ANTIMICROBIAL ACTIVITY, TOXICITY AND PHYTOCHEMICAL SCREENING OF *Lantana trifolia* LEAF EXTRACTS

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DECLARATION

I hereby declare that this thesis is my original work and has not been presented for the award of a degree in any other university.

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DEDICATION

This thesis is dedicated to my loving wife Cynthia, Tony our son, Tania our daughter, my mum Florah and all other family members who have always encouraged me.

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ACRONYMS AND ABBREVIATIONS

ALT	-	Alanine aminotransferase
AMR	-	Antimicrobial resistance
ANOVA	-	Analysis of variance
AST	-	Aspartate aminotransferase
ATCC	-	American type culture collection
BA	-	Blood agar
BW	-	Body weight
LD_{50}	-	Median lethal dose
MFC	-	Minimum fungicidal activity
MCH	-	Mean cell haemoglobin
MCHC	-	Mean corpuscular haemoglobin concentration
MCV	-	Mean corpuscular volume
MIC	-	Minimum inhibitory concentration
OECD	-	Organization of Economic Cooperation
PCV	-	Packed cell volume
RBCs	-	Red blood cells
WBCs	-	White blood cells
WHO	-	World Health Organization

ABSTRACT

Medicinal plants have played a significant role all over the world in preventing and healing a range of diseases. *Lantana trifolia* is a plant used in the management of asthma, common cold, cerebral malaria, epilepsy and tonsillitis. However, empirical data to validate its toxicity profile and safety is lacking. Thus, this study was designed to investigate the phytochemical composition, antimicrobial activity and the acute and sub-acute toxicity of extracts from *L. trifolia* leaves to validate its ethnomedicinal usage. This study provides information on the safety of the plant.

Acute oral toxicity study of the aqueous leaf extract of *L. trifolia* was conducted according to guideline 423 described by the Organization for Economic Co-operation Development (OECD) whereby a single dose of the extract was given to female rats at dose levels 300mg/Kg Bwt and 2000mg/Kg Bwt. Thereafter, the rats were observed individually for the first four hours, then over a period of 24 hours and at least once daily for 14 days.

Sub-acute oral toxicity of the aqueous leaf extract of *L. trifolia* was investigated at three dose levels of 250 mg/Kg Bwt, 500 mg/Kg Bwt, and 1000 mg/Kg Bwt in both female and male Swiss albino mice based on the OECD guideline number 407 for 28 days. The control group received distilled water. Thereafter, body weight, feed consumption and water consumption were monitored. Vital parameters of the blood such as haematological profiles and biochemical profiles were determined at the end of the experiment. Moreover, histopathological examination of the various harvested organs was done.

Qualitative phytochemical analysis was performed on both aqueous and organic extracts to identify compounds of pharmacological value. The extraction yield of the aqueous and organic extracts was 5.2% and 11.2% respectively. Qualitative phytochemical screening of the extracts showed the presence of tannins, saponins, phenolics, terpenoids, flavonoids, alkaloids and reducing sugars in both extracts. In an acute oral toxicity study, the aqueous leaf extract of *L. trifolia* demonstrated a median lethal dose (LD_{50}) of >2000 mg/Kg Bwt, depicting its safety. Following sub-acute oral toxicity, the urea levels in female mice which received 1000 mg/Kg Bwt dose of the aqueous leaf extract of *L. trifolia* were significantly elevated compared to those of the control group mice ($P<0.05$). Moreover, there was no significant difference in the mean body weight between the treated and control groups($P>0.05$). Treatment groups that received 1000mg/kg body weight of the aqueous extract demonstrated diffuse tubular epithelium degeneration, indicating nephrotoxicity and a dose-related hepatocyte degeneration, indicating hepatotoxicity

Staphylococcus aureus (Gram-positive), *Bacillus cereus* (Gram-positive), *Escherichia coli (*Gram-negative) and *Candida albicans*(fungus) were used to determine the antimicrobial activities of aqueous and DCM-methanol extracts. The most susceptible microorganism in the current study was *C. albicans.* The minimum inhibitory concentration and minimum bactericidal concentration values of aqueous and organic leaf extracts of *L. trifolia* for *S. aureus* were 200mg/ml and 400mg/ml; 3.12mg/ml and 6.25mg/ml respectively. The MIC and MFC for *C. albicans* were 100mg/ml and 3.125mg/ml for both aqueous and organic extracts. The aqueous leaf extract of *L.*

trifolia may be relatively non-toxic when administered orally for a short period. This is supported by a higher LD_{50} which was determined to be >2000mg/kg body weight. There is need to monitor the liver, kidneys, haematological and biochemical parameters especially when higher doses are administered for a long time since deleterious dose-dependent effects can result. Various phytochemical constituents were present in the *L. trifolia* leaf extracts hence this could form the basis for the discovery and development of novel drugs.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Medicinal plants are widely used for the management of different human and animal diseases. People living in various parts of Africa have for many years used plant extracts to treat themselves together with their livestock in case of sickness or injuries in their environment (Kokwaro, 2009). According to the World Health Organization (WHO), medicinal plants are classified as important sources of drugs and approximately eighty percent of the people living in the developing world rely on traditional plant medicines for their primary health care needs (Salatino *et al*., 2007). Many drugs that are in use in modern medicine have been derived from various parts of medicinal plants such as seeds, leaves, flowers, barks and roots. Examples of drugs derived from plants include digoxin, morphine and quinine (Arezoo *et al.,* 2016).

Antibiotic chemotherapy is one of the most important medical achievements of the twentieth century. However, constant increase in microbial resistance to antibiotics has been reported. In addition, there are many studies which have revealed numerous adverse effects associated with the synthetic antimicrobials on the immune system (Pankaj *et al.,* 2009; Alothyqi *et al.,* 2016). Consequently, resistance to antimicrobial agents is now reported as a public health problem in the world (Okello *et al.,* 2010).

Therefore, there is need for researchers to come up with new strategies that aim at developing new and safe antimicrobial agents. Medicinal plants that are used in ethnomedicine provide unique and promising alternative for attaining this goal. The plant derived drugs are accessible and have limited side effects.

Many communities especially from the rural parts of Kenya still depend on herbal medicines. Moreover, there are many Kenyans who have confidence in the potency of

medicines that are herbal in nature even though they can access contemporary medicine. Unfortunately, there is limited information concerning literature on the utilization of herbal medicines in Kenya (Kigen *et al.,* 2013). According to Kokwaro (2009), out of 10,000 flora species in Kenya, about 1200 have been identified as medicinal. More than two-thirds of Kenyans turn to medicinal plants for healthcare. This happens due to lack of access to modern medicine. Several households get their income from sale of medicinal trees and shrubs (McMullin *et al.*,2012). *Lantana trifolia* is in Verbenaceae family. Its common name is a three-leaf shrub. In Kenya, it is used to manage several medical conditions such as malaria, chronic cough, tonsillitis and abdominal pain management among different tribes. (Hamill *et al*.,2003; Kisangau *et al.*,2007; Odongo *et al.*,2011; Kipkore *et al.*,2014; Mukungu *et al.*,2016). The current study investigated the antimicrobial activity, safety profile and the major phytochemical constituents of *L. trifolia* which is used in Kenyan ethnomedicine.

1.2 Problem statement

Antimicrobial resistance (AMR) is gradually becoming a significant threat to the public health globally. Thus, it needs appropriate measures to be taken by all government sectors and society. There is emergence of new resistance mechanisms which are spreading worldwide. As a result of antimicrobial resistance, treatment of some of the most common infectious diseases is threatened and this may lead to prolonged duration of illness, development of disability and finally death (WHO, 2017). Various disease-causing pathogens such as fungi and bacteria have shown the potential of developing resistance towards a number of antimicrobial agents that are available commercially. There has been an increase in search for novel antimicrobial agents (Ramesh and Okigbo,2008). Most people who use medicinal plants believe that they are safer than the modern conventional

medicines. Natural products have increasingly become popular because they are easily accessible at low costs. However, when herbal medicines are used inappropriately, they can cause harmful adverse reactions. The reactions could be acutely or chronically manifested. There is limited scientific data on how safe herbal medicines are. Therefore, people use these medicines ignorantly because there is lack of proper information.

1.3 Justification

Since the beginning of civilization, plants with medicinal value have played an important role all over the world in the preventing and healing a range of diseases (Muniappan and Savarimuthu, 2011). Medicinal plants been used to make homemade concoctions that have not been scientifically validated to have antimicrobial activity. In addition, there is a continuous rise in the number of drug resistant microbes. As a result of resistance, successful treatment of infectious diseases is becoming a challenge (Kalyani *et al.,* 2011). Antibiotic resistance has also been observed in Kenya and has contributed to noteworthy morbidity and mortality. This is an impediment to economic development. The safety of medicinal plants has frequently been questioned because of reported illness and death of the test animals (Park *et al.,* 2010). Thus, determining the toxicity of plants is essential. Moreover, it is important for consumers to be aware of safe use of herbal medicines as this will promote collaboration, more training and communication among providers of natural products medicine. *Lantana trifolia* is a plant used in Kenyan ethnomedicine. Currently, there is scanty information in the public domain on the phytochemicals it contains and also its toxicity. Therefore, evaluation of chemical activity and toxicity of medicinal plants extracts is very important in determination of safe and efficient dosages.

1.4 Objectives of the study

1.4.1 General objective

The general objective of this study was to investigate the phytochemical constituents, antimicrobial activity and toxicity profile of *Lantana trifolia* leaf extracts.

1.4.2 Specific objectives

1. To screen for the phytochemical constituents of *L. trifolia* extracts.
2. To determine the antimicrobial activity of *L. trifolia* leaf extracts against selected bacteria strains and fungi
3. To determine the acute and sub-acute toxic effects of *L. trifolia* aqueous leaf extract

CHAPTER TWO

LITERATURE REVIEW

2.1 Medicinal plants

The use of medicinal plants for treating infections in both humans and animals is entrenched in many cultures, for example, among the Maasai in Kenya (Nankaya *et al.*,2019). Plants are known to be a very important source of drugs used in therapeutics. Consequently, many communities depend on the medicinal plants for survival. Moreover, medicinal plants have been used for their medicinal properties to manage diseases, in rituals and also for their aromatic properties as well. Several old civilizations that resorted to medicinal plants are the Muslim, Chinese, Roman and Greek (Montazeri and Sepehri, 2019). Medicinal plants are used in herbal medicine and are known to have chemical constituents that are used in development of drugs. Some of these plants have been used to treat various conditions caused by different microbes. Ease of access, affordable prices and lack of evidence of resistance to the whole plant are some of the advantages of medicinal plants (Ouhaddou *et al.*, 2014).

2.2 Lantana

Lantana is a genus consisting of about one hundred and fifty species occurring in tropical and sub-tropical countries. It is a member of Verbenaceae family. This family comprises of 100 genera and 2600 species that grow as shrubs, herbs or trees (Innocent *et al.*, 2008). Many genera that are in this family seem to have different biological and pharmacological properties. Certain species of *Lantana*, for example *Lantana camara,* are also known to be toxic. The intoxication has been seen in cattle, sheep, buffalo and guinea pigs. Photosensitization, obstructive jaundice, and rise in serum glutamic-oxaloacetic transaminase activity are some of the features observed after poisoning with Lantana. The

intoxication mostly happens when the animals are feeding on pastures where there is some toxic variety of *L.camara (*Kumar *et al.*, 2016).The species that has been studied widely is *L. camara.* Both *L. trifolia* and *L. camara* are in the same family and genus.

2.3 Experimental plant- *Lantana trifolia*

*Lantana trifolia (*Fig 2.1) is one of the plants whose genus is *Lantana* and its family is known as Verbenaceae. It is a scrambling, evergreen, herbaceous shrub that grows uprightly. Its common name is three-leaved lantana due to the arrangement of the leaves in whorls of three on the stem and can grow up to 3 metres tall. The leaves of this plant are usually rough and the petiole has hairs. It produces dark purple fruits and its flowers are lavender- coloured. The plant grows in sub-tropical and tropical regions in places such as disturbed forests, abandoned cultivation and even roadsides (Owembabazi *et al.*, 2017).

2.4 Uses of *Lantana trifolia*

The leaf extracts are used in management of asthma, common cold, madness, cerebral malaria, epilepsy and sickle cell anaemia in Central Uganda (Nalubega *et al.*, 2013). In Southern Uganda, among the Baganda, *L. trifolia* is known as 'Kayuki-yuki.'The stem is used as a tooth brush for maintenance of oral hygiene. The leaves are crushed, mixed with half a litre of water and one teaspoonful salt, and then the patient is given to gargle or swallow to manage tonsillitis. The leaves extracts are also rubbed into the eyes to manage cataracts (Hamill *et al.*, 2003: Odongo *et al.*, 2011). In Tanzania, among the Kihaya,it is known as 'Mushikira' and the leaves are normally boiled and then the decoction is drunk to manage chronic cough(Kisangau *et al.*,2007).It is locally known as 'shimenenwa' among the Luhya of Kakamega county, Kenya. *Lantana trifolia* leaves are boiled in water and taken orally to manage malaria (Mukungu *et al.*, 2016). Among the Marakwet community, it is

known as '*Bekaptarit'* and the leaves are boiled and taken orally to manage abdominal (colic) pain. Its leaves, fruits and twigs are boiled and taken to boost production of milk in breastfeeding mothers (Kipkore *et al.,* 2014).

Figure 2.1: A photograph of the aerial part of *Lantana trifolia*

2.5 Antimicrobial resistance

Development of antibiotics is one of the major advances in modern science and this has saved millions of lives. Despite this progress in antibiotics development, there is an increase in antimicrobial resistance (AMR) which is a threat to this progress and presents noteworthy risks to human health. Notably, increase in the usage of antimicrobial agents in humans and animals that are consumed as food can lead to development of resistant bacteria. The drivers of AMR include genetic factors intrinsic to bacteria, abuse of antimicrobial drugs and their use in human, animal and environmental sectors. In addition, use of antibiotics outside of the health care sector has the potential of causing antimicrobial resistance (Marston *et al.*, 2016). Furthermore, most classes of antimicrobial agents are used in both humans and

animals to treat various infections. Therefore, it is imperative to use all those drugs rationally to prevent development of resistance. Mass treatment of animals with antimicrobial agents which are very important to humans such as the third - and fourth -generation cephalosporins, fluoroquinolones and macrolides is a major concern in animal health and agriculture sectors (Aidara-Kane 2012).

2.5.1 Mechanisms of antimicrobial resistance and the way forward.

The mechanisms of antimicrobial resistance are classified into various groups such as modification of the binding sites of the drugs, inactivation/alteration of the drug, changes in the permeability of the cells leading to reduction in intracellular accumulation of drug, and formation of the biofilms. Some bacteria are known to produce enzymes that modify irreversibly the antibiotics and also inactivate them. Some of the enzymes produced include, aminoglycoside-modifying enzymes, *β*-lactamases, and chloramphenicol acetyltransferases. Beta-lactamases are enzymes that hydrolyze the beta -lactam (β-lactam) ring present in drugs such as penicillins hence altering their activity (Jacoby and Munoz-Price 2005). Target sites of antimicrobial agents can also be modified by some resistant bacteria thus preventing recognition of the drug. The sensitivity of bacteria to a specific antimicrobial agent is determined by the balance of antibiotic absorption and elimination (Tang *et al.*,2014).

Moreover, the other way through which bacteria develop antibiotic resistance is by reducing the amount of antimicrobial agent that can go through the bacteria cell membrane. This is made possible by the efflux pumps which interfere with the amount of drug getting into the cells. In addition, existence of diminished protein channels on the outer membranes also leads to reduction in the amount of drug that enters the cells.

The formation of biofilms which are defined as complex microbial communities that live as a thin layer on biotic or abiotic surfaces can also cause resistance. Biofilms can provide a

biochemical and mechanical shield that provide optimum conditions needed to reduce the activity of drugs, for example, low oxygen, low pH, high carbon dioxide and low water availability. In a healthcare setting, the commonly found pathogens in biofilms are *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa,* and *Acinetobacter baumannii* (Høiby *et al.,* 2010).

Therefore, it is worth to note that antimicrobial resistance in bacteria and other microorganisms is a big challenge. High morbidity and mortality can also be linked to development of antimicrobial resistance in bacteria. Conventional antibiotics may not treat some of the infectious diseases as a result of both Gram-positive and Gram- negative bacteria because of the multidrug resistance patterns in such bacteria. Currently, there is a shortage of therapies that are effective and effectiveness only left to a few new antibiotics. This raises the need to develop new treatment options and alternative therapies (Frieri *et al.,* 2017). However, global antibiotic crisis can be prevented from growing bigger through regulation of antibiotic use. Misperceptions and incomplete knowledge concerning the use of antibiotics must be addressed well so that the issue of AMR is handled successfully (McCullough *et al.*,2016). Healthcare workers need to educate the community on the potential risks that may be associated with antibiotics since people are most likely to trust them because of their medical knowledge. Patients who are diagnosed with an infection that requires treatment with an antibiotic must be given appropriate instructions concerning the antibiotic such as its use, route of administration, proper dose, frequency of dose, duration of treatment and the deleterious effects related to misuse of antibiotics.

According to Bennadi, 2014, improper self -medication, poor patient's compliance to the antibiotic treatment course and medication non adherence in patients are the main reasons that have led to rise in antibiotic resistance crisis and drug-induced diseases. Therefore, the

government should develop and implement new policies to handle antibiotic resistance. The cry for the development of new antimicrobial agents should not be ignored (Ventola, 2015).

2.6 Previous studies on *Lantana trifolia*

2.6.1 Antimicrobial activity

There are not many studies that have been carried out to examine the antimicrobial effects of *L. trifolia* extracts as per the available literature. In a study done in Rwanda to evaluate Rwandan medicinal extracts, the researchers reported that *Lantana trifolia* extracts were active against *Mycobacterium fortuitum* and gram-positive bacteria (Cos *et al.,* 2002). Antibacterial activity of the aqueous extract of *L. trifolia* has also been reported by Kisangau *et al*., 2007 in Tanzania. Apolymethoxylated flavone, a flavonoid, named umuhengerin isolated from *L. trifolia* was found to have antimicrobial activity (Rwangabo *et al.,* 1988).

2.6.2 Antimycobacterial activity

In a study conducted in Brazil, the essential oils isolated from the leaves of *L. trifolia* were found to have activity against *Mycobacterium tuberculosis*. The isolated oils were rich in sesquiterpenes, the major ones being germacrene D, (E)-caryophyllene, bicyclogermacrene and alpha-humulene (Julião *et al.,* 2009).

2.6.3Anti-inflammatory and antinociceptive activity

In Venezuela, the aerial parts of *L. trifolia* are used in management of rheumatism. In a study conducted to assess for anti-inflammatory, anti-nociceptive and antipyretic properties of *L. trifolia,* the methanolic extract of the aerial parts was reported to inhibit the carrageenan-induced edema within the paws of the rats over a dose range of 10-300mg/kg. Furthermore, the extract produced a small but remarkable increase in the response latency of experimental

animals subjected to the hot plate. This is a thermal test that can only detect analgesia by high-efficacy agents (Uzcátegui *et al.,* 2004). Other studies have revealed that the plant has additional actions such as anti-inflammatory and anti-nociceptive activity (Silva *et al.,* 2005).

2.7 Therapeutic activities of medicinal plants

Many countries in the world enjoy a diverse collection of plants with medicinal activities and several studies have ascertained the efficacy of some of these plants. These plants have biologically active substances that are beneficial to humans and animals. Some mainstream medicines are derived from plants, for example quinine (Nyakudya *et al.,* 2020). There are plants with antibacterial, antifungal, antiviral and antiparasitic activities. Extracts derived from leaves, fruits, stems, roots and flowers may possess different biological activities. *Withania somnifera* extracts have been reported to have anti-inflammatory and antioxidant activities (Mazzio *et al.,* 2016). Some of the therapeutic activities of plants are outlined in Table 2.1.

Table 2.1: Summary of selected plants with therapeutic activity

Botanical name of the plant		Therapeutic activity
Moringa oleifera *Argemone Mexicana* *Vernonia amygdalina* *Adansonia digitata L.* *Cola nitida*	*Zingiber officinale* *Thymus vulgaris* *Echinacea purpurea* *Annona senegalensis*	Antibacterial
Mondia whitei *Macaranga bartei*	*Ageratum conyzoides*	Antiviral
Myristica fragrans *Morinda lucida* *Calotropis procera* *Amaranthus spinosus* *Diospyros monbuttensis*	*Mangifera indica* *Momordica charantia* *Cajanus cajan* *Heeria insignis*	Antiparasitic
Azadirachta indica *Morinda lucida* *Bixa Orellana* *Justicia flava* *Cassytha filiformis* *Allophylus africanus*	*Senna alata* *Clerodendrum capitatum,* *Enantia chlorantha* *Landolphia owariensis,* *Allamanda cathartica,*	Antibacterial Antiparasitic Antifungal
Allium sativum L. *Tithonia diversifolia* *Carica papaya* *Microglossa pyrifolia* *Acacia robusta* *Zanthoxylum gilletii* *Toddalia asiatica L*	*Microglossa pyrifolia* *Commiphora africana* *Clerodendrum myricoides* *Plectranthus barbatus* *Rhynchosia elegans* *Zanthoxylum chalybeum*	Antimalarial

Table 2. 2: Common infections in man, causative agents and therapeutics agents

Infection	Causative agent	
Bacterial skin infections; Cellulitis Folliculitis Impetigo Boils	*Pseudomonas aeruginosa* *Staphylococcus aureus* *Streptococcus pyogenes*	Cephalexin Clindamycin Dicloxacillin Flucloxacillin
Upper Respiratory Tract (URT) Infections	*Haemophilus influenzae* *Staphylococcus aureus* *Moraxella catarrhalis* *Streptococcus pyogenes* *Streptococcus pneumoniae (pneumococcus)*	amoxicillin/clavulanate Azithromycin cefuroxime doxycycline levofloxacin moxifloxacin
Bacterial meningitis	*Haemophilus influenzae* *Listeria monocytogenes* *Neisseria meningitidis* *Streptococcus pneumoniae*	Ampicillin Cefepime Cefotaxime Ceftriaxone Meropenem penicillin G vancomycin
Otitis media (OM) subtypes: Acute OM OM with effusion Adhesive OM	*Streptococcus pneumoniae* *Haemophilus influenzae* *Moraxella catarrhalis*	amoxicillin-clavulanate cefuroxime ceftriaxone Levofloxacin NSAIDS
Common Cold	Parainfluenza Virus Respiratory Syncytial Virus (RSV) Rhinovirus	Antihistamines Corticosteroids Cough suppressants NSAIDs Oral decongestants Topical anticholinergics
Infections of the eye	*Chlamydia trachomatis* *Neisseria gonorrhoea* *Staphyloccocus aureus*	Ciprofloxacin Levofloxacin Ofloxacin Gentamicin Cefoxitin Doxycycline
Foodborne bacterial infections; Food Poisoning	*Vibrio cholerae* *Escherichia coli* *Clostridium perfringens* *Salmonella species* *Staphylococcus aureus* *Shigella species* *Campylobacter jejuni*	Metronidazole Hydration (Dextrose 5% and normal saline) Secnidazole Doxycycline Amoxicillin

2.8 Toxicity testing of medicinal plants

Traditional medicine is widely used by many communities in various parts of Africa. This is because it is affordable and many people are low-income earners. Plants play a significant role in herbal medicine and many people who use plant-based medicines assume that they are safe based on their long-standing use. However, the fact remains that certain plants used in herbal medicine are toxic (Mounanga *et al.,* 2015). It has been reported that medicinal plants used to manage diabetes in Nigeria are nephrotoxic while others are hepatotoxic. This was proved by how some liver function enzymes were directly affected (Ezuruike and Prieto 2014). Moreover, adverse reactions have been observed after humans have consumed *Ephedra* and *Aristolochia* (Jordan *et al.,* 2010). Toxicity studies can be performed in vivo using laboratory animals and in vitro using cell lines. Some of the animals that can be used include rats, guinea pigs and monkeys. Systemic or organ toxicity can be studied using in vivo animal models.

Since folk medicine is widely used globally, the need to evaluate the intrinsic activity of extracts of plants arises. This is important for safe treatment and establishing the effects of overdose from the plants' extracts (Nguta *et al.,* 2012). Thus, knowledge on the composition, toxicity and specific efficacy of the plant- based medicines is important. This will help the practitioners manage the patients well.

2.8.1 Acute oral toxicity

Acute, sub-acute and chronic toxicity are some of the various toxicity studies that are normally carried out by pharmaceutical manufacturers when searching for a new drug. Acute oral toxicity is defined as the adverse effects occurring following oral administration of a single dose of a substance, or multiple doses given within 24 hours. The toxic effects that are observed in acute toxicity study should be reported within 14 days after the test substance

has been administered (OECD guidelines, 2001). These studies are important since they help the researchers to establish the dose that produces major adverse effects and determine the minimum dose that causes lethality (Robinson *et al.,* 2007). The dose is also known as LD_{50}. A single dose of a substance that is administered orally and may be expected to kill fifty per cent of animals is what is normally referred to as LD_{50}(OECD, 2001). Acute toxicity is an initial screening step that is performed to assess and evaluate the toxic effects due to test compounds (Akhila *et al.,* 2007).

2.8.2 Sub-acute toxicity

This test is done after the initial information on toxicity has been gotten and the lethal dose determined by acute toxicity testing. In this study, several groups of experimental animals are orally given the test substance daily. Each group of laboratory animals is given a daily one dose level for 28days (OECD, 2008). Major behavioural changes as well as physiological, anatomical and biochemical manifestations due to damaged tissues are determined at the end of the study. It has been reported that those medicinal plants that are toxic usually affect organs such as the liver and the kidneys. The liver acts as a detoxifying organ while the kidney is a major route through which chemical substances are excreted. Therefore, sub-acute toxicity studies are important since the data obtained can be used to show how safe the plant studied is and also the effects of a specific medicinal plant after prolonged exposure to it (Sanyal *et al.,* 2016).

2.9 Phytochemicals in medicinal plants

In general, valuable effects of a medicinal plant are determined by the secondary products found in that plant. Different plants have various compounds whose concentrations vary from one plant to the other. The compounds are normally referred to as secondary

metabolites of plants which include the tannins, steroids, alkaloids, saponins and phenolic compounds. These are synthesized and stored in different parts of the plant (Joseph and Raj 2010).

2.9.1 Classes of phytochemicals

Flavonoids

This is a group of substances that occur naturally with phenolic structures that vary a lot. Flavonoids are present in vegetables, fruits, grains, flowers, barks, stems, roots, and wine. Many herbal medicines have flavonoids. These bioactive compounds interfere with nucleic acids or proteins and have antimicrobial activity (Panche *et al.*, 2016). Moreover, they can be classified as polyphenolic compounds which are present in glycosidic form and they exert many biological activities (Kaume *et al.*, 2012).

Terpenoids

Terpenoids are organic compounds that are formed through condensation of isopentenyl pyrophosphate and dimethylallyl pyrophosphate (Boncan *et al.*,2020). The classification of terpenoids is based on the number of carbons they have and are divided into monoterpenes, sesquiterpenes, diterpenes, triterpenes, tetraterpenes and polyterpenes. This class of compounds easily interacts with membrane proteins and biomembranes. A large number of the terpenoids has been reported to be lipophilic. Essential oils with monoterpenes have antibacterial and antifungal activity. Monoterpenes with phenolic hydroxyl groups or with aldehyde functional groups are active against fungi and bacteria (Wink, 2015).

The mechanism through which group of compounds act is not known but it is thought to involve the disruption of membrane lipophilic compounds (Hamed, 2011).

Tannins

Tannins are a kind of polyphenols in plants and are classified into two groups namely condensed and hydrolysable tannins. Hydrolysable tannins as the name suggests are hydrolyzed by weak acids while the condensed tannins are not readily hydrolyzed. Condensed tannins (often called proanthocyanidins) are polymers of flavanol monomers (Arapitsas 2012). Tannins are astringent substances and have the capacity to precipitate gelatin from solution. They are found in most parts of the plants including the bark, leaves, wood, fruits and roots (Cowan 1999). Some of the plants which are rich in tannins have been found to have antimicrobial activities against various microorganisms. For example, Venkataswamy *et al.,* 2010 studied the antimicrobial activity of leaf extracts of *Lantana indica* and reported the presence of carbohydrates, proteins, tannins and flavonoids. Plant extracts that contain tannins are used as astringents against diarrhea, as diuretics, and against stomach and duodenal tumours (De Bruyne et *al.,* 1999). Apart from tannins being effective against pathogenic microbes, they have been found to have considerable value as a cytotoxic and an antitumor agent (Josh *et al.,* 2013).

Alkaloids

These are heterocyclic nitrogen compounds. They can be produced as secondary metabolites from plants and can also be produced by other organisms including bacteria, fungi and animals (Kittakoop *et al.,* 2014). Alkaloid salts are soluble in water but not in non-polar solvents. Examples of alkaloids include berberine, piperine, palmatine and tetrahydropalmatine. Other examples encompassed under alkaloids are caffeine and nicotine which are neuroactive molecules, and emetine which is used to induce vomiting, therefore fighting oral intoxication and chemotherapeutic drugs such as vinblastine and vincristine (Matsuura and Fett-Neto, 2017). Alkaloids have been found to have many pharmacological properties such as antibacterial, analgesic, antihypertensive, antimalarial, anticancer,

anticholinergic and antiemetic activities (Cushnie and Lamb 2014). Studies conducted in Nigeria on alkaloids extracted from various medicinal plants showed that the extracts had both antibacterial and antifungal activities (Garba and Okeniyi, 2012). Additionally, diterpenoid alkaloids found in Ranunculaceae family have been reported to have antimicrobial properties (Perumal and Gopalakrishnakone, 2010). Harmane, piperine and berberine are highly aromatic planar quaternary alkaloids and are believed to act by intercalating the DNA thus resulting in impaired cell division (Cowan, 1999).

Saponins

Saponins are amphipathic glycosides meaning they have both polar and non-polar parts and are synthesized by different plants. They are high molecular weight compounds and are characterized by a structure that consists of a carbohydrate and either a steroidal or triterpenoid aglycone moieties. Saponins are able to produce foam and also cause haemolysis of blood cells. Moreover, they are said to possess soap-like properties (Juang and Liang, 2020).

Saponins are also found in marine organisms such as sponges, starfish and cucumbers. Since saponins have numerous biological activities such as anti-inflammatory, antimicrobial, hepatoprotective and antitumor activities, they have attracted a lot of attention in the scientific world (Sharma and Paliwal 2013; Moghimipour *et al.,* 2015).

Triterpenoid saponins are mostly derived from dicotyledons while the steroids saponins are generally derived from monocotyledons. Saponins are found in medicinal plants and marine animals as complex mixtures. However, the composition of saponins varies depending on the type of tissue, the age, genetic background, environmental factors and the physiological state of the plant (Szakiel *et al*., 2011).

It is difficult to isolate the complexed saponins from nature because of the scarce amount and structure heterogeneity. Therefore, chemical synthesis is thought to be a powerful tool

that can expand the diversity of saponin's structure thus leading to discovery of promising compounds (Juang and Liang, 2020).

Glycosides

Glycosides are compounds that contain a non-sugar moiety (aglycone also called genin) and a sugar part(glycone). The sugar part is tied up with another chemical at the anomeric carbon via a glycosidic bond. Cardiac glycosides occur naturally as steroidal-like glycosides compounds. The aglycone steroid moiety is the basis on which glycosides are classified. Cardenolides are those with a steroid nucleus with a five membered lactone bound to a sugar moiety while bufadenolides are those with a six membered lactone ring bound to sugar moieties are called (Mijatovic *et al.*,2008).

In folk medicine, cardiac glycosides have been used as abortifacients, heart tonics, arrow poisons, emetics and diuretics as well as in other applications. They are currently used to manage cardiovascular disorders such as cardiac arrythmias, atrial fibrillation and congestive heart failure. Moreover, there is increasing evidence that has shown the potential cytotoxic effects of cardiac glycosides against various types of cancer (El-Seedi *et al.*, 2019).

Reducing sugars

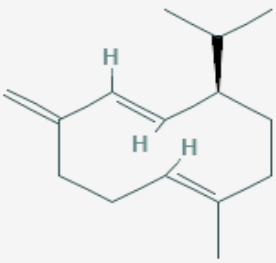
These are carbohydrates that consist of an open-chain with an aldehyde group or a free hemiacetal group. Keto groups and aldehydes reduce Fehling's solution (Campbell and Farrel, 2012). When reducing sugars are heated, complex group of reactions are affected which is termed caramelization, and leads to browning that defines the colour of the final product (Arabhosseini *et al.*, 2011).

It is vital to understand whether a plant contains carbohydrates since it helps to understand the biological activities. The soluble sugar pool in many plants is made up of mainly glucose, sucrose, fructose and other substances like sugar phosphates and uronic acids (Campbell and Farrel, 2012)

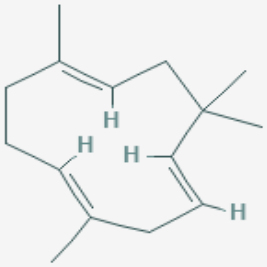
2.9.2 Important compounds in genus *Lantana.*

Lantana, a genus of about 150 species of perennial flowering plants, is traditionally used as antibacterial, stimulant, biologic control, antirheumatic and as ornamental plant (Barreto *et al.*, 2010). The *Lantana* species is rich in essential oils. Essential oils (EOSs) are mixtures of fragrant substances that are heterogeneous in nature and are found in plants in different concentrations. Each essential oil has major compounds whose levels may vary when compared with other compounds already present in small quantities.

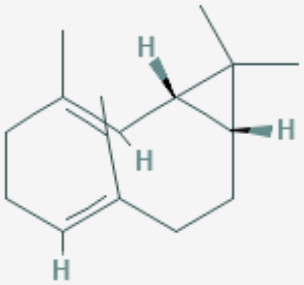
According to a study carried out by Sena Filho *et al.*, 2010, *Lantana* species was reported to have β-caryophyllene in high concentration. The other compounds in low concentration included, cubebene, elixene, and phellandren. The β-caryophyllene is used as a chemical marker for species belonging to the *Lantana* genus. The chemical composition of *Lantana camara* essential oils play an important role in its biological activity; the β-caryophyllene and (*E*)-nerolidol chemotypes showed antimicrobial and cytotoxic activities (Satyal *et al.*,2016). The major essential oils isolated from *L. trifolia* as shown in Figure 2.2 include germacrene D, (E)-caryophyllene, bicyclogermacrene and alpha-humulene (Julião *et al.*, 2009). The chemical structures of some of the antimicrobial compounds are shown in Figure 2.3.



Germacrene D



alpha -humulene



Bicyclogermacrene

Figure 2.2: Structures of some of the essential oils isolated from *Lantana trifolia.*

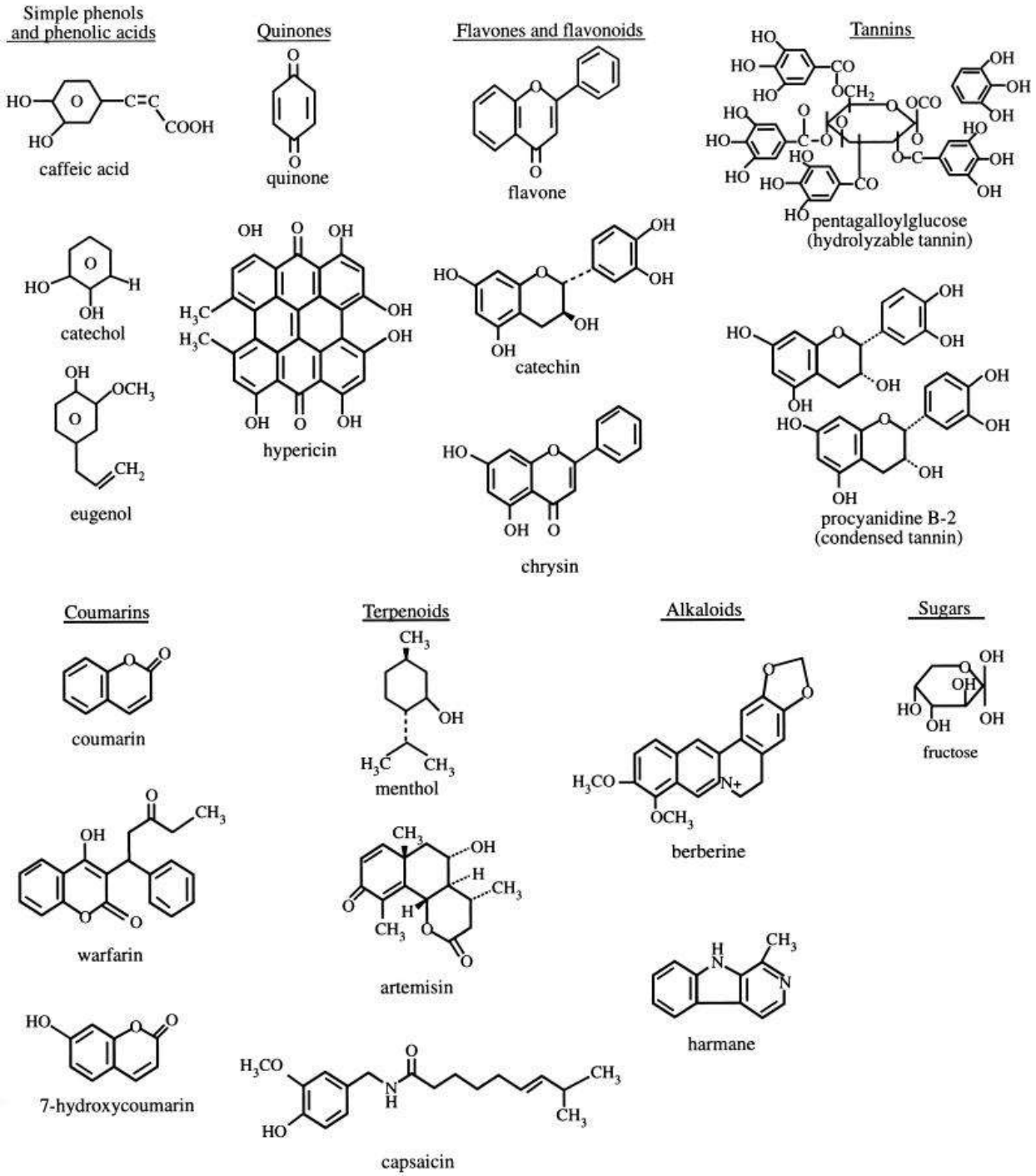


Figure 2.3: Chemical structures of antimicrobial compounds.

Source:(Silver and Fernandes 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample collection and identification

Fresh leaves of *L. trifolia* were collected from Sitatunga, Cherangany sub-county, Trans-nzoia County. Collection was based on the ethnopharmacological use as a plant whose leaf extracts are used for managing eye infections (conjunctivitis), ear infections (otitis), cough and common cold (Rebecca *et al.*,2013). Identification and authentication of the plant was done by a taxonomist at the Department of Land Resource Management and Agricultural Technology (LARMAT) Herbarium, University of Nairobi.

3.2 Preparation of the plant material

The leaves of *L. trifolia* were slightly cleaned with tap water and rinsed with distilled water to remove dust. The leaves were taken to a room that was free from rodents, insects and dust for drying. Thereafter, they were carefully spread on a clean flat surface and let to air dry under room temperature (24±2 ^{0}C) for two weeks. After that, an electric mill was used to grind the dried leaves forming a uniform powder. The powder was stored in sachets in a dry aerated cupboard away from direct sunlight for later aqueous and organic extraction.

3.3 Extraction of plant material

3.3.1 Aqueous extract

The ground material was extracted by cold maceration method. Briefly, 100g of *L. trifolia* leaf powder was correctly weighed using an analytical balance (Mettler PM 4600, Germany) and soaked in 1litre distilled water for 72 hours at room temperature with occasional agitation. The mixture was then filtered through a Whatman filter paper. The filtrate was

transferred into freeze-drying flasks and lyophilized (freeze dried) for 48 hours resulting to a dry powder. The powdered extract was weighed and put into air-tight containers and stored sealed in the refrigerator at 4^0C awaiting analysis. The percentage yield of the extract was assessed on basis of percentage weight by weight (%w/w).

The formula used to calculate the percentage yield is as follows;

Percent (%) of crude extract yield= $\frac{(A2-A1)}{A0} X\ 100$

Where;

A2= Mass of the extract + Mass of the container

A1= Mass of the blank container

A0= Mass of the initial plant sample

3.3.2 Organic extract (Dichloromethane: Methanol)

In brief, 100g of dried leaf powder of *L. trifolia* was weighed on an analytical balance (Mettler PM 4600, Germany) and poured into a 1litre conical flask. Dichloromethane-methanol (ratio 1:1, V/V) mixture was added into the flask until all the powder was submerged. This was followed by maceration for 72 hours with careful intermittent shaking to enhance the efficiency of the extraction process. First, the resulting mixture was filtered through cotton wool then further filtered using Whatman® filter paper (number 1) into a round-bottomed flask. The resulting filtrate was concentrated using vacuum rotary evaporator which was used to remove the excess solvent. For complete drying of the concentrated filtrate, it was placed in a hot sand bath set at 50^0C for four to seven days. The percentage yield of the extract was assessed on basis of percentage weight by weight (%w/w).

The formula used to calculate the percentage yield is as follows;

Percent (%) of crude extract yield= $\frac{(A2-A1)}{A0} X 100$

Where;

A2= Mass of the extract + Mass of the container

A1= Mass of the blank container

A0= Mass of the initial plant sample

This product was then stored under refrigeration in well closed, light resistant bottles awaiting analysis.

3.4 Laboratory animals

A total of 49 rats and mice aged between eight and twelve weeks were required for this study. Nine (9) female Wistar rats (body weight 180-220g) were used in the acute toxicity studies while forty (40) albino mice (20 females and 20 males) weighing 20-30 grams were used to investigate the sub-acute oral toxicity of the plant extract. The experimental animals were obtained from the animal breeding unit at the Department of Public Health Pharmacology and Toxicology (PHPT), University of Nairobi. They were housed in polypropylene cages in the research laboratory at standard conditions; temperature of 25±3°C; 56- 60% relative humidity and a photoperiod of 12 hours light and 12 hours darkness. The animals were fed on standard rodent pellets from a commercial supplier (Unga Group Plc, Kenya) and clean water *ad-libitum.* They were allowed to acclimatize for ten days prior to experimentation The initial weights of the animals were taken and a permanent marker was used to mark their tails.

3.5 Ethical considerations

Ethical clearance was sought and granted from the Faculty of Veterinary Medicine Biosafety, Animal use and Ethics committee (REF: FVM BAUEC/2018/176).

The animals that survived after the 28-day repeated dose administration of aqueous *L. trifolia* leaf extract were euthanized under diethyl-ether then bled aseptically from a cardiac puncture. The mice carcasses were incinerated (OECD 423, 2001).

3.6 Phytochemical screening of leaf extracts of *Lantana trifolia*

The phytochemical constituents of *L. trifolia* were identified using the standard qualitative methods.

3.6.1 Test for alkaloids (Dragendorrf test)

Testing for alkaloids was done using five hundred milligrams of both the aqueous and organic extracts which were dissolved in sufficient amount of distilled water. Five millilitres of concentrated hydrochloric acid were added to each of the solutions. Then resultant mixture was filtered. Two millilitres of the collected filtrate were put in a test tube followed by addition of one milllitre of the dragendorrf's reagent along the inner wall of the test vessels. Formation of reddish-brown precipitate indicated the presence of alkaloids.

3.6.2 Test for saponins

Testing for saponins was done using five hundred milligrams of the extract in a test tube. To the extract, five millilitres of distilled water was added. The mixture was agitated vigorously for stable persistent froth. Formation of an emulsion after addition of three drops of olive oil confirmed the presence of saponins (Harborne, 1998).

3.6.3 Test for phenolics

To test for phenolics, two hundred milligrams of the sample extract were dissolved in two millilitres of distilled water and filtered. To two millilitres of the filtrate, a few drops of 10 % ferric chloride were added. Formation of blue, green or violet colour indicated the presence of phenolic compounds (Trease and Evans, 2002).

3.6.4 Test for tannins

Five hundred (500) milligrams of the sample extract were boiled in ten millilitres of water in a clean test tube followed by filtration. A few drops of 5% ferric chloride were added carefully and observed for brownish green or blue-black colouration which meant that tannins were present/positive (Trease and Evans, 2002).

3.6.5 Test for terpenoids

To test for terpenoids, two (2) millilitres of chloroform were added to 0.5 g of the extract. This was followed by careful addition of three (3) millilitres of concentrated sulphuric acid to the mixture to form a layer. Presence of terpenoids was indicated by formation of a reddish-brown colouration of the interface. (Visweswari *et al*., 2018).

3.6.6 Test for cardiac glycosides

Five hundred (500) mg of both the aqueous and organic extracts was mixed with 5ml distilled water. Then two millilitres of glacial acetic acid were added followed by one drop of ferric chloride ($FeCl_3$) solution. Subsequently, one (1) ml of concentrated sulphuric acid(H_2SO_4) was added along the inner walls. Formation of a brown ring at the interface was an indicator a positive test result (Harbone, 1998).

3.6.7 Test for Flavonoids

About 5 mL of dilute ammonia was added to 2 mL aqueous filtrate of each plant extract. This was followed by addition of 1 mL concentrated sulphuric acid (H_2SO_4). Presence of flavonoids was confirmed by appearance of a yellow coloration that disappears on standing. Another portion of the extract was taken, and into it, a few drops of 10% aluminium were added. A yellow colouration confirmed the presence of flavonoids (Harbone, 1998; Sankhalkar and Vernekar 2016: Visweswari *et al.*, 2018).

3.6.8 Test for reducing sugars

Two hundred milligrams of the extract were shaken with distilled water. One millilitre of the crude extract solution was mixed with a two-millilitre portion of a mixture of Fehling A and Fehling B reagents and boiled gently. A brick red precipitate appearing at the bottom of the test tube indicated the presence of reducing sugars (Harborne, 1998; Odeja *et al.*, 2015).

3.6.9 Test for anthraquinones

Five hundred milligrams of the extract were taken and boiled with 10ml of sulphuric acid and then filtered while hot. The filtrate was shaken in 5 millilitres of chloroform and the chloroform layer was pipetted into another test tube and added one millilitre of dilute ammonia. Presence of anthraquinones was indicated by development of a yellow colour (Trease and Evans, 2002).

3.7 Antimicrobial activity of *Lantana trifolia* extracts

The preliminary studies of the aqueous and organic leaf extracts of *L. trifolia* were done using Agar well diffusion method. The reference strains used for screening were *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Bacillus cereus* (ATCC11778) and *Candida albicans* (ATCC10231). The microorganisms were selected

based on their clinical significance and were obtained from stock cultures from the Bacteriology Laboratory, Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Nairobi, Kenya.

Antimicrobial susceptibility

With the help of an inoculation loop, bacterial stock cultures were sub inoculated on blood agar (Oxoid ®) and incubated for twenty-four hours at 37^0C.The sub cultured bacteria were used later as stock cultures and were maintained at $+4^0$C.The fungal strain (*Candida albican*) was grown in Sabouraud dextrose broth at 35 °C for 48 hours. A sterile loop was used to pick a single colony which was put in 3 millilitres sterile physiological buffer saline. Serial dilutions were made and the density standardized by the McFarland turbidity scale to match tube 0.5. The test strains were suspended in sterile saline to give a final density of 1.5×10^6 colony forming units/mL of bacteria or 1.5×10^5spores/mL of fungi.

The Agar well diffusion medium was prepared by pouring molten Mueller-Hinton Agar (MHA) on petri dishes and letting it to solidify. A single colony of the microorganisms was picked using a sterile loop and streaked on the already pre-prepared Mueller Hinton Agar (Oxoid ®) and incubated at 37^0C for 18 hours. Standard wells measuring one centimetre in diameter were made on the Agar plate. Thereafter, 50 microlitres of both aqueous and organic extracts at different concentrations were added into the wells and incubated for 24 hours at 37^0C. Then the diameters of the zones of inhibition were measured. Antifungal susceptibility assay was carried out with slight modifications as proposed by Zaidi *et al*.,2018. Saboraud dextrose agar (SDA) plates containing inoculums were assayed in the wells that were done using sterile cork borer, to which fifty (50) microlitres of the plant's extracts were also added into the wells and incubated at 37^0C for 48 hours and the zones of inhibitions measured.

3.7.1 Determination of the minimum inhibitory concentration (MIC) of extracts

The inhibitory activity of the plant extract was tested by Broth dilution technique as described by Teh *et al.,* 2017 with minor modifications. Pre-sterilized Muller-Hinton broth (MHB) and Sabouraud dextrose broth were dispensed into sterilized 10ml test tubes using sterile 10ml pipettes. The test tubes were labeled clearly and put in test tube rack. To determine susceptibility, 1.6 g of the crude extract was weighed and mixed with 4mL of sterile MHB and Sabouraud dextrose broth for bacteria and fungi respectively. This produced a concentration of 400mg/ml. Eight; two- fold serial dilutions of the plant extract were made. Thereafter, 0.1ml of bacterial and fungal suspension was dispensed into each of the test tubes. The test tubes were then incubated at 37^{o}C for 24 hours for bacteria and 35 °C for 48 hours for fungi. Two additional tubes containing the bacteria and fungi inoculums without the plant extract were used as negative controls. All experiments were performed in triplicate. Visual turbidity was noted after incubation and used as inference. For the positive controls, Benzyl penicillin, Gentamicin and Amphotericin B were used as standard control drugs for gram-positive bacteria, gram-negative bacteria and fungi respectively. The minimum inhibitory concentration of the extracts and controls was determined from readings on culture plates. In this study, minimum inhibitory concentration (MIC) was taken to be the lowest concentration of the plant extract that inhibited any discernible growth of microorganisms on the culture plates (Wagate *et al.,* 2009; Kalayou *et al.,* 2012).

3.7.2 Minimum bactericidal concentration and minimum fungicidal concentration

In the determination of the minimum bactericidal concentration (MBC), a sterilized pipette was used to take 0.1ml suspension from the non-turbid MIC test tubes. These were sub cultured in Mueller Hinton Agar plates and incubated at 37^{0}C for 24 hours. Thereafter, microbial growth was examined for each plant extract at different test concentrations. The

minimum bactericidal concentration (MBC) was taken as the concentration of the plant extract that did not show any bacterial growth on the freshly inoculated agar plates (Mostafa *et al.,* 2018). On the other hand, the MFC was defined as the lowest concentration of test substance (extracts and controls) that prevented visible growth of the fungus.

3.8 Toxicity studies

3.8.1 Experimental animal model

The protocol previously described in section 3.4 was adopted.

3.8.2 Personal protective equipment and occupational health

Good laboratory practices were observed during handling of the animals to avoid injuries. Laboratory coats, disposable latex gloves and protective masks were used every time when working in the research room. Additionally, anti-rabies and anti-tetanus vaccines were provided to the staff and both were kept under refrigeration.

3.8.3 Housing and feeding conditions

The mice were housed in cages measuring 35cm ×25cm ×18 cm. Wood shavings were used to line the cages and these served as bedding for the animals. The temperature in the experimental room was maintained at 25^0C ($\pm 3^0$C) and the humidity between 30-70%. A photoperiod of 12 hours light and 12 hours darkness sequence was maintained. Water and food were provided ad libitum.

3.8.4 Preparation of animals

The animals used in the studies were selected randomly. Thereafter, they were marked on their tails for ease of identification. They were kept in cages measuring 35cm x 25cmx 18cm for ten days to allow for acclimatisation to the laboratory conditions.

3.8.5 Acute oral toxicity studies of *L. trifolia* aqueous leaf extract

This was done according to the OECD (2001) test guideline 423 with slight modification. The acute toxic class method set out in the guideline is a stepwise procedure whereby 3 animals of a single sex are used per step.

3.8.6 Experimental design for acute oral toxicity

Nine (9) healthy female rats weighing between 180-220g were randomly assigned to three groups (1, 2 and 3). They were individually weighed and marked for ease of identification. Food was withheld overnight before dosing, but drinking water was provided ad libitum. Rats in group 1 were orally administered with 10 ml/Kg Bwt of distilled water and served as the control. The rats in groups 2 and 3 which served as the treatment groups were orally administered 300 mg/Kg Bwt and 2000 mg/Kg Bwt, respectively, of the aqueous leaf extract of *L. trifolia.* Since there was no documented information on the toxicity *L. trifolia,* a beginning dose of 300mg/kg Bwt (Figure 3.1) was selected (OECD 423). A gavage was used to administer the extract orally. Food but not water was withheld for a further 3-4 hours after administration of the extract. The animals were observed individually at least once during the first 30 minutes after dosing, then periodically during first 24 hours. Special attention was given during the first 4hours.All the animals that were still alive were observed up to the 14th day for any abnormal behaviour. All the animals were observed daily for any physiological and behavioral changes. The animals were weighed weekly using an

electronic balance (Mettler PM 4600, Germany). Clinical symptoms were recorded. The observations included changes in eyes and mucous membranes, skin and fur, circulatory, respiratory, central nervous system and autonomic nervous systems, somatomotor activity and behaviour pattern. Other observations included convulsions, tremors, salivation, diarrhoea, lethargy and sleep. These were used as indicators in the determination of acute oral toxicity of *L. trifolia* leaf extract. Those that survived were euthanized using diethyl ether and necropsy performed.

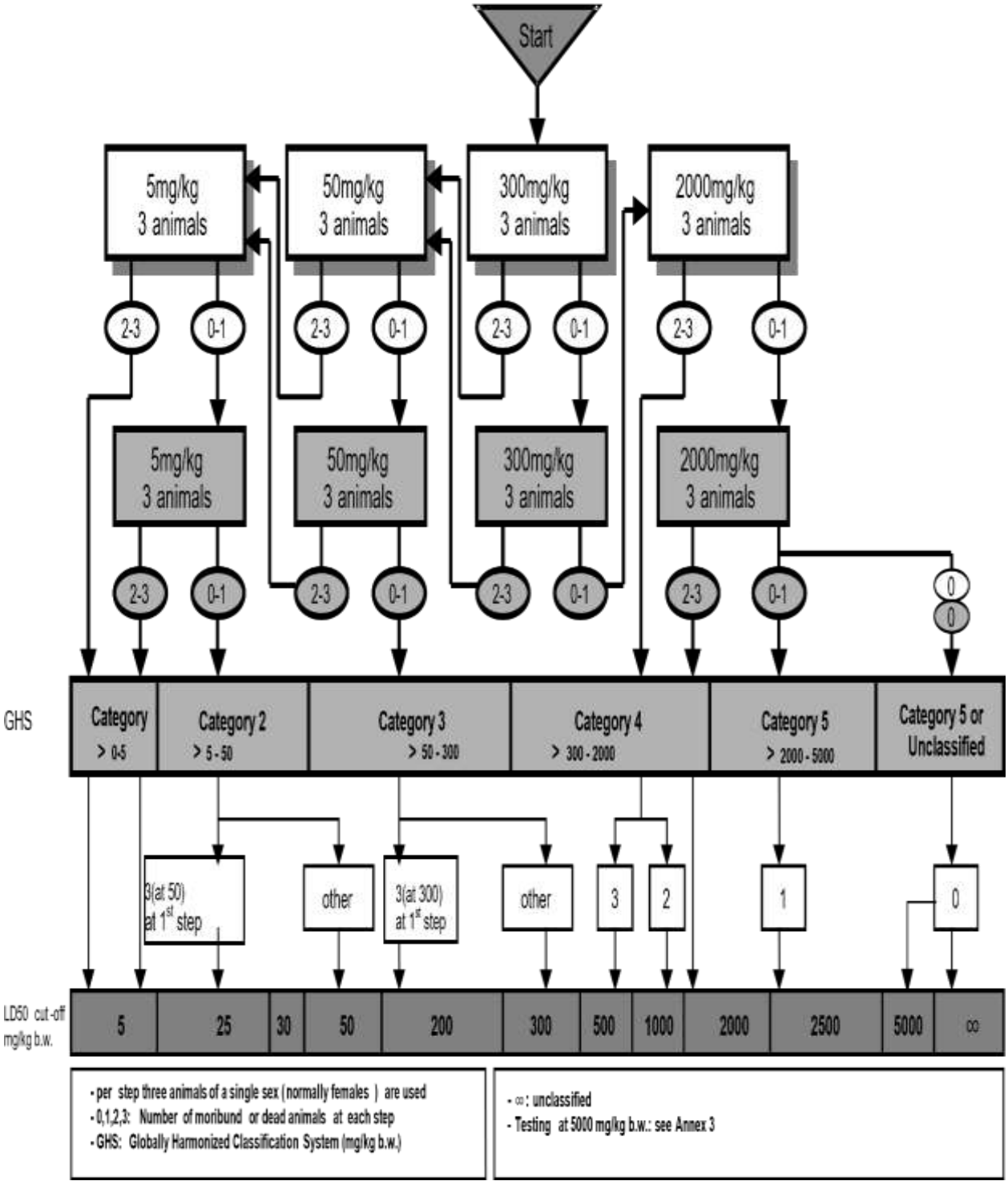


Figure 3.1: Test procedure with a starting dose of 300 mg/kg body weight

3.8.7 Sub-acute toxicity testing of *Lantana trifolia* leaf extract

The mice were grouped by complete randomized design into one control group and three experimental groups with ten (10) animals in each group (5 females and 5 males). The weight of the animals ranged from 20-30g.Group I (control group) received a daily dose of distilled water (10ml/kg body weight) orally, for 28 days. Experimental groups namely, Group II, III and IV received 250mg/kg body weight, 500mg/kg body weight and 1000mg/kg body weight in distilled water, respectively; of the aqueous leaf extract of *L. trifolia* orally once daily for twenty-eight days. The mice were weighed on day one before dosing and then after every seven days using an electronic balance (Mettler PM 4600, Germany). Food and water consumption were monitored throughout the 28-day study period. All the animals were observed daily for any physiological and behavioral changes.

3.8.8 Handling of animals, haematological and biochemical assays

Blood sample was collected on the 29^{th} day from each animal. All surviving animals were anaesthetized in a chamber using diethyl ether and then decapitated. Blood samples were collected from the animals into heparinized (EDTA) tubes for haematological assay and some into plain tubes (non-heparinized) for biochemical assay. The blood in non-heparinized tubes was left to coagulate followed by centrifugation at 3000 revolutions per minute for ten minutes to extract serum which was stored at -20^0C awaiting biochemical analysis. The haematological parameters measured included packed cell volume (PCV), hemoglobin (Hb), red blood cell (RBC) count, white blood cell (WBC) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and thrombocytes. In biochemical analysis, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, urea and total

proteins were assayed for each animal. The haematological and biochemical assays were carried out at Lancet Laboratories.

3.8.9 Histopathological studies

The animals were dissected immediately after blood samples had been collected from them. Various body organs including the heart, liver, spleen and kidney were removed, observed macroscopically and weighed separately. Percentage organ weight ratios were calculated. The organs were then preserved in 10% buffered formalin solution in labelled bottles for histopathological work. The sections of the organs that had been excised were processed and embedded in paraffin wax, followed by sectioning the paraffin-embedded tissue using a microtome. After that, the sections were stained using haematoxylin and eosin (H&E) and observed under the light microscope

$$\text{Relative organ weight} = 100 \times \frac{\text{Absolute organ weight(g)}}{\text{Animal body weight on sacrifice day(g)}}$$

3.9 Statistical analysis

Qualitative phytochemical data was tabulated. The haematological, blood biochemistry and weight measurement results were expressed as mean± standard deviation of the mean. One-Way analysis of variance was performed to determine the significance of differences among means, followed by Tukey's post hoc test for pairwise comparison and separation of means. Differences were considered statistically significant at $P< 0.05$. Acute oral toxicity data were analyzed according to guideline No.423(OECD 2008) and LD_{50} recorded. The results of the zones of inhibition were presented as the mean± standard deviation of the mean for each of the experiments. The IBM Statistical Package for the Social Sciences (SPSS version 21.0) software was used for analysis. $P<0.05$ was considered significant.

CHAPTER FOUR

RESULTS

4.1 Extraction yields of aqueous and organic extracts of *Lantana trifolia* leaves.

The yield of the aqueous leaf extract was 52 grams (5.2%) while that of the organic (Dichloromethane-methanol) extract was 33.6 grams (11.2%). The appearance of the extracts is as described below (Table 4.1). The aqueous extract produced the minimum yield while the highest yield was obtained from the organic extract.

4.1.1 Extracts' yield

Table 4.1: Appearance and the percentage yield of *Lantana trifolia* leaf extracts

Solvent	Weight taken (g)	Amount after drying (g)	Percentage yield	Appearance of the extract
Distilled water	1000	52	5.2	Brown powder
Organic (DCM/M)	300	33.6	11.2	Dark-green mass

4.2 Phytochemical composition of *Lantana trifolia* leaf extracts

The phytochemical constituents present in the extracts are as shown in Table 4.2. Qualitative phytochemical screening revealed presence of chemical compounds such as tannins, saponins, phenolics, terpenoids, flavonoids, alkaloids and reducing sugars.

Table 4.2: Results of the phytochemical analysis of *Lantana trifolia* leaf extracts

Test for:	**Aqueous extract**	**Organic extract**
Tannins	+ve	+ve
Saponins	+ve	+ve
Phenolics	+ve	+ve
Terpenoids	+ve	+ve
Cardiac glycosides	-ve	-ve
Flavonoids	+ve	+ve
Alkaloids	+ve	+ve
Reducing sugars	+ve	+ve
Anthraquinones	-ve	-ve

Key;

+ ve: present

- ve: Absent

4.3 Antimicrobial activity of extracts of *Lantana trifolia*

Table 4.3 shows a summary of the diameter of zones of inhibition noted when aqueous and DCM-methanol extracts are tested against four standard test organisms at the highest working concentration of 400mg/ml. Both gram-positive and gram-negative bacteria used in the study were sensitive to both extracts. In addition, *C. albicans*, was also sensitive to both extracts. The zones of inhibitions for *S. aureus, B. cereus, E. coli* and *C. albicans* were 29.00 ± 3.46mm, 24.67 ± 0.58 mm, 22.33± 0.58mm, 22.33± 0.58mm and 29.67 ±0.58 for the aqueous extract; and 31.33 ± 3.79mm, 25.33 ± 0.58mm, 27.67± 0.58 mm and 32.33 ± 0.58 mm for DCM-methanol extract.

Table 4. 3: The antimicrobial activity of the extracts showing zones of inhibition

Test organism	**Aqueous extract(mm)**	**DCM-Methanol extract(mm)**
Staphylococcus aureus	29.00 ± 3.46	31.33 ± 3.79
Bacillus cereus	24.67 ± 0.58	25.33 ± 0.58
Escherichia coli	22.33± 0.58	23.67± 1.16
Candida albicans	29.67 ±0.58	32.33 ± 0.58

The antimicrobial activity of the aqueous leaf extract of *L. trifolia* against selected test organisms at different concentrations is shown in Table 4.4. The minimum inhibitory concentration for *S. aureus, B. cereus* and *E. coli* was 200mg/ml. The MIC for *C. albicans* was 100mg/ml.

Table 4. 4: Minimum inhibitory concentration of the aqueous extract against the microbes.

	Aqueous extract (mg/ml)									**MIC**
Test	400	200	100	50	25	12.5	6.25	3.125	1.5625	
Staphylococcus aureus	NG	G*	G	G	G	G	G	G	G	200mg/ml
Bacillus cereus	NG	G*	G	G	G	G	G	G	G	200mg/ml
Escherichia coli	NG	G*	G	G	G	G	G	G	G	200mg/ml
Candida albicans	NG	NG	NG	G*	G	G	G	G	G	50mg/ml

KEY: **NG - no growth**
G- growth
G*-MIC

Table 4.5 shows a summary of the antimicrobial activity of DCM-methanol leaf extract of *L. trifolia* against selected test organisms at different concentrations. The MIC for *S. aureus* and *B. cereus* was 3.125mg/ml. The MIC for *E. coli* and *C. albicans* were 6.25mg/ml and 1.5625mg/ml respectively.

Table 4.5: Minimum inhibitory concentration of the DCM-methanolic extract against the microbes.

	DCM-methanol (mg/ml)									MIC
Test	400	200	100	50	25	12.5	6.25	3.125	1.5625	
Staphylococcus aureus	NG	NG	NG	NG	NG	NG	NG	G*	G	3.125 mg/ml
Bacillus cereus	NG	NG	NG	NG	NG	NG	NG	G*	G	3.125 mg/ml
Escherichia coli	NG	NG	NG	NG	NG	NG	G*	G	G	6.25 mg/ml
Candida albicans	NG	NG	NG	NG	NG	NG	NG	NG	G*	1.5625 mg/ml

KEY: **NG - no growth**
G- growth
G*-MIC

Table 4.6 shows a summary of minimum inhibitory concentration when aqueous and DCM-methanol extracts of *Lantana trifolia* are tested against selected test organisms.

Table 4.6: Minimum inhibitory concentrations (MIC) mg/ml for aqueous and DCM-methanolic extracts of leaves of *Lantana trifolia*

	Aqueous extract	**DCM-methanol**	**Benzylpenicillin**	**Gentamicin**	**Amphotericin B.**
Staphylococcus aureus	200	3.125	0.625	-	-
Bacillus cereus	200	3.125	0.625	-	-
Escherichia coli	200	6.25	-	0.0049	-
Candida albicans	50	1.5625	-	-	0.0125

Table 4.7 shows a summary of minimum bactericidal/fungicidal concentration (MBC/MFC) when aqueous and DCM-methanol extracts of *Lantana trifolia* are tested against various test organisms.

Table 4.7: Minimum bactericidal/fungicidal concentration (MBC/MFC) in mg/ml of the extracts of *Lantana trifolia* against the microbes.

	Aqueous extract	**DCM-methanol**	**Benzylpenicillin**	**Gentamicin**	**Amphotericin B.**
Staphylococcus aureus	400	6.25	0.625	-	-
Bacillus cereus	400	6.25	0.625	-	-
Escherichia coli	400	12.5	-	0.0049	-
Candida albicans	100	3.125	-	-	0.0125

Figure 4.1 shows diameter of zones of inhibition of microbial growth for extracts on Agar well diffusion.

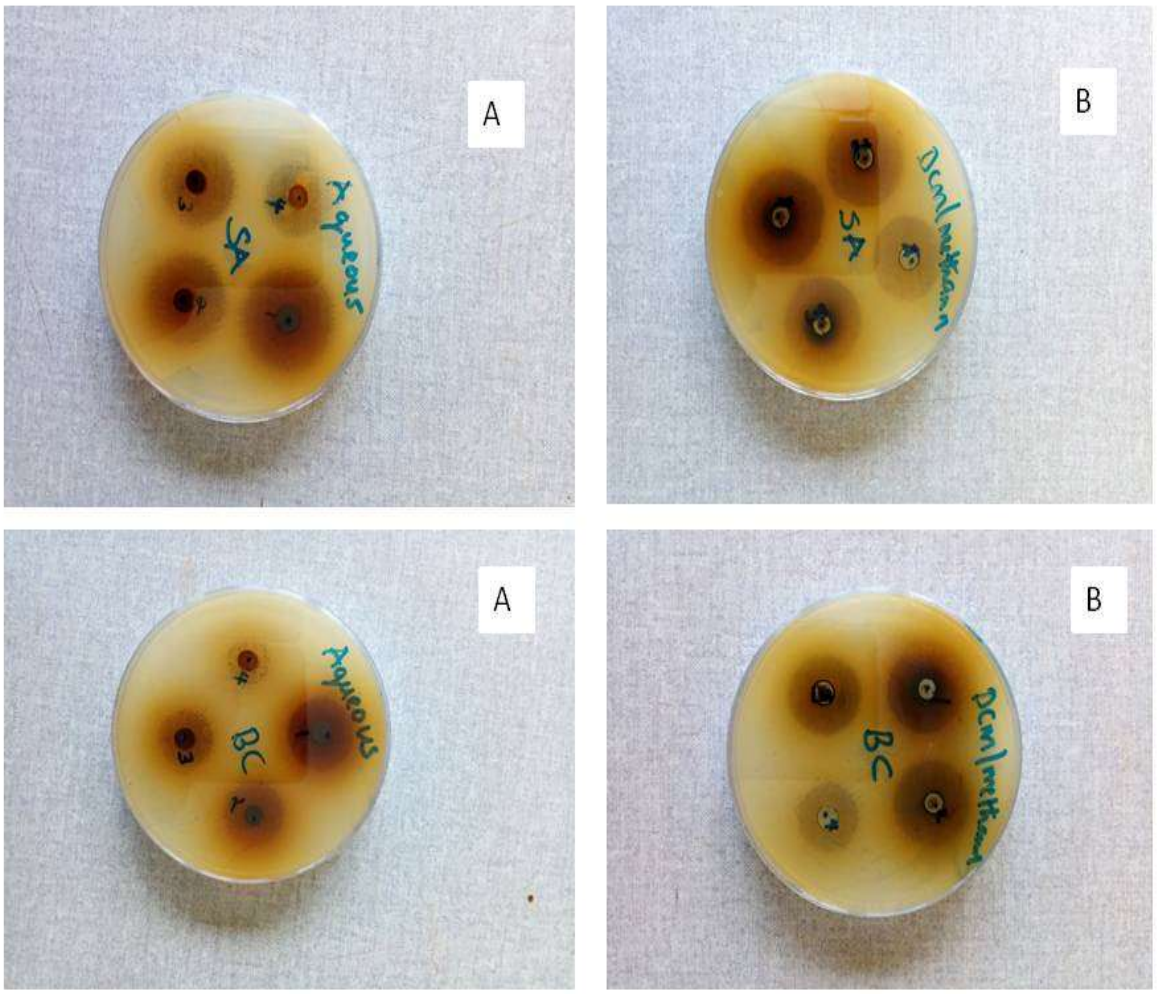


Figure 4.1: Culture plates showing diameters of zones of inhibitions of microbial growth for; A) aqueous extract; B) DCM-Methanol extract of *Lantana trifolia* against *Staphylococcus aureus* and *Bacillus cereus.*

4.4 Acute and Sub-acute toxicity of the aqueous *Lantana trifolia* leaf extract

4.4.1 Oral Acute Toxicity Testing

This study was undertaken for a period of two weeks. Parameters such as physico-clinical changes and mortality were used in the evaluation of the acute toxicity of the aqueous leaf extract of *L. trifolia* after the animals' oral administration of the extract.

4.4.1.1 Physico-clinical changes and mortality

The results of acute oral toxicity showed that administration of the aqueous leaf extract of *L. trifolia* at 300mg/kg and 2000mg/kg did not produce any changes in the eyes, mucus membranes, fur and skin colour. Moreover, there were no noteworthy changes in respiratory system, central nervous system and behaviour pattern. There were also no signs of convulsions, tremors, salivation, diarrhoea, urinary incontinence, sleep and coma. The behaviour of the animals during the study period was generally normal as shown in Table 4.8 below. There was no mortality throughout the two weeks of the study.

Table 4. 8: Effect of single dose of aqueous leaf extract of *Lantana trifolia* on physical and behavioural parameters

Observation	Control	Aqueous 300mg/kg	Aqueous 2000mg/kg
Respiratory changes	-	-	+
Circulatory changes	-	-	-
Gripping strength	+	+	+
signs of aggression	-	-	-
Skin and fur changes	-	-	-
Response to sound	+	+	+
Salivation	-	-	-
Urinary incontinence	-	-	-
Defaecation	+	+	+
Diarrhoea	-	-	-
Sedation	-	-	+
Convulsions	-	-	-
Mortality	-	-	-
Post mortem changes	-	-	-

Key: + = Present - = Absent

4.4.1.2 Mean Body Weight Change in the Acute Oral Toxicity Study

There was a progressive increase in the mean body weights of the control group and the treatment groups that received the aqueous leaf extract of *Lantana trifolia* over the 14 days of acute toxicity study. Furthermore, no significant differences (P>0.05) were observed in mean body weights between the control group and the treatment groups over the 14 days (Table 4.9 and Figure 4.2). This was backed by the P-values of 0.99 and 1.00 for aqueous extract 300mg/kg Bwt and aqueous extract 2000mg/kg Bwt respectively in relation to the control.

Table 4. 9: *Lantana trifolia* aqueous leaf extract single dose effect on the weekly mean body weights of Wistar albino rats

Treatment group (n=3)	Mean weights (g)			P-values
	Day 0	Day 7	Day 14	
Control	203.04±18.22	229.14 ±43.03	240.59±41.90	-
300mg/kg	209.33±1.04	232.71±14.49	247.12±15.99	0.99
2000mg/kg	205.09±19.97	219.64±17.82	223.70±17.79	1.00

Note: All values are expressed as mean ± standard deviation, n= three animals.

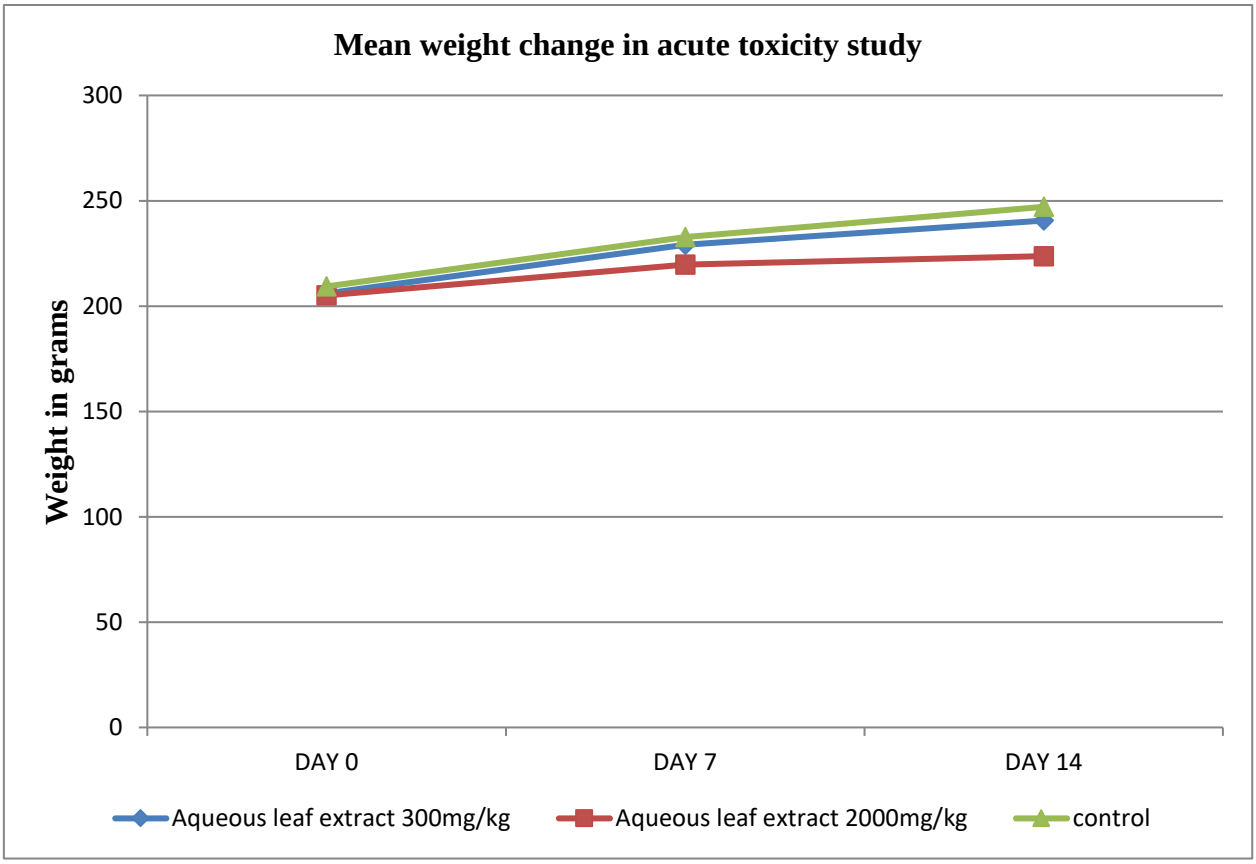


Figure 4.2: Effects of the aqueous leaf extract of *Lantana trifolia* on mean body weights of Wistar rats in acute toxicity study.

4.4.2 Sub-Acute Toxicity Studies

4.4.2.1 Mean body weights

There were no significant changes related to the dose in mean body weights of both female and male mice treated with aqueous extract compared with the controls that received distilled water, as shown in Table 4.10.

Table 4. 10: The effects of aqueous leaf extract of *Lantana trifolia* graded doses on the weekly mean body weights of the of Swiss albino mice over a 28-day period

Day	Sex	Control	250mg/kg	P Value	500mg/kg	P Value	1000mg/kg	P Value
0	**F**	28.54±1.84	24.87±2.92	0.09	24.79±2.05	0.08	24.61±2.14	0.06
	M	23.99±1.72	28.38±1.63	0.11	24.28±1.61	0.99	27.60±0.82	0.08
7	**F**	26.87±3.33	24.56±2.58	0.56	24.90±2.26	0.68	25.51±2.76	0.86
	M	25.00±1.42	28.77±2.78	0.13	26.13±3.01	0.89	27.16±2.69	0.55
14	**F**	26.66±3.72	24.86±3.71	0.77	24.90±2.29	0.62	26.66±3.72	0.78
	M	25.50±1.20	29.40±2.45	0.08	27.92±1.68	0.39	27.48±3.45	0.56
21	**F**	25.52±3.08	24.39±3.39	0.91	25.79±1.22	1.00	23.54±2.65	0.66
	M	25.78±1.92	29.80±3.10	0.14	25.40±3.12	1.00	27.79±2.82	0.67
28	**F**	25.59±3.06	24.19±3.64	0.83	25.65±0.73	1.00	23.11±2.33	0.48
	M	26.26±1.98	30.84±3.07	0.12	25.17±3.94	0.94	26.58±2.79	1.00

Note: All values are expressed as mean ± standard deviation of five animals.

4.4.2.2 Average feed consumption

Table 4.11 shows the average feed consumption of mice treated with graded doses of aqueous extracts of *L. trifolia* plants and distilled water (control) over a 28-day study. There were no significant changes in feeding in both sexes due to treatment with extracts at 250mg/ kg, 500mg/kg and 1000mg/kg body weights when compared with the control group. This is supported by the P-values, which are greater than 0.05 in Table 4.11.

Table 4. 11: The effects of aqueous leaf extract of *Lantana trifolia* graded doses on feed consumption in Swiss albino mice over a 28-day period

Treatment	(gms**)**					
Group	**Sex**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**P-value**
Control	**M**	5.26	3.99	3.85	3.01	**-**
	F	7.89	6.92	5.64	5.63	**-**
250mg/kg	**M**	5.53	4.74	3.86	3.42	**1.00**
	F	5.56	5.41	4.94	4.47	**0.06**
500mg/kg	**M**	4.46	4.9	4.17	3.17	**0.98**
	F	6.04	5.61	5.68	5.66	**0.40**
1000mg/kg	**M**	4.17	4.94	4.26	3.27	**1.00**
	F	6.04	5.61	5.68	5.66	**0.40**

Note: Values are recorded in grams per mouse per day

F- Females **M** -Males

4.4.2.3 Average water consumption

There were no significant changes observed in water consumption behaviour of both sexes treated with 250mg/kg, 500mg/kg and 1000mg/kg of the extract compared to the control group as shown in Table 4.12, P> 0.05.

Table 4. 12: Effect of graded doses of the aqueous leaf extract of *Lantana trifolia* on water consumption in mice over a 28-day period

Treatment		(ml)				
Group	**Sex**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**P-value**
Control	**M**	4.23	4.86	4.57	5.29	-
	F	4.86	4.43	3.86	4.14	-
250mg/kg	**M**	6.00	6.29	5.57	5.71	0.80
	F	3.71	4.00	3.86	3.86	0.14
500mg/kg	**M**	5.71	4.86	4.00	4.29	0.48
	F	4.23	4.14	4.71	5.00	0.81
1000mg/kg	**M**	6.29	5.71	4.14	4.57	0.77
	F	4.00	4.29	3.00	3.00	0.06

Values are recorded in average milliliters per mouse per day

Normal reference range – 3-7ml per mouse per day (Derelanko, 2018)

4.4.2.4 Haematological analysis in sub-acute toxicity studies of *Lantana trifolia*

The effect of oral administration of graded doses of aqueous leaf extract of *L. trifolia* on haematological parameters at day 28 in sub-acute toxicity of mice is shown in Table 4.13 and Figure 4.3.

Both significant and non-significant changes were observed in the parameters of both male and female mice when compared with the control group. There was no significant difference in RBC, WBC, Haematocrit and MCV in both male and female groups administered with 250mg/kg,500mg/kg and 1000mg/kg of the aqueous leaf extract of *L. trifolia* relative to the control, $P>0.05$. However, there was significant elevation of the platelets count in both male and female treatment groups that received the aqueous leaf extract of *L. trifolia* compared to the control group, $P<0.05$.

In both female and male treatment groups, a significant decrease ($P<0.05$) was noted with MCH at the doses of 250mg/kg body weight and 500mg/kg body weight. Moreover, there was a significant difference $P<0.05$ in MCHC in males that received the dose of 1000mg/kg body weight.

Table 4. 13: Effects of the aqueous leaf extract of *Lantana trifolia* on Haematological parameters of experimental mice following Sub-Acute toxicity study.

parameter	Sex	Control	250mg/kg	P-value	500mg/kg	P-value	1000mg/kg	P-value
RBC (10^6/uL)	M	9.52±0.96	11.25±0.56	0.06	11.08±0.59	0.09	9.95±1.50	0.9
	F	8.39±3.60	10.34±0.54	0.12	10.46±0.74	0.35	10.02±0.93	0.55
WBC (10^3/uL)	M	12.06±3.72	13±6.35	0.96	11.20±3.21	0.99	7.21±2.74	0.31
	F	5.96±2.25	12.05±4.27	0.62	13.8±11.09	0.42	17.74±10.14	0.13
Platelets (10^3/uL)	M	326±122.75	847±138.20	0.00	849±73.24	0.00	980±290.75	0.00
	F	312±226.66	792±203.66	0.02	895±118.76	0.03	1129±295.19	0.00
Haematocrit %	M	40.34±3.48	45.56±0.52	0.10	46.24±2.90	0.06	43.10±4.83	0.57
	F	35.74±14.39	45.12±0.69	0.23	43.54±1.41	0.37	42.82±2.98	0.45
Hb (g/dL)	M	15.28±0.99	15.32±0.39	0.10	15.86±1.00	0.91	14.04±2.41	0.52
	F	12.66±5.11	15.82±0.46	0.30	14.54±0.49	0.70	14.10±1.83	0.84
MCV (fL)	M	42.48±1.33	40.56±1.92	0.40	40.06±1.85	0.22	43.54±2.33	0.81
	F	43.54±3.33	40.04±2.10	0.15	41.74±2.68	0.66	42.96±1.26	0.98
MCH (pg)	M	16.20±2.39	13.64±0.59	0.03	13.72±0.50	0.03	14.08±0.61	0.08
	F	15.46±1.20	14.00±0.31	0.04	13.94±0.64	0.04	14.08±0.74	0.06
MCHC (g/dL)	M	37.64±4.97	33.62±0.65	0.15	34.30±0.98	0.27	32.38±2.29	0.04
	F	35.56±1.84	35.08±1.18	0.97	33.38±0.65	0.19	32.82±2.34	0.07

Key: All Values are expressed as mean± standard deviation (n=5 females and 5 males), Hb (Haemoglobin), Red blood cells (RBC), White blood cells (WBC), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC).

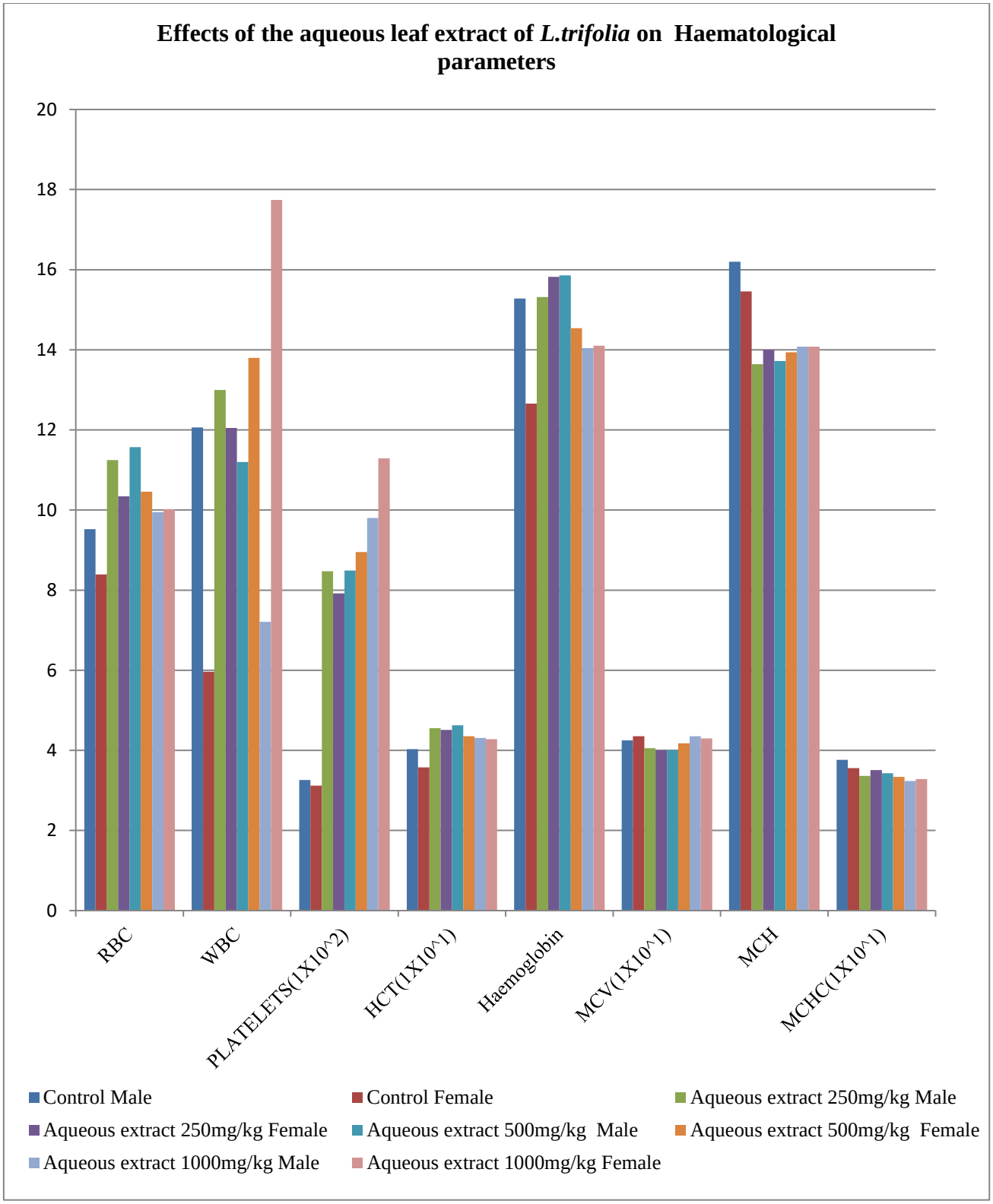


Figure 4.3: Effects of *Lantana trifolia* extracts on Haematological parameters of mice following sub-acute toxicity study.

4.4.2.5 Biochemical parameters in sub-acute toxicity studies of *L. trifolia*

The effects of oral administration of various doses of aqueous leaf extract of *L. trifolia* on biochemical parameters are shown in Table 4.14 and Figure 4.4, respectively. No significant differences ($P>0.05$) among the alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total protein (TP) levels of experimental mice treated with the aqueous leaf extract of *L. trifolia* at all three dose levels compared with those of the control group of mice were observed. However, the levels of urea in the females that received 1000mg/kg Bwt dose of the aqueous extract were significantly elevated relative to the control, $P<0.05$. Moreover, the results showed that the mean serum creatinine levels increased significantly in male mice treated with 1000mg/kg Bwt of the extract compared to the control group that received distilled water.

Table 4. 14: Effects of the aqueous leaf extract of *Lantana trifolia* on Biochemical parameters of experimental mice following Sub-Acute Toxicity Study.

	Sex	Control	250mg/kg	P value	500mg/kg	P-value	1000mg/kg	P value
ALT (IU/L)	**M**	156.29± 112.06	67.76± 18.78	0.45	92.48± 37.08	0.70	157.34± 141.67	1.00
	F	85.70± 8.23	112.02± 26.82	0.77	81.51± 8.32	1.00	135.50± 81.79	0.30
AST (IU/L)	**M**	526.00± 382.16	349.40± 27.97	0.78	414.32± 153.92	0.93	553.72± 419.57	1.00
	F	214.28± 42.39	293.96± 97.46	0.96	305.02± 60.65	0.93	499.05± 484.51	0.31
UREA (mg/dl)	**M**	7.30± 1.51	8.52± 1.71	0.80	7.67± 1.86	0.99	10.10± 3.01	0.20
	F	6.81± 1.05	8.06± 1.72	0.76	7.76± 0.80	0.88	10.79± 3.41	0.03
Creatinine (mg/dl)	**M**	35.60± 3.13	37.00± 3.00	0.59	43.80± 1.92	0.07	52.80± 5.07	0.00
	F	40.00±6.65	35.60± 2.61	0.42	43.40± 4.09	0.63	40.20± 3.49	1.00
T.P (g/L)	**M**	50.59± 5.62	54.64± 5.86	0.84	57.81± 8.13	0.48	59.58± 10.57	0.30
	F	59.48± 2.09	51.92± 5.35	0.31	56.21± 6.17	0.86	67.62± 10.20	0.25

Key: All tabulated values are expressed as mean± standard deviation, n=5 females and 5 males, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Total protein (TP)

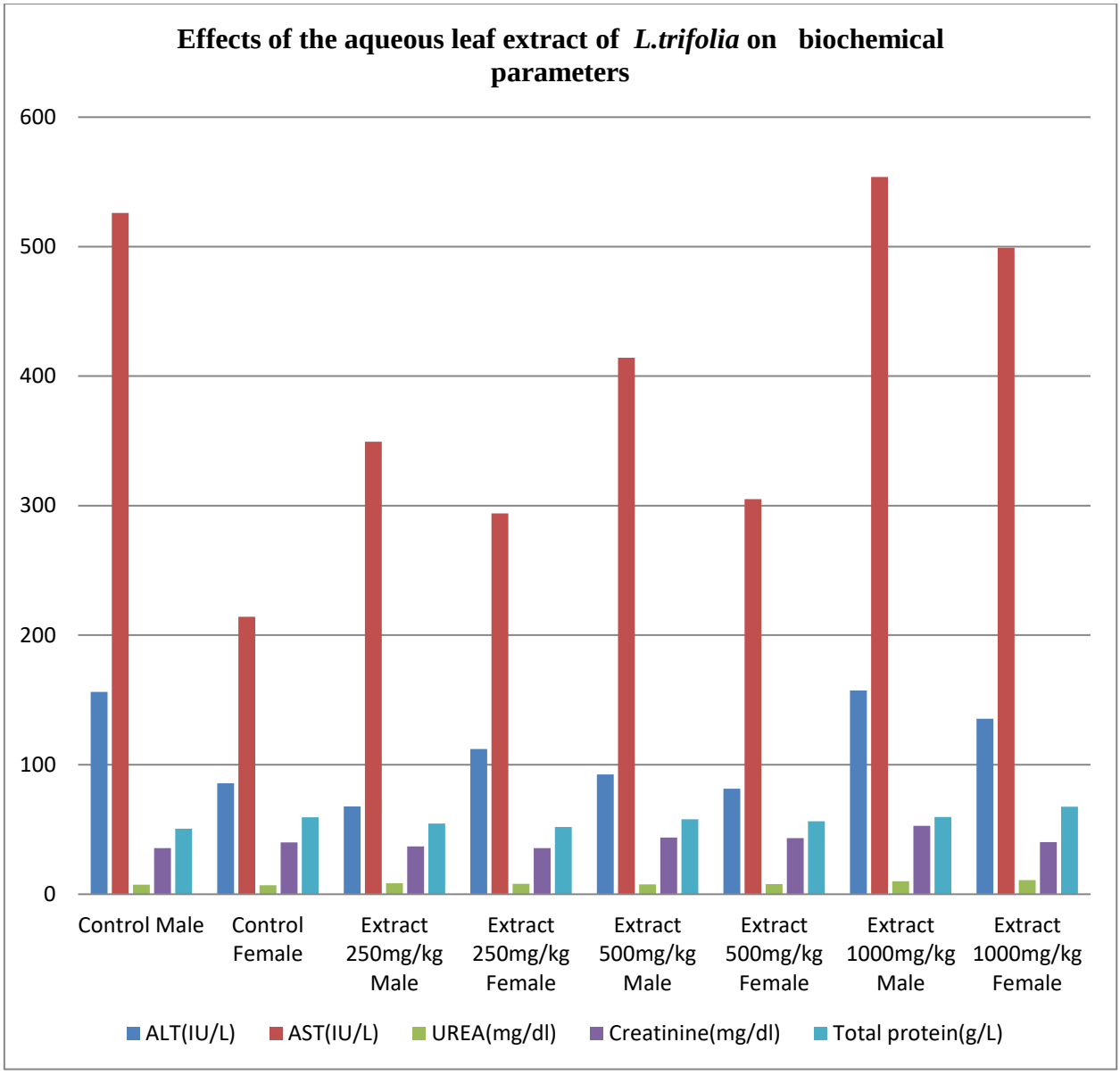


Figure 4.4: Biochemical parameters after the 28- day administration of the aqueous *L. trifolia* extracts

4.4.2.6 Relative organ weight

The data on relative organ weights are presented in Table 4.15 and Figure 4.5. Sub -acute administration of the aqueous leaf extract of *L. trifolia* did not produce significant difference ($P>0.05$) in relative organ weights in all the doses for the liver, kidney, heart and spleen after 28 days of administration.

Table 4. 15: The effects of aqueous leaf extract of *Lantana trifolia* graded doses on the relative mean organ weight in Swiss albino mice over a 28-day period.

Organ weight (grams)	Sex	Control	250mg/kg	P value	500mg/kg	P value	1000mg/kg	P value
Liver	M	5.72±1.06	5.13±0.61	0.56	4.50±0.53	0.43	5.26±0.48	0.73
	F	5.88±0.33	5.51±0.20	0.82	5.89±0.86	1.00	5.87±0.78	1.00
Kidneys	M	1.56±0.13	1.48±0.11	0.82	1.39±0.20	0.31	1.60±0.15	0.96
	F	1.24±0.12	1.41±0.10	0.63	1.35±0.14	0.98	1.48±0.14	0.19
Heart	M	0.51±0.06	0.50±0.02	1.00	0.50±0.08	1.00	0.59±0.08	0.23
	F	0.55±0.09	0.56±0.06	1.00	0.54±0.08	1.00	0.62±0.15	0.69
Spleen	M	0.84±0.15	0.73±0.14	0.81	1.00±0.33	0.42	0.99±0.35	1.00
	F	0.94±0.07	0.83±0.32	0.90	1.02±0.15	1.00	0.85±0.18	0.56

Note: All Values are expressed as mean±standard deviation, n=5 females and 5 males

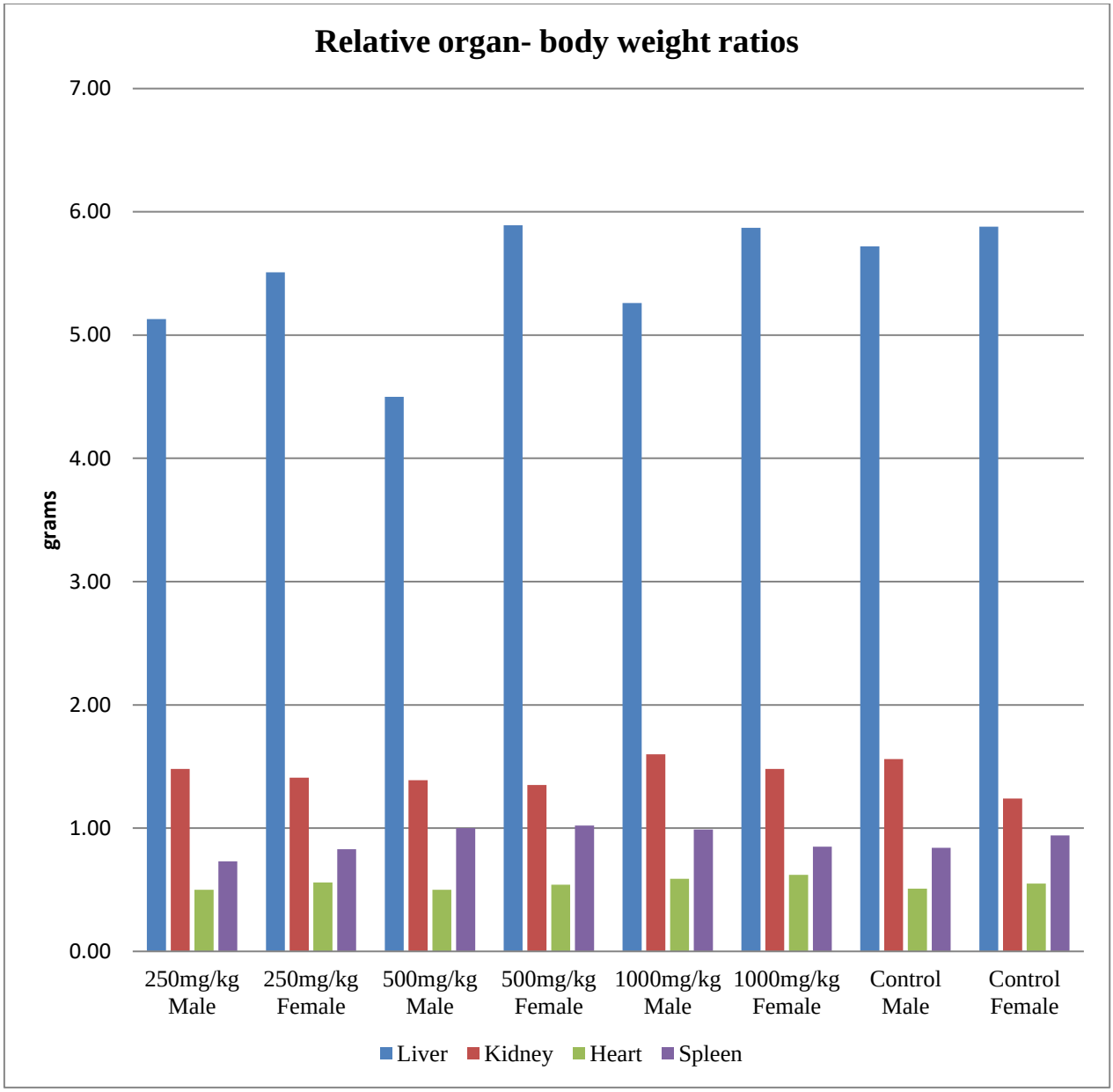


Figure 4.5: Effects of oral administration of aqueous extract at graded doses daily for 28 days on the percent relative organ to body weights in mice.

4.4.2.7 Histopathological examination of the kidney and liver sections of mice treated with aqueous Leaf extract of *Lantana trifolia*

a. Effects *of Lantana trifolia* aqueous leaf extract on the liver

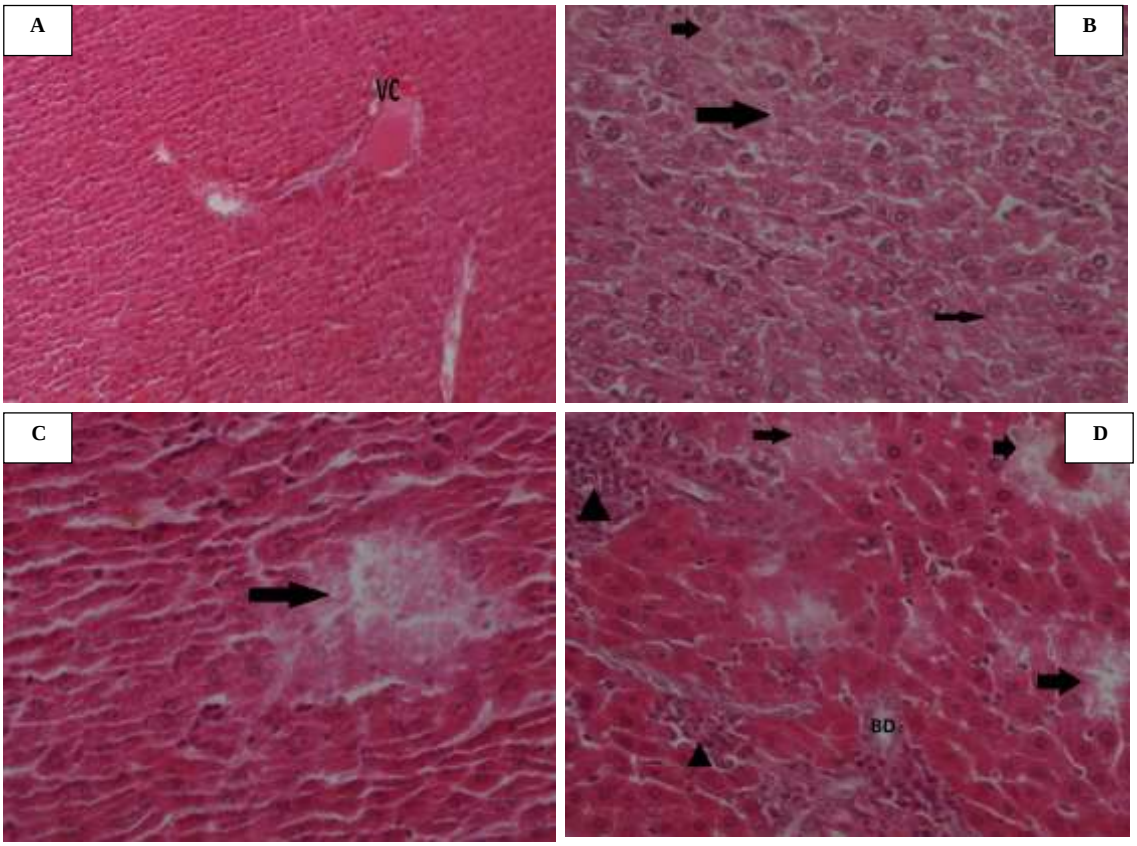


Figure 4.6: Photomicrographs showing liver sections from mice treated with different doses for 28 days (H&E X400). Key: VC congestion of the hepatic blood vessels, BD Bile duct proliferation

A. Liver section from a mouse treated with distilled water(control) **B.** Liver section from a mouse that received a daily dose of 250mg/kg Bwt of aqueous leaf extract of *L. trifolia* for 28 days. **C**. Liver section from a mouse that received a daily dose of 500mg/kg Bwt of aqueous leaf extract of *L. trifolia* for 28 days. **D**. Liver section from a mouse that received a daily dose of 1000mg/kg Bwt of aqueous leaf extract of *L. trifolia* for 28 days.

Liver section A was characterized by normal parenchymal architecture with normal hepatic cells that were evenly distributed and separated by sinusoids. Liver section B was characterized by focal hepatocyte swelling. Liver section C was characterized by hepatocyte necrosis and focal hepatocyte swelling. Liver section D was characterized by kupffer cell proliferation, neutrophil proliferation, diffuse hepatocyte necrosis and karyolysis.

b. Effects *of Lantana trifolia* aqueous leaf extract on the kidneys

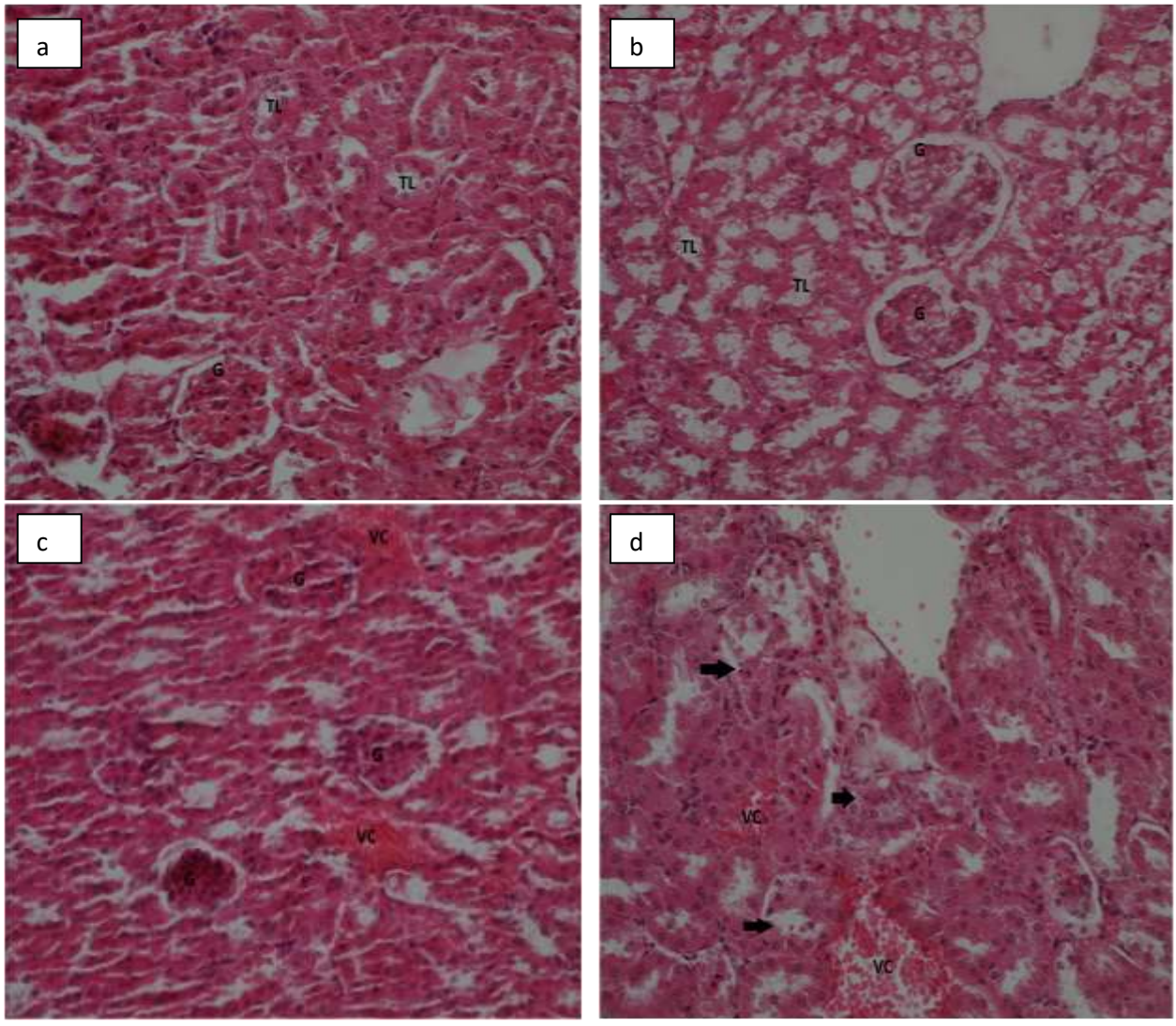


Figure 4. 7: Photomicrographs showing kidney sections from mice treated with different doses for 28 days (H&E X400). Key G- glomerular, VC- Vascular congestion, TL- Tubular lumen

a. Kidney section from a mouse that received distilled water (control) b. Kidney section from a mouse that received 250mg/kg Bwt of aqueous leaf extract of *L. trifolia* for 28 days. c. Kidney section from a mouse that received 500mg/kg Bwt of aqueous leaf extract of *L. trifolia* for 28 days. d. Kidney section from a mouse that received 1000mg/kg Bwt of aqueous leaf extract of *L. trifolia* for 28 days.

Kidney sections (a), (b) and (c) were characterized by normal tubules and regular glomeruli. Kidney section (d) showed diffuse tubular epithelium degeneration which was characterized by cell swelling and necrosis (Arrow G).

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

Herbal products obtained from the natural sources such as medicinal plants have become famous in the developing world. The bioactive compounds that are normally present in the traditional medicinal plants are assumed to be safe even without a clear and better understanding of their long-term effects on one's health (Vaghasiya *et al.,* 2011). Therefore, the current study was meant to investigate the antimicrobial activity of selected extracts of *L. trifolia* and also describe its safety profile.

According to Chemat *et al.,* 2012, the phytochemical constituents present in the organic or aqueous extracts show physicochemical differences which can be associated with the process of extraction. The presence of certain phytochemical compounds in a plant extract determines its biological activity (Mazid *et al*., 2011). Phytochemical compounds play a vital role in the prevention of chronic diseases such as diabetes mellitus, coronary artery disease and cancer. Moreover, they are important in the treatment of certain diseases. *L. trifolia* has tannins, saponins, alkaloids, terpenoids, phenolics and flavonoids. These phytochemicals generally have biological activity in the cells and tissues (Ghasemzadeh *et al.,* 2018). Presence of more than one secondary metabolite and the quantity of the phytochemical plant extracts determines the nature and extent of the biological activity of the plant extract (Wang *et al*., 2010).

Lantana trifolia contains tannins which have been found to have anti-inflammatory activity. Moreover, tannins have antioxidant, antimicrobial, anticarcinogenic, antimutagenic, antidiabetic and would healing activities (Sieniawska and Baj, 2017). This may explain why the plant is used to manage abdominal pain due to gastritis, esophagitis, enteritis, and irritating bowel disorders (Ashok and Upadhyaya 2012). According to Anderson *et al.*, 2012,

tannins' antimicrobial activity is attributed to their ability to inhibit membrane bound enzymes.

Phenolic compounds were also present in *L. trifolia.* According to Priya,2014, phenolics have good antioxidant activity, therefore they have a role in degenerative diseases such as cancer, cardiovascular diseases and also in inflammation that results from oxidative damage. Studies conducted recently have revealed that presence of several subgroups in phenolic compounds hinder the proliferation of different cancer cells (Jafari *et al.,* 2014).

L.trifolia contains saponins. According to Juan and Liang 2020, saponins that are derived from natural sources have been used for a long time in herbal and traditional medicines. In addition, they can cause cell membrane permeabilization (Thakur *et al.,* 2011). Most saponins also exhibit surface-active properties and can bind cholesterol leading to the formation of insoluble complexes which can be extracted in bile. They have been shown to display various biological and pharmacological activities such as antiallergic, cytotoxic, antiviral, antihepatotoxic, antibacterial, molluscicidal, antiparasitic and antifungal activities, and immunoadjuvant activities (Barbosa 2014).

Terpenoids were also identified. These compounds have been reported to have toxic effects on the cell wall of gram-positive bacteria. Moreover, they are also toxic to gram-negative bacteria. Terpenoids interact with proteins of the intracellular constituents and cell membrane leading to disruption on the structure of the membrane. Consequently, there is degradation, both in function and structure, of the cytoplasmic membrane. Furthermore, damaging the cytoplasmic membrane causes cytoplasm coagulation and increased membrane permeability which leads to leakage of important intracellular substances and reducing ATP synthesis. The process eventually leads to cell death. Terpenoids have also been found to have anti-cancer, antimicrobial, anti-nociceptive, hepatoprotective, anti-spamosdic and anti-inflammatory activities (Ludwiczuk *et al.,* 2017; Afidati *et al.,* 2019).

Flavonoids were also detected in the extracts of *Lantana trifolia*. Flavonoids are reported to decrease the prevalence of upper respiratory tract infections (URTIs) because of several physiologic effects in humans which include anti-inflammatory, antiviral, cytotoxic, antimicrobial and antioxidant (González-Gallego *et al.,* 2010). Moreover, flavonoids possess hepatoprotective activity, free radical scavenging capacity and are useful in the prevention of coronary heart disease. In plant systems, flavonoids regulate growth and also assist in combating oxidative stress (Cui *et al.*,2020). Therefore, flavonoids are critical compounds in health promotion and amelioration of several disease symptoms. In addition, anxiolytic and sedative effects have been associated with presence of flavonoids in plant extracts (Aguirre-Hernández *et al.,* 2016). The presence of flavonoids in *L. trifolia* explains the use of the plant in the treatment of some of the infections of the respiratory tract, for example, cough, tonsilitis and sinusitis.

Lantana trifolia also contains alkaloids. Several alkaloids have been shown to have toxic effects and also potent pharmacological capacity. This class of secondary metabolites has been used lawfully and illicitly as pharmaceuticals, stimulants and narcotics. Studies have shown that alkaloids exhibit anti-tumor, anti-viral (anti-HIV), anti-microbial, anti-inflammatory and anti-cholinesterase activities (Moreira 2018).

Staphylococcus aureus is a dangerous microorganism that has been reported to cause nosocomial infections. Moreover, it also causes community acquired infections and therefore, it is a major burden in healthcare. This microorganism can attach to the medical implants and the host tissue too and establish a mature biofilm which plays a role in the persistence of chronic infections. During an infection, cells from the biofilm can spread to various sites in the body hence worsening the infection (Lister and Horswill 2014).

Bacillus cereus, gram-positive bacterium, causes diarrhoea in humans. People can be infected mostly after they have consumed food that is contaminated. *B.cereus* is ubiquitous

in nature and has the ability to occur in several types of foods. The onset of this infection is usually about 8-16 hours and lasts for 12-24hours (Banerjee *et al.*,2011).

Escherichia coli, a gram-negative bacterium, is found in the lower intestinal tract of animals and human beings. It is the main organism that causes urinary tract infections (UTIs). There are reports that *E. coli* also causes neonatal sepsis and nosocomial infections. The organism is becoming resistant to commonly used antimicrobials such as fluoroquinolones (Mellata 2013; Jang *et al.*,2017).

Candida albicans, generally, does not cause harm. However, when the immune status of the host is compromised, it can cause infections that range from superficial infections of the mucosa (oral/vaginal thrush) to life threatening systemic conditions (Motaung *et al.*,2015).

The results obtained from the antimicrobial activity study show that plants used in folk medicine can have both antibacterial and antifungal activities. In the present study, both the aqueous and organic leaf extracts of *Lantana trifolia* exhibited varying degree of antimicrobial activity against the selected test microorganisms. Similar results with other plant extracts against gram positive, gram negative and fungi have been reported by other researchers (Saraf *et al.*,2011). It is important to note that the type of solvent used during extraction determined how potent *Lantana trifolia* is against the microbes. This was also reported by Agwu,2019. In this study, the DCM-methanolic extract was found to be more potent than the aqueous extract against the tested microorganisms.

Gram-positive bacteria were more susceptible than gram-negative ones. This is supported by previous studies which have revealed that, in general, plant extracts are generally more active against Gram-positive bacteria than Gram-negative bacteria and this could be due to the difference in the structure of the cell wall of these classes of bacteria. The additional outer membrane that surrounds the cells of Gram-negative bacteria provides them with a surface which is hydrophilic in nature that functions as a permeability barrier for many

substances that includes natural compounds (Briers and Lavigne 2015; Da Silva *et al.*,2016). Both aqueous and organic extracts showed activity against *Candida albicans.* Fungal Infections caused by *Candida spp* are very common in patients who are immunosuppressed. People who are *immunocompromised* have a reduced ability to fight infections and other diseases and causes of this condition include cancers, HIV infection, organ transplantation and surgeries especially in developing countries (Pitman *et al.*,2011). Related to this, the growing microbial resistance and also lack of new antifungals make these results relevant (Odom,2014).

Substances with antimicrobial properties are said to be bacteriostatic agents when the ratio MBC/MIC >4 and bactericidal when the ratio MBC/MIC ≤ 4 (Gatsing et *al.,* 2006). Following the stated criterion, it can be suggested that both extracts were bactericidal in nature. The extracts of *L. trifolia* have demonstrated significant biological activity against the test microorganisms.

The minimum inhibitory concentration (MIC) of the extracts against the gram-positive *Staphylococcus aureus* ranged from 3.125 to 200mg/ml while the minimum bactericidal concentration ranged from 6.25 to 400mg/ml. The observed MIC and MBC values were higher than those recorded by Kim *et al., (*2020) when they studied 239 traditional Chinese extracts and reported minimum inhibitory concentration values ranging from 0.10 to 12.5 mg/ml and MBC values ranging from 0.78 to 25 mg/ml. The minimum inhibitory concentration for *Candida albicans* was 50mg/ml for the aqueous extract and 1.563 mg/ml for the organic extract. These findings differ from the study findings of essential oils from *Nigella sativa, Syzgum animaticum-Thymus vulgaris* mixture, and *Origanum vulgare* whereby the MIC values were high and ranged from 0.3 to 48.8 mg/ml (Najee *et al.*,2018). The minimum bactericidal activity was 100mg/ml and 3.125mg/ml for the organic extract.

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) exhibited for the aqueous and organic plant extracts on different microbes at different concentrations revealed that the leaves of *L. trifolia* have antimicrobial properties and therefore is a useful medicinal plant as reported by Nalubega *et al.*,2013. Moreover, these findings agree with Saraf *et al*.,2013 and Agwu,2019 who reported that *Lantana* sp. has antimicrobial properties. Therefore, the results support the use of the plant in the management of different infections.

The main objective of safety assessment of any plant with medicinal activity is to find out if the plant causes any adverse effect and also establish the dose level that causes the observed effect (Ibrahim *et al.*,2016). In the acute oral toxicity study using a single dose of the aqueous leaf extract of *L. trifolia*, no significant clinical signs of toxicity or death were observed in the rats, which survived throughout the 14 days observation period. Our findings are similar to those of Pour *et al.,* 2011 who reported absence of clinical toxic signs in mice after administration of *L. camara* (Verbenaceae) leaf extract. The LD_{50} value was found to be greater than 2000mg/kg. This suggests that the aqueous leaf extract of *L. trifolia* has low toxicity and is essentially safe after oral administration (Prasanth *et al.,* 2015).

Lack of signs of toxicity and death after laboratory animals have been treated with a certain test dose indicates that the median lethal dose (LD_{50}) is greater than the test dose (Husna *et al.,* 2013). Furthermore, OECD criteria under its Globally Harmonised Classification System (GHS) for chemical substances and mixtures categorizes substances with LD_{50}>2000-5000mg/kg body weight as unclassified or category five (OECD 2001).

A decrease in locomotor activity which is a sign of sedation was observed after administration of the extract at a dose of 2000mg/kg but it later normalised after ten minutes. According to Santana *et al.,* 2010, the sedative effect of *L. trifolia* extracts in animals may be attributed to flavones and phenylpropanoids. Studies have also been done on *Lantana*

camara which is also found in the Verbenaceae family. Jawonisi and Adoga, 2017 reported the LD_{50} of *L. camara* to be >5000mg/kg body weight hence may be considered safe at lower doses and if used for a short period.

In the current study, there was no significant change in body weight of the treated group compared to the control group after administration of single doses of aqueous extract of *L. trifolia* leaves. However, there was continuous gain of body weight without significant differences between the animals given the aqueous extracts of *L. trifolia* and the control group. This shows that the extract did not affect metabolism of fats, proteins and carbohydrates meaning it did not have inhibitory effect on the growth of the animals (Klaassen, 2001).

One of the systems which is mostly targeted by toxic compounds is the haematopoietic system. Therefore, it is an important index of physiological and pathological status in man and animals (Klaassen 1996). Classification of anaemia is based on the red blood cells indices which include the mean corpuscular haemoglobin, mean corpuscular volume and the mean haemoglobin concentration. Biochemical parameters that are sensitive indicators of organ function or metabolic defects may be altered in the presence of a disease or toxic substances. The liver plays the most important role in the metabolism and detoxification of compounds. Therefore, it is the prime target organ for toxic substances and drugs (Shah *et al*., 2011).

When a substance is available at toxic levels in any biological media, it can cause changes in the blood parameters and this could be due to its toxic effects in the spleen or in the bone marrow resulting in possible alteration in haemopoiesis or even interfering with blood forming precursors (Arika *et al.,* 2016). The laboratory assessment of the haematological parameters indicated that *L. trifolia* did not cause any significant effect on the red blood cells, white blood cells, haematocrit, haemoglobin, mean corpuscular volume, mean

corpuscular haemoglobin and mean corpuscular haemoglobin concentration. This could suggest that these doses did not induce anaemia in the mice. However, the mean corpuscular haemoglobin concentration (MCHC) increased significantly in the male mice that were given a dose of 1000mg/kg aqueous leaf extract *of L. trifolia* compared to the control. This could be due to an error arising from the analyzer since the mean corpuscular haemoglobin concentration values have been found to be very sensitive to the aforesaid errors (Bull *et al.*, 2018).

There was a significant increase in platelets in all groups of mice that received aqueous leaf extract *of L. trifolia* compared to the control. This implies that the aqueous extract could find use in promoting healing of wounds and also be useful in cases of thrombocytopenia as a result of diseases like Dengue fever. (Subenthirian *et al.,* 2013; Atik *et al.,* 2018).

Liver and kidney functions assessment is crucial in the evaluation of the toxic effects of both traditional and modern medicines because these organs have a vital role in metabolism of xenobiotics in the body. According to Koffi *et al.,* 2014, the liver is important in the detoxification of any substance that may cause harm to the body while the kidneys maintain the homeostasis via reabsorption of vital substances and excretion of waste materials. The status of the liver can be determined through performance of serum liver function tests. When tissues have been injured, they tend to release certain enzymes into the circulatory system and some of the enzymes present in the cells of the liver are alanine aminotransferase and aspartate aminotransferase. When an injury occurs within the hepatocytes, the liver releases the two enzymes and this can aid in the assessment of the extent of liver injury (Ike *et al.,* 2016). Alanine transaminase (ALT) is more specific to liver injury while Aspartate transaminase (AST) is a non-specific enzyme whose concentration can vary as a result of injury to several important organs in the body such as liver, muscles, heart and kidney. (McGill, 2016; Mujahid *et al.,* 2017).

In the present study, there were no significant differences in alanine aminotransferase and aspartate aminotransferase levels in mice treated with all the three doses of the aqueous leaf extract of *L. trifolia* compared to the control after the twenty-eight days of the study. However, the non-significant increase in alanine aminotransferase (ALT) and aminotransferase (AST) could imply that there was damage to the liver due to accumulation of toxic compounds such as triterpenoids (Asadu *et al.*,2015). On the contrary, other studies have reported significant elevation of aspartate transaminase, alanine transaminase and alkaline phosphatase after extracts of *L. camara* for a prolonged period (Saini *et al.*,2007).

In the Sub-acute toxicity studies, renal function tests are very important because they help in determining whether the extract has the potential of causing kidney damage. Urea and creatinine are vital parameters that are used as glomerular filtration rate indicators. Therefore, significant changes in the concentrations of urea and creatinine could mean that the extract is nephrotoxic. Kidney damage may affect the glomerular filtration and this may lead to accumulation of creatinine and urea which are also known to be end products of metabolism of proteins (Krstic *et al.*,2016).

In this study, there was a significant increase in urea levels in female mice treated with the aqueous extract at 1000mg/kg as compared to the control group. This could be explained by sex differences. Moreover, there was a significant elevation of creatinine levels in males that received a dose of 1000mg/kg Bwt of the aqueous extract. The levels of creatinine and urea may increase in rats with renal dysfunction especially when the rate of glomerular filtration is reduced. An increase in the creatinine level may suggest that the aqueous extract caused a decrease in the rate of glomerulus filtration which is used as a parameter for assessing the renal function (Olaniyan *et al.*, 2016). However, the findings showed that was no significant difference in the levels of total protein in both female and male mice treated with the extract ($P>0.05$).

The toxic action of a particular chemical can be indicated by the pathological picture observed during the toxicity studies. The liver plays a key role in metabolic processes and is first affected after oral dosing because the chemical has to go through it after absorption and undergo first pass metabolism (Frank and Robert, 2005). Therefore, it is vital to investigate the effects of new drugs on the liver since most of the drugs that cause liver injury during the preclinical studies do not proceed to clinical trials. The build -up of toxic compounds in the liver causes liver damage and this can be assessed through determination of serum enzymes over and above total proteins measurement ((Araujo *et al.,* 2017). Therefore, histopathological studies are important since they provide supportive evidence for haematological and biochemical observations.

In the current study, the livers of the mice in the control group had normal architecture. In addition, the shapes of the hepatocytes were normal, however, there was some scanty congestion of the hepatic veins. The livers from the male mice treated with 250mg/kg body weight were generally normal though there was slight congestion of the hepatic blood vessels. However, the female mice that were treated with 250mg/kg body weight revealed some very mild hepatic injury characterized by focal hepatic swelling and necrosis. This shows that the extract affects both males and females differently. The livers of the animals treated with 500mg/kg body weight also displayed mild form of injury characterized by neutrophil infiltration of the portal areas, diffuse hepatocyte necrosis and scanty congestion of the hepatic vessels. The livers of both male and female mice that received a dose of 1000mg/kg body weight of the aqueous leaf extract of *L. trifolia* appeared normal with scanty congestion of the hepatic blood vessels. The aqueous leaf extract of *L. trifolia* caused focal hepatocyte swelling, neutrophils infiltration and congestion of hepatic vessels. These observations could be attributed to active chemicals present in the extract such as tannins, alkaloids, phenolics and flavonoids which are said to induce inflammatory processes

(Benouadah *et al.,* 2016). Although medicinal plants are natural, they contain bioactive constituents which can cause adverse effects hence the need for toxicity studies.

5.2 CONCLUSIONS

The following outlined conclusions were made from the findings of these studies;

1. *Lantana trifolia* leaves have flavonoids, tannins, saponins, phenolics, terpenoids, alkaloids and reducing sugars which may be responsible for the plant's biological properties hence its use in traditional medicine.
2. The LD_{50} of the aqueous leaf extract of *Lantana trifolia* is above 2000mg/kg body weight. Therefore, this extract may be considered safe on oral administration at doses below 2000mg/kg body weight.
3. The aqueous and organic extracts of *Lantana trifolia* have antibacterial activity against *Staphylococcus aureus, Bacillus cereus and Escherichia coli.* Moreover, both extracts have antifungal activity against *Candida albicans.*
4. The study demonstrates that folk medicine can be as effective as modern medicine in combating pathogenic microorganisms. It reveals the potential of plants being a good source of drugs and validates the use of *Lantana trifolia* in ethnomedicine.

5.3. RECOMMENDATIONS

1. Chronic toxicity studies should be conducted to evaluate the potential of this plant to induce genotoxicity and carcinogenicity.
2. The tested *Lantana trifolia* extracts were found to inhibit growth of *Candida albicans* (fungus), gram -positive and gram-negative bacteria. Therefore, there is need to carry out detailed investigations at molecular level to unravel the actual mechanisms of action and also identify the drug targets in microorganisms tested.
3. The presence of bioactive plant constituents supports the use of *Lantana trifolia* in traditional medicine. Therefore, further isolation, identification, purification and structural elucidation of individual bioactive principle is recommended as this will form the basis for drug discovery and development.

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APPENDICES

Appendix 1: Approval letter

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Dr Ivayo Ronny Musinya
C/o Dept. of PHP&T

REF: FVM BAUEC/2018/176
04/10/2018

Dear Dr Musinya,

RE: Approval of Proposal by Biosafety, Animal use and Ethics committee

Phytochemical screening , antimicrobial activity and toxicity studies of *Lantana trifolia* leaf extracts

By Ivayo Ronny Musinya (J56/87712/2016)

We refer to the above MSc proposal that you re-submitted to our committee for review and approval. We have now reviewed the proposal and are satisfied that you have addressed the issues that had been raised including: reduction in animal numbers, clarifying the issue of early end-points to the experiments, animal husbandry and restraint of animals.

We expect you to work closely with your supervisors who are veterinarians, on the animal work. We hereby approve your work as per your revised proposal.

Rodi O. Ojoo BVM M.Sc Ph.D
Chairman, Biosafety, Animal Use and Ethics Committee,
Faculty of Veterinary Medicine

Appendix 2: Published article from the research findings.

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Research Article

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Acute and Sub-acute toxicity of the aqueous leaf extract of *Lantana trifolia* (Verbenaceae) in experimental rodents

Ronny Ivayo Musinya*, James Mucunu Mbaria, Isaac M Ole-Mapenay

ABSTRACT

Lantana trifolia, a plant of the Verbenaceae family, is traditionally used to treat several diseases; however, empirical data to validate its toxicity profile and safety is lacking. Thus, this study investigated the qualitative phytochemical composition, acute and sub-acute toxicity of the aqueous leaf extract of *L. trifolia* to validate its ethnomedicinal usage. **Methods:** Qualitative phytochemical analysis of the studied plant extract was performed based on standard procedures to appraise its pharmacological value. Acute oral toxicity of the study extract was investigated at dose levels of 300 mg/Kg BW and 2000 mg/Kg BW according to guideline 423 described by the Organization for Economic Co-operation (OECD) for 14 days. Sub-acute oral toxicity of the studied plant extract was investigated at three dose levels (250 mg/Kg BW, 500 mg/Kg BW, and 1000 mg/Kg BW) in Swiss albino mice based on the OECD guideline number 407 for 28 days, after which haematological, biochemical, and histological traits were determined. **Results:** Phytochemical screening revealed the presence of tannins, saponins, phenolics, terpenoids, flavonoids, alkaloids, and reducing sugars. In an acute oral toxicity study, the aqueous leaf extract of *L. trifolia* demonstrated a median lethal dose (LD_{50}) of >2000 mg/Kg BW, depicting its safety. Following sub-acute oral toxicity, the urea levels in female mice which received 1000 mg/Kg BW of the aqueous leaf extract to *L. trifolia* were significantly elevated compared to those of the control group mice ($P<0.05$). Also, significantly higher platelet counts were observed in all the extract-treated mice compared with those of the control group mice ($P<0.05$). Additionally, the mice administered with 1000 mg/Kg BW of the studied plant extracts demonstrated diffuse tubular epithelium degeneration, indicating nephrotoxicity and a dose-related hepatocyte degeneration, indicating hepatotoxicity. **Conclusions:** The aqueous leaf extract of *L. trifolia* may be relatively non-toxic when administered orally for a short period. The aqueous leaf extract of *L. trifolia* induces nephrotoxicity and hepatotoxicity in experimental mice when administered sub-acutely at a dose of ≥1000 mg/Kg BW.

Keywords: *Lantana trifolia*; Acute oral toxicity; Sub-acute oral toxicity; Haematological, Biochemical, and histological traits.

INTRODUCTION

Medicinal plants have been used to manage different human and animal diseases by approximately 80% of the global populations in developing nations [1, 2]. Over the last two decades, the demand and access to traditional medicine have grown exponentially [3]. Despite the widespread use of medicinal plants, there is a lack of precise chemical classification, dosage regimens for various diseases, toxicity profiles, and safety data to appraise their usefulness [4]. Therefore, this study was designed to investigate the qualitative phytochemical composition, acute and sub-acute oral toxicity of the aqueous leaf extract of *Lantana trifolia* to appraise its safety.

Lantana trifolia is a three-leaved, scrambling, an evergreen herbaceous shrub of the Verbenaceae family, which grows uprightly up to 3 metres tall [5]. It grows in the subtropical and tropical regions. Especially on disturbed forests, abandoned cultivated lands, and even roadsides [6]. In Kenya, it is known as "*Shimenenwa*" among the Luhya community and "*Bekaptarit*" among the Marakwet community [7,8]. Some ethnomedical claims associated with various parts of *L. trifolia* include treatment of asthma, common cold, malaria, epilepsy, eye cataracts, conjunctivitis, among other maladies [7, 9]. In addition, it is claimed to boost lactation in breastfeeding mothers [8].

Previous studies reveal that extracts of *L. trifolia* have antimicrobial effects against *Mycobacterium fortuitum* and *Mycobacterium tuberculosis* and anti-inflammatory and antinociceptive efficacy [10, 11]. Besides, some major essential oils, including germacrene D, (E)-caryophyllene, bicyclogermacrene, and alpha-humulene, have been isolated [10]. However, there is insufficient empirical data to validate the ethnopharmacological applications and safety of *L. trifolia*, hence the present study.