0.11
		Lungs	0.33±0.10	0.13±0.20	0.03±0.10*	0.30±0.10			0.09±0.11	0.05±0.22	0.02±1.22*	0.21±0.12
		Spleen	0.18±0.01	0.18±0.01	0.18±0.01	0.18±0.01			0.16±0.23	0.16±0.23	0.15±0.03	0.18±0.13
	R (%)	Liver	1.24±0.02	1.60±0.03	1.04±0.03*	1.98±0.11		R (%)	1.56±0.04	1.48±0.13	1.28±0.15	1.98±0.11
		Heart	0.22±0.04	0.23±0.04	0.18±0.04	0.26±0.05			0.27±0.14	0.26±0.16	0.25±0.16	0.26±0.12
		Kidney	0.77±0.05	0.63±0.05	0.60±0.05	0.61±0.15			0.25±0.17	0.29±0.17	0.38±0.17	0.33±0.17
		Lungs	0.61±0.02	0.58±0.02	0.60±0.06	0.63±0.03			0.44±0.18	0.45±0.18	0.48±0.18	0.46±0.18
		Spleen	0.27±0.07	0.18±0.07	0.28±0.07	0.18±0.07			0.26±0.19	0.18±0.19	0.19±0.19	0.19±0.17

Values are expressed as mean± SD. A: absolute organ weight (g); R: relative organ weight (%). * P< 0.05 compared with the control (0mg/kg).

Table 15: Effect of ethyl acetate extract of *L. kituiensi* on absolute (A) and relative organ weight (R %) of mice after 14 days of treatment

Sex		**Organ**	**Dose mg/kg n = 5**				**Sex**		**Organ**	**Dose mg/kg n = 5**			
			300mg/kg	600mg/kg	1500mg/kg	0mg/kg				300mg/kg	600mg/kg	1500mg/kg	0mg/kg
F	A(g)	Liver	1.23±0.11	0.90±0.01*	0.60±0.31*	1.30±0.10	M	A(g)	Liver	1.52±0.09	1.41±0.02	1.04±0.01*	1.68±0.01
		Heart	0.14±0.21	0.13±0.40	0.14±0.01	0.13±0.21			Heart	0.16±0.15	0.16±0.10	0.17±0.17	0.18±0.10
		Kidney	0.40±0.12	0.44±0.01	0.43±0.11	0.31±0.01			kidney	0.28±0.10	0.20±0.21	0.07±0.11*	0.28±0.11
		Lungs	0.25±0.10	0.04±0.10	0.01±0.10*	0.30±0.10			lung	0.18±0.11	0.28±0.10	0.35±0.13	0.21±0.12
		Spleen	0.17±0.01	0.12±0.01	0.11±0.01*	0.18±0.01			Spleen	0.17±0.12	0.17±0.13	0.18±0.13	0.18±0.13
	R (%)	Liver	1.46±0.03	0.85±0.23	0.79±0.03	1.62±0.01		R (%)	Liver	1.69±0.15	1.69±0.13	1.99±0.15	1.88±0.01
		Heart	0.25±0.02	0.26±0.14	0.48±0.04	0.26±0.10			Heart	0.42±0.16	0.42±0.13	0.42±0.16	0.46±0.10
		Kidney	0.46±0.05	0.48±0.15	0.48±0.15	0.48±0.11			Kidney	0.58±0.12	0.51±0.11	0.47±0.17	0.48±0.11
		Lung	0.24±0.06	0.26±0.06	0.26±0.06	0.21±0.12			Lung	0.25±0.12	0.25±0.18	0.23±0.18	0.21±0.12
		Spleen	0.17±0.07	0.19±0.07	0.19±0.07	0.19±0.13			spleen	0.16±0.19	0.17±0.19	0.18±0.19	0.19±0.12

Values are expressed as mean± SD. A: absolute organ weight (g); R: relative organ weight (%). * P< 0.05 compared with the control (0mg/kg)

Table 16: Effect of methanolic extract of *L. kituiensis* on absolute (A) and relative organ weight (R %) of mice after 14 days of treatment

Sex		**Organ**	**Dose mg/kg n = 5**				**Sex**		**Organ**	**Dose mg/kg n = 5**			
			300mg/kg	600mg/kg	1500mg/kg	0mg/kg				300mg/kg	600mg/kg	1500mg/kg	0mg/kg
F	A(g)	Liver	1.05±0.01	1.05±0.11	1.00±0.01	1.30±0.10	M	A(g)	Liver	1.05±0.09	1.57±0.01	1.46±0.12	1.68±0.01
		Heart	0.14±0.21	0.13±0.01	0.12±0.21	0.13±0.21			Heart	0.16±0.11	0.15±0.10	0.15±0.12	0.18±0.10
		Kidney	0.30±0.01	0.41±0.01	0.21±0.01	0.31±0.01			Kidney	0.19±0.14	0.05±0.13	0.09±0.12	0.18±0.11
		Lungs	0.28±0.14	0.33±0.12	0.20±0.13	0.30±0.10			Lungs	0.18±0.11	0.09±0.12	0.05±0.12	0.18±0.12
		Spleen	0.15±0.11	0.16±0.21	0.15±0.01	0.18±0.01			Spleen	0.17±0.12	0.19±0.11	0.13±0.13	0.18±0.13
	R (%)	Liver	1.38±0.03	1.16±0.03	1.29±0.03	1.67±0.03		R (%)	Liver	1.25±0.25	1.17±0.11	1.18±0.15	1.98±0.09
		Heart	0.22±0.02	0.25±0.04	0.24±0.04	0.29±0.04			Heart	0.24±0.13	0.21±0.14	0.24±0.16	0.19±0.10
		Kidney	0.62±0.02	0.61±0.05	0.48±0.05	0.65±0.03			Kidney	0.24±0.12	0.26±0.17	0.29±0.17	0.28±0.11
		Lung	0.28±0.06	0.33±0.06	0.28±0.06	0.17±0.03			Lung	0.45±0.13	0.38±0.18	0.48±0.18	0.44±0.12
		Spleen	0.18±0.07	0.18±0.07	0.18±0.07	0.18±0.07			Spleen	0.16±0.19	0.17±0.18	0.18±0.17	0.19±0.13

Values are expressed as mean± SD. A: absolute organ weight (g); R: relative organ weight (%). * P< 0.05 compared with the control.

5.3.3 Sub-acute toxicity study

The sub-acute toxicity study of chloroform, ethyl acetate and methanolic extract of the tested plant extract was determined as reported by Muhammad *et al.* (2015) where the limit dose of 500 mg/kg was used. No treatment-related toxic symptom or mortality was observed after oral administration of the tested plants (*C. meturiferus* and *L. kituiensis*) as shown in Table 17. No significant change in general behavior of the treated animals and control group was observed. None of the two plant extracts produced mortality after giving a dose of 150 mg/kg/, 300 mg/kg and 500 mg/kg body weight. All rats given the extracts survived for 28 days period of administration and observation and were sacrificed on the 29 days.

Table 17: General appearance and behavioral observations of sub acute toxicity study for control and treated groups of *C. meturiferus* and *L. kituiensis*

PARAMETER	**DOSE(mg/kg)**			
	Control	**300 mg/kg**	**600 mg/kg**	**1500 mg/kg**
Lacrimation	No	No	No	No
Salivation	No	No	No	No
Piloerection	No	No	No	No
Drowsiness	No	No	No	No
Tremors	No	No	No	No
Convulsions	No	No	No	No
Fur	Normal	Normal	Normal	Normal
Food intake	Normal	Normal	Normal	Normal
Water taking	Normal	Normal	Normal	Normal
Mortality	No	No	No	No
Breathing	Normal	Normal	Normal	Normal
Temperature	Normal	Normal	Normal	Normal
Sedation	Normal	No effect	No effect	No effect
Diarrhea	Normal	No present	Not present	Not present
Eye color	Normal	No effect	No effect	No effect
Change skin	Normal	No effect	No effect	No effect
Urination	Normal	No effect	No effect	No effect
Coma	Not present	No effect	No effect	No effect
Death	No	Alive	Alive	Alive

5.3.4 General clinical symptom and mortality of rats

In sub-acute toxicity study of *C. metuliferus* and *L. kituiensis*, no mortality and clinical symptoms were observed during the experimental period at any of the three doses. No differences in general behavior were observed between the control and the treated groups.

5.3.5 Effect of plants on body weight of rats in the sub-acute study

The gain in body weight of rats treated with *C. metuliferus* and *L. kituiensis* were recorded and compared with the control group as shown in Table 18 and Table 19.The mean body weight of male and female rats treated with chloroform, ethyl acetate and methanolic extract of both plants significantly increases in dose level 150 mg/kg, 300 mg/kg and 500 mg/kg as compared to the control.

Table 18: Effect of *C. metuliferus* extract on body weight in sub-acute study

			Body weight(g)						Body weight(g)					
Sex	**Extract**	**Dose**	**1st day**	**7th day**	**14th day**	**21st**	**28th**	**Weight gain (g)**	**1st day**	**7th day**	**14th day**	**21st**	**28th**	**Weight gain (g)**
F	**Chloroform**	0	65.3±0.2	72.0±0.4	75.00±0.4	79.33±0.4	82.4±0.5	16.91±0.8	65.0±0.2	69.9±0.0	74 ±0.0	79.±0.21	85 ±0.21	19.8±0.1
		150	66.6±0.4	69.6±0.2	73.8±0.2	78.3±01	84.0±0.1	19.4±0.4*	60.1±0.1	66.3±0.0	72 ±0.1	78.9±0.0	83.4±0.13	25 ±0.1*
		300	55.9±0.5	62.2±03	66.8±07	74.0±02	79.1±0.2	20.1±0.5*	63.1±0.1	68.0±0.1	74 ±0.3	79.9±0.3	83.9±0.4	20.8±0.4
		500	52.1±0.3	57.0±0.2	63.7±0.9	68.1±0.1	76.9±0.2	19.8±0.6*	66.1±0.1	68.0±0.1	75 ±0.3	79.9±0.4	83.9±0.5	24.1±0.5*
	Ethyl acetate	0	65.3±0.2	72.0±0.4	75.0±0.4	79.3±0.4	82.4±0.5	16.1±0.81	65.0±0.2	69.0±0.0	74 ±0.0	79.9±0.1	85.9±0.21	19.8±0.12
		150	51.9±0.7	57.1±0.1	63.1±0.1	66.1±0.1	70.9±1.2	19.9±0.1*	59.9±0.2	65.09±0.2	71 ±0.1	76.2±0.2	83.9±0.23	24.0±0.9*
		300	54.8±0.1	59.3±0.1	63.1±0.1	73.1±01	75.2±01	19.04±0.2*	58.9±0.2	66.08±0.2	72 ±0.1	77.2±0.3	84.9±0.24	26.9±0.1*
		500	50.8±1.2	55.21±04	61.08±01	66.09±03	69.0±04	20.0±0.5*	62.9±0.2	66.05±0.2	73 ±0.1	75.2±0.4	79.9±0.25	25.9±0.1*
		0	65.3±0.2	72.90±03	75.0±0.4	79.3±04	82.4±0.5	16.1±0.1	65.0±0.2	69.90±0.0	69 ±0.0	81.9±0.1	83.0±0.21	19.3±0.12
	Methanolic	150	61.9±0.8	67.1±0.1	70.9±0.1	75.2±03	81.9±0.1	19.9±0.2*	64.9±0.9	67.20±0.1	73 ±0.2	75.6±0.23	84.9±0.23	24.7±0.3*
		300	61.3±0.9	65.1±0.2	72.9±0.2	76.2±0.4	83.7±0.2	22.04±0.1*	65.9±0.1	66.0±0.1	74.0±0.2	78.6±0.2	83.9±0.2	26.7±0.4*
		500	61.4±0.0	67.01±0.3	73.7±03	76.2±0.5	82.1±0.3	21.1±0.6*	60.1±0.1	68.3±0.1	71.9±0.2	77.7±0.3	84.1±0.3	20.27±0.4

Values are expressed as mean ± SD. P > 0.05 when compared to control group. * = values are significantly different (p < 0.05) from that of the control) compared to the control.

Table 19: Effect of *L. kituiensis* extract on body weight

Sex	Extract	Dose	Body weight(g)							Body weight(g)					
			1st	7th	14th	21st	28th	Weight gain (g)		1st	7th	14th	21st	28th	Weight gain (g)
F	Chloroform	0	65.43±0.6	72.90±0.3	75.00±0.3	79.33±0.4	82.34±0.3	16.91±0.81	M	65.90±0.23	69.90±0.2	74.90±0.5	79.09±0.2	85.09±0.2	19.38±0.12
		150	68.56±0.4	71.66±0.9	74.80±0.2	76.30±0.1	82.40±91	20.84±0.44*		61.01±0.13	66.93±0.1	72.09±0.7	76.09±0.0	82.04±0.1	23.03±0.13*
		300	57.89±0.5	63.02±0.0	66.88±0.7	72.90±0.2	79.21±0.1	23.31±0.55*		65.01±0.14	68.90±0.1	76.09±0.3	79.09±0.0	83.09±0.3	24.08±0.24*
		500	52.31±03	57.90±0.7	62.67±09	68.01±0.1	76.99±0.2	19.68±0.56*		66.01±0.15	68.90±0.1	74.09±0.3	78.09±0.0	83.09±0.3	23.01±0.15*
	Ethyl acetate	0	65.43±0.6	72.90±0.3	75.00±0.3	79.33±0.4	82.34±0.3	16.91±0.81*		65.90±0.23	69.90±0.2	74.90±0.0	79.09±0.2	85.09±0.2	19.38±0.12
		150	53.09±0.5	59.71±0.1	63.81±90	66.81±0.1	71.09±12	22.90±0.18*		61.09±0.22	65.09±0.1	72.09±0.1	75.22±0.2	82.99±0.2	25.90±0.09*
		300	55.98±0.1	58.03±0.1	65.81±0.1	73.01±0.2	75.02±0.1	21.04±0.22*		59.09±0.23	66.08±0.2	70.89±0.1	76.22±0.2	82.99±0.2	23.09±0.10*
		500	49.88±1.0	55.21±0.0	64.08±0.1	68.09±0.1	69.90±0.4	23.90±0.25*		63.09±0.24	64.05±0.3	70.09±0.1	75.22±0.2	79.99±0.2	21.9±0.11*
	Methanolic	0	65.43±0.6	72.90±0.3	75.00±0.3	79.33±0.4	82.34±0.3	16.91±0.81*		65.90±0.23	69.90±0.4	69.90±0.0	81.09±0.2	83.09±0.2	19.38±0.12
		150	61.09±0.7	67.01±0.2	72.09±0.0	75.02±0.2	83.09±0.2	19.09±0.20*		65.09±0.99	69.20±0.1	72.90±0.2	75.66±0.2	84.09±0.2	24.57±0.33*
		300	60.03±0.1	65.01±0.1	72.09±0.2	77.02±0.2	82.07±1	21.04±0.11*		67.19±0.10	66.20±0.1	73.90±0.2	76.66±0.2	82.09±0.2	23.37±0.04*
		500	61.04±0.8	67.01±0.2	72.07±0.0	75.02±0.2	83.09±0.2	20.09±0.62*		63.09±0.10	68.30±0.1	72.90±0.2	77.66±0.2	84.09±0.2	24.27±0.5*

Values are expressed as mean ± SD. P > 0.05 when compared to control group. * = values are significantly different (p < 0.05) from that of the control); from that of the control

5.3.6 Effect of *C. meturiferus* and *L. kituiensis* extract on hematological parameters in a sub-acute test

The results of hematological investigations in Table 20, 21, 22, 23, 24 and 25 conducted on 29th day for sub-acute toxicity study revealed significant changes in the values of red blood cell (RBC), white blood cell (WBC), hemoglobin (Hb), hematocrit (HCT), lymphocytes (LYM), neutrophil granulocytes (NEUT), differential count, mean corpuscular volume (MCV) mean corpuscular hemoglobin (MCH), mean-corpuscular hemoglobin concentration (MCHC) and Red Blood Cells Distribution Width (RDW), of treated groups when compared with the respective control rats in dose levels 300 mg/kg and 500 mg/kg. For chloroform extract of *C. metulifures* the result showed a significant increase in WBCs count, RBCs, HCT, MCV, MCH, MCHC, RDW, and HB of male rats and female rats WBCs count, HCT, MCV, MCH, MCHC, RDW, and HB in a dose of 300 mg/kg and 500 mg/kg (Table 20).

The rats treated with ethyl acetate extract showed a significant increase of WBCs and RBCs count, MCV, HCT, MCH, MCHC, HB and RDW in dose level 500 of mg/kg. For female rats, the WBCs count raises significantly in a dose level of 500 mg/kg as compared to the control. RBCs count and MCV, MCH and MCHC were also significantly increased as compared to control in dose level of 500 mg/kg as shown in Table 21. For methanolic extract, a significant increase of WBCs and RBCs count in dose levels of 300 mg/kg and 500 mg/kg was observed. And all MCV, HCT, MCH, MCHC, RDW and HB were significantly increased in dose level of 500 mg/kg with that of the control. For female rats, WBCs count increased significantly at a dose of 300 mg/kg and 500 mg/kg as that of the control group. RBCs and HCT, MCV, MCH, MCHC and RDW were significant increases as that of control in dose level of 500 mg/kg as shown in Table 22.

For *L. kituiensis* plant the rats treated with chloroform extract revealed significant increased of WBCs, RBCs, MCV, HCT, MCH and MCHC in a dose level 500 mg/kg as compared to the with control, and for female rats significantly increased of WBCs, RBCs count and HCT, MCV, MCH and MCHC in dose levels 300 mg/kg and 500 mg/kg was observed as compared to controls (Table 23). The results obtained in rats treated with ethyl acetate extract showed a significant increase of WBC, LYMP, MON, NEU, RBC, MCV, HCT and MCHC in dose levels of 300 mg/kg and 500 mg/kg for male rats and WBCs, LYMP, NEU, RBC, MCV, HCT and MCHC for female rats in a dose levels of 300 mg/kg and 500 mg/kg when compared with control (Table 24).

And rats administered with methanolic extract, the hematological result of male and female rats' showed a significant increase of WBC, LYMP, MON, NEU, RBC and HCT in dose levels of 300 mg/kg and 500 mg/kg as compared to the control group (Table 25).

Table 20: Effect of chloroform extract of *C. meturiferus* on hematological parameters in rate after 4 weeks of treatment

Sex	**Parameter**	**Dose mg/kg n = 5**				**Sex**	**Dose mg/kg n = 5**			
		150mg/kg	**300mg/kg**	**500mg/kg**	**0mg/kg**		**150mg/kg**	**300mg/kg**	**500mg/kg**	**0mg/kg**
F	WBC	91.43±0.09	97.44±0.12*	109.2±0.1*	88.23±0.01	M	72.12±0.02	85.82±0.02*	90.61±1.2*	75.23±0.02
	LYMP	66.40±0.02	69.00±0.13*	79.02±0.5*	66.00±0.02		65.02±0.11	68.00±0.01*	71.0±0.12*	62.0±0.50
	MON	18.00±0.12	20.04±0.14*	21.40±0.10*	16.03±0.08		13.10±0.12	14.80±0.11*	15.60±0.03*	12.20±1.02
	NEU	7.53±0.02	8.90±0.15*	8.00±0.09*	6.20±0.04		2.00±0.10	3.02±0.05*	3.01±0.12*	1.03±0.03
	RBC	7.57±0.15	8.72±0.11*	9.90±0.12*	7.37±0.01		6.21±0.04	6.92±0.01*	7.33±0.01*	5.90±0.03
	MCV	59.70±0.02	63.22±0.17	66.01±0.3*	55.23±0.06		57.11±0.02	60.23±0.07	63.23±1.07*	58.0±0.03
	HCT	42.7±0.13	43.3±0.12	48.32±0.12*	41.90±0.07		39.13±0.10	41.12±0.01	45.23±0.09*	39.18±0.03
	MCH	23.4±0.02	26.2±0.19	27.93±0.01*	24.23±08		18.01±0.01	20.21±0.18	19.22±0.00	19.31±0.01
	MCHC	35.52±0.02	35.04±0.2	38.05±0.90*	32.40±0.01		29.02±0.17	32.21±0.09	34.23±0.03	30.05±0.02
	RDW	15.34±0.01	17.21±0.21	19.05±0.10*	16.21±1.10		15.0±0.6	18.81±0.10	20.12±1.10*	16.91±0.05
	HB	15.31±0.01	14.01±0.25	17.13±0.10*	14.23±1.01		15.22±0.09	15.8±0.23	17.12±0.01*	13.34±0.01

Values are expressed as mean ± SD. P > 0.05 when compared to control group. * = values are significantly different ($p < 0.05$) from that of the control); White blood cell, red blood cell, hematocrit, hemoglobin, mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, lymphocytes, monocytes, neutrophil granulocytes, red blood cells distribution width.

Table 21: Effect of ethyl acetate extract of *C. meturiferus* on hematological parameters in rate after 4 weeks of treatment

Sex	**Parameter**	**Dos mg/kg n = 5**				**Sex**	**Dos mg/kg n = 5**			
		150mg/kg	**300mg/kg**	**500mg/kg**	**0mg/kg**		**150mg/kg**	**300mg/kg**	**500mg/kg**	**0mg/kg**
F	WBC	90.40±0.09	93.44±0.12	100.42±0.51*****	93.23±0.01	M	75.12±0.02	85.82±0.02*	91.61±1.02*	78.23±0.02
	LYMP	64.40±0.02	67.00±0.13	72.02±0.15*****	65.00±0.02		64.02±0.11	68.00±0.01*	71.00±0.12*	62.0±0.50
	MON	18.00±0.12	18.04±0.14	20.00±0.10*****	16.03±0.08		14.10±0.12	15.80±0.11*	18.60±0.03*	12.20±1.02
	NEU	8.00±0.02	8.40±0.15	8.02±0.09	6.20±0.04		3.00±0.10	3.02±0.05	4.01±0.12*	1.03±0.03
	RBC	8.57±0.15	9.32±0.11	9.90±0.12*****	7.37±0.01		6.21±0.04	6.92±0.01*	7.33±0.01*	5.90±0.03
	MCV	60.7±0.02	58.22±0.17	61.1±0.31*	55.23±0.06		57.11±0.02	60.23±0.07	63.23±1.07*	58.0±0.03
	HCT	44.9±0.13	43.3±0.12	49.32±0.12*	41.90±0.07		39.13±0.10	41.12±0.01	45.23±0.09*	39.18±0.03
	MCH	21.4±0.02	25.2±0.19	27.23±0.01*	24.23±08		18.01±0.01	20.21±0.18	23.22±0.00*	19.31±0.01
	MCHC	34.52±0.02	35.04±0.20	39.05±0.90*	32.40±0.01		29.02±0.17	32.21±0.09	35.23±0.03*	30.05±0.02
	RDW	16.34±0.01	16.21±0.21	20.05±0.10	16.21±1.10		14.0±0.6	16.81±0.10	21.12±1.10*	16.91±0.05
	HB	16.31±0.01	15.01±0.25	16.13±0.10	14.23±1.01		15.22±0.09	15.8±0.23	19.12±0.01*	13.34±0.01

Values are expressed as mean ± SD. P > 0.05 when compared to control group. * = values are significantly different ($p < 0.05$) from that of the control. White blood cell, red blood cell, hematocrit, hemoglobin, mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, lymphocytes, platelet, monocytes, neutrophil granulocytes, basophils, red blood cells distribution width.

Table 22: Effect of methanol extract of *C. meturiferus* on hematological parameters in rate after 4 weeks of treatment

Sex	Parameter	Dose mg/kg n = 5				Sex	Parameter	Dose mg/kg n = 5			
		150mg/kg	**300mg/kg**	**500mg/kg**	**0mg/kg**			**150mg/kg**	**300mg/kg**	**500mg/kg**	**0mg/kg**
F	WBC	85.40±0.06	99.44±0.12*	102.42±0.51*	93.23±0.01	**M**	WBC	74.12±0.02	85.82±0.02*	89.61±1.02*	75.23±0.02
	LYMP	58.40±0.02	69.00±0.13*	68.02±0.15*	65.00±0.02		LYMP	64.02±0.11	67.00±0.01*	69.00±0.1*	62.0±0.50
	MON	20.00±0.12	24.04±0.14	25.00±0.10	20.03±0.08		MON	14.10±0.12	15.80±0.11*	17.60±0.03*	12.20±1.02
	NEU	7.00±0.02	8.40±0.15	9.02±0.09*	6.20±0.04		NEU	2.00±0.10	3.02±0.05*	3.01±0.12*	1.03±0.03
	RBC	6.57±0.15	7.72±0.11	9.90±0.12*	7.37±0.01		RBC	6.21±0.04	6.92±0.01	7.53±0.01*	5.90±0.03
	MCV	58.7±0.02	59.22±0.17	65.01±0.31*	55.23±0.06		MCV	59.11±0.02	60.23±0.07	66.23±1.07*	58.0±0.03
	HCT	43.9±0.13	43.3±0.12	49.32±0.12*	41.90±0.07		HCT	40.13±0.10	40.12±0.01	45.23±0.09*	39.18±0.03
	MCH	25.4±0.02	26.2±0.19	29.23±0.01*	24.23±08		MCH	19.01±0.01	22.21±0.18	24.22±0.00*	19.31±0.01
	MCHC	34.52±0.02	35.04±0.2	38.05±0.90*	32.40±0.01		MCHC	27.02±0.17	33.21±0.09	35.23±0.03*	30.05±0.02
	RDW	18.34±0.01	18.21±0.21	23.05±0.10*	16.21±1.10		RDW	14.0±0.6	19.81±0.10	22.12±1.10*	16.91±0.05
	HB	15.31±0.71	16.01±0.25	17.13±0.10	14.23±1.01		HB	15.22±0.09	16.8±0.23	19.12±0.01*	13.34±0.01

Values are expressed as mean ± SD. P > 0.05 when compared to control group. * = values are significantly different ($p < 0.05$) from that of the control); White blood cell, red blood cell, hematocrit, hemoglobin, mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration, lymphocytes, monocytes, neutrophil granulocytes, red blood cells distribution width.

Table 23: Effect of chloroform extract of *L. kituiensis* on hematological parameters in rate after 4 weeks of treatment

Sex	**Parameter**	**Dose mg/kg n = 5**				**Sex**	**Dose mg/kg n = 5**			
		150mg/kg	**300mg/kg**	**500mg/kg**	**0mg/kg**		**150mg/kg**	**300mg/kg**	**500mg/kg**	**0mg/kg**
F	WBC	91.40±0.09	97.44±0.12*	99.42±0.51*	93.23±0.01	M	69.12±0.02	80.82±0.02	88.91±1.02*	78.23±0.02
	LYMP	65.40±0.02	68.00±0.13*	68.02±0.15*	65.00±0.02		55.02±0.11	65.00±0.01	70.00±0.12*	62.0±0.50
	MON	19.00±0.12	21.04±0.14*	22.00±0.10*	16.03±0.08		12.10±0.12	14.80±0.11	15.60±0.30	12.20±1.02
	NEU	7.00±0.02	8.40±0.15	9.02±0.09*	6.20±0.04		2.00±0.10	2.02±0.05	3.01±0.12*	2.03±0.03
	RBC	7.57±0.15	8.72±0.11*	8.90±0.12*	7.37±0.01		6.21±0.04	6.92±0.01	7.93±0.01*	5.90±0.03
	MCV	53.7±0.02	63.22±0.17*	66.01±0.31*	55.23±0.06		59.11±0.02	60.23±0.07	64.23±1.07*	58.0±0.03
	HCT	42.9±0.13	46.0±0.12*	49.32±0.12*	41.90±0.07		40.13±0.10	41.12±0.01	46.23±0.09*	39.18±0.03
	MCH	24.54±0.02	28.72±0.19*	30.83±0.01*	24.23±08		19.01±0.01	20.21±0.18	22.22±0.0*	19.31±0.01
	MCHC	33.52±0.02	37.04±0.2*	38.05±0.90*	32.40±0.01		30.02±0.17	33.21±0.09	36.23±0.03*	30.05±0.02
	RDW	13.34±0.01	18.21±0.21	19.05±0.10*	16.21±1.10		13.0±0.6	14.81±0.10	15.12±1.10	16.91±0.05
	HB	14.31±0.01	15.01±0.25	15.13±0.10	14.23±1.01		13.22±0.09	14.8±0.23	14.12±0.01	13.34±0.01

Values are expressed as mean ± SD. P > 0.05 when compared to control group. * = values are significantly different ($p < 0.05$) from that of the control): White blood cell (WBC), red blood cell (RBC), hematocrit (HCT), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), lymphocytes (LYM), monocytes (MON), neutrophil granulocytes (NEUT), red blood cells distribution width (RDW).

Table 24: Effect of ethyl acetate extract of *L. kituiensis* on hematological parameters in rate after 4 weeks of treatment

Sex	Parameter	Dose mg/kg n = 5				Sex	Parameter	Dose mg/kg n = 5			
		150mg/kg	300mg/kg	500mg/kg	0mg/kg			150mg/kg	300mg/kg	500mg/kg	0mg/kg
F	WBC	92.40±0.09	98.74±0.12*	105.52±0.51*	93.23±0.01	**M**	WBC	80.16±0.02	90.82±0.02*	99.81±1.02*	78.23±0.02
	LYMP	65.40±0.02	70.00±0.13*	75.02±0.15*	65.00±0.02		LYMP	72.06±0.11*	70.07±0.01*	74.00±0.12*	62.0±0.50
	MON	19.07±0.12	20.76±0.14	22.40±0.10	16.03±0.08		MON	14.10±0.12	17.80±0.11*	18.60±0.03*	12.20±1.02
	NEU	8.00±0.02	8.70±0.15*	8.80±0.09*	6.20±0.04		NEU	3.00±0.10*	3.02±0.05*	3.01±0.12*	2.03±0.03
	RBC	7.07±0.15	7.92±0.11*	8.40±0.11*	7.37±0.01		RBC	5.21±0.04	6.92±0.01*	7.93±0.01*	5.90±0.03
	MCV	58.7±0.02	60.22±0.17*	65.01±0.31*	55.23±0.06		MCV	56.11±0.02	59.93±0.07	63.93±1.07*	58.0±0.03
	HCT	44.9±0.13	44.3±0.12*	49.37±0.12*	41.90±0.07		HCT	40.13±0.13	44.12±0.01*	47.23±0.09*	39.18±0.03
	MCH	21.4±0.05	23.2±0.10	25.23±0.51	24.23±08		MCH	20.01±0.01	21.31±0.18	24.22±0.00*	19.31±0.01
	MCHC	33.52±0.02	36.04±0.2*	38.05±0.90*	32.40±0.01		MCHC	31.02±0.17	34.21±0.07	35.83±0.03*	30.05±0.02
	RDW	14.34±0.03	15.21±0.21	16.05±0.10	16.21±1.10		RDW	12.0±0.60	13.81±0.10	14.12±1.10	16.91±0.05
	HB	16.31±0.01	15.01±0.25	16.13±0.10	14.23±1.01		HB	13.22±0.06	15.8±0.23	16.12±0.01	13.34±0.01

Values are expressed as mean ± SD. P > 0.05 when compared to control group. * = values are significantly different ($p < 0.05$) from that of the control): White blood cell (WBC), red blood cell (RBC), hematocrit (HCT), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), lymphocytes (LYM), monocytes (MON), neutrophil granulocytes (NEUT) red blood cells distribution width (RDW).

Table 25: Effect of methanolic extract of *L. kituiensis* on hematological parameters in rate after 4 weeks of treatment

Sex	**Parameter**	**Dose mg/kg n = 5**				**Sex**	**Parameter**	**Dose mg/kg n = 5**			
		50mg/kg	**00mg/kg**	**00mg/kg**	**mg/kg**			**150mg/kg**	**300mg/kg**	**500mg/kg**	**0mg/kg**
F	WBC	95.70±0.09	99.64±0.12*	103.2±0.51*	93.23±0.01	M	WBC	79.32±0.04	89.72±0.02*	99.91±1.02*	75.23±0.02
	LYMP	67.70±0.02	71.60±0.13*	71.02±0.15*	65.00±0.02		LYMP	61.02±0.11	71.00±0.01*	78.00±0.42*	62.0±0.50
	MON	20.00±0.12	20.04±0.14	22.00±0.10*	16.03±0.08		MON	15.30±0.12	16.70±0.11	18.90±0.06*	12.20±1.02
	NEU	8.00±0.02	8.40±0.15	8.02±0.09	6.20±0.04		NEU	1.00±0.70	3.02±0.05*	3.01±0.12*	1.03±0.03
	RBC	7.57±0.15	8.72±0.11*	9.90±0.12*	6.3±0.01		RBC	6.71±0.04	7.92±0.01*	8.33±0.01*	5.90±0.03
	MCV	58.07±0.2	63.82±0.17*	65.41±0.31*	55.23±0.06		MCV	59.81±0.02	60.83±0.07	63.93±1.07*	58.0±0.03
	HCT	41.9±0.13	44.3±0.12*	48.32±0.12*	41.90±0.07		HCT	40.13±0.10	43.12±0.01*	46.23±0.09*	39.18±0.03
	MCH	20.4±0.02	23.2±0.19	26.23±0.01*	24.23±08		MCH	19.91±0.01	21.81±0.18	23.22±0.0*	19.31±0.01
	MCHC	31.52±0.02	33.04±0.2	36.05±0.90*	32.40±0.01		MCHC	28.02±0.17	30.21±0.09	33.23±0.03*	30.05±0.02
	RDW	12.34±0.01	13.21±0.21	16.05±0.10	16.21±1.10		RDW	12.0±0.6	13.81±0.10	14.62±1.10	16.91±0.05
	HB	14.31±0.01	14.01±0.25	16.13±0.10	14.23±1.01		HB	12.72±0.09	12.48±0.23	14.12±0.01	13.34±0.01

Values are expressed as mean ± SD. P > 0.05 when compared to control group. * = values are significantly different (p < 0.05) from that of the control): White blood cell (WBC), red blood cell (RBC), hematocrit (HCT), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), lymphocytes (LYM), monocytes (MON), neutrophil granulocytes (NEUT)and red blood cells distribution width (RDW)

5.3.7 Effect of *C. meturiferus* and *L. kituiensis* extract on absolute and relative organ body weight in the sub-acute study

At the end of the study, data on the weights of vital organs (heart, lungs, liver, kidneys, and spleen) were assessed. The mean absolute and relative organ weights of male and female rats (spleen, heart, lungs, liver and kidneys) treated with chloroform, ethyl acetate and methanolic extract in dose levels of 150 mg/kg, 300 mg/kg and 500 mg/kg were determined as shown in Table 26, 27, 28, 29, 30 and 31.

The liver and heart inmale rats treated with chloroform extract of *C. metuliferus* showed significant different dose levels of 300 mg/kg and 500 mg/kg while kidney and lungs in a dose level of 500 mg/kg when compared with control. For female rats, the organ weights of liver, heart, kidney, and lungs showed significantly increase in dose levels of 300 mg/kg and 500 mg/kg when compared with the controls as shown in Table 26.

For rats treated with ethyl acetate extract the organ weights of the liver and lungs of male rats showed significant increased in dose level of 500 mg/kg and kidney in dose levels of 300 mg/kg and 500 mg/kg. For female rats, the significant increase of liver, kidney and spleen in dose level of 500 mg/kg and lungs in a dose of 300 mg/kg and 500 mg/kg was observed as shown in Table 27.

While the rats treated with methanolic extract, the mean organ weights of male and female rats are shown in Table 28. For male treated rats, the significant increase of liver, heart, kidney and lungs in dose level 500 mg/kg was observed as compared to the control. While female rats, the results shows a significant increase of liver and heart in dose level 300 mg/kg and 500 mg/kg, kidney, and spleen in dose level 500 mg/kg as compared to the control.

For *L. kituiensis* plant the rats treated with chloroform extract revealed significant reduction of liver, lungs and kidney weight in dose level of 500 mg/kg in male rats and in female rats, the significant reduction of liver in a dose of 300 mg/kg and 500 mg/kg was observed while lungs and kidney the significant reduction was recorded in dose level 500 mg/kg as compared to the control group (Table 29).

For the rats treated with ethyl acetate extract, showed significantly reduced of vital organ, kidney, lungs and spleen in dose level 500 mg/kg in male rats and female rats significant

reduced of kidney and lungs in dose level 300 mg/kg, and 500 mg/kg and liver in dose level 500 mg/kg was observed when compared with the control group (Table 30).

While the rats treated with methanolic extract, showed the significant reduction of liver, kidney and lungs weight in dose level 500 mg/kg for male and female rats when compared with the control (Table 31).

Table 26: Effect of chlorofom extract of *C. metuliferus* on absolute (a) and relative organ weight R (%) of mice after 28 days of treatment

Sex		**Organ**	**Dose mg/kg n = 5**				**Sex**		**Organ**	**Dose mg/kg n = 5**			
			150mg/kg	**300mg/kg**	**500mg/kg**	**0mg/kg**				**150mg/kg**	**300mg/kg**	**500mg/kg**	**0mg/kg**
F	A(g)	Liver	5.85±0.05	7.96±0.31*	8.93±0.11*	6.51±0.01	M	A(g)	Liver	5.70±0.01	7.77±0.09*	8.53±0.06*	5.98±0.02
		Heart	0.65±0.21	0.89±0.41*	0.96±0.01*	0.75±0.21			Heart	0.71±0.01	1.55±0.10*	1.80±0.11*	0.85±0.13
		Kidney	1.03±0.11	1.70±0.21*	1.87±0.31*	1.35±0.11			kidney	1.05±0.31	0.95±0.14	1.73±0.06*	1.20±0.21
		Lungs	1.53±0.15	1.73±0.15*	1.95±0.10*	1.60±0.14			lungs	1.50±0.01	1.36±0.12	1.69±0.12*	1.32±0.02
		Spleen	0.35±0.05	0.24±0.07	0.07±0.01	0.35±0.51			Spleen	0.46±0.10	0.50±0.12	0.50±0.13	0.59±0.14
	R (%)	Liver	5.79±0.56	6.10±0.06	6.35±0.03	5.89±0.03		R (%)	Liver	6.30±0.25	6.02±0.11	6.92±0.14	6.99±0.13
		Heart	1.44±0.02	1.46±0.40	1.47±0.24	1.47±0.04			Heart	1.42±0.14	1.52±0.15	1.63±0.16	1.53±0.12
		Kidney	2.06±0.01	2.48±0.15	2.53±0.25	2.57±0.05			Kidney	1.40±0.17	1.77±0.16	1.61±0.14	1.60±0.17
		Lung	1.52±0.06	1.60±0.11	1.63±0.01	1.60±0.06			Lung	1.77±0.18	1.87±0.14	1.65±0.11	1.75±0.02
		Spleen	0.16±0.07	0.17±0.07	0.17±0.07	0.18±0.07			spleen	0.55±0.14	0.43±0.15	0.74±0.15	1.16±0.15

Values are expressed as mean± SD. A: absolute organ weight (g); R: relative organ weight (%). * P< 0.05 compared with the control (0mg/kg).

Table 27: Effect of ethyl acetate extract of *C. metuliferus* on absolute (a) and relative organ weight R (%) of rats after 28 days of treatment

Sex	**Organ**		**Dose mg/kg n=5**				**Sex**	**Organ**		**Dose mg/kg n=5**			
			150mg/kg	300mg/kg	500mg/kg	0mg/kg				150mg/kg	300mg/kg	500mg/kg	0mg/kg
F	A(g)	Liver	5.50±0.05	6.84±0.31	7.90±0.11*	6.51±0.01	M	A(g)	Liver	5.40±0.01	6.10±0.09	7.03±0.06*	5.98±0.02
		Heart	0.67±0.21	0.65±0.41	0.77±0.01	0.75±0.21			Heart	0.71±0.01	0.80±0.20	0.80±0.12	0.82±0.12
		Kidney	1.25±0.11	1.38±0.11	1.67±0.31*	1.35±0.11			kidney	1.45±0.11	1.75±0.14*	1.93±0.06*	1.20±0.21
		Lungs	1.61±0.19	1.80±0.15*	1.92±0.10*	1.60±0.14			lungs	1.30±0.01	1.33±0.12	1.75±0.12*	1.32±0.02
		Spleen	0.35±0.05	0.34±0.07	0.67±0.01*	0.35±0.51			Spleen	0.44±0.10	0.50±0.12	0.60±0.13	0.59±0.14
	R (%)	Liver	5.89±0.56	6.20±0.06	6.09±0.03	5.89±0.03		R (%)	Liver	5.30±0.25	5.02±0.11	6.32±0.14	6.99±0.13
		Heart	1.04±0.02	1.21±0.04	1.09±0.24	1.47±0.04			Heart	1.12±0.14	1.32±0.15	1.63±0.16	1.53±0.12
		Kidney	1.06±0.01	1.38±0.15	1.23±0.25	1.57±0.05			Kidney	1.50±0.17	1.67±0.16	1.01±0.14	1.60±0.17
		Lung	1.46±0.06	1.30±0.11	1.34±0.01	1.60±0.06			Lung	1.67±0.18	1.87±0.14	1.25±0.11	1.75±0.02
		Spleen	0.18±0.07	0.18±0.07	0.18±0.07	0.18±0.07			spleen	0.52±0.19	0.42±0.15	0.71±0.15	1.18±0.19

Values are expressed as mean± SD. A: absolute organ weight (g); R: relative organ weight (%). * P< 0.05 compared with the control (0mg/kg).

Table 28: Effect of methanolic extract of *C. metuliferus* on absolute (a) and relative organ weight R (%) of rats after 28 days of treatment

Sex	**Organ**		**Dose mg/kg n=5**				**Sex**	**Organ**		**Dose mg/kg n=5**			
			150mg/kg	**300mg/kg**	**500mg/kg**	**0mg/kg**				**150mg/kg**	**300mg/kg**	**500mg/kg**	**0mg/kg**
F	A(g)	Liver	6.55±0.05	7.90±0.31*	8.43±0.11*	6.51±0.01	M	A(g)	Liver	5.40±0.01	5.70±0.09	7.83±0.16*	5.98±0.02
		Heart	0.97±0.21	1.08±0.41*	1.12±0.01*	0.75±0.21			Heart	0.71±0.01	0.80±0.20	1.30±0.12*	0.82±0.12
		Kidney	1.03±0.11	1.58±0.11	1.93±0.31*	1.35±0.11			kidney	0.65±0.11	1.48±0.14	1.93±0.06*	1.20±0.21
		Lungs	1.53±0.19	1.80±0.15	1.90±0.10	1.60±0.14			lungs	1.33±0.01	1.26±0.12	1.94±0.12*	1.32±0.02
		Spleen	0.35±0.05	0.97±0.07*	0.99±0.01*	0.35±0.51			Spleen	0.46±0.10	0.20±0.12	0.90±0.13	0.59±0.14
	R (%)	Liver	5.89±0.56	5.20±0.06	4.69±0.03	5.89±0.03		R (%)	Liver	5.40±0.25	6.02±0.11	6.92±0.14	6.99±0.13
		Heart	1.04±0.02	1.01±0.40	1.09±0.24	1.47±0.04			Heart	1.52±0.14	1.52±0.15	1.63±0.16	1.53±0.12
		Kidney	1.36±0.01	1.48±0.15	1.53±0.25	1.57±0.05			Kidney	1.60±0.17	1.67±0.16	1.61±0.14	1.60±0.17
		Lung	1.42±0.06	1.50±0.11	1.47±0.01	1.60±0.06			Lung	1.77±0.18	1.77±0.54	1.85±0.11	1.75±0.02
		Spleen	0.18±0.07	0.18±0.07	0.18±0.07	0.18±0.07			spleen	0.42±0.19	0.42±0.15	0.11±0.15	1.18±0.19

Values are expressed as mean± SD. A: absolute organ weight (g); R: relative organ weight (%). * P< 0.05 compared with the control (0mg/kg)

Table 29: Effect of chloroform extract of *L. kituiensis* on 5absolute (A) and relative organ weight R (%) of rats after 28 days of treatment

Sex		**Organ**	**Dose mg/kg n = 5**				**Sex**		**Organ**	**Dose mg/kg n = 5**			
			150mg/kg	**300mg/kg**	**500mg/kg**	**0mg/kg**				**150mg/kg**	**300mg/kg**	**500mg/kg**	**0mg/kg**
F	A(g)	Liver	5.50±0.07	4.00±0.02*	3.10±0.01*	6.51±0.01	M	A(g)	Liver	5.30±0.02	5.20±0.01	3.04±0.20*	5.98±0.02
		Heart	0.70±0.01	0.54±0.01	0.44±0.01	0.75±0.21			Heart	0.77±0.15	0.77±0.10	0.78±0.12	0.82±0.12
		Kidney	1.30±0.01	1.00±0.01	0.60±0.01*	1.35±0.11			kidney	1.30±0.13	1.00±0.11	0.70±0.13*	1.20±0.21
		Lungs	1.30±0.06	1.30±0.10	1.00±0.10*	1.60±0.14			Lung	1.33±0.12	1.28±0.12	0.69±0.12*	1.32±0.02
		Spleen	0.38±0.05	0.33±0.15	0.33±0.01	0.35±0.51			Spleen	0.50±0.13	0.51±0.13	0.67±0.12	0.59±0.14
	R (%)	Liver	6.10±0.03	6.20±0.01	6.98±0.01	6.89±0.03		R (%)	Liver	5.90±0.12	7.96±0.15	7.56±0.13	6.99±0.13
		Heart	1.20±0.02	1.42±0.02	1.89±0.04	1.47±0.04			Heart	1.44±0.12	1.34±0.12	1.55±0.16	1.53±0.12
		Kidney	1.89±0.02	2.10±0.02	2.68±0.05	2.57±0.05			Kidney	1.40±0.13	1.50±0.11	1.90±0.17	1.60±0.17
		Lung	1.60±0.03	1.85±0.01	1.87±0.6	1.90±0.06			Lung	1.66±0.13	1.78±0.13	1.98±0.18*	1.76±0.02
		Spleen	0.71±0.04	0.66±0.12	0.76±0.13	0.18±0.07			spleen	0.70±0.11	0.71±0.14	1.31±0.18	1.18±0.19

Values are expressed as mean± SD. A: absolute organ weight (g); R: relative organ weight (%). * P< 0.05 compared with the control (0mg/kg).

Table 30: Effect of ethyl acetate extract of *L. kituiensis* on absolute (A) and relative organ weight R (%) of rats after 28 days of treatment

Sex	**Organ**		**Dose mg/kg n = 5**				**Sex**	**Organ**		**Dose mg/kg n = 5**			
			150mg/kg	**300mg/kg**	**500mg/kg**	**0mg/kg**				**150mg/kg**	**300mg/kg**	**500mg/kg**	**0mg/kg**
F	A(g)	Liver	5.50±0.07	5.20±0.02	3.80±0.01*	6.51±0.01	M	A(g)	Liver	5.80±0.02	4.80±0.01	3.04±0.20*	5.98±0.02
		Heart	0.74±0.01	0.68±0.01	0.64±0.01	0.75±0.21			Heart	0.77±0.35	0.59±0.10	0.28±0.12*	0.82±0.12
		Kidney	1.30±0.01	0.80 ±0.1*	0.60±0.21*	1.35±0.11			kidney	1.320±0.13	1.00±0.11	0.50±0.13*	1.20±0.21
		Lungs	1.50±0.06	0.90±0.10*	0.63±0.10*	1.60±0.14			lung	1.23±0.12	1.00±0.12	0.72±0.12*	1.32±0.02
		Spleen	0.35±0.05	0.33±0.15	0.23±0.01	0.35±0.51			Spleen	0.50±0.13	0.41±0.13	0.27±0.12	0.59±0.14
	R (%)	Liver	6.10±0.03	6.20±0.01	6.98±0.01	5.89±0.03		R (%)	Liver	5.60±0.12	5.96±0.15	6.66±0.13	6.99±0.13
		Heart	1.21±0.02	1.32±0.02	1.39±0.04	1.47±0.04			Heart	1.44±0.12	1.54±0.12	1.55±0.16	1.53±0.12
		Kidney	2.39±0.02	2.10±0.02	2.68±0.05	2.57±0.05			Kidney	1.70±0.13	1.60±0.11	1.70±0.17	1.60±0.17
		Lung	1.60±0.03	1.85±0.01	1.87±0.60	1.90±0.06			Lung	1.67±0.13	1.77±0.13	1.88±0.18	1.76±0.02
		Spleen	0.61±0.04	0.67±0.12	0.86±0.13	0.18±0.07			spleen	0.50±0.11	0.61±0.14	1.41±0.18	1.28±0.19

Values are expressed as mean± SD. A: absolute organ weig`ht (g); R: relative organ weight (%). * P< 0.05 compared with the control (0mg/kg).

Table 31: Effect of methanolic extract of *L. kituiensis* on absolute (A) and relative organ weight R (%) of rats after 28 days of treatment

Sex		**Organ**	**Dose mg/kg n = 5**				**Sex**		**Organ**	**Dose mg/kg n = 5**			
			150mg/kg	**300mg/kg**	**500mg/kg**	**0mg/kg**				**150mg/kg**	**300mg/kg**	**500mg/kg**	**0mg/kg**
F	A(g)	Liver	6.50±0.02	5.30±0.02	4.10±0.01*	6.51±0.01	M	A(g)	Liver	5.50±0.12	4.90±0.21	3.44±0.20*	5.98±0.02
		Heart	0.70±0.01	0.74±0.01	0.74±0.01	0.75±0.21			Heart	0.75±0.15	0.70±0.10	0.68±0.12	0.82±0.12
		Kidney	1.30±0.01	0.98±0.01	0.80±0.02*	1.35±0.11			kidney	1.30±0.13	0.85±0.11	0.30±0.13*	1.20±0.21
		Lungs	1.50±0.06	1.50±0.10	0.64±0.10*	1.60±0.14			lung	1.33±0.12	1.68±0.12	0.79±0.12*	1.32±0.02
		Spleen	0.35±0.15	0.34±0.25	0.35±0.11	0.35±0.51			Spleen	0.50±0.13	0.51±0.13	0.47±0.12	0.59±0.14
	R (%)	Liver	6.20±0.03	6.50±0.01	6. 74±0.01	5.89±0.03		R (%)	Liver	5.80±0.22	5.66±0.15	5.56±0.13	6.99±0.13
		Heart	1.30±0.02	1.32±0.02	1.39±0.04	1.47±0.04			Heart	1.54±0.12	1.00±0.12	0.95±0.16	1.53±0.12
		Kidney	1.89±0.02	2.10±0.02	2.68±0.05	2.57±0.05			Kidney	1.45±0.13	1.70±0.11	1.80±0.17	1.60±0.17
		Lung	1.62±0.13	1.85±0.01	1.86±0.60	1.90±0.06			Lung	1.66±0.23	1.58±0.13	1.98±0.18	1.76±0.02
		Spleen	0.01±0.24	0.16±0.12	0.16±0.13	0.18±0.07			spleen	0.80±0.11	0.81±0.14	1.91±0.18	1.18±0.19

Values are expressed as Mean± SD. A: absolute organ weight (g); R: relative organ weight (%). * P< 0.05 compared with the control (0mg/kg)

5.3.8 Effect on biochemical parameters of *C. metuliferus and L. kituiensis* in sub-acute study

The results on the biochemical tests of experimentally treated animals are summarized in Table 32, 33, 34, 35, 36 and 37. Oral administration of *C. metuliferus* and *L. kituiensis* extact revealed significant changes in serum biochemical parameters such as Alanine transaminase (ALT), Alkaline phosphatase (ALP), Aspartate transaminase (AST), Albumin (ALB), Total bilirubin (TB), Total protein (TP), Creatinine (CREA), urea (UREA), Triglyceride (TG), Total cholesterol (TCHO) and Glucose (GLU) in dose level 300 mg/kg and 500 mg/kg .

The administration of chloroform, ethyl acetate methanolic extract of *C. metuliferus* revealed significant increases in serum aspartate aminotransferase (AST), alkaline phosphatase (ALP) and Alanine aminotransferase (ALT) activities ($P < 0.05$) in a dose-dependent level 300 mg/kg and 500 mg/kg for both male and female when compared to the control as shown in Table 32, 33 and 34.

Chloroform, ethyl acetate methanolic extract of *L. kituiensis* revealed a significant decrease in serum aspartate aminotransferase (AST), alkaline phosphatase (ALP) and Alanine aminotransferase (ALT) activities ($P < 0.05$) in a dose-dependent level 300 mg/kg and 500 mg/kg for both male and female when compared to the control as shown in Table 35, 36 and 37.

The result in kidney parameters (blood urea) of rats treated with chloroform extract of *C. metuliferus* revealed a significant increase in dose level 500 mg/kg in male rats, while in dose level 150 mg/kg and 300 mg/kg remain normal as control. And in female rats' significant increase was observed in dose level 150 mg/kg, 300 mg/kg and 500 mg/kg as shown in Table 32. For the rats treated with ethyl acetate extract revealed significant increases in dose level 500 mg/kg both male and female rats as shown in Table 33. Methanolic extract the result revealed a significant increase in dose level of 300 mg/kg and 500 mg/kg when compared with control both female and male rats as shown in Table 34.

For the rats treated with chloroform extract of *L. kituiensis* revealed significant increase at a dose of 500 mg/kg of male and female rats significantly increase was observed at 300 mg/kg and 500 mg/kg when compared to control as shown in Table 35. The rats treated with ethyl acetate extract, the significant increase was observed in dose level 500 mg/kg in both male and female as shown in Table 36, and the rats treated with methanolic extract the significant

increase was observed in a dose of 300 mg/kg and 500 mg/kg in male and female as compared to control as shown in Table 37.

Creatinine is another kidney parameter, the rats treated with chloroform extract of *C. metuliferus* revealed significant increases in dose level 500 mg/kg and 300 mg/kg both males and females as compared to control as shown in Table 32. And the rats treated with ethyl acetate extract significantly increases of creatinine was observed in all dose levels in male and female rats as shown in Table 33. While the rats treated with methanolic extract revealed significant increase in dose level 500 mg/kg in male and 300 mg/kg and 500 mg/kg in female as compared with the control as shown in Table 34. The rats treated with chloroform extract of *L. kituiensis* in dose level 300 mg/kg and 500 mg/kg in Table 35, while in males no significant changes were observed as compared to control. The rats that treated with ethyl acetate extract revealed a significant increase in dose level 300 mg/kg and 500 mg/kg in male and 150 mg/kg, 300 mg/kg and 500 mg/kg in female rats as shown in Table 36. While the rats treated with methanolic extract revealed a significant increase in dose level 500 mg/kg in male and female as compared with the control as shown in Table 37.

Albumin blood serum is another kidney parameter tested in this study, for the rats treated with chloroform extract of *C. metuliferus* revealed significant decreases in dose level of 500 mg/kg for male and female when compared with control as shown in Table 32. No significant change observed in male and female rats treated with ethyl acetate when compared to the control as shown in Table 33. For the rats treated with methanolic extract, the significant decrease was observed in dose level 300 mg/kg and 500 mg/kg body weight for male and female when compared to the control group as shown in Table 34. For rats treated with *L. kituiensis* chloroform, ethyl acetate and methanolic extract significant decrease was observed in dose level 500 mg/kg for male and female respectively when compared with the control group as shown in Table 35, 36 and 37.

Moreover, the study tested for lipid parameters**,** for triglyceride level the animal treated with chloroform, ethyl acetate and methanolic extract of *C. metuliferus* revealed significant decrease in dose level 150 mg/kg, 300 mg/kg and 500 mg/kg when compared with control. The cholesterol level of treatment dose150 mg/kg, 300 mg/kg and 500 mg/kg decreases significantly in rats treated with chloroform, ethyl acetate and methanolic extract when compared to control both male and female rats as shown in Table 32, 33 and 34. And total protein the animal treated with chloroform extract revealed no significant change in male while

in the female the significant decrease was observed in dose level 300 mg/kg and 500 mg/kg as shown in Table 32. No significant change was observed in animals treated with ethyl acetate and methanolic extract as shown in Table 33 and Table 34. For the rats treated with *L. kituiensis* the rat's male and female treated with chloroform, ethyl acetate and methanolic extract shows a significant decrease of triglyceride level in dose level 150 mg/kg, 300 mg/kg and 500 mg/kg as compared to control. The cholesterol level of treatment dose150 mg/kg, 300 mg/kg and 500 mg/kg showed significant decreases in rats treated with chloroform, ethyl acetate and methanolic extract as compared to the control in the male and female rat as shown in Table 35, 36 and 37. Total protein was another parameter tested in this study, the rats treated with chloroform extract revealed no significant change in male while in the female the significant decrease was observed in dose level 300 mg/kg and 500 mg/kg as shown in Table 35. While the animal treated with ethyl acetate and methanolic extract, revealed significant decrease in a dose of 500 mg/kg body weight as shown in Table 36 and Table 37.

Glucose level is another parameter tested and the animal treated with chloroform extract of *C. metuliferus* revealed a significant increase in dose 500 mg/kg both for male and female rats (Table 32). The animal treated with ethyl acetate extract revealed significant increases in dose 500 mg/kg for male rats and 300 mg/kg and 500 mg/kg for female rats as compared to control Table 33. While the rats treated with methanol extract significant increases were observed at dose level 500 mg/kg in male rat and 150 mg/kg, 300 mg/kg and 500 mg/kg in females as compared to control as shown in Table 34. For animals treated with chloroform extract of *L. kituiensis* in dose level 500 mg/kg in both male and female as shown in Table 35. The rats treated with ethyl acetate extract shows a significant increase in treatment dose 500 mg/kg in male and 300 mg/kg and 500 mg/kg in females as compared to control Table 36. While the animal treated with methanolic extract significant increase were observed in dose level 500 mg/kg in male and 300 mg/kg and 500 mg/kg in female when compared to the control as shown in Table 37.

Lastly total bilirubin was tested the animal treated with chloroform extract of *C. Metuliferus* revealed a significant increase in dose 500 mg/kg both male and female as compared to control (Table 32). While the animal treated with ethyl acetate extract revealed, significantly increases in the treatment dose 500 mg/kg in dose level 500 mg/kg for male and 300 mg/kg and 500 mg/kg for females as compared to control as shown in Table 33. And the rats treated with methanol extract, a significant change was observed at a dose of 300 mg/kg and 500 mg/kg

both for male and female when compared to the control group as shown in Table 34. The animals treated with chloroform extract of *L. kituiensi* revealed a significant increase in dose level 500 mg/kg in both male and female as shown in Table 35. The rats treated with ethyl acetate extract, the treatment dose 500 mg/kg significantly increases in male and 300 mg/kg and 500 mg/kg in females as compared to the control group as shown in Table 36. While the animals treated with methanol extract, no significant change was observed as shown in Table 37.

Table 32: Effect of the chloroform extract of *C. meturiferus* in biochemical parameters in rate after 4 weeks of treatment

Sex	Parameter	Dose mg/kg n = 5				Sex	Dose mg/kg n = 5			
		150mg/kg	**300mg/kg**	**500mg/kg**	**0mg/kg**		**150mg/kg**	**300mg/kg**	**500mg/kg**	**0mg/kg**
F	ALP(U/L)	44.5±1.50	64.5±1.02*	77.9±1.01*	46.8±1.10	M	62.3±1.10	68.5±0.7*	78.4±1.01*	59.6±1.0
	AST(U/L)	49.40±0.1	53.3±1.01*	55.3±1.11*	48.5±0.01		45.7±1.01	66.6±0.1*	75.7±1.0*	55.3±0.13
	TP(g/L)	7.40±0.21	6.80±0.41*	5.9±0.01*	7.40±0.21		6.18±0.11	6.29±0.11	6.38±0.11	6.40±0.15
	ALB (U/L	2.70±0.10	2.60±0.40	3.20±1.1*	2.80±0.30		3.00±0.50	2.80±0.4	2.40±0.10*	3.20±0.50
	ALT(U/L)	33.6±0.21	43.6±0.01*	56.4±0.21*	31.10±0.1		34.9±0.10	39.5±1.10*	43.4±0.15*	35.4±0.9
	GLU (mmoI/L	53.5±0.80	58.8±1.10	59.2±1.01*	54.8±1.00		55.20±0.9	58.2±1.10	61.5±0.15*	57.0±0.12
	CREA (µmol/L	33.3±0.20	35.5±0.12*	39.2±0.02*	25.7±0.90		25.4±1.10	28.5±0.13	37.7±0.40*	28.4±0.15
	TCHO (mmol/L	93.3±0.16*	90.0±0.08*	83.8±1.3**	117.2±2.9		66.3±0.16*	48.0±0.1**	31.5±1.50**	96.1±0.80
	TG (mmol/L	76.3±1.10*	71.50±1.5*	57.6±0.90**	94.1±1.60		74.4±0.01*	61.3±0.01*	53.5±0.01**	94.0±2.40
	UREA (µmol/L	24.57±0.4*	21.6±1.01*	17.18±1.0*	28.06±0.2		23.04±0.6	25.4±0.9	19.29±0.1*	29.73±0.4
	TB (mg/dl)	0.60±0.10	0.70±0.10	0.90±0.10*	0.70±0.10		0.50±0.20	0.80±0.14	1.60±0.23*	0.70±0.20

Values are expressed as mean± SD, * = values are significantly different ($p < 0.05$) from that of the control); from that of the control. Alanine transaminase (ALT), Alkaline phosphatase (ALP), Aspartate transaminase (AST), Albumin (ALB), Total bilirubin (TB), Total protein (TP), Creatinine (CREA), urea (UREA), Triglyceride (TG), Total cholesterol (TCHO) and Glucose (GLU).

Table 33: Effect of ethyl acetate extract of *C. meturiferus* on biochemical parameters in rate after 4 weeks of treatment

Sex	**Parameter**	**Dose mg/kg n = 5**				**Sex**	**Parameter**	**Dose mg/kg n = 5**			
		150mg/kg	**300mg/kg**	**500mg/kg**	**0mg/kg**			**150mg/kg**	**300mg/kg**	**500mg/kg**	**0mg/kg**
F	ALP(U/L)	53.6±1.85*	54.6±1.02*	54.7±1.06*	46.8±1.0	M	ALP (U/L)	68.3±1.10*	70.3±0.70*	71.20±1.0*	59.6±1.0
	AST(U/L)	44.7±0.10	47.10±1.0	53.7±1.10*	48.5±0.01		ASP (U/L	58.7±0.06	62.9±0.10*	65.4±1.10*	55.3±0.13
	TP(g/L)	7.10±0.01	6.81±0.01	5.11±0.01*	6.41±0.01		TP (g/L	6.54±0.10	6.30±0.13	6.18±0.11	6.40±0.15
	ALB (U/L	2.70±0.70	3.0±0.60	3.80±0.50*	2.80±0.30		ALB (U/L)	3.50±0.50	3.20±0.40	3.80±0.10	3.20±0.50
	ALT(U/L)	44.3±1.60*	45.0±1.01*	47.9±0.01*	31.1±1.0		ALT (U/L)	38.9±0.09	43.9±1.01*	48.9±1.10*	35.4±0.90
	GLU (mmoI/L	52.3±1.0	57.1±1.15*	63.8±1.71*	54.80±1.0		GLU (mmoI/L)	56.20±0.90	58.2±1.90	62.5±0.15*	57.7±0.12
	CREA (µmol/L	25.9±0.20*	35.9±0.10*	44.5±0.02*	25.7±0.90		CREA (µmol/L	33.40±1.10*	42.4±0.3**	48.6±0.4**	28.4±0.15
	TCHO (mmol/L	97.8±0.16*	88.8±1.10*	62.5±1.30*	117.2±2.9		TCHO (mmol/L)	68.60±0.70*	58.2±0.90*	45.8±1.02*	96.1±0.8
	TG (mmol/L	83.2±1.5*	74.2±1.4*	58.1±0.90*	94.1±1.60		TG (mmol/L	82.10±0.01*	74.90±1.5*	62.20±0.9*	94.0±2.4
	UREA (µmol/L	32.95±1.4	25.60±84	23.00±0.1*	28.06±0.2		UREA (µmol/L	28.55±0.90	26.34±0.6	22.60±0.1*	29.73±0.4
	TB (mg/dl)	0.60±0.10	1.70±0.10*	1.80±0.20*	0.70±0.01		TB (mg/dl)	0.70±0.10	0.70±0.1	1.30±0.20*	0.70±0.20

Values are expressed as mean± SD, * = values are significantly different ($p < 0.05$) from that of the control); ** = values are more significantly different ($p < 0.01$) from that of the control. Alanine transaminase (ALT), Alkaline phosphatase (ALP), Aspartate transaminase (AST), Albumin (ALB), Total bilirubin (TB), Total protein (TP), Creatinine (CREA), urea (UREA), Triglyceride (TG), Total cholesterol (TCHO) and Glucose (GLU).

Table 34: Effect of methanolic extract of *C. meturiferus* on biochemical parameters in rate after 4 weeks of treatment

Sex	**Parameter**	**Dose mg/kg n = 5**				**Sex**	**Parameter**	**Dose mg/kg n = 5**			
		150mg/kg	**300mg/kg**	**500mg/kg**	**0mg/kg**			**150mg/kg**	**300mg/kg**	**500mg/kg**	**0mg/kg**
F	ALP(U/L)	37.5±1.20*	49.3±1.05*	55.5±1.02*	46.8±1.0	M	ALP (U/L)	53.3±1.10	58.4±0.10	65.3±1.0*	59.6±1.0
	AST(U/L)	44.6±0.70	52.6±0.06*	55.8±1.10*	48.5±0.01		AST (U/L	57.2±0.90	62.1±0.10*	68.9±1.0*	55.3±0.13
	TP(g/L)	0.13±0.01	0.11±0.01	0.01±0.01	0.11±0.01		TP (g/L	0.18±0.11	0.18±0.11	0.18±0.11	0.18±0.11
	ALB (U/L	2.60±0.20	2.00±0.40*	1.70±1.10*	2.80±0.30		ALB (U/L)	3.20±0.50	2.6±0.10*	2.4±0.10*	3.2±0.50
	ALT(U/L)	52.9±0.70*	54.6±0.60*	57.0±0.50*	31.1±0.09		ALT (U/L)	35.1±1.50	39.4±0.70*	43.3±0.10*	35.4±0.9
	GLU (mmoI/L	59.5±0.8*	62.8±1.50*	69.0±1.07*	54.8±1.0		GLU (mmoI/L)	59.2±0.9	60.8±1.1	68.4±0.15*	57.0±0.12
	CREA (µmol/L	31.0±0.50*	32.6±0.12*	34.7±0.02*	25.7±0.9		CREA (µmol/L	25.2±1.10	29.5±0.15*	33.7±0.40*	28.4±0.15
	TCHO (mmol/L	72.7±0.16*	65.4±0.6**	52.8±1.3**	117.2±2.9		TCHO (mmol/L)	97.4±0.12	77.1±0.9*	65.1±0.50*	96.1±0.8
	TG (mmol/L	65.5±1.14*	53.9±1.4**	46.2±0.9**	94.1±1.6		TG (mmol/L	85.5±0.01*	84.0±1.50*	78.3±0.10*	94.0±2.4
	UREA (µmol/L	28.44±0.4	24.39±1.0*	20.58±1.0*	28.06±0.2		UREA (µmol/L	22.58±0.6*	21.67±0.9*	18.29±0.8*	29.73±0.4
	TB (mg/dl)	0.8±0.1	0.8±0.10	0.9±0.1	0.7±0.0		TB (mg/dl)	0.60±0.20	0.8±0.1	0.80±0.20	0.70±0.20

Values are expressed as mean± SD, * = values are significantly different ($p < 0.05$) from that of the control); ** = values are more significantly different ($p < 0.01$) from that of the control. Alanine transaminase (ALT), Alkaline phosphatase (ALP), Aspartate transaminase (AST), Albumin (ALB), Total bilirubin (TB), Total protein (TP), Creatinine (CREA), urea (UREA), Triglyceride (TG), Total cholesterol (TCHO) and Glucose (GLU)

Table 35: Effect of chloroform extract of *L. kituiensis* in biochemical parameters in rate after 4 weeks of treatment

Sex	Parameter	Dose mg/kg n=5				Sex	Dose mg/kg n=5			
		150mg/kg	**300mg/kg**	**500mg/kg**	**0mg/kg**		**150mg/kg**	**300mg/kg**	**500mg/kg**	**0mg/kg**
F	ALP(U/L)	42.6±1.1	38.9±0.01*	35.4±1.0*	46.8±1.1	M	55.6±0.1	58.3±0.09	59.6±1.0	59.6±0.1
	AST(U/L)	45.6±0.1	36.1±1.0*	34.9±0.9*	48.5±0.1		38.9±1.1*	31.2±1.10*	28.9±0.9*	55.3±0.10
	TP(g/L)	7.10±0.01	7.31±0.01	6.98±0.1*	7.40±0.01		6.40±0.11	6.2±0.11	5.2±0.11*	6.40±0.11
	ALB (U/L	2.8±0.10	2.7±0.40	2.1±0.8*	2.8±0.30		3.1±0.7	2.9±0.20	2.1±0.80*	3.2±0.50
	ALP (U/L)	2.2±0.11	1.8±0.41	1.5±0.9*	2.8±0.40		3.1±0.8	3.8±0.3*	4.3±0.90*	3.2±0.60
	GLU (mmoI/L	64.3±1.1	66.8±1.0*	74.6±1.0*	54.8±0.02		54.6±1.4	63.2±1.1*	66.8±1.01*	57.0±0.60
	CREA (µmol/L	28.4±1.0	31.8±1.0*	34.2±1.7*	25.7±0.90		25.4±1.1	24.6±0.6	21.7±0.01	28.4±1.0
	TCHOL (mmol/L	97.3±1.1*	67.9±0.9**	57.2±1.5**	117.2±2.9		96.5±1.5	83.2±0.1*	81.3±0.80*	96.1±0.30
	TG (mmol/L	75.3±0.05*	53.1±0.7**	34.6±0.3**	94.1±1.60		88.9±0.1*	83.3±0.8*	54.2±0.10**	94.8±0.17
	UREA (µmol/L	24.31±0.1	24.36±0.8	26.25±1.5	28.06±0.2		28.19±0.7	25.64±0.9	23.29±1.0*	29.06±0.2
	TB (mg/dl)	0.8±0.1	0.9±0.1	1.5±0.4*	0.7±0.01		0.4±0.1	0.8±0.1	1.3±0.01*	0.7±0.20

Values are expressed as mean± SD, * = values are significantly different ($p < 0.05$) from that of the control); ** = values are more significantly different ($p < 0.01$) from that of the control. Alanine transaminase (ALT), Alkaline phosphatase (ALP), Aspartate transaminase (AST), Albumin (ALB), Total bilirubin (TB), Total protein (TP), Creatinine (CREA), urea (UREA), Triglyceride (TG), Total cholesterol (TCHO) and Glucose (GLU).

Table 36: Effect of ethyl acetate extract of *L. kituiensis* in biochemical parameters in rate after 4 weeks of treatment

Sex	**Parameter**	**Dose mg/kg n = 5**				**Sex**	**Parameter**	**Dose mg/kg n = 5**			
		150mg/kg	**300mg/kg**	**500mg/kg**	**0mg/kg**			**150mg/kg**	**300mg/kg**	**500mg/kg**	**0mg/kg**
F	ALP(U/L)	45.8±0.9	38.4±0.01*	36.5±1.0*	46.8±1.1	M	ALP (U/L)	54.3±0.10	48.0±0.07	42.3±1.0*	59.6±0.1
	AST(U/L)	45.8±0.1	53.8±1.0*	58.9±0.9*	48.5±0.1		AST (U/L	48.3±1.10*	45.7±1.10*	43.9±0.9*	55.3±0.10
	TP(g/L)	7.70±0.01	7.10±0.91	6.11±0.1*	7.4±0.01		TP (g/L	6.50±0.1	6.0±0.01*	5.81±0.1*	6.40±0.15
	ALB (U/L	2.7±0.50	2.6±0.50	2.0±0.40*	2.8±0.3		ALB (U/L)	3.4±0.20	3.2±0.20	2.3±0.80*	3.2±0.50
	ALP (U/L)	2.8±0.11	1.9±0.41*	1.8±0.9*	2.8±0.4		ALP (U/L)	3.10±0.80	2.5±0.30*	2.10±0.9*	3.2±0.60
	GLU (mmoI/L	56.7±1.1	58.8±1.0*	65.5±1.0*	54.8±0.02		GLU (mmoI/L)	55.3±1.4	59.8±1.1	69.8±1.2*	57.0±0.6
	CREA (µmol/L	30.5±1.0*	32.5±1.0*	35.3±1.7*	25.7±0.9		CREA (µmol/L	29.5±1.2	31.06±0.6*	35.4±0.1*	28.4±1.0
	TCHO (mmol/L	88.4±1.1*	71.9±0.9*	65.4±1.5**	117.2±2.9		TCHO (mmol/L)	81.7±1.5*	74.5±1.1*	53.1±0.5**	96.1±0.3
	TG (mmol/L	77.5±1.0*	71.6±0.7*	68.2±1.5**	94.1±1.6		TG (mmol/L	64.8±0.1**	61.3±0.9**	42.6±0.1**	94.18±0.7
	UREA (µmol/L	27.16±1.0	25.31±0.8	18.7±1.0*	28.6±0.2		UREA (mmol/L	29.51±0.7	25.93±0.9	21.22±1.0*	29.06±0.2
	TB (mg/dl)	0.6±0.1	0.7±0.1	0.9±0.4	0.7±0.0		TB (mg/dl)	0.7±0.20	0.9±0.10	1.5±0.01*	0.70±0.20

Values are expressed as mean± SD Values are expressed as mean± SD, * = values are significantly different ($p < 0.05$) from that of the control);** = values are more significantly different ($p < 0.01$) from that of the control, Alanine transaminase (ALT), Alkaline phosphatase (ALP), Aspartate transaminase (AST), Albumin (ALB), Total bilirubin (TB), Total protein (TP), Creatinine (CREA), urea (UREA), Triglyceride (TG), Total cholesterol (TCHO) and Glucose (GLU),

Table 37: Effect of methanolic extract of *L. kituiensis* in biochemical parameters in rate after 4 weeks of treatment

Sex	**Parameter**	**Dose mg/kg n = 5**				**Sex**	**Parameter**	**Dose mg/kg n = 5**			
		150mg/kg	**300mg/kg**	**500mg/kg**	**0mg/kg**			**150mg/kg**	**300mg/kg**	**500mg/kg**	**0mg/kg**
F	ALP(U/L)	54.7±1.10*	65.3±1.01*	68.6±1.0*	46.8±1.1	M	ALP (U/L)	54.6±0.10*	60.6±0.09*	68.9±1.01*	59.6±0.1
	AST(U/L)	48.3±0.10	55.5±1.0*	58.4±0.9*	48.5±0.1		AST (U/L	53.4±1.10	62.4±1.10*	68.4±0.90*	55.3±0.10
	TP(g/L)	7.26±0.10	6.88±0.61*	6.08±0.1*	7.4±0.21		TP (g/L	6.34±0.41	6.06±0.21	5.21±0.61*	6.18±0.11
	ALB (U/L	3.3±0.200	2.60±1.10	1.40±0.4*	2.8±0.30		ALB (U/L)	3.10±0.20	2.90±0.20	2.10±0.80*	3.2±0.50
	ALT (U/L)	32.4±0.11	45.3±0.41*	48.5±0.9*	31.1±0.9		ALT (U/L)	32.4±0.80	35.8±0.90	39.3±0.60*	35.4±0.90
	GLU (mmoI/L	54.4±1.10	58.7±1.2*	63.6±1.5*	54.8±0.2		GLU (mmoI/L)	54.3±1.40	57.0±1.17	63.10±1.0*	57.0±0.60
	CREA (µmol/L	25.4±1.01	29.4±1.09*	32.2±1.4*	25.7±0.90		CREA (µmol/L	24.9±1.10	28.9±0.60	35.6±0.10*	28.4±1.01
	TCHO (mmol/L	85.2±1.10*	72.3±0.90*	67.3±1.50*	117.2±2.9		TCHO (mmol/L)	65.4±1.50*	58.0±0.10*	43.3±0.80*	96.1±0.31
	TG (mmol/L	77.5±1.01*	66.0±0.70*	54.2±0.30**	94.1±1.60		TG (mmol/L	79.3±0.90*	72.6±0.80*	60.9±0.10**	94.0±0.17
	UREA (µmol/L	23.31±0.1	22.25±0.8*	18.74±0.9*	28.06±0.2		UREA (mmol/L	27.07±0.8	25.86±0.9*	18.81±1.0*	29.06±0.2
	TB (mg/dl)	0.5±0.1	0.8±0.1	1.9±0.40*	0.7±0.01		TB (mg/dl)	0.60±0.10	0.70±0.10	1.8±0.01*	0.70±0.20

Values are expressed as mean± SD, Values are expressed as mean± SD, * = values are significantly different ($p < 0.05$) from that of the control); from that of the control Alanine transaminase (ALT), Alkaline phosphatase (ALP), Aspartate transaminase (AST), Albumin (ALB), Total bilirubin (TB), Total protein (TP), Creatinine (CREA), urea (UREA), Triglyceride (TG), Total cholesterol (TCHO) and Glucose (GLU).

5.3.9 Histopathological examination

The results of histopathological examination of liver, kidney, lungs, and spleen section in rats treated with chloroform, ethyl acetate and methanolic extract of *C. metuliferus* and *L. kituiensis*.

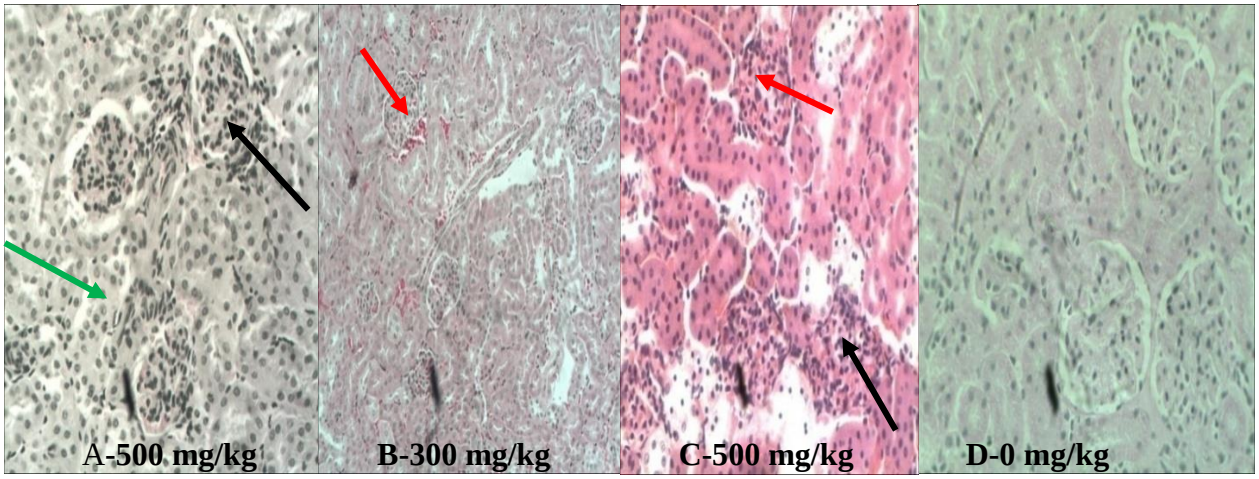
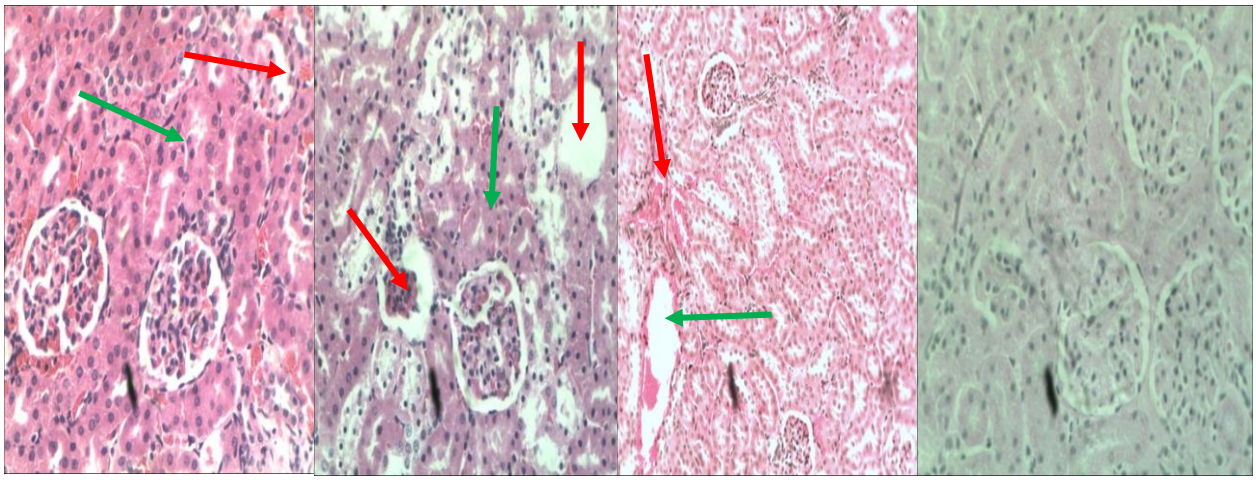


Figure 3: Photomicrograph of kidney section of rats treated with chloroform extract of *C. metuliferus* (male and female) 40x magnifications

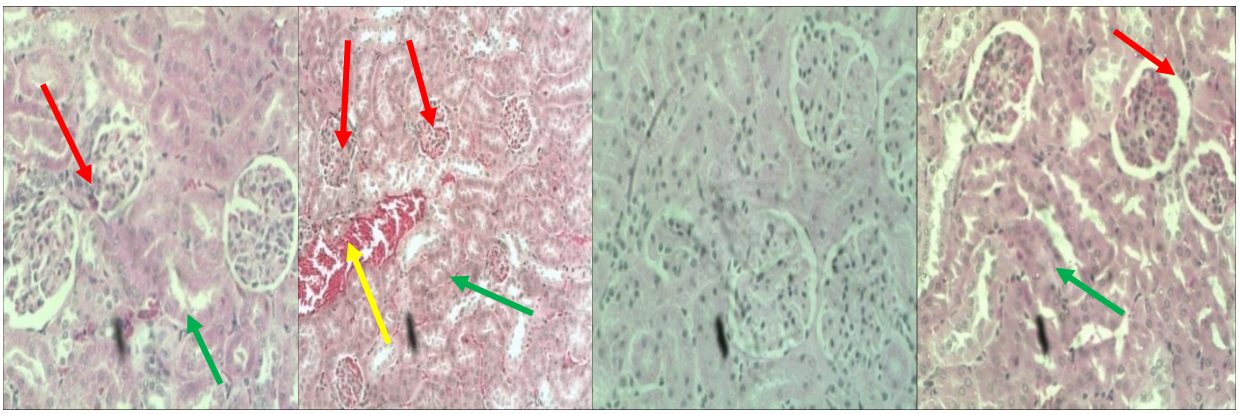
The results of histopathological examination of kidney section in rats treated with chloroform extract of *C. metuliferus* are shown in Fig. 3. The kidney in rats administered with 300 mg/kg body weight presented the distracted glameli red arrow and normal of tubules (Fig. 3B). For the kidney in rats administered with 500 mg/kg body weight shows mild tubular necrosis (green arrow), the distraction of glomerula and Bowman capsule as shown in Fig. 3C (red arrow). While the female rats treated with 500 mg/kg body weight showed shrinkage of glomerula, inflammations black arrow and degeneration of tubular cells as shown in Fig. 3A when compared to control (Fig. 3D).



A-300 mg/kg B-500 mg/kg C-500 mg/kg D-0 mg/kg

Figure 4: Photomicrograph of kidney section of rats treated with methanolic extract of *C. metuliferus* male and female 40x magnifications

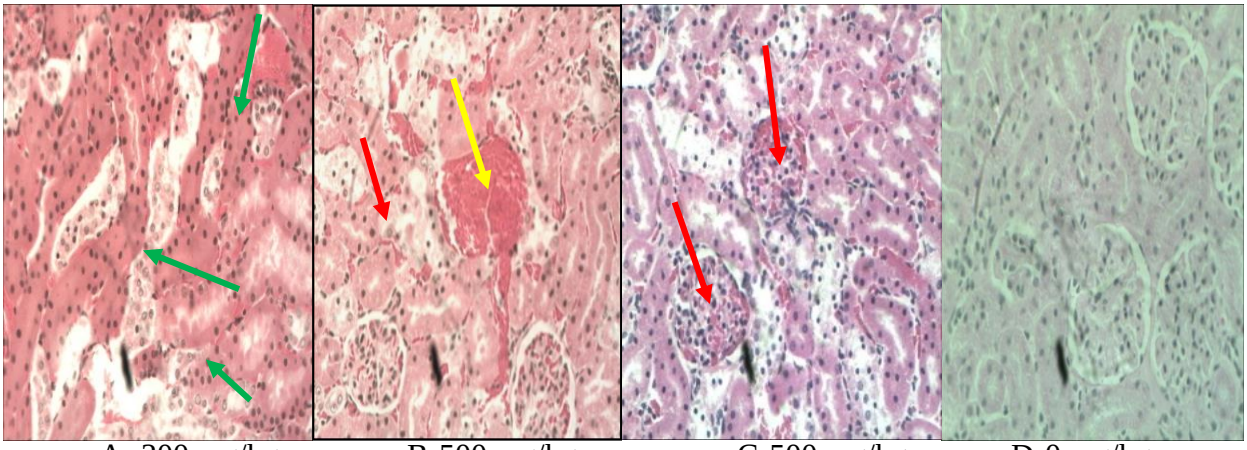
Photomicrographs of the kidney section of rats administered with methanolic extract at a dose of 300 mg/kg, 500 mg/kg body weight are shown in Fig. 4. The kidney in rat's adiministed with 300 mg/kg bodyweight of methanolic extract of *C. metuliferus* showed distraction of bowman capsule and tubules (Fig. 4A) red arrow as compared to controls (Fig. 4D). While the rats administered with 500 mg/kg body weight showed mild tubular necrosis Fig. 4C green arrow and distraction of glomerula and bowman capsule (Fig.4B) red arrow as compared to the control. And the kidney in rat's adimistered with dose level 500 mg/kg showed shrinkage of glomerular and moderate cortical necrosis (Fig. 4B) red arrow was compared to control (Fig. 4D).



A-500 mg/kg B-500 mg/kg C-0 mg/kg D-300 mg/kg

Figure 5: Photomicrograph of kidney section of rats treated with chloroform extract of *L. kituiensis* (male and female) 40x magnifications

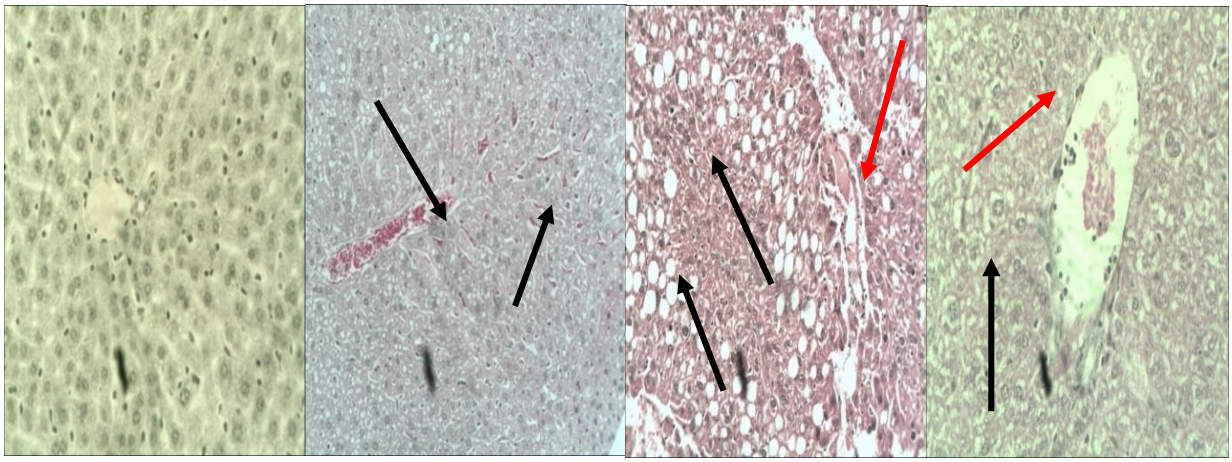
The results of histopathological examination of kidney section in rats treated with chloroform extract of *L. kituiensis* are shown in Fig. 5. The kidney in rats administered with 300 mg/kg showed degeneration of tubular cells and intact glomerula (Fig. 5A & D). For the kidney in rats administered with chloroform extract in a dose of 500 mg/kg body showed the distraction of glomerula and bowman capsule red arrow and congestion yellow arrow (Fig. 5B). While 500 mg/kg body weight showed shrinkage of glomerula, inflammations, and degeneration of tubular cells as shown in Fig. 5A as compared to control (Fig. 5C).



A- 300 mg/kg B-500 mg/kg C-500 mg/kg D-0 mg/kg

Figure 6: Photomicrograph of kidney section of rats treated with ethyl acetate extract of *L. kituiensis* (male and female) 40x magnifications

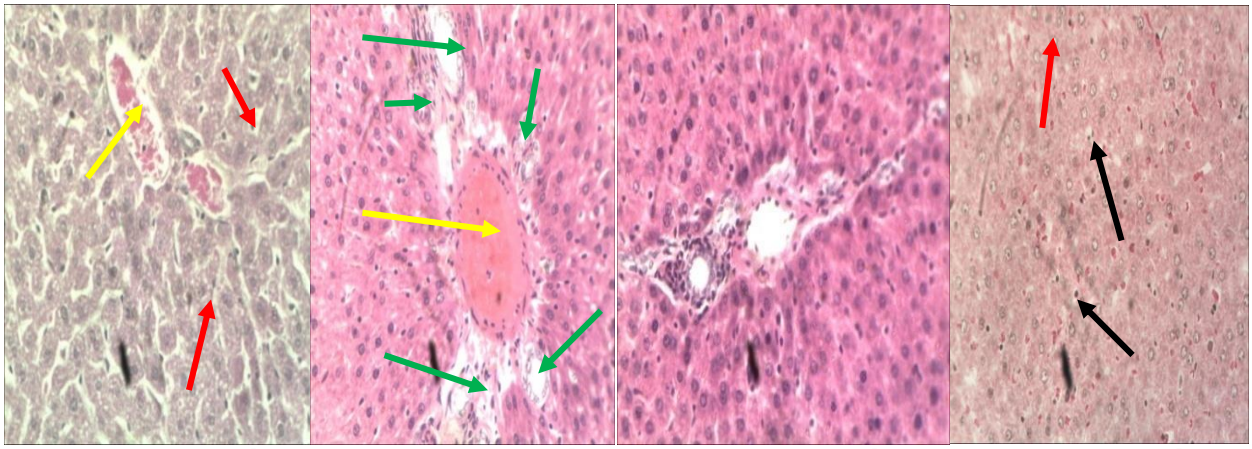
The photomicrograph of the kidney section in rats administered with ethyl acetate at a dose of 300 mg/kg showed degeneration of tubular and tubular necrosis (Fig. 6A) -green arrow. The kidney in rats treated with 500 mg/kg body weight showed mild tubular necrosis, destruction of glomerular and bowman's capsule (Fig. 6C) red arrow. And the rat administered with 500 mg/kg body weight shrinkage of Bowman capsule space, degeneration of tubular cells, mild to moderate necrosis and blood congestion yellow arrow was observed in Fig. 6B as compared to control (Fig. 6D).



A – 500 mg/kg B-0 mg/kg C -300 mg/kg D-500 mg/kg

Figure 7: Photomicrograph of liver section of rats treated with chloroform extract of *C. metuliferus* (male and female) 40x magnifications.

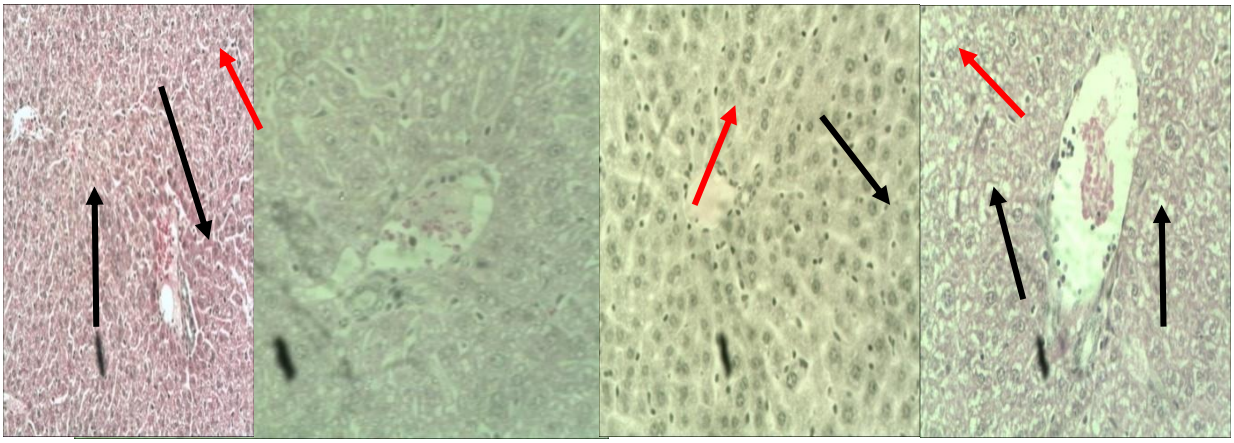
The results of histopathological examination of the liver section in rats treated with chloroform extract of *C. metuliferus* are shown in Fig. 7. The liver in rats administered with dose level 300 mg/kg in all extract showed mild necrosis, portal congestion and vacuolation as shown in Fig. 7C. For the liver in rats administered with 500 mg/kg body weight showed hepatic necrosis, bile lakes and shrinkage of cell in the liver was observed in both male and female and vacuolation (Fig.7D). While the liver in rats treated with dose level 500 mg/kg body weight showed bile duct hyperplasia in the portal triad, moderate hepatic necrosis, and portal congestion as shown in Fig. 7A as compared to control (Fig. 7B).



A- 300 mg/kg B-500 mg/kg C- 0 mg/kg D-500 mg/kg

Figure 8: Photomicrograph of liver section of rats treated with methanolic extract of *C. metuliferus* (male and female) 40x magnifications.

The results of histopathological examination of the liver section in rats treated with chloroform extract of *C. metuliferus* are shown in Fig. 8. The liver in rats administered with 300 mg/kg body weight showed mild necrosis, congestion yarrow arrow and vacuolation black arrow. While the liver in rats treated with dose level 500 mg/kg body weight showed bile duct hyperplasia in the portal triad green arrow, moderate hepatic necrosis red arrow, and portal congestion as shown in Fig. 8A as compared to control (Fig. 8B). And the rats administered with dose level, 500 mg/kg showed severe necrosis (Fig. 8D).



A -500 mg/kg B-0 mg/kg C -300 mg/kg D-300 mg/kg

Figure 9: Photomicrograph of liver section of rats treated with chloroform extract of *L. kituiensis* (male and female) 40x magnifications.

The results of histopathological examination of the liver section in rats treated with chloroform extract of *L. kituiensis* are shown in Fig. 9. The liver in rats administered with 300 mg/kg for 28 days presented the normal portal triad with vacuolation black arrow and mid necrosis as shown in Fig. 9D. For the liver in rats administered with 500 mg/kg body weight showed mild hepatic necrosis red arrow, vacuolation black arrow and normal portal triad as shown in Fig. 9A, while the rats treated with 500 mg/kg body weight showed hepatic necrosis as shown in Fig. 9C.

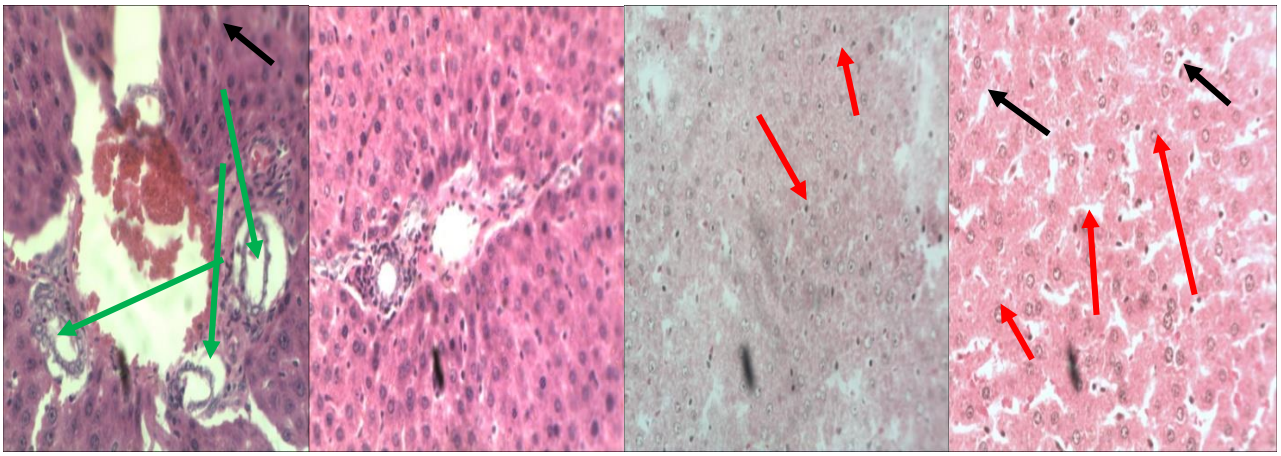


Figure 10: Photomicrograph of liver section of rats treated with methanolic extract of *L. kituiensis* (male and female) 40x magnifications.

The results of histopathological examination of the liver section in rats treated with methanolic extract of *L. kituiensis* are shown in Fig. 10. The liver in rats administered with 300 mg/kg of methanolic extract showed normal portal triad and mid necrosis as shown in Fig. 10C red arrow. For the liver in rats administered with 500 mg/kg body weight of methanolic extract showed mild hepatic necrosis, vacuolation black arrow and normal portal triad as shown in Fig 10D, while 500 mg/kg body weight showed hepatic necrosis red arrow and congestion and bile duct hyperplasia (Fig. 10A) green arrow

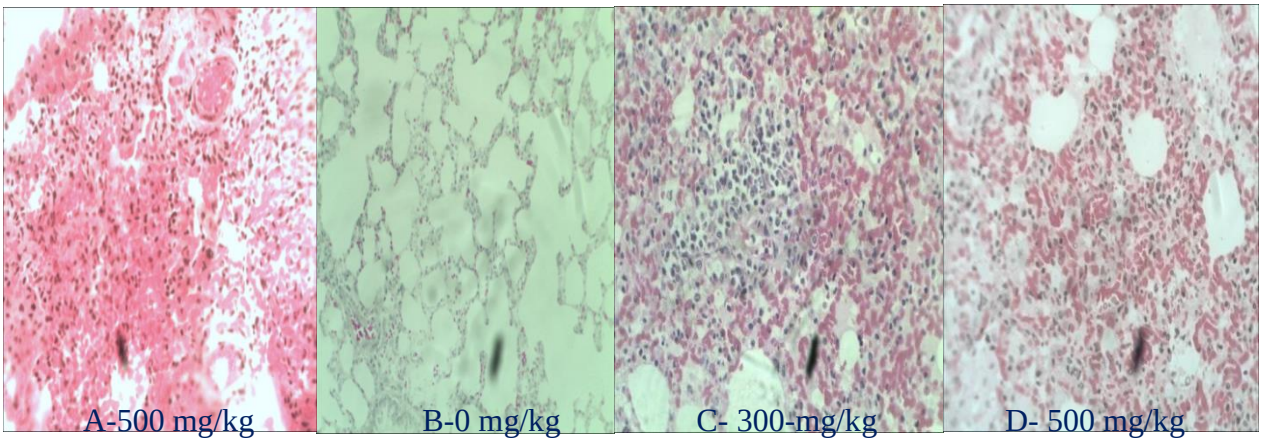
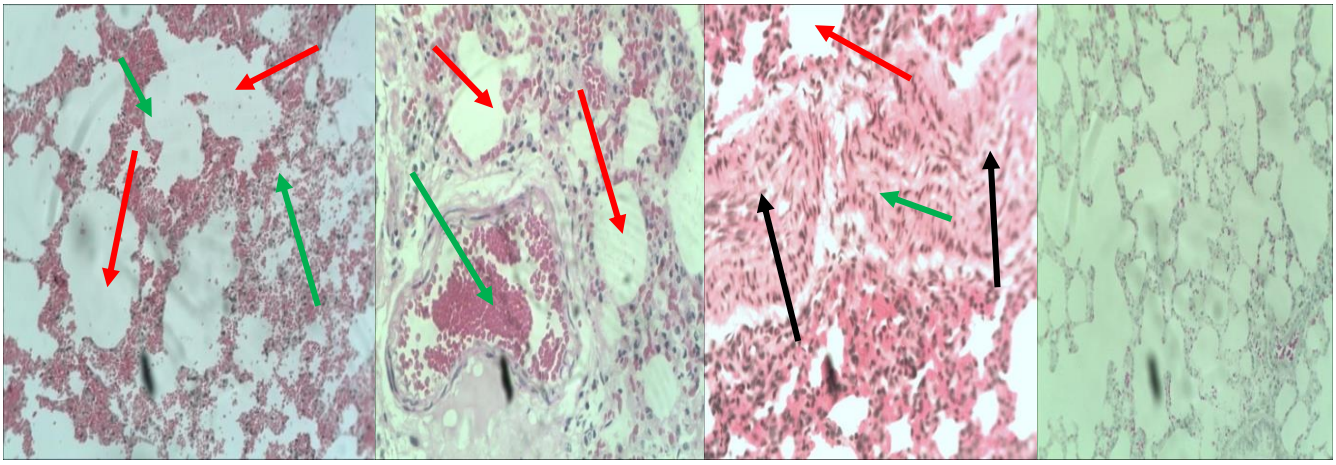


Figure 11: Photomicrograph of lungs section of rats treated with chloroform extract of *C. metuliferus* (male and female) 40x magnifications

The results of histopathological examination of the lung section in rats treated with chloroform extract of *C. metuliferus* are shown in Fig. 11. The lungs in rats administered with 300 mg/kg

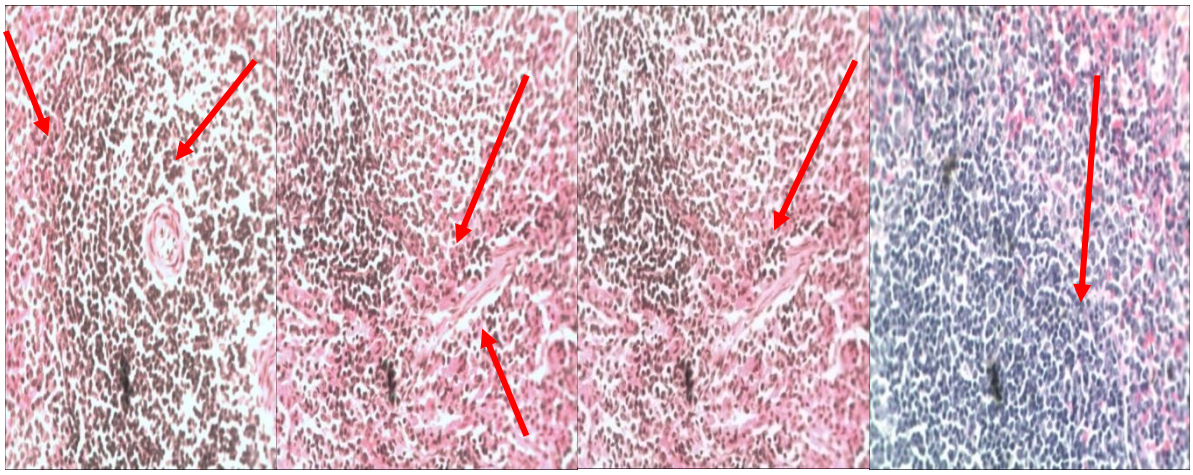
body weight of chloroform extract showed thickened alveolar wall, narrow respiratory ducts and congestion (Fig. 11A, B & C) as compared to control (Fig. 11D).



A-500 mg/kg B-500 mg/kg C- 300 mg/kg D- 0 mg/kg

Figure 12: Photomicrograph of lung section of rats treated with ethyl acetate extract of *L. kituiensis* (male and female) 40x magnifications.

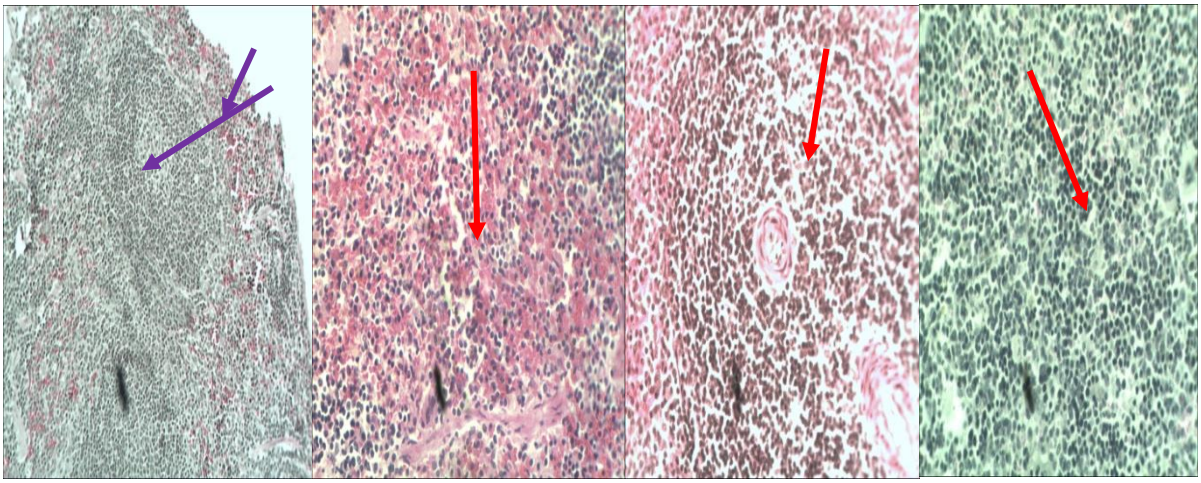
The results of histopathological examination of the lungs section in rats treated with ethyl acetate extract of *L. kituiensis* are shown in Fig. 12. The lungs in rats administered with 300 mg/kg and 500 mg/kg body weight of ethyl acetate extract showed thickened alveolar wall and narrow respiratory ducts red arrow, hemorrhage (green arrow) and congestion (black arrow) (Fig.12A, B & C) as compared to control (Fig. 12D).



A-300 mg/kg B- 500 mg/kg C-500 mg/kg D-500 mg/kg

Figure13: Photomicrograph of spleen section of rats treated with chloroform extract of *C. metuliferus* (male and female) 40x magnifications

The results of histopathological examination of the spleen section in rats treated with chloroform extract of *C. metuliferus* are shown in Fig. 13. The spleen in rats administered with 300 mg/kg showed mid distraction of red and white pulp and 500 mg/kg body weight of chloroform extract showed distraction of red and white pulp appearance (Fig. 13A, B & C) as compared to control (Fig. 13D).



0 mg/kg A A500 mg/kg B 300 mg/kg C 500 mg/kg D

Figure14: Photomicrograph of spleen section of rats treated with methanolic extract of *L. kituiensis* (male and female) 40x magnifications

The results of histopathological examination of the spleen section in rats treated with methanolic extract of *C. metuliferus* were shown in Fig. 14. The spleen in rats administered with 300 mg/kg and 500 mg/kg body weight of methanolic extract showed the distraction of red and white pulp appearance (Fig. 14B, C & D) red arrow as compared to control (Fig. 14A) purple.

5.4 Discussion

Toxicity is an expression of being poisonous, indicating the state of adverse effects led by the interaction between toxicants and cells (Das *et al.*, 2015). The acute toxicity study of *C. meturiferus* and *L. kituiensis* extract was carried out as per Muhammad *et al.* (2015). The single oral dose administration of *C. meturiferus* and *L. kituiensis* in albino rats at a dose of 300 mg/kg, 600 mg/kg and 1500 mg/kg revealed neither mortality nor clinical signs, like changes in fur and skin, drowsiness, tremors, Lacrimation, salvation, temperature, diarrhea, eyes, respiratory rate were observed. No significant reduction in feed and water intake of the treated rats in either sex through the 14-day study; this is a pointer to the fact that the diet and water were well

accepted by the animals, suggesting that the extracts did not interfere with the metabolism of in the treated animals. This report is in agreement with the work reported by Akhila *et al*. (2007) and therefore this supports the traditional usage of the plant by the oral route. Insignificant difference in the bodyweight gain of all the animals treated with chloroform, ethyl acetate and methanolic extract of *C. meturiferus* and *L. kituiensis* in all doses 300 mg/kg, 600 mg/kg and 1500 mg/kg were observed. This suggests that extracts did not interfere the normal body metabolism of the animals as the increment in food and water intake is synonymous with an increase in body weight. This result is in agreement with the findings reported by Raza and Al-shabanah (2002). The assessment of blood hematology provides an insight into possible damage brought about by the extract in the hematopoietic system (Rasekh *et al.,* 2008). No significant change was observed in organ weight and hematological parameters white blood cell (WBC), red blood cell (RBC), hematocrit (HCT), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), lymphocytes (LYM), monocytes (MON), neutrophil granulocytes (NEUT), Red blood cells distribution Width (RDW) in rats treated with chloroform, ethyl acetate and methanolic extract of *C. meturiferus* and *L. kituiensis* in all doses 300 mg/kg, 600 mg/kg and 1500 mg/kg body weight. This report is in agreement with the work reported by Clarke *et al*. (2011). The LD_{50} value was established to be not greater than 2000 mg/kg, throughout the repeated dose 28-day administration of *C. meturiferus* and *L. kituiensis* no morbidity or mortality no clinical signs, like changes in fur, skin were observed in the extract treated rats. This result align with the findings reported by Olorunnisola (2012). In addition to that no significant reduction in feed and water intake of the treated rats in male and female sex was observed; this is a pointer to the fact that the diet and water were well accepted by the rats, suggesting that the extracts did not alter the metabolism processes. This may also signify that the nutritional status of weight gain and appetite stability of the animal did not adversely affect by the extracts. This corroborates the traditional usage of the plant by the oral route.

The body weight changes may reflect the general health status of animals (Michael *et al*., 2015). However, the body weight gain witnessed in all the animals treated with *C. metuliferus* and *L. kituiensis* in dose level 150 mg/kg, 300 mg/kg and 500 mg/kg of chloroform, ethyl acetate and methanolic extract suggests that the extracts did not interfere with normal body metabolism of the animals as the increment in food and water intake is synonymous with an increase in body weight. This result corroborates with the findings reported by Sireeratawong (2008), and this supports the traditional usage of this plants.

Organ weight is one of the most sensitive drug toxicity indicators (Yano *et al*., 2007). Based on the results, the toxicant present in the plant greatly affects the heart, liver, kidney and spleen, which were presented with an absolute difference. The rats treated with chloroform, ethyl acetate and methanolic extract of *C. meturiferus* shows a significant increase in heart, liver, kidney and spleen in dose level 300 mg/kg and 500 mg/kg body weight. This result is in agreement with the findings reported by Sireeratawong (2010). While the rats treated with chloroform, ethyl acetate and methanolic extract of *L. kituiensis* revealed a significant decrease in heart, liver, kidney and spleen in dose level 300 mg/kg and 500 mg/kg body weight. The alteration of organ weight provides an insight into possible damage of organs brought by the extract in the hepatic and renal functions. The alterations in liver weight may suggest treatment-related changes including hepatocellular hypertrophy (enzyme induction or peroxisome proliferation or lipidosis) however; elevation of liver weight signifies enzyme-induction, which is one marked activity of a terpene. Changes in kidney weight may reflect renal toxicity. Spleen is one of the histopathological indicators for possible immune toxicity; the depressing weight of spleen suggests cell depletion and is viewed as a potentially immunotoxic effect but still requires more definitive testing (Firenzuoli *et al*., 2008).

Blood parameters analysis is relevant to risk evaluation as the hematological system has a higher predictive value for toxicity in humans when assay involves animals (Olson *et al.*, 2000). Blood is an important index of physiological and pathological status in both animals and humans and the parameters usually measured are red blood cell count (RBC), white blood cell count (WBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), lymphocytes (LYM), and monocytes (MON). In this study, the rats treated with chloroform, ethyl acetate and methanolic extract of *C. meturiferus* and *L. kituiensis* shows a significant increase in in the above-mentioned blood parameters in dose level 300 mg/kg and 500 mg/kg body weight. From this result, it is possible that the extract contains agents that stimulate the bone marrow to produce neutrophils and release them into the blood. Neutrophils are the major granulocytes to be activated when the body is invaded by bacteria and they provide the first line of defense against invading microorganisms. The granules of the neutrophil contain many enzymes, which makes it a powerful and effective killing machine. This effect on neutrophil count may be partly responsible for the claim that *C. metuliferus* and *L. kituiensis* have antibacterial actions.

The study also revealed a significant rise in lymphocytes which signify the potential usefulness of the plant as immune system stimulant, a factor that may justify the use of the plant in the treatment of cancer (Hoffmann, 2003). In addition, the significant increase in lymphocytes in the tested animals suggests that the extract may have immune stimulating properties (Bruneton, 1995; Govid *et al*., 2012; Hoffmann, 2003). According to Lissoni (2006) the plants that show anticancer chemotherapies tend to raise lymphocyte numbers by producing interleukine2 that modulates cytokines that promote differentiation of lymphocyte precursor cells into mature lymphocytes, due to that these two plants may have anticancer effects that triggered the rise of WBC (Lissoni *et al*., 2006; Lisson *et al*., 1999). In general, this result suggests that the extract did not change the hemopoietic sites that produce white blood cells in treated groups. Moreover, red blood cell distribution width (RDW) is a measure of erythrocyte size variability; it has been used as a good indicator of renal disease (Solak *et al*., 2014). The RDW is a change in RBC shapes, caused by several factors like nutrition, heart diseases, iron deficiency and chemotherapy, in humans. In this study, *C. metuliferus* showed a significant increase of RDW in rats treated with chloroform, ethyl acetate and methanolic extract; this increase may be associated with renal impairment (Yakubu *et al*., 2007; Tekce *et al*., 2014).

The assessment of clinical biochemistry provides an insight into possible damage brought about by the extract in the hepatic and renal functions. In toxicity studies, assessment of liver and kidney functions is germane because both organs are essential for the survival of an organism (Olorunnisola*et al*., 2012). In this study Alanine transaminase (ALT), Alkaline phosphatase (ALP), Aspartate transaminase (AST), Albumin (ALB), Total bilirubin (TB), Total protein (TP), Creatinine (CREA), urea (UREA), Triglyceride (TG), Total cholesterol (TCHO) and Glucose (GLU) were measured. Alanine aminotransferase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) are sensitive enzymes used in assessing the severity of liver damage (Ramaiah *et al*., 2011). Elevated activities of these enzymes are associated with liver or heart damage (Wasan *et al*., 2001; Brawtbar *et al*., 2002). The rats treated with Chloroform, ethyl acetate and methanolic extract of *C. metuliferus* in 300 mg/kg and 500 mg/kg showed significant increases in ALT and AST of the treated animals in both sexes compared with control. This could suggest that *C. metuliferus* may have hepatotoxic effect, while the increase of ALP might suggest obstruction of the biliary tract which may be present in the liver. An elevation in the activity of liver enzymes (ALT, AST and ALP) is conventionally an indicator of liver injury. This result aligns with findings reported by Tarkang

(2010) on the sub acute and subchronic toxicity of the aqueous and ethanol leaf extracts of *Carica papaya* in Wistar rats.

While the rats treated with *L. kituiensis* revealed a significant reduction of ALT, AST and ALP in dose level 300 mg/kg and 500 mg/kg, this could suggest that *L. kituiensis* that may not have a hepatotoxic effect. Measurement of blood urea and creatinine concentration reflects the likelihood of renal problems or dysfunction (Lameire *et al*., 2005). Creatinine is a by product of muscle cellular metabolism for energy production and is removed by kidneys to a level that can be accommodated by the body (BioSystems, 2016). The results in this study reveal a significant increase of blood urea to the rats treated with chloroform, ethyl acetate and methanolic extract of *C. metuliferus* and *L. kituiensis* in dose level 300 mg/kg and 500 mg/kg. Destruction of the glomeruli causes a significant decrease in the glomerular filtration rate and that results to increase in blood urea and creatinine resulting in chronic renal failure. Creatinine is not supposed to be reabsorbed but all creatinine that is filtered in the glomerular filtrate passes on through the tubular system and is excreted in the urine. In this situation, creatinine is reabsorbed rather than excreted in the urine. The abnormal values of kidney parameters such as blood urea nitrogen (BUN) and creatinine suggest that sub-acute administration of *C. metuliferus* and *L. kituiensis* can course damage to the kidney. The result of this study is consistent with the findings of Builders *et al*. (2011).

Albumin is a big part of the serum protein produced from the liver and excreted through the kidneys (Townsed *et al*., 1990). In this study the rats treated with chloroform, ethyl acetate and methanolic extract of *C. meturiferus* and *L. kituiensis* shows a significant decrease in dose level 300 mg/kg and 500 mg/kg body weight. A reduction in albumin is an indication of impaired hepatocellular function (Thapa *et al*., 2008). However, a significant difference in the serum level of these parameters upon prolonged administration of *C. metuliferus* and *L. kituiensis,* when compared with the control further, corroborates the fact that the extracts destroy the secretory functions of the liver. Therefore, a decrease of albumin can directly be linked to liver damage and kidney disfunction (Thapa & Walia, 2007).

Triglycerides and Cholesterol make up the body lipids, in this study the results showed a significant decrease in dose level 300 mg/kg and 500 mg/kg in both plants as compared to the control group. This result signifies that the two plants may have hypolipidemic effects, and this result is in agreement with that reported by Khanna *et al*. (2010). In addition to that, abnormalities in the concentration of major lipids like cholesterol (TCHO) and triglycerides

(TG) can give useful information on the lipid metabolism as well as the predisposition of the animals to atherosclerosis and its complications.

In this study, total protein was also tested, the results of total protein revealed a significant decrease in rats treated with chloroform, ethyl acetate and methanolic extract of *C. metuliferus* and *L. kituiensis* in dose level 300 mg/kg and 500 mg/kg. The observed a decrease in total protein in tested rats compared to controls might be due to liver and kidney malfunction because of this extract, so the organs failed to produce the required amount of protein (Burtis *et al.,* 2008). This is also confirmed with slides that show abnormality in liver cells and kidney glomeruli that may hinder protein synthesis and elimination (Ozer *et al*., 2008; Burtis *et al*., 2008; Yaqub *et al*., 2013).

Glucose level is another parameter measured; the significant increase was observed in rats treated with chloroform, ethyl acetate and methanolic extract of *C. metuliferus* and *L. kituiensis* in treatment dose 300 mg/kg and 500 mg/kg body weight. The increase in glucose level in the blood signifies that the extract had an influence on the metabolism of carbohydrates into glucose. Glucose level increases are normally observed in animals that are suffering from impairment of pancreas function that leads to the production of inadequate or dysfunctional insulin. In this study, the extracts reduce the ability of insulin to act on peripheral tissues like skeletal, adipose and liver tissues (Goji *et al*., 2009). The result of this study is consistent with the findings reported by Pieme (2006).

Bilirubin is a yellow pigment that is produced after the breakdown of the erythrocyte hemoglobin in the liver resulting in protein separating into globin and heme, heme is further broken to biliverdin that is reduced into bilirubin, this occurs in the reticular endothelial system of the liver, spleen and bone marrow (Kakadiya, 2009). The result revealed significant increases in rats treated with *C. metuliferus* and *L. kituiensis* in dose level 300 mg/kg and 500 mg/kg body weight. Elevated bilirubin levels are an indication of distorted liver functions and a small elevation is an important indicator of liver damage in laboratory animals or could be a sign of biliary duct obstruction. In order to assess the synthetic capacity of the liver, determination of plasma proteins like albumin is required and decrease in plasma proteins, therefore, tend to reflect chronic damage (Burtis *et al*., 2008).

Histopathological studies provide supportive evidence for biochemical and hematological observations. The above-mentioned biochemical investigations were in correlation with the

histopathological studies. Liver section of rats administered with 300 mg/kg and 500 mg/kg body weight of chloroform, ethyl acetate and methanolic extracts revealed inflammation, necrosis, and duct hyperplasia in the portal triad, moderate hepatic necrosis, and portal congestion in both plants. And kidney section of rats administered with 300 mg/kg and 500 mg/kg body weight revealed that shrinkage of glomerula, inflammations, degeneration of tubular cells and distraction of glomeruli were observed indicating that *C. metuliferus* and *L. kituiensis* extract causes kidney damage. However, animals in the control group had intact hepatocytes, portal vein, glomeruli and intact tubules. The occurrence of lymphocytic infiltration in organs has been attributed to the presence of glycosides as reported by Adedapo (2003). The result of this study is consistent with the findings of Builders (2012) who investigated the toxicity of *Parkiabiglobosa* stem bark extracts in rats.

The lungs of the rats treated with chloroform, ethyl acetate and methanolic extract of *C. metuliferus* at a dose of 300 mg/kg and 500 mg/kg revealed thickened alveolar wall and narrow respiratory ducts and congestion. While rats treated with chloroform, ethyl acetate and methanolic extract *L. kituiensis* at a dose of 300 mg/kg and 500 mg/kg showed thickened alveolar wall and narrow respiratory ducts and hemorrhage as compared to control. While the micrograph for histological sections of the spleen showed normal, red and white pulp appearance with the appearance of extramedullary hemopoiesis in male and female spleen of *C. metuliferus* and *L. kituiensis*, this may indicate damage of spleen as compared to control. The result of this study is consistent with the findings of Pudoe *et al*. (2010). It was reported that the toxicity of some of the herbal medications might be a result of phytochemical constituents. Muhammad *et al*. (2015). It also reported that a large intake of tannins may cause kidney and liver damage (Kifayatullah *et al*., 2015).

5.5 Conclusion

The result from this chapter describes the adverse effects or safety of a substance that result either from a single exposure or multiple exposures in a short space of time. The results obtained in the acute toxicity test showed fluctuations in organ weight over the 14 days of survival observation period this maybe suggestive of chronic toxicity. In sub acute study the plants showed severe toxic to animals. The study suggest isolation of pure compound should be taken as a promising alternative for exploring therapeutic and pharmaceutical interest of *C. metuliferus* and *L. kituiensis* extract with a reduction of possible adverse effects.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 General discussion

This chapter presents the concluding remarks from the obtained findings, research contribution, and future research directions. The aim of this study was to evaluate antimalarial activity two medicinal plants namely *C. metuliferus* and *L. kituiensis* used traditionally to treat malaria in Tanzania. Furthermore, the study provided scientific evidence of the efficacy of these plants through in *vivo*-Acute and Sub-acute toxicity in an animal model, that all plant materials entailed for medical purposes should be thoroughly tested for their efficacy and safety before being approved for human consumption. Additionally, this study established the volatile phytochemicals present in the *C. metuliferus* and *L. kituiensis* leaf extract. The major findings, outcomes and recommendations are summarized in this chapter.

The results of this study showed that the leaves of *C. metuliferus* and *L. kituiensis* possess highly antimalarial activity. Comparatively, the extracts of *C. metuliferus* exhibited higher antimalarial activity than extracts of *L. kituiensis.* This result has established the rationale for the traditional use of the plants in the treatment of malaria and showed that medicinal plants which have reputations for antimalarial properties can be screened to ascertain their efficacy and determine their potentials as sources of new antimalarial drugs.

The phytochemical screening of methanolic, chloroform and ethyl acetate of *C. metuliferus* and *L. kituiensis* showed the presence of steroids, alkaloids, flavonoids, tannins, glycosides, amino acids, and phenol compounds. The gas chromatography-mass spectroscopy result revealed the presence of 11 major compound namely Cyclotrisiloxanehexamethyl; Z-Z-6, 28-Heptatriactontadien-2-one; D-Manitol, 1, 1, 1’-o-1,16-hexadecanediylbis; Trans, CIS-1, 8-Dimethylspiro(4, 5)-Decane and L-(+)-Ascorbic acid 2, 6-dihexadecanoate, 3, 7, 11, 15-Tetramethyl-2-Hexadecen-1-ol; Oleyl alcohol tri fluoroacetate; 1-Pentatriacontanol; N-propyl 11-octadecenoate, fucosterol, Artemiseole compound and other usefully compound. It is thereforepossible that the antimalarial effects observed may be attributed by these compounds observed. The antimalarial effects produced by crude extracts of both experimental plants validate their use in traditional medical practice. It also gives them curative advantage as antimalarials

Extracts of *C. metuliferus* and *L. kituiensis* have been found to be toxic and therefore not safe for malaria therapy and perhaps for the treatment of other ailments. The acute administration of *C. metuliferus* and *L. kituiensis* at the three dose levels showed fluctuations in organ weight over the 14 days of survival observation period, this be suggestive that the intake of high doses of this plant's extracts may exhibit organs toxicity in Swiss albino mice. In sub acute study all experimental rats were alive and normal for all 28 days of experimentation period. No mortality or signs of toxicity were observed. However, at a dose of 300 mg/kg and 500 mg/kg *C. metuliferus* and *L. kituiensis* caused elevation of body weight, organ weight, hematology, biochemical parameters and histopathological changes in target organs of toxicity (liver, kidney, lungs and spleen). These plants can cause severe toxic to animals.

6.2 Conclusion

Malaria is a major public health problem that presently is complicated by the increasing resistance of *P. falciparum* against the current drugs. This has necessitated the search for novel antimalarial agents that have a unique mechanism of action. As a result, a number of medicinal plants have been screened for antimalarial activity. In this study, extracts from *C. metuliferus* and *L. kituiensis* have been established to exhibit antimalarial activity against *plasmodium berghei*. The medicinal properties of *L. kituiensis* and *C. metuliferus* observed might therefore due to the presence of these groups of phytochemicals. Additionally, the studies provide strong evidence that the plant can cause severe toxicity to heath tissue.

6.3 Recommendations

Based on these findings the following recommendations are considered:

(i) Isolation and characterization of antimalarial compounds are recommended in order to provide a stepping-stone for further detailed study and development of antimalarial herbal products.

(ii) The study suggests further pharmacological studies should be carried out in order to characterize secondary metabolites with toxic properties for the purpose of suppressing toxicity effect.

(iii) A similar research work should be conducted on toxicity using extracts of the same experimental plants, involving more animal models over a longer period of time to determine the safety of these plants.

(iv) The study also recommends for evaluation of antioxidants and anticancer compounds.

(v) Since medicinal plants have been used as alternative drugs for the management of malarial, plants have been over-exploited, thus increasing risks of their extinction. Therefore, appropriate strategic plans on the proper utilization of medicinal plants should be put forward in order to meet the needs of the current and future generations.

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APPENDICES

Appendix 1: Ethical clearance

UNITED REPUBLIC OF

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TENGERU, P.O Box 447
ARUSHA

CLEARANCE CERTIFICATE FOR CONDUCTING MEDICAL RESAERCH IN TANZANIA

This is to certify that the research entitled: Antimalarial, toxicity and phytochemicals evaluation of *Lippia kituiensis* and *Cucumis metuliferus* species found in Tanzania (Mzena et al) has been granted ethical clearance to be conducted in Tanzania.

The Principal Investigator of the study must ensure that the following conditions are fulfilled:

1. Progress report is submitted to the Ministry of Health and the National Institute for Medical Research, Regional and District Medicinal Officers after every six months.
2. Permission to publish the result is obtained from National Institute for Medicinal Research.
3. Copies of final publication are made available to the Ministry of Health &Social Welfare and the National Institute for Medical Research
4. Any research, who contravenes or fails to comply with these conditions, shall be guilt of an offence and shall be liable on conviction to a fine. NIMR Act No.23 of 1979,PART III Section 10(2).
5. Sited: at The Nelson Mandela African Institute of Science and Technology

Approval is for one year: 09th March 2016 to 07th March 2017

Name: Dr Mwelecele N Malecela
Signature
For CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Prof. Muhammed Bakari Kambi
Signature
CHIEF MEDICAL OFFICER
MIN. OF HEARTH CDGE&CHILDREN

CC: RMO
DED
DMO