STUDIES ON ETHNOPHARMACOLOGY, ANTIMICROBIAL ACTIVITY AND TOXICITY OF *CATHA EDULIS* (VAHL.) FORSSK.EX ENDL. (CELESTRACEAE) IN SPRAGUE DAWLEY RATS

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A THESIS SUBMITTED IN PARTIAL FULFILMENT FOR THE REQUIREMENTS OF THE AWARD OF MASTER OF SCIENCE DEGREE IN PHARMACOLOGY AND TOXICOLOGY

DEPARTMENT OF PUBLIC HEALTH, PHARMACOLOGY AND TOXICOLOGY,

FACULTY OF VETERINARY MEDICINE,

UNIVERSITY OF NAIROBI.

2022

DECLARATION

This Thesis is my original work and has not been presented for an award of a degree in any other University.

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J56/11246/2018

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TITLE OF THE WORK: STUDIES ON ETHNOPHARMACOLOGY, ANTIMICROBIAL ACTIVITY AND TOXICITY OF *CATHA EDULIS* (VAHL.) FORSSK.EX ENDL., IN SPRAGUE DAWLEY RATS

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DEDICATION

I wish to thank my lovely wife, Dr Yvonne Njeri Kariuki for her tireless efforts and guidance. I wish also to thank my parents, Mr George Githua and Susan Githua for believing in me and may God continue blessing you all.

ACKNOWLEDGEMENTS

I would like to acknowledge and sincerely thank my supervisors, Prof T. Maitho and Dr J.M Nguta for their generous guidance, constant encouragement and immense support throughout the study period. It has been an honour and pleasure working with you all on this study.

I am very grateful to my respondents and I would like to thank them for their time and consent to participate in this study.

I would also like to appreciate and thank the staff of the Pharmacology and Toxicology laboratory, Microbiology laboratory and Animal house in the Department of Public Health, Pharmacology and Toxicology of the University of Nairobi for their great input to this study.

I would also like to acknowledge Prof P. Mbuthia of the Department of Veterinary Pathology, Microbiology and Parasitology of the University of Nairobi for his guidance during this study.

I would also like to thank Dr Okumu Mitchel for his assistance and input during this study.

Above all, I am very grateful to Almighty God for making this study possible.

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LIST OF ABBREVIATIONS AND ACRONYMS

ALP	Alkaline Phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ANOVA	Analysis of Variance
AR	Analytical Reagent
BWT	Body Weight
CTMDR	Center for Traditional Medicine Research
Fl	Femtolitres
G/dl	Grams per decilitre
G/l	Grams per litre
HB	Haemoglobin
HPLC	High performance Liquid Chromatography
KEMRI	Kenya Medical Research Institute
LYM	Lymphocytes
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration
MHA	Muller Hinton Agar
MHB	Muller Hinton Broth
NACOSTI	National council of science, technology and innovation
PHPT	Public Health, Pharmacology and Toxicology

PLT	Platelets
RBC	Red Blood Cells
TB	Total Bilirubin
TSA	Tryptone Soy Agar
U/L	Units per litre
µmol/L	Micromole per litre
WBC	White Blood Cells

ABSTRACT

Catha edulis (Vahl.) Forssk. ex Endl. (Celastraceae) is a plant which is predominantly used for its euphoretic and stimulant actions. Other indigenous uses of the plant which have been reported include; treatment of Helminthiasis, toothache, asthma, gonorrhea, heartburn, diarrhoea and fatigue. Information on the antimicrobial efficacy and safety of *Catha edulis* (Vahl.) Forssk. ex Endl., from Embu County is limited. This study aimed at documenting the Ethnobotanical uses of *Catha edulis* (Vahl.) Forssk. ex Endl., which is grown in Embu County, antimicrobial properties and toxicity. Ethnobotanical data on *Catha edulis* (Vahl.) Forssk. ex Endl., was collected from 35 key informants using semi-structured questionnaire between July and November 2020. Antimicrobial efficacy of *Catha edulis* Acetone extracts (CEAC), *Catha edulis* Aqueous extracts (CEAQ), and *Catha edulis* Methanol extracts (CEMET) against *Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans* were determined by using micro broth dilution and agar well diffusion techniques. Changes in body weights, Haematological, and Biochemical parameters of female and male *Sprague Dawley* rats over a 28-day period were used to determine the toxicity of the plant extracts. The Haematological parameters which were evaluated included; White Blood Cells (WBC), Red Blood Cells (RBC), Lymphocytes (LYM), Haemoglobin (HGB), Mean Corpuscular Haemoglobin Concentration (MCHC), Platelets (PLT) and the Mean Corpuscular Volume (MCV). The Biochemical parameters which were evaluated included; Kidney function tests (KFT's) such as Creatinine (CR), Urea (UR), Total protein (TP), and Albumin (ALB) and Liver function tests (LFT's) such as Alanine amino transferase (AST), Alkaline phosphatase (ALP), Alanine transferase (ALT), Direct bilirubin (DB) and Total bilirubin (TB). All informants were married males who were over 50 years old in age and had at least a Primary level of education. The majority of the informants, who were mostly from Kithunthuri Sub Location had been practicing Traditional medicine for more than ten years and had received no formal training. The sources, local names, preparation, storage conditions, indications for Human and Veterinary uses, frequency of use, dosage, and side effects of *Catha edulis* (Vahl.) Forssk. ex Endl., were documented. The Aqueous, Methanol and Acetone plant extracts were ineffective against gram negative *E.coli*, *P. aeruginosa* and *C. albicans* (fungi). Moreover, the extracts had limited efficacy against gram positive *B. cereus* and *S. aureus.* The mean weight gains in female and male rats which were given low, intermediate or high doses of CEAQ orally were not significant statistically ($p<0.05$). Female rats demonstrated significantly greater ($p<0.05$) mean levels of DB and CR than male rats after receiving a 250 mg/kg dosage of CEAQ. Male rats, on the other hand, had significantly higher ($p<0.05$) average levels of UR, ALP, AST, ALT, and TP than female rats. There was no substantial difference in the mean levels of TB and Albumin in male and female rats at a dose of 250 mg/kg of CEAQ. The mean level of ALB was significantly greater *($p<0.05$)* in female rats than male rats at a dose of 500 mg/kg of CEAQ. However, the average levels of ALP, AST, and ALT in male rats were significantly greater *($p<0.05$)* than in female rats. There was no difference in the mean levels of UR, CR, TB, DB and TP in male and female rats at a dose of 500 mg/kg of CEAQ. At a dose of 1000 mg/kg of CEAQ, female rats had substantially greater (*$p<0.05$)* mean levels of UR, TB, and ALB than male rats. However, the average levels of CR, ALP, AST, and ALT, and TP in male rats were significantly greater *($p<0.05$)* than in female rats. There was no statistically substantial change in the mean levels of DB and TB in male and female rats at a dose of 1000 mg/kg of CEAQ. The mean levels of WBC and LYM were significantly greater *($p<0.05$)* in male rats than female rats at low and

intermediate doses of CEAQ. There was no substantial statistically change in the average levels of PLT, HGB, MCHC, RBC, and MCV in male and female rats at low and intermediate doses of CEAQ. The mean levels of WBC, LYM, and MCV were significantly greater *($p<0.05$)* in male rats than female rats at a 1000 mg/kg dose of CEAQ. The average levels of PLT, HGB, MCHC, and RBC were significantly greater *($p<0.05$)* in male rats than female rats at a 1000 mg/kg dose of CEAQ. Histopathological examination of the liver, spleen, kidney, heart, and testes of male and female rats revealed that high, intermediate and low doses of CEAQ resulted in local congestion of the cardiac and hepatic vessels. Moreover, high, intermediate and low doses of CEAQ resulted in localized interstitial connective tissue proliferation, multifocal kidney interstitial haemorrhage and localized tubular epithelium necrosis in the kidney in some female and male rats. However, there were no adverse effects on lungs and testes. The Ethnopharmacological relevance of *Catha edulis* (Vahl.) Forssk. ex Endl., which was collected from the Mbeere community was documented. The limited antimicrobial efficacy and observed toxicity limit the use of leaves for medicinal purposes from *Catha edulis* (Vahl.) Forssk. ex Endl.

CHAPTER ONE

INTRODUCTION

1.1. Background Information

Medicinal plants have been utilized in the treatment of diseases since ancient times. Their use dates back to prehistoric times with the first incidence having been reported in France where plants were first accepted for healing purposes. Consequently, the World Health Organization (WHO) recognizes ethnic medicine as a main Healthcare service which guarantees better Healthcare provision to the users (Van Wyk & Wink, 2018). One of the plants with medicinal value is *Catha edulis* (Vahl.) Forssk. ex Endl. (Celastraceae) which is commonly referred to as *Muguka* by the local Mbeere South community of Embu County. *Muguka* is a variant to *Catha edulis* Forssk also referred to as *Khat* (Ongeri et al., 2019). The leaves of *Muguka* are chewed for psychostimulatory purposes while for *Khat* it's the stem. Unlike, *Khat*, *Muguka* is a relatively short shrub and is produced in Embu County while *Khat* is mostly grown in Meru County (Michuki & Kivuva, 2013). *Khat* has been in use by Ethiopian communities since ancient times. Its use later spread throughout East and South Africa (Berihu et al., 2017; Getahun et al., 2010). *Catha edulis* Forssk (Celastraceae) was reported first in 1697 by a French scientist Barthélémy d'Herbelot de Molainville, after his first travel to Yemen. He highlighted that it was made up of a seed that was not known and had been forbidden by Yemen Doctors due to its effects on the brain. However, it was not until 1975 when the U.S laboratories first gave an insight of the composition of *Catha edulis* Forsk when they revealed that it contained cathinone as the main biochemical active ingredient (Al-Juhaishi et al., 2012). The growing use of *Catha edulis* Forsk in overseas Countries is attributed to African emigration into these Countries which brings forth

its spread and use (Ageely, 2009). Further, the use of *Catha edulis* Forsk has spread to Australia, Asia and the U.S (Berihu et al., 2017; Stefan & Mathew, 2005).

Catha edulis (Vahl.) Forssk. ex Endl., is a plant belonging to the family Celastraceae and is mainly grown in the horn of Africa to South Africa and Yemen. Moreover, the plant has a native origin of Southern Arabia and East Africa. Its leaves are chewed for stimulatory effects (Osman & Söderbäck, 2011; Wabe, 2011). According to statistical data, the shrub is chewed daily by over 20 million people in these Countries (Riyaz et al., 2014a). The taste of the plant's leaves is slightly sweet and astringent. Similarly, the plant is hardy and seedless and can grow in a variety of soils and climatic regions (Wabe, 2011). Its leaves produce an aromatic odour. As a consequence, the chewing habit for *Catha edulis* (Vahl.) Forssk. ex Endl., has been heightened in males thereby culminating in the spread of its use (Riyaz et al., 2014a). The World Health Organization classifies *Catha edulis* (Vahl.) Forssk. ex Endl., as a potential drug of abuse, however, its addictive capacity is less than that of tobacco and alcohol (Al-Juhaishi et al., 2012). Moreover, *Catha edulis* (Vahl.) Forssk. ex Endl., contains cathinone as the main active ingredient which forms the heart of its psychostimulatory effects (Alsalahi et al., 2012). In the sentiments reiterated by Ongeri *et al*., (2019), an average 100 g *Catha edulis* leaf contains 120 mg of Cathine, 36 mg of cathinone and 8 mg of norepinephrine. The plant also contains an array of Pharmacological compounds which include; Terpenoids, Alkaloids, Sterols, Tannins, Vitamins, Minerals, Glycosides, and Amino acids. Environmental and climatic conditions are the principal factors which dictate the chemical profile of the leaves of this plant (Wabe, 2011). Previous studies concerning the Pharmacological and Toxicological effects of *Catha edulis* reveal that it has potential to prompt cytotoxic effects in kidney and liver cells of rabbits (Abdul-Mughni et al., 2018). Similar studies have also demonstrated the capacity of the plant to act on

the Central Nervous System and the Gastrointestinal System. Its main effects in the Gastrointestinal System include; constipation and urine retention whereas in the Central Nervous System it constitutes alertness, psychiatric symptoms, tolerance and dependence (Wabe, 2011). Regardless of the medicinal effects of *Catha edulis* (Vahl.) Forssk. ex Endl., several toxic effects accompany its use such as tachycardia, anorexia, insomnia, constipation, increased blood pressure, irritability, impaired sexual potency in males, migraine, and malaise (Wabe, 2011).

In Kenya, *Catha edulis* (Vahl.) Forssk. ex Endl., is a highly valued horticultural crop which accounts for 60% of the total exports (Ongeri et al., 2019). It is cultivated in Eastern parts of Kenya and has remained a common commercial crop in the Country (Ongeri et al., 2019). The region of Mbeere South, Embu County, where *Muguka* cultivation is prevalent, is of great concern to the public. Several varieties of *Catha edulis* Forsk grown in Embu County have been categorized into *Muguka, Kibwe, Gitu, Mutimutiri, Mugwathiri,* and *Mugumo* according to Unweighted Pair Group Method with Arithmetic Mean (UPGMA) classification (Kiunga et al., 2016). This study focused on *Muguka* as it forms one of the most consumed varieties in the County due to its inebriating effects and its low cost.

1.2. Problem Statement

Bacterial infections still remain a vital cause of human health illnesses in Kenya (Karambu et al., 2013). As such, most people have resorted to Traditional medicine which is a cheaper and user-friendly way of disease treatment when compared to the use of modern-day chemotherapy. Moreover, the problem of resistance to antibiotics has led to the limited treatment options for bacterial infections (Lee Ventola, 2015). However, with the use of Traditional medicines like *Catha edulis* (Vahl.) Forssk. ex Endl., being in place, the knowledge about the efficacy and toxicity of these medicines is scanty and remains to be elucidated. More than 70% of Kenyans

rely heavily on traditional medicine as the primary source of disease treatment (Okumu et al., 2017). As such, Scientists have a better perception of the rationale use of *Catha edulis* (Vahl.) Forssk. ex Endl., and the side effects it can impact on the users by conducting laboratory experiments and procedures. As one of the Sustainable Development Goals (SDGs) of the United Nations more specifically SDG 3 which emphasizes on healthy lives and promotion of well-being for all at all ages, it is crucial for Scientists to study and discover new and alternate Pharmacological aspects of plants (Organization, 2016). The present research aimed at establishing the antimicrobial effects against fungi, Gram negative and Gram-positive bacteria, Sub-acute Toxicity and Ethnopharmacological uses of *Muguka.* In addition, the current study aimed at building on the literature of *C. edulis* (Vahl.) Forssk. ex Endl., (Muguka) and provide more knowledge on what is known about the plant.

1.3. Justification

Different traditional varieties of *Catha edulis* Forsk have different Pharmacological actions (Al-thobhani et al., 2008). However limited information is known of the variety *Muguka* except for its psychostimulant abuse and hence necessitating a thorough investigation on its Pharmacological action especially the Ethnopharmacological use, Antimicrobial aspect, and Toxic effects of the plant.

1.4. Objectives

1.4.1. Overall Objective

The overall objective of the study was to investigate the local traditional uses, antimicrobial activity and toxicity of *Catha edulis* (Vahl.) Forssk. ex Endl., (Muguka) a shrub which is commonly used for its stimulatory effects by the Mbeere South community of Embu County in Kenya.

1.4.2. Specific Objectives

The specific objectives of the study were;

i. To investigate the Ethnopharmacological uses of *C.edulis* (Vahl.) Forssk. ex Endl., in Mbeere South Sub County, Embu County.

ii. To evaluate the antimicrobial properties of *C.edulis* (Vahl.) Forssk. ex Endl., on Gram negative bacteria, Gram positive bacteria and fungi.

iii. To determine Sub-acute Toxic effects of *C.edulis* (Vahl.) Forssk. ex Endl., in *Sprague Dawley* rats.

CHAPTER TWO

LITERATURE REVIEW

2.1. *Catha edulis* (Vahl.) Forssk. ex Endl. (Celastraceae)

Celastraceae family contains shrubs, lianas, and trees. One of the members of the Celastraceae family is *Catha edulis* (Vahl.) Forssk. ex Endl., an evergreen flowering plant which is mostly found in Arabia and East Africa. It is chewed daily by over 20 million people in East Africa and Southern Arabia (Riyaz et al., 2014). Moreover, *Catha edulis* (Vahl.) Forssk. ex Endl., is an evergreen flowering dicotyledonous plant which has psychostimulant properties (Alfaifi et al., 2017). The chewing habit of *Catha edulis* (Vahl.) Forssk. ex Endl., has grown viral especially in East Africa and Asia. Similarly, the rampart importation and distribution of *Catha edulis* (Vahl.) Forssk. ex Endl., has led to its spread use in U.S.A and Europe (Manghi et al., 2009). *Catha edulis* (Vahl.) Forssk. ex Endl., has a long history of traditional use coupled with Government regulation policy on its trade (Anderson & Carrier, 2009). The plant grows at high altitude in Eastern Africa, and the Middle East (Al'Absi & Grabowski, 2012).

Figure 2.1: Photograph of *Catha edulis* (Vahl.) Forssk. ex Endl. (Muguka) plant. The picture was taken in Mbeere South Sub County, Embu County by Kevin Kariuki.

2.2. Ethnopharmacological uses of *Catha edulis* (Vahl.) Endl.

Several Ethnopharmacological uses of *Catha edulis* (Vahl.) Forssk. ex Endl., have been documented in Kenya. For instance, in Embu and Meru Counties 13 Ethnopharmacological uses of *Catha edulis* have been reported which include; treatment of Helminthiasis, toothache, asthma, erectile dysfunction, general body pain, gonorrhoea, heartburn, influenza, pneumonia, stomach upset, coughing, diarrhoea and fatigue (Kiunga et al., 2016). *Catha edulis* (Vahl.) Forssk. ex Endl., leaves form the main parts which have been shown to possess Medicinal value. Nonetheless, the young shoots and stems have been shown to exhibit scanty Medicinal and Pharmacological properties. The application of *Catha edulis* (Vahl.) Forssk. ex Endl., in the

management of erectile dysfunction has been supported by studies conducted in baboons, which revealed that *Catha edulis* (Vahl.) Forssk. ex Endl., increases the levels of testosterone, erection and libido (Mwenda et al., 2003). Several other studies conducted by (Nyongesa et al., 2008) revealed contradicting results which indicated that *Catha edulis* (Vahl.) Forssk. ex Endl., reduces testosterone levels. In addition, the chewing habit of *Catha edulis* (Vahl.) Forssk. ex Endl., has been show to decrease the cases of periodontal disease and gingivitis (Al-hebshi & Al-ak'hali, 2010). *Catha edulis* (Vahl.) Forssk. ex Endl., is also held responsible for the treatment of stomach upset (Kokwaro, 2009).

2.3. Phytochemical composition of *Catha edulis* (Vahl.) Endl.

Catha edulis (Vahl.) Forssk. ex Endl., contains amino acids, minerals, vitamins, glycosides, forty alkaloids and tannins (Gambaro et al., 2012; Wabe, 2011). The chemical profile of its leaves is determined by the environmental and climatic conditions. It also contains cathinone, an alkaloid with an amphetamine-like activity which is considered to be responsible for euphoria, loss of appetite and excitement (Al-Juhaishi et al., 2012). Further, cathine and norephedrine comprise the other major constituents of *Catha edulis (Vahl.) Forssk. ex Endl.*,(Alfaifi et al., 2017). During maturation of the plant, there is metabolism of cathinone to cathine [(+)-norpseudoephedrine] and (-)-norephedrine)] in a ratio of 4:2:10. Other classes of phenylalkylamines alkaloids of *Catha edulis* Forssk include; merucathinone, merucathine, phenylalkylamines, and pseudomerucathines (Wabe, 2011). During harvesting, cathinone undergoes decomposition due to instability. The decomposition process results in a dimer (3, 6-dimethyl-2, 5-diphenylpyrazine) and other minor fragments (Wabe, 2011). The composition of phenylpropylamines varies between the regions occupied by the plant and the Country of origin (Wabe, 2011).

2.4. Pharmacological effects of *Catha edulis* (Vahl.) Forssk. ex Endl.

Cathinone and cathine are reported to be the most active Pharmacological constituents in *Catha edulis* (Vahl.) Forssk. ex Endl., (Wabe, 2011). Cathinone is a chemical constituent which is also called alpha –amino propiophenone and is amphetamine-like in structure. It is mainly a Sympathomimetic agent (Al-Juhaishi et al., 2012)**.** The structure of amphetamine and cathinone are similar hence they share similar Pharmacological actions. However, cathinone has lower potency. Cathine which is referred to as norpseudoephedrine and phenylpropanolamine has been shown to be 7 times less potent than cathinone (Ali et al., 2011). Both cathine and cathinone share similar psychostimulatory effects on the Nervous System. The different compounds in *Catha edulis* (Vahl.) Forssk. ex Endl., mediate its Pharmacological effect (Muema et al., 2016). Most of the Pharmacological outcomes of *Catha edulis* (Vahl.) Forssk. ex Endl., are centered on the Gastrointestinal System and the Nervous System (Wabe, 2011). These Pharmacological responses include; constipation, dependence, tolerance, increased alertness, and urine retention (Mwenda et al., 2006).

2.4.1. Mechanisms of action of Cathinone and Cathine

Cathinone and cathine interact with both serotonergic and dopaminergic receptors which are found in the Peripheral and Central Nervous System for their actions (Geisshüsler & Brenneisen, 1987; Odenwald et al., 2005). Moreover, cathinone acts by the stimulation of the Sympathetic-Autonomic Nervous System. The effects of *Catha edulis* (Vahl.) Forssk. ex Endl., are similar to those of 0.8mg/kg of cathinone dose. The maximum concentration in plasma of *Catha edulis* (Vahl.) Forssk. ex Endl., is reached after 127 minutes with a half-life of 260 minutes on average (Al-Juhaishi et al., 2012).

Cathinone acts by enhancing the release of catecholamines from presynaptic sites coupled with their reuptake leading to their spatial presence in the presynaptic sites (Banjaw et al., 2006). Cathinone causes the release of serotonin and dopamine either directly or indirectly by acting on dopaminergic and serotonergic transporters. Consequently, this results in the effects of its impacts on the Neurobehavioural Systems (Berihu et al., 2017). Cathinone has also been shown to cause indirect dopamine antagonism by enhancing the release of dopamine at presynaptic cleft as well as the reuptake of dopamine inhibitor (Wabe, 2011). Nonetheless, *Catha edulis* (Vahl.) Forssk. ex Endl., causes acute effects which involve increased blood pressure, increased heart rate, increased alertness, and low appetite (Ali et al., 2011). The mechanisms of action of cathinone on the brain results in behavioural effects which involve stereotype behaviours, excitation and increased locomotor activity (Al-Juhaishi et al., 2012; Hadi M. Mujlli, 2005).

2.5. Toxicological effects of *Catha edulis* (Vahl.) Forssk. ex Endl.

The acute effects of *Catha edulis* are similar to those of methamphetamine and cocaine. In most cases, there is transient disorientation and confusion, a phenomenon linked to psychosis (Sheikh et al., 2014). In cases of acute intoxication, sources demonstrate that it emanates into Sympathetic arousal (al'Absi et al., 2014). The chronic usage of *Catha edulis* (Vahl.) Forssk. ex Endl., increases the risks of diseases like Gastro-intestinal, Genitourinary and Cardiovascular diseases (Al-Juhaishi et al., 2012). Studies conducted by Berihu *et al*., (2017) demonstrate that *Catha edulis* (Vahl.) Forssk. ex Endl., has effects on memory discrepancy. Similar studies conducted by Alsalahi *et al.*, (2012) revealed that its consumption results in neuropsychological disturbances like neurosis and increased diastolic pressures (Corkery et al., 2011). In addition, gastritis, duodenal ulcers, and haemorrhoids have shown to be prevalent among the users.

2.6. Antimicrobial effects of *Catha edulis* (Vahl.) Forssk. ex Endl.

Five studies to date have demonstrated the effects of *Catha edulis* on both intestinal and oral microbiota. Studies indicate that *Catha edulis* has effects on periodontal bacteria such as *Streptococcus mutans* by interfering with the formation of biofilms which are adherent to the oral mucosa. Similar studies have also showed the effects of *Catha edulis* on *Viellonella parvula* and *Bacteroides forsythus* (Al-Maweri et al., 2018). Al-Hebshi et al. (2010) found out that the chewing habit of *Catha edulis* reduces the total burden of pathogens among the pathogenic and normal oral microbiota, rather than increasing bacterial colonization of gingival plague. Furthermore, *Catha edulis* has been shown to be active against some gram-positive bacteria like *Staphylococci spp* and *Bacillus spp* (Al-Maweri et al., 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Materials

3.1.1 Chemicals, drugs and microorganisms

Acetone 99.5% AR, Methanol 99.8% HPLC grade, Dimethyl Sulfoxide AR grade and Diethyl ether were bought from Skytech Africa Supplies Limited, Kenya. Fluconazole 150mg capsule (Universal corp limited), Ciprofloxacin SD142-ICT (Himedia) and Erythromycin SD013-ICT (Himedia) were also bought from Skytech Africa Supplies Limited, Kenya. *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778), and *Pseudomonas aeruginosa* (ATCC 27853) were obtained from the stock cultures in the Microbiology Laboratory of the Department of Public Health, Pharmacology and Toxicology (PHPT) of the University of Nairobi. Muller Hinton Broth (MHB) and Muller Hinton Agar (MHA) were purchased from Chemoquip limited, Kenya.

3.1.2 Instruments

The instruments which were used in the study included; Mettler top Pan Balance, Heidolph Magnetic stirrer, Rotary Evaporator, Humastar 100 automated analyzer, Swelab haematology analyzer and Labconco freeze dryer.

3.1.3 Experimental animals

Twenty young healthy female and 20 young healthy male *Sprague Dawley* rats aged between 7 and 9 weeks were purchased from Kenya Veterinary Laboratory Services and were housed at the PHPT animal house in Polypropylene cages measuring 35L × 25W × 18H. Soft wood shavings were used as the beddings and were spread evenly in the cages. The animals were fed on a regular diet of Unga feed pellets and water *ad libitum.* The temperature of the housing was 24±2

°C and relative humidity was between 41% and 58%. With 12 daylight hours and 12 darkness hours, the lighting was natural.

3.2. Study area

The fresh leaves of *Catha edulis* (Vahl.) Forssk. ex Endl., were obtained from 0°36'23.7"S latitude and 37°31'14.3"E longitude in Mbeere South Sub County of Embu County. The County is located in Central part of Kenya and has four Constituencies. The County has a total area of 2,818 square kilometres and an estimated population of 577,390 persons as reported in the 2009 National Census. The temperature of Mbeere South region is approximately 28 ^{0}C on average and has an annual rainfall of between 781 mm and 1210 mm. The area has an altitude of 1350 meters above sea level. Laboratory work was undertaken in the Department of Public Health, Pharmacology and Toxicology of the University of Nairobi and Kenya Medical Research Institute (KEMRI) Nairobi. Figure 3.2.1 illustrates the Geographical location of Mbeere South Sub County in Embu County, Kenya.

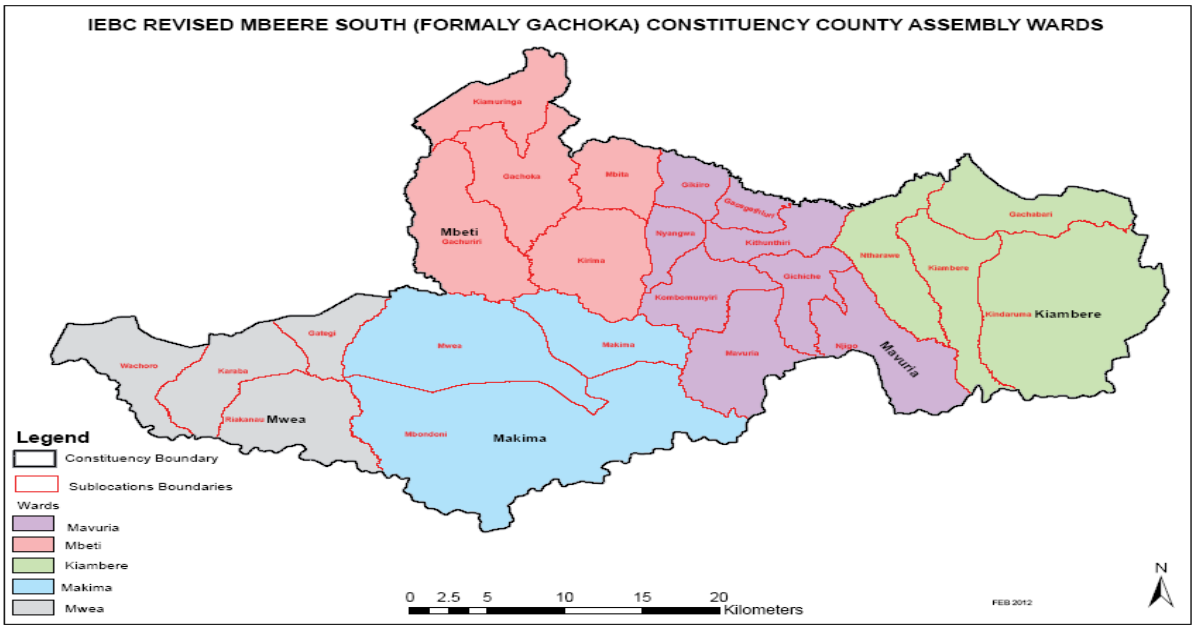


Figure 3.1: Map of Mbeere South Sub County, Embu County, Kenya (Mwita et al., 2016)

3.3 Methods

3.3.1 Ethnopharmacological field survey and collection of plant materials

Snowballing sampling technique as described by (Kiunga et al., 2016) with a few modifications was used in this survey. Information on the Traditional uses of *C.edulis* (Vahl.) Forssk. ex Endl., was generated strictly from the community of Mbeere South through open and closed ended questionnaires targeting the local Herbalists and *Muguka* farmers.

Collection of the plant leaves was conducted by a team which comprised of a research team, a local farmer and a Herbalist with a good knowledge of the area and Ethnopharmacological uses of *C.edulis* (Vahl.) Endl. A sample of the freshly plucked *Muguka* leaves were put in between newspapers, packaged in a carton and transported to the National Museum of Kenya Herbarium for voucher specimen identification, authentication and deposition. The specimen was identified as *Catha edulis* (Vahl.) Forssk. ex Endl., with a reference number of NMK/BOT/CTX/1/2. The rest of the other leaves were packaged in woven bags and were transported to the PHPT laboratory for drying.

3.3.2 Preparation of Plant Extract

The leaves were kept in a well-ventilated room with a natural lighting at the Department of PHPT and were left to air dry for four weeks. The leaves were then pulverized in an Electric Mill, and the powder was stored in brown paper bags that were firmly sealed while awaiting extraction.

Figure 3.2: Photograph of *Catha edulis* (Vahl.) Forssk. ex Endl. (Muguka) leaves air drying. The picture was taken in the PHPT Laboratory University of Nairobi by Kevin Kariuki.

3.3.3 Acetone extract

A total of 1214.8 grams of *Muguka* leaves powder was weighed using a top Pan Balance and put in an extraction jar. A total of 3000 ml of 99.5% AR grade Acetone was added to the powder in an extraction chamber and stirred using a glass rod. The preparation was left to macerate for 72 hours under constant stirring using a Magnetic stirrer. The mixture was then filtered into a round bottomed flask using Whatman filter paper No.1. The filtrate was put on a Rotary Evaporator for concentration and evaporated at 35 ^{0}C for 2 hours. The resulting content was poured into two amber glass bottles, sealed with aluminium foil, and were placed in a sand bath for further drying at 40°C.The Acetone extract yielded 19 g after one week of sand bath drying and was later kept in a refrigerator at 4 ^{0}C to 8 ^{0}C.

Figure 3.3: Photograph of a Rotary Evaporator. The picture was taken in the PHPT Laboratory of the University of Nairobi by Kevin Kariuki.

3.3.4 Methanol extract

A total of 300 g of the *Muguka* powder extract was weighed using a top Pan Balance and put in an extraction jar. A total of 1000 ml of 99.8% HPLC grade Methanol was added to the extraction jar and the mixture was stirred using a glass rod. The mixture was then put in a Magnetic Stirrer for 72 hours and then filtered into a round bottomed flask using Whatman filter paper No.1. The filtrate was put on a Rotary Evaporator for evaporation and concentration at 50 ^{0}C and the process took 2 hours. The resulting content was poured into an amber glass bottle, sealed with aluminium foil, and was placed in a sand bath for further drying at 40°C. The sample which was

obtained after one week of drying at the sand bath was 18.16 g and it was kept in a refrigerator at 4^0C to 8^0C.

3.3.5. Aqueous extract

This process was carried out at KEMRI Center for Traditional Medicine Research (CTMDR) Laboratory. A total of 1180 g of the powdered extract was soaked in 6500 ml of distilled water. The mixture was heated in a water bath for 2 hours at 70^0C in order to activate extraction. The mixture was filtered using a cotton gauze and then passed through a cotton wool. A total of 5.1 litres of the filtrate which was obtained was put in freeze drying flasks and freeze-dried using carbon ice. A 99.5% AR grade Acetone was added to the dry ice pellets in order to enhance quick solidification of the extract. The solidified extract was put in a freeze dryer for 48 hours in order to separate the extract from the water. The sample obtained was 86.45 g which was transferred to PHPT Laboratory in a plastic bottle and was stored in a freezer.

3.4. Antimicrobial assay

3.4.1 Preparation of the culture media

A total of 6.3 g of Muller Hinton Broth (MHB) was mixed with 300 ml of distilled water. The solution was then warmed using a Microwave for about 2 minutes in order to help the MHB to dissolve well. A total of 2 ml of the solution was poured into test tubes which were then sterilized using a pressure Cooker for 15 minutes. After sterilization, the test tubes were left to cool and were stored in a cool dry place.

3.4.2 Sub culturing of the microorganism specimens

The microorganisms which were used included; *Staphylococcus aureus* and *Bacillus cereus* as the gram-positive bacteria, *Pseudomonas aeruginosa* and *Escherichia coli* as the gram-negative bacteria and *Candida albicans* as the fungus.

Two loopfuls of each microorganism was streaked using a sterilized wire loop on Tryptone Soy Agar (TSA) plates and was incubated for 24 hours at 37 ^{0}C apart from *Candida albicans* which was incubated at room temperature of 24.4 ^{0}C for 24 hours. The cultures were then suspended in 10 ml of sterile physiological saline to reach a concentration of 0.5 MacFarland Standards.

3.4.3 Microbroth dilution

The method described by the Clinical and Laboratory Standards Institute (CLSI) (Wayne, 2014) was used. A total of 3.2 g of the Acetone, Methanol and Aqueous extracts were dissolved in 4ml of MHB in order to achieve a concentration of 800 mg/ml. A series of 8 culture tubes containing 2ml of MHB were lined up in a rack in duplicates per each extract. The Two-fold serial dilution was done from the stock solution and the concentrations which were achieved were 800 mg/ml, 400 mg/ml, 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml. The tubes were then inoculated with 100 µl of the respective bacterial and fungal suspension using a 1 ml micropipette and were incubated overnight at 35 ^{0}C except for the fungal samples which were kept at room temperature for 72 hours. A positive control was done where the test tubes included the microorganisms and commercially available antimicrobial drugs. The antimicrobial drugs used were Ciprofloxacin, Erythromycin and Fluconazole. Dimethyl Sulphoxide (DMSO) was used as the negative control.

3.4.4 Agar well diffusion

The procedure recommended by (Wayne, 2014) was used. Mueller Hinton Agar (MHA) was prepared according to the Manufacturer's instructions and was sterilized at 121 ^{0}C for 15 minutes. The MHA was then poured onto sterilized Petri dishes. The petri dishes were later inoculated with the respective microorganisms using cotton swabs. A total of 4 holes of about 6mm in diameter were punched aseptically using a sterile cork borer in each petri dish and were numbered. A total of 100 µl of the plant extract solution at different desired concentrations was added into the numbered wells. The plates were incubated at 37 ^{0}C for 24 hours except the ones containing the fungal strain which were incubated at room temperature of 24.4 ^{0}C for 24 hours. A positive control containing the respective antimicrobial drugs and a negative control containing Dimethyl Sulphoxide (DMSO) were maintained in this experiment. The diameter of the zones of inhibition was measured in mm and later used to determine the microbial growth. Zones of inhibition diameter of ≤6.00 mm was interpreted as no activity and >6.00 mm as susceptible to the plant extracts.

3.4.5 Determination of Minimum Bactericidal Concentration and Minimum Inhibitory Concentration

The test tubes with the least concentration which did not record any growth or show turbidity from the microbroth dilution were recorded as the Minimum Inhibitory Concentration (MIC) and were cultured on MHA petri dishes through pour plate method and were incubated overnight at 35 ^{0}C. The Minimum Bactericidal Concentration (MBC) which indicated 99.5 percent kill of the initial inoculum was defined as the lowest concentration with no observable growth. The MIC and MBC were determined according to Bloomfield (1991) by plotting natural logarithm of concentration in x- axis and inhibition zone square value in y-axis.

3.5 Sub-Acute toxicity assay

3.5.1 Experimental animals

The protocol which was described previously in section 3.1.3 was used in the study.

3.5.2 Preparation of animals

The Organisation for Economic Co-operation and Development (OECD) guidelines 407 (2008) was used. Random selection of *Sprague Dawley* rats was done and they were kept in cages of five animals each according to their sex. The animals were left for 10 days feeding on rat pellets and water *ad libitum* in order to acclimatize. After acclimatization, the rats underwent an overnight fasting before commencement of the study. The rats were then weighed using a top Pan Balance and their tails were labelled with a red marker for identification purposes after which they were assigned randomly to four treatment groups i.e., the Control group, High dose group (1000mg/kg BW), Medium dose group (500mg/kg BW) and Low dose group (250mg/kg BW) each comprising of 5 animals.

3.5.3 Sub-acute toxicity assay of Aqueous plant extract

Sub-acute toxicity was assessed using the OECD guidelines 407 (2008) and Aqueous *Catha edulis* (Vahl.) Forssk. ex Endl., extracts were used in the study. The formula used in the study to do the dose calculation was adopted from (Okumu et al., 2017) as described below ;

$$\text{Volume of extract required (ml)} = \frac{\text{Dosage of extract (mg / kg)} \times \text{weight of rat (kg)}}{\text{Concentration (mg / ml)}}$$

The Aqueous extract was administered daily through intragastric gavage using a curved gavage needle to the rats in the high dose, medium dose and low dose groups and then monitored for any behavioural, morbidity and mortality changes. The control group received distilled water as the placebo and this was also weight dependent dosing. The weight was measured weekly and the

dosing was adjusted according to the weights of the rats. Approximately, 200 g of rats' pellets and 500 ml of water were measured and initially given to each cage. This was monitored and adjusted daily and the consumption was recorded weekly. The temperature and humidity of the animal room where the animals were kept were recorded daily.

3.5.4 Blood collection and organ harvesting

After the 28 days study, the rats were monitored for an extra day in case of any delayed changes. They were fasted overnight, weighed and anaesthetized using Diethyl ether for purposes of blood collection through the retro orbital vein using capillary tubes. Two sets of blood samples were collected, one set for the Haematological assay was collected in EDTA tubes and the other set for Biochemistry assay was collected in clot activator tubes. The rats were later euthanized using Diethyl ether in a glass chamber and were observed macroscopically for signs of gross lesions on organs which were harvested. The organs which were collected included; liver, lungs, kidney, heart and gonads.

3.5.5 Haematological assay

The blood samples which were collected in EDTA tubes were transported to Mama Lucy Teaching and Referral Hospital where they were analysed using a Swelab Haematology analyzer. The parameters which were examined included; White Blood Cells (WBC), Platelets (PLT), Haemoglobin (HGB), Lymphocytes (LYM), Red Blood Cells (RBC), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Volume (MCV).

3.5.6 Biochemistry assay

The blood samples which were collected in the clot activator tubes were left to stand for 15 minutes in order to allow time for clotting. The tubes were later centrifuged for 15 minutes in order to obtain the supernatant. A Pasteur Pipette was used to transfer the serum into Eppendorf

tubes and these were transported to Embu Level 5 Hospital in an ice box. The analysis was done using a Humastar 100 analyzer. The parameters which were examined included; Urea, Creatinine, Total Bilirubin, Bilirubin Direct, Alkaline Phosphatase, Alanine Aminotransferase, Aspartate Aminotransferase, Total Protein and Albumin.

3.5.7 Histopathology

After necropsy, the organs which were harvested were preserved in containers containing 10% Formalin and they were transported to the Histology Laboratory in the Department of Veterinary Pathology, Microbiology and Parasitology of the University of Nairobi for analysis. Biopsies which were obtained were fixed in 10% neutral Formalin, processed and embedded in paraffin wax. Tissue blocks of 5 microns (5μ) were sectioned using a Microtome and were stained using Haematoxylin and Eosin stain and were observed using a light Microscope.

Absolute organ weights were taken before preservation in formalin and the relative organ weights were calculated as follows;

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Weight of the rat at sacrifice (g)}} \times 100$$

3.6 Disposal of carcasses

The rat carcasses were put in red biohazard bags and were disposed in the Disposal Pit of the Department of Veterinary Pathology, Microbiology and Parasitology of the University of Nairobi according to the University of Nairobi Ethical committee guidelines.

3.7. Data analysis

The collected data was entered into MS Excel and was loaded to GenStat version 15 and was analysed. The descriptive statistics for the variables were presented using frequency Tables and Graphs. The data on food and water consumption, Biochemical and Haematological parameters

were summarized as mean± standard deviation and Tukey's multiple comparison test and one-way Analysis of Variance (ANOVA) were used to analyze the data. Significant values of $p<0.05$ were considered significant.

3.8. Ethical consideration

The approval for conducting the study was sought from the Faculty of Veterinary Medicine Biosafety, Animal Use and Ethics Committee under a letter reference number FVM BAUEC/2020/256 and National Commission for Science, Technology and Innovation (NACOSTI) Ref No. 760323. Confidentiality was upheld throughout the study.

CHAPTER FOUR

RESULTS

4.1 Extraction yield.

Table 4.1 presents the results of the plant extraction yields.

Table 4.1: Summary of the extraction percentage yield of three *Catha edulis* (Vahl.) Forssk. ex Endl., extraction methods.

Plant extracts	Dry weight of sample in the solvent (g)	Weight of the product (g)	Extraction yield (%w/w)
Aqueous extract	1180 g in 6,500 ml distilled water	86.45	7.33
Methanol extract	300 g in 1,000 ml methanol	18.16	6.05
Acetone Extract	1214.8 g in 3,000 ml acetone	19.00	1.56

The Aqueous extraction method yielded the highest percentage of the extract with Acetone producing the least.

4.2 Ethnopharmacological uses of *Catha edulis* (Vahl.) Forssk. ex Endl.

Table 4.2 shows the Demographic characteristics of the informants interviewed in Embu County.

Table 4.2: Demographic characteristics of informants (n=35) interviewed in Embu County.

Variable (n=35)	Frequency (percentage)
Gender	
Male	35 (100.0)
Female	0 (0.0)
Marital status	
Single	3 (8.6)
Married	31 (88.6)
Not specified	1 (2.8)
Age (years)	
18-34	1 (2.9)
35-49	7 (20)
>50	27 (77.1)
Level of education	
Illiterate	8 (22.9)
Primary	20 (60)
Secondary	6 (17.1)
Graduate	1 (2.9)
Years of practice	
0-5	2 (5.7)
6-10	11 (31.4)
11-20	12 (34.3)
>20	6 (17.2)
Not captured	4 (11.4)
Received formal training	
Yes	3 (8.6)
No	32 (91.4)
Sub location	
Kithunthuri	9 (25.7)
Gacegithiuri	6 (17.1)
Nyangwa	4 (11.4)
Kianjiru	3 (8.5)
Mbita	3 (8.5)
Gikiiro	2 (5.7)
Kirima	1 (2.9)
Machangia	1 (2.9)
Mariari	1 (2.9)
Mavuria	1 (2.9)
Mombo munyiri	1 (2.9)
Mulindi	1 (2.9)

Mutuobare	1 (2.9)
Tigoo	1 (2.9)

All the informants interviewed in Embu County were males (Table 4.2). Most informants were married and were over 50 years in age. Most informants had a Primary level of education and they had practiced Traditional medicine for 11 to 20 years. Majority of the informants had not received any formal training and they came from Kithunthuri Sub location (Table 4.2).

The results of the Ethnopharmacological uses of *Catha edulis* (Vahl.) Forssk. ex Endl are given in Table 4.3.

Table 4.3: Local names, parts used, sources, indications, preparation, frequency of use, dosage and duration, side effects, and storage of *Catha edulis* (Vahl.) Forssk. ex Endl.

Local names of the plant	Miraa, Muguka, Mugombe, Mutimutiri, Mugumo, Mutamucii, Muraa, Mugwathingi, Gitune, Mwirugi, Gituu, Karuki, Muruti, Mutamuai
Parts used	Leaves, roots
Source of the plant	Local/personal farms; Kiritiri, Nyangwa, Kerwa, Gikondi and Gacegethiuri
Human indications	Stomachache, cough, heartburn, fatigue, diarrhoea, fever, chest congestion, gonorrhea, constipation and stress relief
Livestock indications	Constipation in cows and Diarrhoea in goats and sheep
Preparation	Fresh leaves can be chewed or the leaves can be macerated in water and the filtrate used.
Frequency of use	When necessary, once, twice or thrice
Dosage and duration	**Fresh leaves:** 2 handfuls, when necessary, 5-20 leaves, 10 leaves ground into powder, 30 leaves **Liquid:** 50 ml for 2 days, 250 ml when necessary, 50 mls+2 drops of Aloe Vera for 3 days, 50 ml for 3 days, 200 ml for 3 days and 30 ml for 2 days **Syringe:** 10 ml or 20 ml **Tablespoonful:** Once daily for 5 days, 2 Tablespoonful for 3 days and 2 Tablespoonful for 5 days **Glass/cup:** 1 glass of crude preparation, 1 cup for 5 days, ¼ of a glass for 3 days, 100 ml using a measuring cup and ½ a glass for 3 days. (The dosages and treatment duration for the different indications varied amongst the informants) **Teaspoonful:** 2 teaspoonful's for 4 days or 2 teaspoonful's for 5 days
Side effects	Constipation, ulcers, discoloration and cracking of teeth
Management of side effects	Ulcers (*Kiathaa* is used), constipation (*Mucee* is used), patients are advised to brush their teeth regularly or after chewing the leaves, eat cabbage, or drink plenty of water
Management of addiction	Clients were are advised to reduce intake, stop medication after the treatment period and they were observed closely. They were also advised to have self-discipline
Storage	The preparations in plastic bottles were kept in a cool dry place or kept in a cupboard. The fresh leaves were stored in opaque bottles, polythene bags, woven bags and were wrapped using newspapers. The leaves were also mixed with lemon in order to avoid spoilage. In

general, all the preparations in plastic bottles and fresh leaves were kept away from direct sunlight.

4.3 Antimicrobial assay

The results of the zones of inhibition of *Catha edulis* (Vahl.) Forssk. ex Endl., extracts, the positive and negative controls are given in Table 4.4

Table 4.4: Zone of inhibition values of *Catha edulis* (Vahl.) Forssk. ex Endl., extracts, the positive and negative controls.

		Zone of inhibition (mm)				
Microorganism	Concentration (mg/mL)	Acetone extract	Aqueous extract	Methanol extract	Positive control	Negative control
					Ciprofloxacin	**DMSO**
Bacillus cereus	3.125	6.00±0.00	6.00±0.00	6.00±0.00	32.00±0.00	6.00±0.00
	6.25	6.00±0.00	6.00±0.00	6.00±0.00		
	12.50	6.00±0.00	6.50±0.71	6.00±0.00		
	25.00	8.50±3.54	8.00±0.00	0.00±0.00		
	50.00	12.00±4.24	9.00±0.00	9.00±0.00		
	100.00	15.00±8.49	11.50±0.71	12.00±0.00		
	200.00	12.00±2.83	12.50±0.71	12.50±0.71		
	400.00	14.00±1.41	14.50±0.71	15.50±0.71		
	800.00	18.00±1.41	16.00±0.00	17.00±0.00		
Staphylococcus aureus	3.125	6.00±0.00	6.00±0.00	6.00±0.00	32.00±0.00	6.00±0.00
	6.25	6.00±0.00	6.00±0.00	6.00±0.00		
	12.50	6.00±0.00	6.00±0.00	6.00±0.00		
	25.00	7.50±0.71	6.00±0.00	6.00±0.00		
	50.00	7.50±0.71	6.00±0.00	7.50±0.71		
	100.00	8.50±2.12	6.50±0.71	9.50±0.71		
	200.00	9.50±0.71	7.50±0.71	10.50±0.71		
	400.00	14.50±0.71	11.00±0.00	11.00±1.41		
	800.00	15.00±0.00	16.00±0.00	18.00±1.41		
					Erythromycin	
Pseudomonas aeruginosa	800	6.00±0.00	6.00±0.00	6.00±0.00	15.00±0.00	6.00±0.00
Escherichia coli	800	6.00±0.00	6.00±0.00	6.00±0.00	14.50±0.71	6.00±0.00
					Fluconazole	

Candida albicans	800	6.00±0.00	6.00±0.00	6.00±0.00	56.50±0.71	6.00±0.00

Catha edulis (Vahl.) Forssk. ex Endl., has moderate efficacy against the gram-positive bacteria *Bacillus cereus* and *Staphylococcus aureus* but no activity against *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans* (Table 4.4).

The results of the MIC and MBC values of the plant extracts are given in Table 4.5

Table 4.5: Comparison of the MIC and MBC values of the plant extracts.

	Bacillus cereus		*Staphylococcus aureus*	
	MIC	**MBC**	**MIC**	**MBC**
Acetone	1.79 (0.04)[a]	7.16 (0.18)[a]	1.60 (0.04)[a]	6.41 (0.16)[a]
Aqueous	1.56 (0.26)[a]	6.25 (1.10)[a]	1.19 (0.02)[a]	4.74 (0.11)[b]
Methanol	1.99 (0.07)[a]	7.96 (0.30)[a]	1.87 (0.08)[a]	7.46 (0.32)[c]

At $p<0.05$, means with distinct superscripts along the columns differ considerably. Values in parenthesis are standard deviation

No statistically significant change ($p>0.05$) was observed in the MIC values of Acetone, Aqueous, and Methanol extracts of *Catha edulis* (Vahl.) Forssk. ex Endl., against *Bacillus cereus.*

No statistically significant change ($p>0.05$) was found in the MIC values of Acetone, Aqueous, and Methanol extracts of *Catha edulis* (Vahl.) Forssk. ex Endl., against *Staphylococcus aureus.*

The MBC of the Methanol extract of *Catha edulis* (Vahl.) Forssk. ex Endl., against *Staphylococcus aureus* was significantly greater *($p<0.05$)* than the MBC of Acetone and Aqueous extracts of *Catha edulis* (Vahl.) Endl. Moreover, the MBC of the Acetone extract of *Catha edulis* (Vahl.) Forssk. ex Endl., *was* significantly greater ($p<0.05$) than the MBC of the Aqueous extract of *Catha edulis* (Vahl.) Forssk. ex Endl.

4.4 Subacute toxicity assay in *Sprague Dawley* rats.

4.4.1 Food and water consumption

The results of the food and water consumption which were given to the rats are given in Table 4.6.

Table 4.6: Food consumption patterns of male and female rats treated with the Aqueous extracts of *Catha edulis* (Vahl.) Forssk. ex Endl.

	Food consumption (in grams)	
Duration	**Males**	**Females**
Week 1	790.20 (36.12)[a]	722.70 (42.13)[a]
Week 2	701.80 (61.26)[a]	672.20 (33.10)[a]
Week 3	722.00 (39.26)[a]	698.50 (33.12)[a]
Week 4	779.60 (34.45)[a]	631.50 (25.27)[a]

Means with the same superscripts along the columns are not significantly different from each other at $p<0.05$. Values in parenthesis represent the standard error of the mean

The amount of food which was consumed by male *Sprague Dawley* rats on week one of treatment with the Aqueous extracts of *Catha edulis* was not significantly different from the amount of food which was consumed by the rats on week 2, 3, and 4 (Table 4.6)**.** Moreover, the amount of food which was consumed by female *Sprague Dawley* rats on week one of treatment with the Aqueous extracts of *Catha edulis* (Vahl.) Forssk. ex Endl., was not significantly different from the amount of food which was consumed by the rats on week 2, 3, and 4 (Table 4.6).

The results of the water consumed by the female and male rats are given in the Table 4.7.

Table 4.7: Water consumption patterns of female and male rats which were given the Aqueous extracts of *Catha edulis* (Vahl.) Forssk. ex Endl.

	Water consumption (in milliliters)	
Duration	**Males**	**Females**
Week 1	1175.00 (101.40)[a]	1018.00 (47.15)[a]
Week 2	1065.00 (83.02)[a]	928.00 (50.23)[a]
Week 3	1175.00 (78.05)[a]	1072.00 (95.16)[a]
Week 4	1212.00 (113.20)[a]	992.00 (100.50)[a]

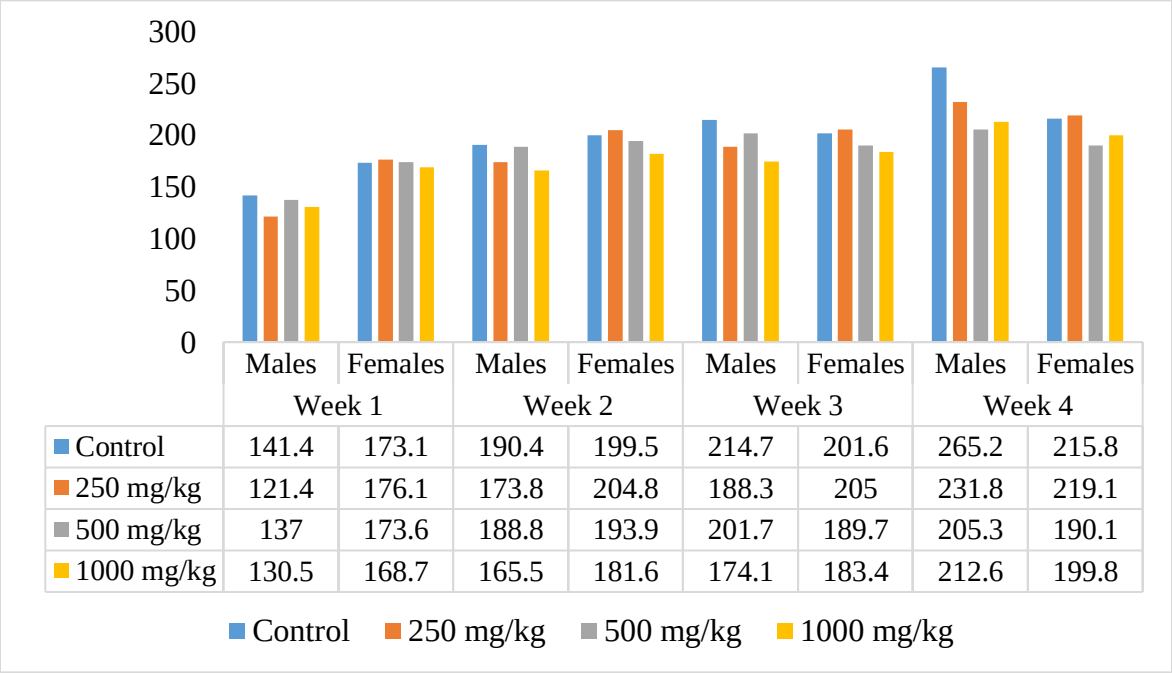
Means with the same superscripts along the columns are not significantly different from each other at $p<0.05$. Values in parenthesis represent the standard error of the mean

The amount of water which was consumed by male *Sprague Dawley* rats on week one of treatment with the Aqueous extracts of *Catha edulis* (Vahl.) Forssk. ex Endl., was not significantly different from the amount of water which was consumed by the rats on week 2, 3, and 4 (Table 4.7)**.** Moreover, the amount of water which was consumed by female *Sprague Dawley* rats on week one of treatment with the Aqueous extracts of *Catha edulis* (Vahl.) Forssk. ex Endl., was not significantly different from the amount of water which was consumed by the rats on week 2, 3, and 4 (Table 4.7).

4.4.2 Weight changes in *Sprague Dawley* rats

The weight changes in rats are given in Figure 4.1. Weight in mg is shown in the Y axis.

4.4.2.1 Changes in animal weights over the 28-day treatment period



	Week 1		Week 2		Week 3		Week 4	
	Males	Females	Males	Females	Males	Females	Males	Females
Control	141.4	173.1	190.4	199.5	214.7	201.6	265.2	215.8
250 mg/kg	121.4	176.1	173.8	204.8	188.3	205	231.8	219.1
500 mg/kg	137	173.6	188.8	193.9	201.7	189.7	205.3	190.1
1000 mg/kg	130.5	168.7	165.5	181.6	174.1	183.4	212.6	199.8

Figure 4.1: Summary of the changes in the average weights of *Sprague Dawley* rats treated with different doses of the Aqueous extract of *Catha edulis* (Vahl.) Forssk. ex Endl., over a 28-day period.

The mean weights of female *Sprague Dawley* rats were greater than the mean weights of male rats after week 1, 2, and 3 of treatment with *Catha edulis* (Vahl.) Forssk. ex Endl., (Figure 4.1). However, the mean weights of male rats were greater than the mean weights of female rats on week 4 of treatment with *Catha edulis* (Vahl.) Forssk. ex Endl., (Figure 4.1).

4.4.3 Differences in the mean weights gain over the treatment period.

Table 4.8 shows the results of the differences in mean weight gain across the treatment period.

Table 4.8: Summary of the effects of different doses of *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts on weight gain in *Sprague Dawley* rats.

	Week 1 and Week 2		Week 1 and Week 3		Week 1 and Week 4	
Treatment	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
Control	49.05±8.90^{a}	22.48±3.46^{b}	73.27±16.42^{a}	28.58±5.57^{b}	123.80±25.16^{a}	42.71±8.18^{b}
250 mg/kg	52.46±7.10^{a}	28.72±4.15^{b}	66.91±12.76^{a}	28.88±5.34^{b}	110.40±13.80^{a}	42.99±6.92^{b}
500 mg/kg	51.74±17.02^{a}	20.32±6.34^{ab}	64.64±32.62^{a}	16.18±11.16^{ab}	112.20±38.37^{a}	16.57±16.94^{a}
1000 mg/kg	35.04±15.93^{a}	12.85±5.13^{a}	43.57±24.73^{a}	14.70±5.08^{a}	82.10±33.52^{a}	31.04±5.95^{ab}

At ($p<0.05$), the means with distinct superscripts along the columns are significantly different.

Week 1 and 2 of treatment

The mean weight gain of male rats which were treated with distilled water and the mean weight gain of male rats which were given a low, intermediate, or high dose of the Aqueous extracts of *Catha edulis* (Vahl.) Forssk. ex Endl., were not significant statistically ($p>0.05$) (Table 4.8). The mean weight gain in female rats which were given a high dose of the Aqueous extracts of *Catha edulis* (Vahl.) Forssk. ex Endl., was significantly lower *($p<0.05$)* than the mean weight gain in female rats which received distilled water (Table 4.8). The mean weight gain in female rats which were given a high dose of the Aqueous extracts of *Catha edulis* (Vahl.) Forssk. ex Endl., was significantly lower *($p<0.05$)* than the mean weight gain in female rats which received a low dose of the extracts (Table 4.8). The mean weights gain in female rats which were given distilled water and female rats which received a low or intermediate dose of the extracts were not significantly different (Table 4.8).

Treatment weeks 1 and 3

There was no statistically significant change ($p>0.05$) between the mean weight gain of male rats which were given distilled water and the mean weight gain of male rats which received a low, intermediate, or high doses of *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts (Table 4.8). The mean weight gain in female rats which were given a high dose of the Aqueous extracts of *Catha edulis* (Vahl.) Forssk. ex Endl., was significantly lower *($p<0.05$)* than the mean weight gain in female rats which were given distilled water, a low dose of the extracts, or a high dose of the extracts (Table 4.8). There was no significant change between the average weight gain in female rats which were given distilled water and the average weight gain in female rats which were given a low or intermediate doses of the extracts (Table 4.8).

Week 1 and 4 of treatment

No significant statistically change ($p>0.05$) was found between the mean weight gain of male rats which were given distilled water and the mean weight gain of male rats which were given a low, moderate or high dose of the Aqueous extracts of *Catha edulis* (Vahl.) Forssk. ex Endl., (Table 4.8). The mean weight gain in female rats which were given an intermediate dose of the Aqueous extracts of *Catha edulis* (Vahl.) Forssk. ex Endl., was significantly lower *($p<0.05$)* than the mean weight gain in female rats which were treated with distilled water, or a low dose of the extracts (Table 4.8). There was no substantial difference in the mean weight gain in female rats which were given distilled water and those which received a low dose of the extracts or a high dose of the extracts (Table 4.8).

4.5 Haematological Assay in *Sprague Dawley* rats.

Table 4.9 shows the results of the Haematological assay in male rats.

Table 4.9: Summary of the Haematological Parameters in the male *Sprague Dawley* rats.

Treatment (n=5)	WBC	LYM	HGB	MCHC	RBC	MCV	PLT
Control	11.06±1.79[a]	7.97±1.53[a]	15.84±0.73[a]	35.72±0.85[a]	7.89±0.46[a]	56.28±2.43[a]	581.40±94.08[a]
250 mg/kg	13.40±5.48[a]	9.92±4.94[a]	15.56±2.04[a]	35.86±1.15[a]	7.59±0.93[a]	57.20±2.84[a]	539.40±186.10[a]
500 mg/kg	13.74±4.29[a]	11.42±4.16[a]	15.02±0.90[a]	34.92±0.70[a]	7.52±0.64[a]	57.50±3.63[a]	549.20±95.92[a]
1000 mg/kg	11.84±4.87[a]	9.34±4.94[a]	14.98±1.12[a]	34.96±0.67[a]	7.35±0.78[a]	58.56±3.23[a]	475.20±97.00[a]

Means with different subscript along the columns are significantly different at $p<0.05$

WBC

No statistically significant change ($p>0.05$) was found between the mean WBC levels of male rats which were given distilled water and the mean WBC levels in male rats which were given a low, intermediate, or high doses of *Catha edulis* (Vahl.) Forssk. ex Endl.

LYM

The mean LYM levels of male rats which were given distilled water compared to male rats which were given low, intermediate, or high doses of *Catha edulis* (Vahl.) Forssk. ex Endl., were not statistically significant ($p>0.05$) (Table 4.9).

HGB

Between the mean HGB levels of male rats which were given distilled water and the mean HGB levels of male rats which were given low, intermediate, or high doses of *Catha* edulis (Vahl.) Forssk. ex Endl., there was no statistically significant change ($p>0.05$) (Table 4.9).

MCHC

Between the mean MCHC levels of male rats which were given distilled water and the mean MCHC levels of male rats which were treated with low, intermediate, or high doses of *Catha edulis* (Vahl.) Forssk. ex Endl., there was no statistically significant change ($p>0.05$) (Table 4.9).

RBC

The mean RBC levels of male rats which were given distilled water and male rats which were given low, intermediate, or high doses of *Catha edulis* (Vahl.) Forssk. ex Endl., showed no statistically significant change ($p>0.05$) (Table 4.9).

MCV

The mean MCV levels of male rats which were given distilled water and male rats which were given low, intermediate, or high doses of *Catha edulis* (Vahl.) Forssk. ex Endl., showed no statistically significant change ($p>0.05$) (Table 4.9).

PLT

The mean PLT levels of male rats which were given distilled water and male rats which were given low, intermediate, or high doses of *Catha edulis* (Vahl.) Forssk. ex Endl., showed no statistically significant change ($p>0.05$) (Table 4.9).

Table 4.10 shows the results of the Haematological assay in female rats.

Table 4.10: Summary of the Haematological Parameters in the female *Sprague Dawley* rats.

Treatment (n=5)	WBC	LYM	HGB	MCHC	RBC	MCV	PLT
Control	9.04±3.39[a]	6.56±2.60[a]	13.58±2.32[a]	39.10±0.52[a]	6.28±1.13[a]	55.46±1.78[a]	497.20±121.10[a]
250 mg/kg	8.74±2.77[a]	6.68±2.06[a]	15.60±0.57[a]	33.90±7.32[a]	7.45±0.46[ab]	55.58±2.30[a]	607.40±97.80[a]
500 mg/kg	8.14±1.59[a]	6.14±1.08[a]	15.58±0.45[a]	36.82±0.43[a]	7.53±0.18[b]	56.16±0.88[a]	559.20±107.20[a]
1000 mg/kg	7.18±1.59[a]	5.23±1.00[a]	16.05±0.52[a]	37.48±0.69[a]	7.78±0.48[b]	55.10±2.17[a]	634.60±21.31[a]

Means with different subscript along the columns are significantly different at $p<0.05$

WBC

No significant statistically change ($p>0.05$) was seen between the mean WBC levels of female rats which were given distilled water and the mean WBC levels in female rats which were given a low, intermediate, or high doses of *Catha edulis* (Vahl.) Forssk. ex Endl.,(Table 4.10).

LYM

No significant statistically change ($p>0.05$) was seen between the mean LYM levels of female rats which were given distilled water and the mean LYM levels in female rats which were given a low, intermediate, or high doses of *Catha edulis* (Vahl.) Forssk. ex Endl.,(Table 4.10).

HGB

No significant statistically change ($p>0.05$) was observed between the mean HGB levels of female rats which were given distilled water and the mean HGB levels in female rats which were given a low, intermediate, or high doses of *Catha edulis* (Vahl.) Forssk. ex Endl.,(Table 4.10).

MCHC

There was no significant statistically change ($p>0.05$) between the mean MCHC levels of female rats which were given distilled water and those which were given a low, intermediate, or high doses of *Catha edulis* (Vahl.) Forssk. ex Endl., (Table 4.10).

RBC

The mean RBC levels of female rats which were given distilled water and the mean RBC levels of female rats which were given a low dose of *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts did not significantly differ statistically ($p>0.05$) (Table 4.10). However, the average levels of RBC in female rats which were given an intermediate dose of *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts were significantly higher than the mean levels of RBC in female rats which were given distilled water (Table 4.10). Furthermore, the mean RBC levels in female rats which were given a high dose of *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts were significantly higher than the mean RBC levels in female rats which received distilled water. (Table 4.10).

MCV

No significant statistically change ($p>0.05$) was seen between the mean MCV levels of female rats which were given distilled water and the mean MCV levels in female rats which were given a low, intermediate, or high doses of *Catha edulis* (Vahl.) Forssk. ex Endl. (Table 4.10).

PLT

No significant statistically change ($p>0.05$) was noted between the mean PLT levels of female rats which were given distilled water and the mean PLT levels in female rats which were given a low, intermediate, or high doses of *Catha edulis* (Vahl.) Forssk. ex Endl. (Table 4.10).

Table 4.11 shows the results of the mean levels of Haematological parameters in *Sprague Dawley* rats given a low dose of Aqueous *Catha edulis* extracts.

Table 4.11: Summary of the mean levels of the Haematological Parameters in *Sprague Dawley* rats which were treated with 250 mg/kg of *Catha edulis (Vahl.)* Forssk. ex Endl., Aqueous extracts.

	Gender	
Parameter	**Male**	**Female**
WBC (10^9/L)	13.40*	8.74
LYM (10^9/L)	9.92*	6.68
HGB (g/dL)	15.56	15.60
MCHC (g/dL)	35.86	33.90
RBC (10^{12}/L)	7.59	7.45
MCV (fl)	57.20	55.58
PLT (10^9/L)	539.40	607.40

The values with * are significantly higher

The average levels of WBC and Lymphocytes in male rats which were given 250 mg/kg of *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts were substantially greater ($p<0.05$) than the average levels of WBC and Lymphocytes in female rats which received the same doses of extracts.

The mean values of Platelets in male and female rats which were given 250 mg/kg of *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts showed no significant change (Table 4.11).

No significant statistically difference ($p>0.05$) was observed between the mean levels of Hemoglobin and Mean corpuscular hemoglobin concentration in male rats and female rats which were given 250 mg/kg of *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts (Table 4.11).

There was no statistically significant change ($p>0.05$) in RBC levels between male and female rats which were given 250 mg/kg of *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts (Table 4.11).

The Mean corpuscular volume levels of male and female rats which were given 250 mg/kg of *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts showed no significant statistically change ($p>0.05$) (Table 4.11).

The results of the means levels of the Haematological Parameters in female and male rats which were given an intermediate dose of Aqueous *Catha edulis* extracts are presented in Table 4.12.

Table 4.12: Summary of the mean levels of the Haematological Parameters in male and female *Sprague Dawley* rats treated with 500 mg/kg of *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts

	Gender	
Parameter	**Male**	**Female**
WBC (10^9/L)	13.74*	8.14
LYM (10^9/L)	11.42*	6.14
HGB (g/dL)	15.02	15.58
MCHC (g/dL)	34.92	36.82*
RBC (10^{12}/L)	7.52	7.53
MCV (fl)	57.50	56.16
PLT (10^9/L)	549.20	559.20

The values with * are significantly higher

The mean PLT levels of male and female rats which were given 500 mg/kg of *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts showed no significant statistically change ($p>0.05$) (Table 4.12). The average levels of WBC and LYM in male rats which were given 500 mg/kg of

Catha edulis (Vahl.) Forssk. ex Endl., Aqueous extracts were significant higher ($p<0.05$) than the average levels of WBC and LYM in female rats which were administered with the same dose of the extracts (Table 4.12).

There was no statistically significant change ($p>0.05$) in mean HGB levels between male and female rats which were given 500 mg/kg of *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts. However, the mean levels of MCHC in the female rats were substantially greater ($p<0.05$) than the male rats which received the same dose of the extracts (Table 4.12).

The average RBC values of male and female rats which were given 500 mg/kg of *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts showed no significant statistically change ($p>0.05$) (Table 4.12).

There was no significant statistically change ($p>0.05$) between the mean MCV levels of male and female rats which received a 500 mg/kg dosage of *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts (Table 4.12).

The results of the means levels of the Haematological Parameters in female and male rats which received a high dose of Aqueous *Catha edulis* (Vahl.) Forssk. ex Endl., extracts are presented in Table 4.13

Table 4.13: Summary of the average values of the Haematological Parameters in the male and female *Sprague Dawley* rats which were given 1000 mg/kg dose of the Aqueous extracts of *Catha edulis* (Vahl.) Forssk. ex Endl.

	Gender	
Parameter	**Male**	**Female**
WBC (10^9/L)	11.84*	6.78
LYM (10^9/L)	9.34*	4.90
HGB (g/dL)	14.98	15.88*
MCHC (g/dL)	34.96	37.60*
RBC (10^{12}/L)	7.35	7.71*
MCV (fl)	58.56*	54.84
PLT (10^9/L)	475.2	634.6*

The values with * are significantly higher

The average levels of WBC, LYM, and MCV in male *Sprague Dawley* rats which were given 1000 mg/kg of *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts were substantially greater ($p<0.05$) than those in female rats which were given the same doses of extracts (Table 4.13).

The average levels of HGB, MCHC, RBC, and PLT in female rats which were given a 1000 mg/kg dose of *Catha edulis* were significantly greater ($p<0.05$) than in male rats which were given the same dose of extracts (Table 4.13).

4.6 Biochemistry Assay in *Sprague Dawley* rats.

Table 4.14 shows the results of the Biochemistry parameters in male rats.

Table 4.14: Summary of the mean levels of Albumin, AST, ALT, ALP, Creatinine, Direct Bilirubin, Total Bilirubin, Total Protein and Urea in male rats which were given distilled water and graded doses of the Aqueous extracts of *Catha edulis* (Vahl.) Forssk. ex Endl.

Treatment	Albumin	ALP	ALT	AST	Creatinine	Direct Bilirubin	Total Bilirubin	Total Protein	Urea
Control	3.80±0.19[a]	265.40±59.34[a]	111.40±24.99[a]	210.60±42.77[a]	35.41±5.44[a]	0.68±0.46[a]	11.82±6.13[a]	70.40±4.49[a]	8.75±0.64[a]
250 mg/kg	3.73±0.32[a]	427.80±240.10[a]	118.20±15.53[a]	231.60±17.67[a]	39.29±4.84[a]	1.13±0.12[a]	8.45±3.83[a]	71.85±5.27[a]	8.28±1.27[a]
500 mg/kg	3.76±0.11[a]	349.40±127.70[a]	120.00±17.07[a]	257.80±39.79[a]	40.83±5.02[a]	1.24±0.56[a]	15.47±10.69[a]	73.13±3.43[a]	9.34±1.13[a]
1000 mg/kg	3.73±0.19[a]	473.20±150.60[a]	125.60±13.13[a]	254.80±35.49[a]	39.12±2.57[a]	1.92±1.40[a]	16.41±11.34[a]	72.66±4.54[a]	8.39±1.35[a]

Means with different superscripts along the columns are significantly different at $p<0.05$

No statistically significant difference ($p>0.05$) was observed between the mean levels of Albumin, AST, ALT, ALP, Creatinine, Direct Bilirubin, Total Bilirubin, Total Protein and Urea in male rats which were given distilled water or graded doses (250 mg/kg, 500 mg/kg, and 1000 mg/kg) of the Aqueous extracts of *Catha edulis* (Vahl.) Forssk. ex Endl. (Table 4.14).

Table 4.15 shows the results of the Biochemistry Parameters in the female rats.

Table 4.15: Summary of the mean levels of Albumin, AST, ALT, ALP, Creatinine, Direct Bilirubin, Total Bilirubin, Total Protein and Urea in female rats which were given distilled water and graded doses of the Aqueous extracts of *Catha edulis* (Vahl.) Forssk. ex Endl.

Treatment	Albumin	ALP	ALT	AST	Creatinine	Direct Bilirubin	Total Bilirubin	Total Protein	Urea
Control	3.97±0.14^{a}	106.60±39.70^{a}	90.61±12.68^{a}	179.20±29.52^{a}	42.02±4.21^{a}	1.27±0.47^{a}	11.98±4.00^{ab}	69.72±1.52^{a}	7.17±0.74^{a}
250 mg/kg	3.91±0.12^{a}	140.00±52.60^{a}	87.01±11.81^{a}	197.20±38.57^{a}	42.41±3.63^{a}	1.38±0.23^{a}	8.61±1.33^{a}	68.77±2.84^{a}	7.48±0.84^{ab}
500 mg/kg	3.80±0.50^{a}	209.60±229.40^{a}	94.01±16.37^{a}	215.60±51.91^{a}	40.83±3.63^{a}	1.22±0.15^{a}	15.15±3.93^{ab}	70.81±3.65^{a}	9.35±1.72^{b}
1000 mg/kg	3.89±0.12^{a}	143.50±46.08^{a}	94.51±14.80^{a}	231.50±20.27^{a}	36.16±1.42^{a}	1.92±1.15^{a}	18.95±8.47^{b}	72.02±2.35^{a}	8.76±0.79^{ab}

Means with different superscripts along the columns are significantly different at $p<0.05$

No statistically significant difference ($p>0.05$) was observed between the mean levels of Albumin, AST, ALT, ALP, Creatinine, Direct Bilirubin, or Total Protein in female rats which were given distilled water or graded doses (250 mg/kg, 500 mg/kg, and 1000 mg/kg) of the Aqueous extracts of *Catha edulis* (Vahl.) Forssk. ex Endl. (Table 4.15).

The mean levels of TB in female rats which were given Aqueous extracts of *Catha edulis* (Vahl.) Forssk. ex Endl., at a dose of 250 mg/kg were substantially lower *($p<0.05$)* than the average values of TB in female rats which were given a high dose of Aqueous the extracts (Table 4.15). The average Urea levels in female rats which were given 500 mg/kg *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts were substantially higher than those in female rats which were given distilled water. There was no significant statistically change in average levels of Urea in female rats which were given distilled water versus female rats which were given 250 mg/kg or 1000 mg/kg of *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts. (Table 4.15).

There was no statistically significant change in the average levels of Urea in female rats which were given a low dose of *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts and the average levels of Urea in female rats which were given 1000 mg/kg *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts (Table 4.15).

The results of the comparison of the mean levels of Creatinine, Urea, Total Bilirubin, and Direct Bilirubin in *Sprague Dawley* rats which were given a low dose of Aqueous *Catha edulis* (Vahl.) Forssk. ex Endl., extracts are shown in Figure 4.2.

250 mg/kg of the Aqueous extracts of *Catha edulis* (Vahl.) Forssk. ex Endl.

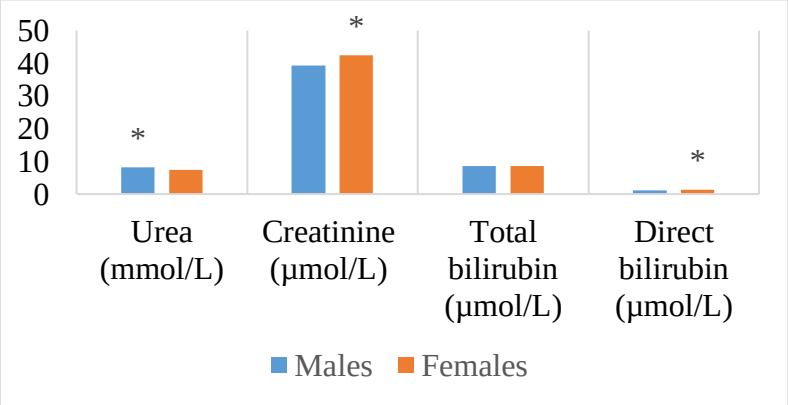


Figure 4.2: Comparison of the mean levels of Creatinine, Urea, Total Bilirubin, and Direct Bilirubin in *Sprague Dawley* rats which were given 250 mg/kg dose of *Catha edulis* (Vahl.) Forssk. ex Endl.

The average levels of Urea in male rats which were given 250 mg/kg *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts were considerably higher ($p<0.05$) than in female rats which were given the same dose of extracts (Figure 4.2). The average levels of Creatinine and Direct Bilirubin in female rats which were given 250 mg/kg *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts were substantially higher ($p<0.05$) than the average levels of Creatinine and Direct Bilirubin in male rats which received the same dose of the extracts (Figure 4.2). There was no significant statistically change ($p>0.05$) between the mean Total Bilirubin levels in male and female rats which were given a 250 mg/kg dose of *Catha edulis* (Vahl.) Forssk. ex Endl. (Figure 4.2).

The results of the comparison of the mean levels of AST, ALT and ALP in *Sprague Dawley* rats which were given a low dose of Aqueous *Catha edulis* (Vahl.) Forssk. ex Endl extracts are shown in Figure 4.3.

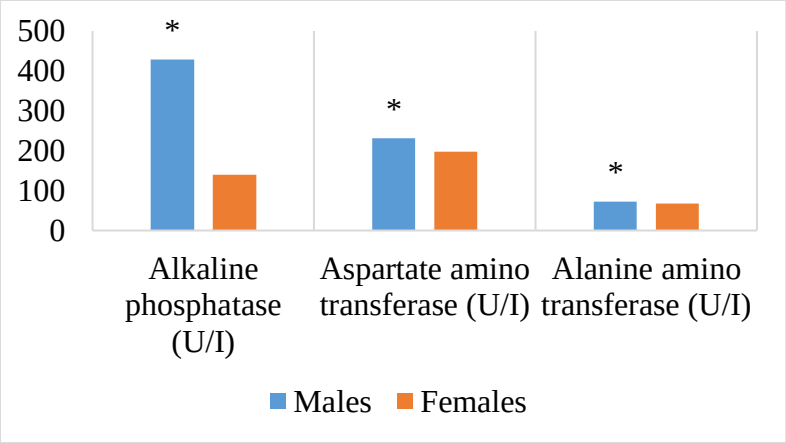


Figure 4.3: Comparison of the average levels of AST, ALT and ALP in *Sprague Dawley* rats which were given 250 mg/kg dose of *Catha edulis* (Vahl.) Forssk. ex Endl.

The average levels of AST, ALT and ALP in male rats which were given 250 mg/kg *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts were substantially higher *($p<0.05$)* than the average levels of AST, ALT and ALP in female rats which received the same dose of the extracts (Figure 4.3).

The results of the comparison of the average levels of Albumin and Total protein in female and male rats which were given 250 mg/kg dose of Aqueous *Catha edulis* (Vahl.) Forssk. ex Endl., extracts are shown in Figure 4.4.

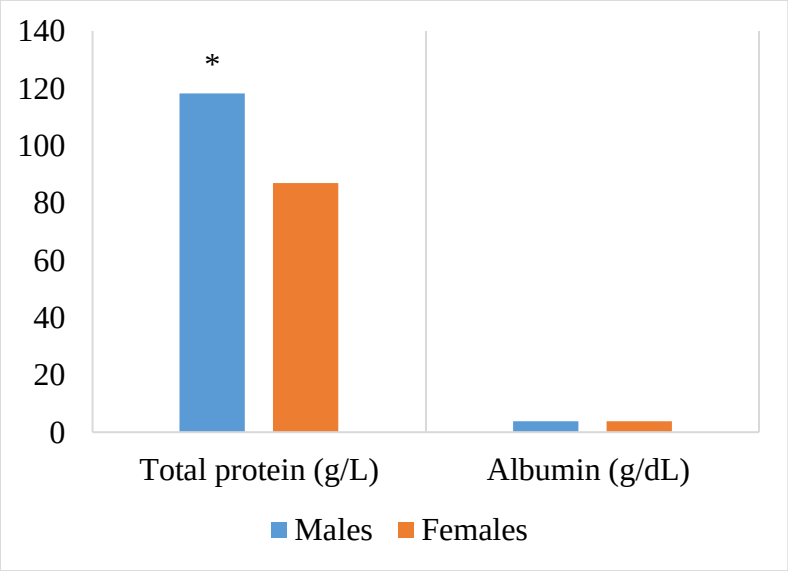


Figure 4.4: Comparison of the average levels of Albumin and Total protein in female and male rats which were given 250 mg/kg dose of *Catha edulis* (Vahl.) Forssk. ex Endl.

The average amounts of Total protein in male rats which were given 250 mg/kg *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts were substantially greater ($p<0.05$) than those in female rats which were given the same dose of extracts (Figure 4.4). The average amounts of Albumin in male and female rats which were given 250 mg/kg *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts showed no statistically significant difference (Figure 4.4).

The results of the comparison of the average levels of Urea, Creatinine, Total Bilirubin, and Direct Bilirubin in female and male rats which were given an intermediate dose of *Catha edulis* (Vahl.) Forssk. ex Endl., are shown in Figure 4.5.

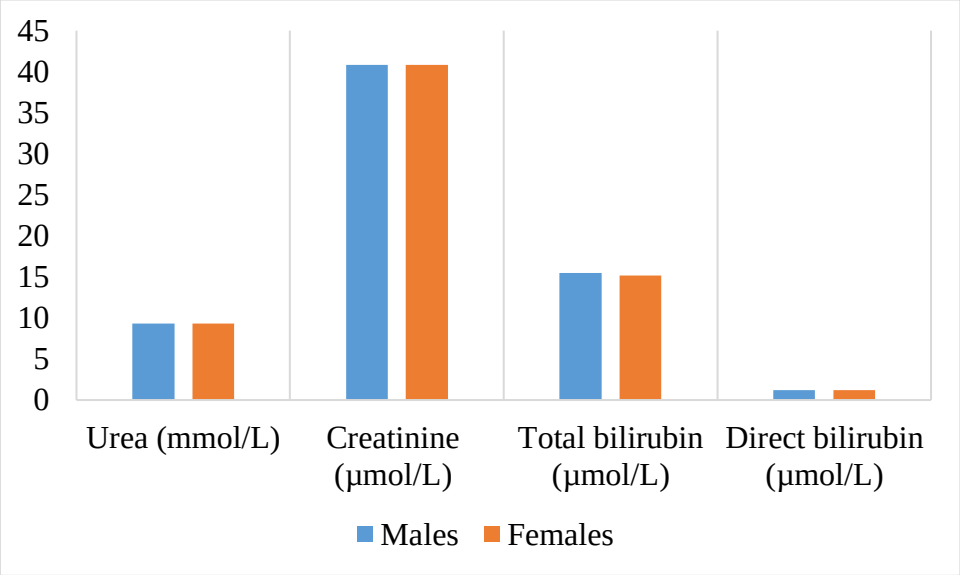


Figure 4.5: Comparison of the average levels of Total Bilirubin, Creatinine, Urea, and Direct Bilirubin in female and male *Sprague Dawley* rats which were given 500 mg/kg dose of *Catha edulis* (Vahl.) Forssk. ex Endl.

No significant statistically change ($p>0.05$) was observed in the average values of Total Bilirubin, Urea, Creatinine and Direct Bilirubin in male and female rats which were given 500 mg/kg *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts (Figure 4.5).

The results of the comparison of the mean amounts of AST, ALP and ALT in *Sprague Dawley* rats which received an intermediate dose of *Catha edulis* (Vahl.) Forssk. ex Endl., are shown in Figure 4.6.

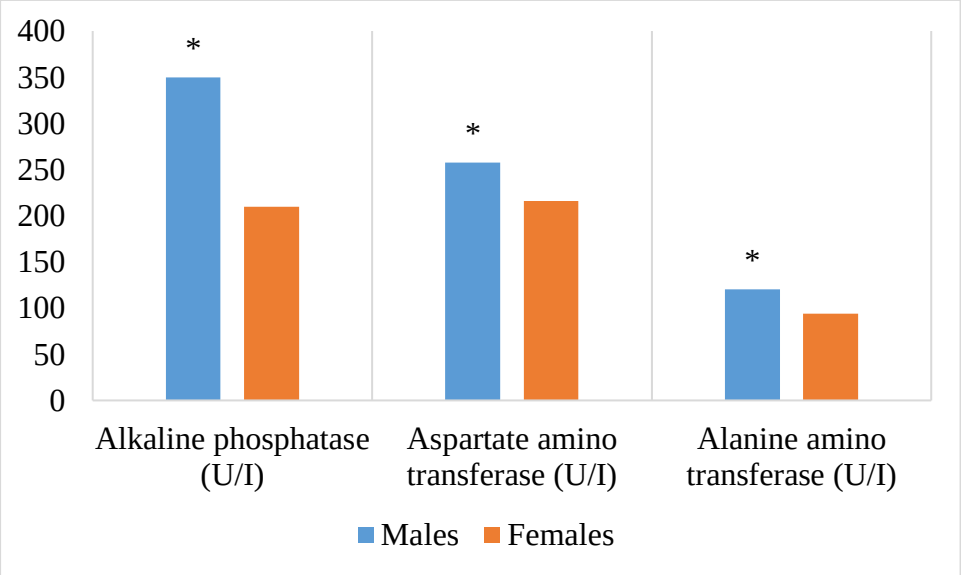


Figure 4.6: Comparison of the mean amounts of AST, ALP and ALT in *Sprague Dawley* rats which were given 500 mg/kg dose of *Catha edulis* (Vahl.) Forssk. ex Endl.

The mean amounts of AST, ALP and ALT in male rats which were given 500 mg/kg *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts were substantially higher *($p<0.05$)* than the mean amounts of AST, ALP and ALT in female rats which received the same dose of the extracts (Figure 4.6).

The results of the comparison of the average levels of Albumin and Total Protein in female and male rats which were given an intermediate dose of *Catha edulis* (Vahl.) Forssk. ex Endl., are shown in Figure 4.7.

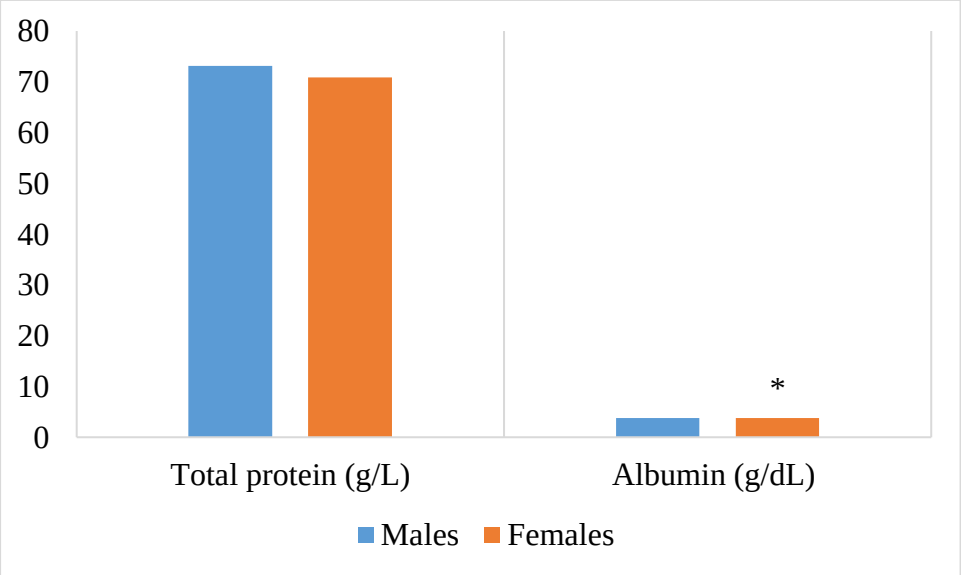


Figure 4.7: Comparison of the average levels of Albumin and Total Protein in female and male rats which were given an intermediate dose (500 mg/kg) of *Catha edulis* (Vahl.) Forssk. ex Endl.

The average levels of Albumin in female rats which were given 500 mg/kg *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts were considerably higher ($p<0.05$) than those in male rats which were given the same dose of extracts (Figure 4.7). The average levels of Total protein in male and female rats which were given 500 mg/kg *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts showed no significant statistically change *($p>0.05$)* (Figure 4.7).

The results of the comparison of the mean levels of Total Bilirubin, Creatinine, Urea and Direct Bilirubin in female and male rats which were given a high dose of *Catha edulis* (Vahl.) Forssk. ex Endl., are shown in Figure 4.8.

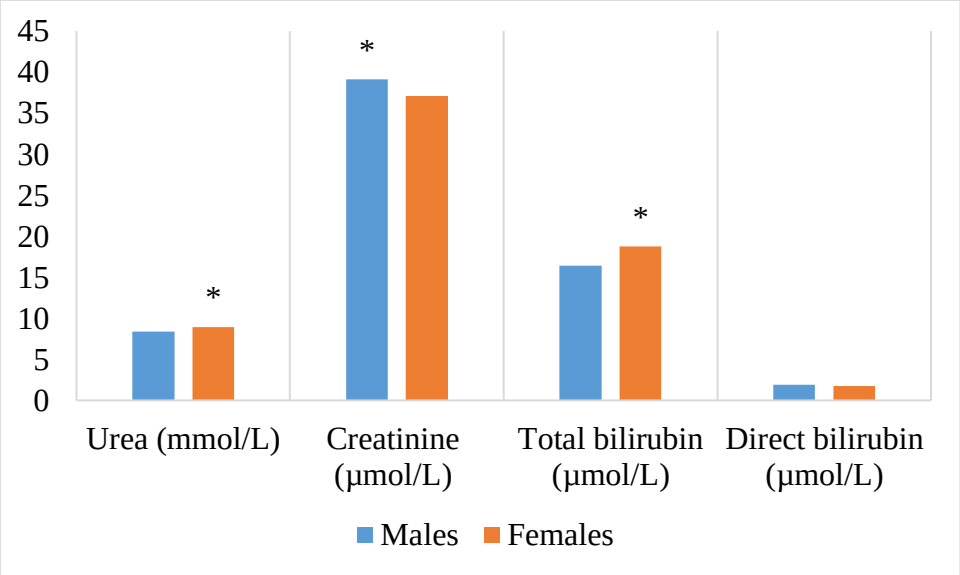


Figure 4.8: Comparison of the average levels of Total Bilirubin, Creatinine, Urea and Direct Bilirubin in female and male *Sprague Dawley* rats which were given 1000 mg/kg dose of *Catha edulis* (Vahl.) Forssk. ex Endl.

The mean levels of TB and Urea in female rats which were given 1000 mg/kg *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts were substantially higher *($p<0.05$)* than the average levels of TB and Urea in male rats which were given the same dose of the extracts (Figure 4.8). The average levels of Creatinine in male rats which were given 1000 mg/kg *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts were substantially higher *($p<0.05$)* than the average levels of Creatinine in female rats which received the same dose of the extracts (Figure 4.8).

The average values of Direct Bilirubin in male and female rats which were treated with 1000 mg/kg *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts showed no statistically significant change ($p>0.05$) (Figure 4.8).

The results of the comparison of the mean levels of AST, ALP and ALT in *Sprague Dawley* rats which were given a high dose of *Catha edulis* (Vahl.) Forssk. ex Endl., are shown in Figure 4.9.

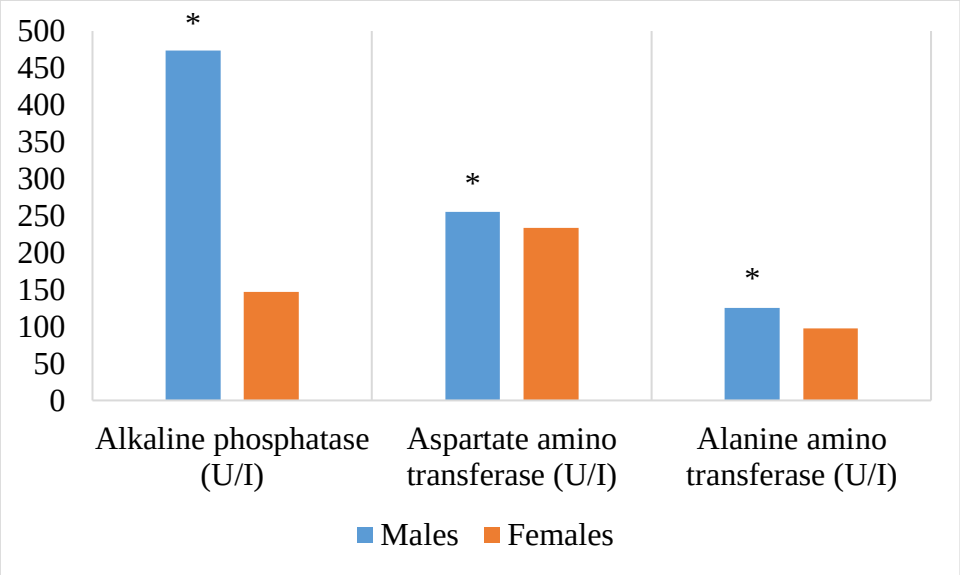


Figure 4.9: Comparison of the average levels of AST, ALP and ALT in *Sprague Dawley* rats which given 1000 mg/kg *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts.

The mean amounts of AST, ALP and AST in male rats which were given 1000 mg/kg *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts were substantially higher *($p<0.05$)* than the mean amounts of AST, ALP and AST in female rats which received the same dose of the extracts (Figure 4.9).

The results of the comparison of the mean levels of Albumin and Total Protein in female and male rats which were given a high dose of *Catha edulis* (Vahl.) Forssk. ex Endl., are shown in Figure 4.10.

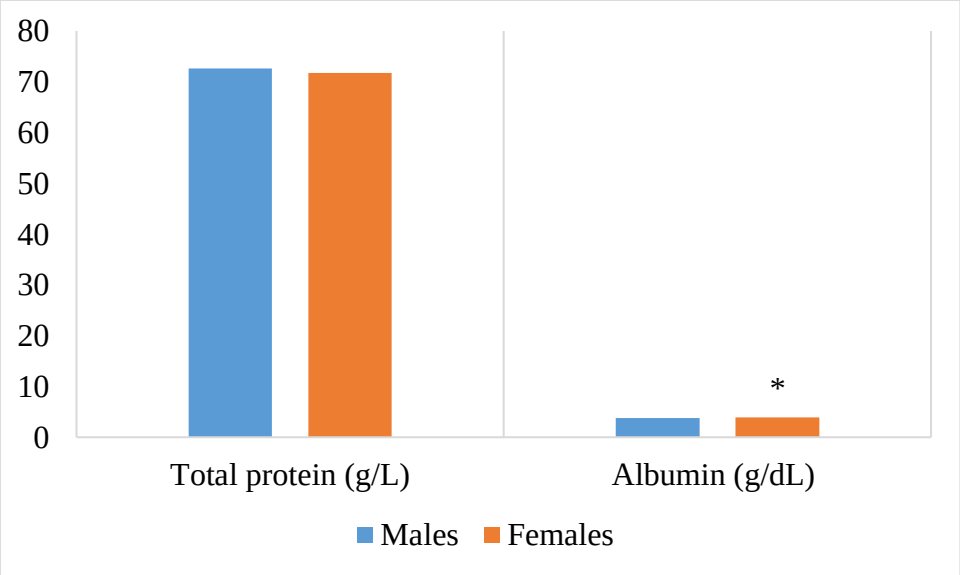


Figure 4.10: Comparison of the average levels of Albumin and Total Protein in female and male *Sprague Dawley* rats which were given a 1000 mg/kg dose of *Catha edulis* (Vahl.) Forssk. ex Endl.

The average levels of Albumin in female rats which were given 1000 mg/kg *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts were substantially greater *($p<0.05$)* than the average levels of Albumin in male rats which received the same dose of the extracts (Figure 4.10).

The average amounts of Total Protein in male and female rats which were treated with 1000 mg/kg *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts showed no statistically significant change ($p>0.05$) (Figure 4.10).

4.7 Histopathology Assay in Sprague Dawley rats

Table 4.16 shows the findings of the histopathology examination.

Table 4.16: Summary of the histopathological findings of the harvested body organs of the female and male *Sprague Dawley* rats which were treated with *Catha* edulis (Vahl.) Forssk. ex Endl., Aqueous extract.

	ID	LIVER	LUNG	KIDNEY	HEART	TESTES	REMARK
1.	**CLE 1 F (I)**	Normal	Normal	C	C		Normal
2.	**CLE 1 F (III)**	C	C	C	Normal		Normal
3.	**CLE 1 F (IV)**	Normal	Normal	C	Normal		Normal
4.	**CLE 2 F(I)**	C	Normal	C	C		Normal
5.	**CLE 2 F(II)**	Normal	Normal	Normal	Normal		Normal
6.	**CLE 2 F(III)**	Normal	Normal	Localized interstitial Connective tissue proliferation multifocal Kidney Interstitial haemorrhage	C		Chronic renal injury (fibrosis)
7.	**CLE 3 F(II)**	Normal	Normal	Localized tubular epithelium necrosis	C		Renal injury
8.	**CLE 3 F(IV)**	Normal	Normal	Localized interstitial connective tissue proliferation	Normal		Renal fibrosis
9.	**CLE 3 F(V)**	Normal	Normal	Localized interstitial connective tissue proliferation	Normal		Renal fibrosis
10.	**CLE 4 F(I)**	Normal	Normal	C	Normal		Normal
11.	**CLE 4 F(III)**	C	Normal	Normal	Normal		Normal
12.	**CLE 4 F(V)**	C	C	C	Normal		Normal
13.	**CLE 1 M(I)**	Normal				Normal	Normal
14.	**CLE 1 M(III)**	Normal	Slight localized interstitial haemorrhage	Localized interstitial Connective tissue proliferation multifocal Kidney Interstitial haemorrhage	C	Normal	Chronic renal injury
15.	**CLE 1 M(V)**	Normal	C	C	Normal	Normal	Normal

16.	**CLE 2 M(II)**	Normal	C	C	Normal	Normal	Normal
17.	**CLE 2 M(III)**	Normal	C	Normal	Normal	Normal	Normal
18.	**CLE 3 M(IV)**	Normal	Normal	Normal	Normal	Normal	Normal
19.	**CLE 3 M(V)**	Normal	C	C	Normal	Normal	Normal
20.	**CLE 4 M(II)**	Normal	Normal	C	Normal	Normal	Normal
21.	**CLE 4 M(III)**	Normal	C	C	Normal	Normal	Normal

KEY

CLE *Catha edulis* (Vahl.) Forssk. ex Endl., extract **C** Tissue congestion

CLE 1 High dose **M** Males

CLE 2 Intermediate dose **F** Females

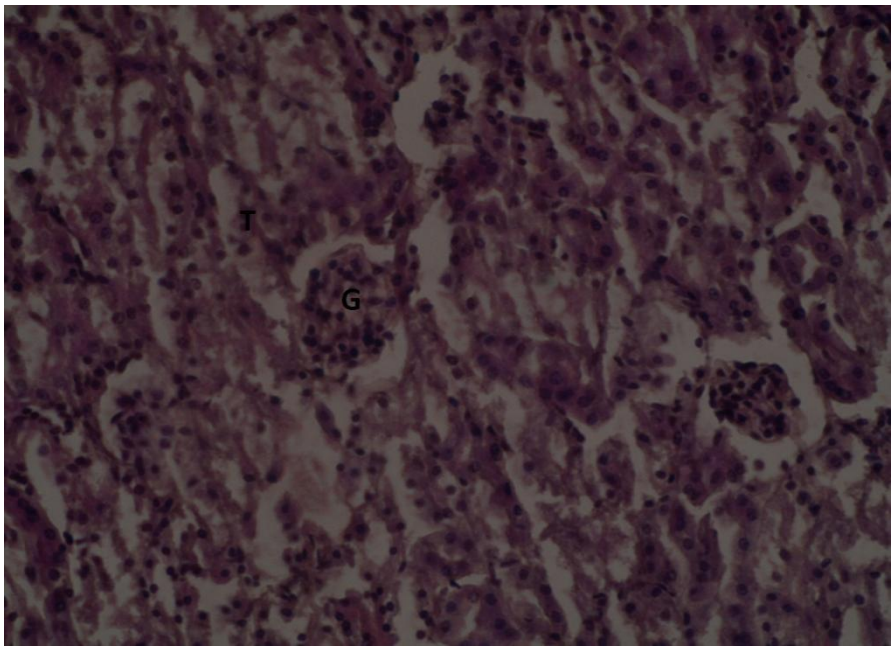
CLE 3 Low dose **Normal** Tissues okay with no significant finding

CLE 4 Control group

All tissues were normal except the kidneys of the female rats in the intermediate and low dosage groups which showed chronic renal injury (Table 4.16). The gonads of all the male rats were normal (Table 4.16).

The effects of the distilled water on the histological section of the kidneys in the female *Sprague Dawley* rat are shown in Plate 4.1.

4.7.1 *Catha edulis* (Vahl.) Forssk. ex Endl., effects of on the histological sections of the kidneys in the female Sprague Dawley rats.



T: Renal tubules **G**: Glomerulus

Plate 4.1: Normal renal parenchyma of the control group. (X400).

The effects of an intermediate dose of *Catha edulis* (Vahl.) Forssk. ex Endl., on the histological section of the kidney in the female *Sprague Dawley* rat is shown in Plate 4.2

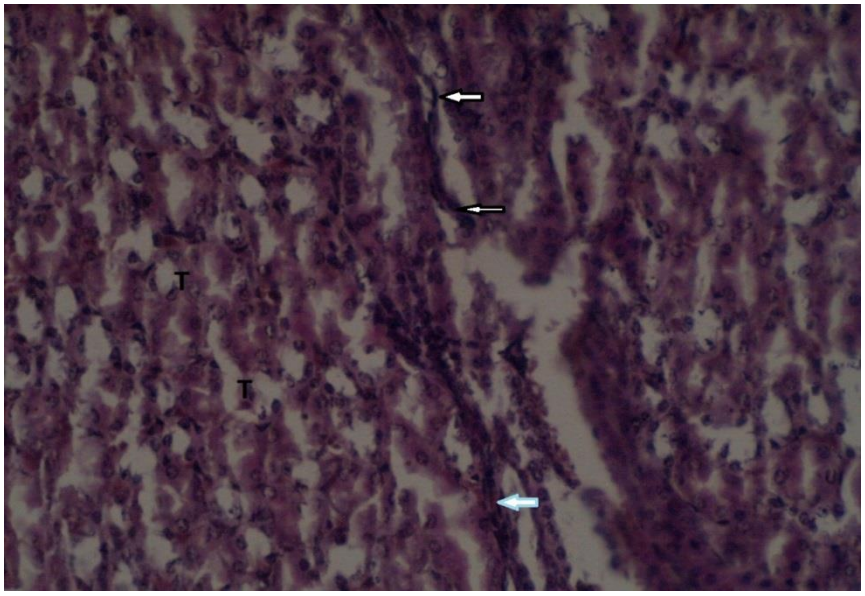


Plate 4.2: Localized interstitial connective tissue proliferation shown by the arrow indicating multifocal kidney interstitial hemorrhage in the intermediate dose group. (X400).

The effects of a low dose of *Catha edulis* (Vahl.) Forssk. ex Endl., on the histological section of the kidney in the female Sprague Dawley rat is shown in Plate 4.3.

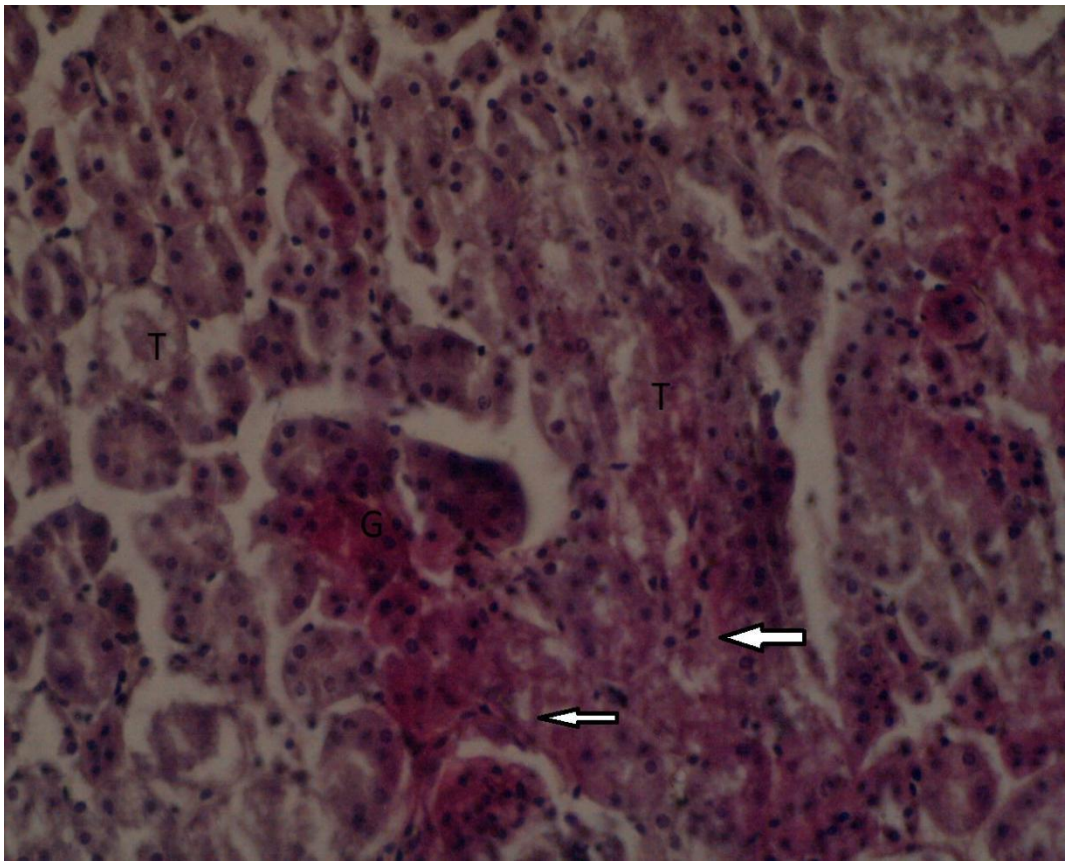


Plate 4.3: Localized tubular epithelium necrosis shown by the arrow in the low dose group. (X400).

4.8 Relative Organ Weights of male and female *Sprague Dawley* rats

The results of the mean relative organ weights of the male *Sprague Dawley* rats which were given Aqueous *Catha edulis* (Vahl.) Forssk. ex Endl., extracts are given in Table 4.17

Table 4.17: Comparison of the relative mean organ weights in male rats which were given the Aqueous extracts of *Catha edulis* (Vahl.) Forssk. ex Endl.

	Males				
Treatment	Relative mean lung weight (g)	Relative mean heart weight (g)	Relative mean gonad weight (g)	Relative mean liver weight (g)	Relative mean kidney weight (g)
Control	0.86 (0.11)[a]	0.64 (0.08)[a]	2.01 (0.20)[a]	4.85 (0.25)[a]	0.95 (0.09)[a]
250 mg/kg	1.07 (0.32)[a]	0.37 (0.02)[a]	1.90 (0.34)[a]	4.90 (0.53)[a]	0.92 (0.17)[a]
500 mg/kg	1.07 (0.29)[a]	0.37 (0.04)[a]	2.00 (0.38)[a]	4.84 (0.54)[a]	0.90 (0.05)[a]
1000 mg/kg	1.45 (0.65)[a]	0.63 (0.17)[a]	2.06 (0.19)[a]	4.80 (0.55)[a]	0.97 (0.09)[a]

Means with different superscripts along the columns are substantially different at $p<0.05$. Values in parenthesis are standard deviation.

No significant statistically change ($p>0.05$) was noted in the relative mean lung weight, relative mean heart weight, relative mean gonad weight, relative mean liver weight and relative mean kidney weight of male rats which were given distilled water and male rats which were given 250 mg/kg, 500 mg/kg, or 1000 mg/kg of the Aqueous extracts of *Catha edulis* (Vahl.) Forssk. ex Endl. (Table 4.17).

The results of the mean relative organ weights of the female *Sprague Dawley* rats which were given Aqueous *Catha edulis* (Vahl.) Forssk. ex Endl., extracts are given in Table 4.18

Table 4.18: Comparison of the relative mean organ weights in female rats which were given the Aqueous extracts of *Catha edulis* (Vahl.) Forssk. ex Endl.

	Females				
Treatment	Relative mean lung weight (g)	Relative mean heart weight (g)	Relative mean gonad weight (g)	Relative mean liver weight (g)	Relative mean kidney weight (g)
Control	0.75 (0.11)^a	0.59 (0.07)^a	0.28 (0.06)^a	4.41 (0.45)^a	0.87 (0.07)^a
250 mg/kg	0.82 (0.13)^a	0.63 (0.11)^a	0.22 (0.06)^a	4.13 (0.25)^a	0.89 (0.04)^a
500 mg/kg	1.36 (1.23)^a	0.65(0.16)^a	0.18 (0.04)^a	4.40 (0.35)^a	0.90 (0.08)^a
1000 mg/kg	0.99 (0.09)^a	0.62 (0.08)^a	0.24 (0.07)^a	4.17 (0.50)^a	0.90 (0.11)^a

Means with different superscripts along the columns are substantially different at $p<0.05$. Values in parenthesis are standard deviation.

No significant statistically change ($p>0.05$) was noted in the relative mean lung weight, relative mean heart weight, relative mean gonad weight, relative mean liver weight and relative mean kidney weight of female rats which were given distilled water and female rats which were given 250 mg/kg, 500 mg/kg, or 1000 mg/kg of the Aqueous extracts of *Catha edulis* (Vahl.) Forssk. ex Endl. (Table 4.18).

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

Ethnomedicinal relevance of *Catha edulis* (Vahl.) Forssk. ex Endl.

Studies on the Ethnomedicinal value of *Catha edulis* (Vahl.) Forssk. ex Endl., are very scarce. Between September 2014 and February 2015, Kiunga et al. (2016) undertook a survey of Traditional *Catha edulis* medicinal usage in Kenya's Meru and Embu Counties. They reported that most of the informants in the study were males (32/42; 76.19%), aged 45-84 years, and had a low level of education (Kiunga et al., 2016). This compares well with the findings of the present study where all 35 informants interviewed were males aged above 50 years and most had only a Primary level of education. These observations in both studies are not surprising considering that the cultivation and use of *Catha edulis* is mainly a preserve of men in African and Arabian Societies (Al-Motarreb et al., 2002; Kiunga et al., 2016; Siddiqui, 2012). Kiunga and colleagues also found that the leaves of *Catha edulis* were the main parts which were used and this was similar to the findings of the current study where leaves and roots were reported to be the main parts which were utilized by people. They also reported that masticating fresh material was the major method of crude drug preparation (Kiunga et al., 2016) and this was comparable to the observations of the present study where the informants reported that the medication from *Catha edulis* (Vahl.) Forssk. ex Endl., was primarily prepared by chewing fresh leaves or macerating the leaves with water and using the filtrate. Kiunga et al. (2016) reported that diarrhoea, gonorrhea, and toothache were the main indications of *Catha edulis*. In contrast, the present study delineated Human and Livestock indications of *Catha edulis* (Vahl.) Forssk. ex Endl. Some of the indications in the present study overlap with those of Kiunga et al. (2016) but more indications are provided in the present study including the use of *Catha edulis* (Vahl.) Forssk. ex Endl., in stomach-ache,

cough, fatigue, fever, heartburn, chest congestion, constipation and stress relief. Kiunga and colleagues observed that *Catha edulis* leaves were administered mostly orally with no proper dosage for any given ailment and that specific varieties had negative health effects (Kiunga et al., 2016). In the present study, preparations of *Catha edulis* (Vahl.) Forssk. ex Endl., were administered orally and the dosages, frequency, and duration of use were clearly spelt out but there was no uniformity in the treatment regimens. The storage conditions of the preparations, the side effects and their management were also observed.

In summary, the observations of the present study contribute to the body of knowledge on *Catha edulis* documented previously by Kiunga and colleagues particularly as far as the plant parts which were used, preparation of crude drugs, indications, varieties of *Catha edulis*, dosages, frequency, the duration of use, side effects and their management.

Antimicrobial properties of *Catha edulis* (Vahl.) Forssk. ex Endl.

The Aqueous, Acetone, and Methanol extracts of *Muguka* were ineffective against *Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans*. Moreover, the extracts had limited efficacy against *Bacillus cereus* and *Staphylococcus aureus* relative to the standard antimicrobial agents.

(Kithinji et al., 2021) evaluated the antimicrobial effects of the Aqueous twig and leaf extracts of *Catha edulis* from Ithanja village in Meru County against *Streptococcus pneumoniae*, *Escherichia coli*, *Candida albicans, Streptococcus pyogenes* and *Staphylococcus aureus* and reported that all the tested concentrations significantly limited the growth of the bacteria pathogens except *Escherichia coli* and was ineffective against *Candida albicans.* Siddiqui and colleagues evaluated the anti-acanthamoebic and antibacterial properties of the crude Methanolic leaf and shoot extracts of *Catha edulis* procured from a Somali shop on Edgware Road, London, UK (Siddiqui, 2012). Their study established that the crude Methanolic leaf and shoot extracts of *Catha edulis* were

amoebicidal against *Acanthamoeba castellanii*, had potent antibacterial activity against *Brevundimonas diminuta*, *Bacillus magaterium* and *Micrococcus luteus* (Siddiqui, 2012). However, it was not effective against *Escherichia coli* and yeast (*Aspergillus varicoluor, Penicillum solitum,* and *Penicillum brevicompactum*) (Siddiqui, 2012). Limited sensitivity of *Porphyromonas gingivalis* and *Tannerella forsythensis* and lack of sensitivity of *Veillonella parvula*, *Actinomyces israelii*, and some *Streptococci spp* to the Aqueous extracts of *Catha edulis* prepared from three cultivars in Yemen has also been reported (Al-hebshi et al., 2006). Moreover, Fatima and co-workers evaluated the GC-MS phytochemical profile and antimicrobial properties of Aqueous, methanol, and dimethyl sulfoxide (DMSO) extracts of *Catha edulis* cultivated in Saudi Arabia and reported that these extracts had good antimicrobial activity against *Proteus mirabilis, Escherichia coli, Klebsiellae pneumoniae, Streptococcus pyogenes, Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans* (Fatima et al., 2017).

Gram negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* have complex cell walls which comprise of peptidoglycan and an outer membrane of lipopolysaccharides and lipoproteins (Parhusip & Sitanggang, 2011; Lesage & Bussey, 2006). It could be argued that this complex cell may have limited the capacity of the Acetone, Aqueous, and Methanol extracts of *Catha edulis* (Vahl.) Forssk. ex Endl., to interact with important intracellular components of the bacteria (Fan et al., 1975; Parhusip & Sitanggang, 2011). The ineffectiveness of the Acetone, Aqueous, and Methanol leaf extracts of *Muguka* towards *Candida albicans* was associated with the presence of Chitin on the cell wall of the fungus which contributes to the cell wall strength and stability thereby impairing the penetration of the prepared extracts (Parhusip & Sitanggang, 2011; Yokoi et al., 1998). The ineffectiveness of the extracts towards *Pseudomonas aeruginosa* may possibly be due to the inability of the

prepared extracts to interfere with the permeability of the cytoplasmic membrane of the pathogen (Farkas, 1979).

Toxicity of *Catha edulis* (Vahl.) Forssk. ex Endl.

Estimates suggest that up to 20 million people chew *Catha edulis* on a daily basis Worldwide and many more rely on the plant as a source of livelihood (Bogale et al., 2016; Pennings et al., 2008). Despite the fact that such a huge population of people is exposed to *Catha edulis* habituation, there are very few studies which are available in literature to shed light on its toxicity. It is therefore important to evaluate the Toxicological properties of *Catha edulis* (Vahl.) Forssk. ex Endl., in suitable animal models.

The present investigation used a 28-day repeated dose experimental design in order to evaluate the effects of different doses (250 mg/kg bwt, 500 mg/kg bwt, 1000 mg/kg bwt) of the Aqueous leaf extract of *Muguka* on food and water consumption, weight, liver and kidney histology, Biochemical and Haematological parameters of female and male *Sprague Dawley* rats. The results of Toxicity evaluation of the Aqueous leaf extracts of *Muguka* were a mixed bag. Many of the parameters evaluated suggested that the extracts were safe but the results of a few parameters may be of concern. Female and male *Sprague Dawley* rats which received the Aqueous leaf extracts of *Muguka* exhibited a non-significant reduction in food intake after the first seven days of treatment. Similar observations were made by Al-Mamary and colleagues with the exception that the New Zealand White rabbits studied regained their appetite (Al-Mamary et al., 2002). There was no significant decrease in the water intake of female and male rats in the second week of treatment. A substantial reduction in the weight of female rats which received the highest dose of the Aqueous leaf extracts of *Muguka* were observed between week one and week two as well as between week one and week three. Decreased food and water intake appear not to be the cause of weight loss suggesting that the plant could potentially have some anti-obesity effects. Decrease in body weight could be

related to the decrease in leptin level (Alsalahi et al., 2012). Al-Shaggha and colleagues carried out a scoping review of animal and human studies on *Catha edulis* and concluded that there was acceptable evidence which suggested that extracts of the plant produced changes in terms of weight, fat mass, appetite, lipid biochemistry and hormonal levels in both humans and animals (Al-Meshal et al., 1985). It was further reported that the changes were more pronounced at higher doses and longer durations of interventions (Al-Meshal et al., 1985). The observation that female rats were more vulnerable to weight loss than their male counterparts is in agreement with OECD guidelines (Guidelines et al., 2013).

Blood is a constantly circulating fluid which provides the body with nutrition, oxygen and it helps in waste removal. It is therefore a focal point of exposure to foreign substances some of which may have untoward effects (Chibuogwu et al., 2021). By analyzing the different components of blood, it may be possible to have a sense of the Physiological and Pathological status of the body (Chibuogwu et al., 2021). Haematological analysis in this study suggests that there were no significant differences in the WBC, LYM, HGB, MCHC, MCV, and PLT levels in female and male rats which were given graded doses of *Catha edulis* (Vahl.) Forssk. ex Endl., relative to the rats in the control group. However, it was observed that the mean levels of RBC in female rats which were given the highest dose of *Catha edulis* (Vahl.) Forssk. ex Endl., were substantially greater than the mean levels in the control group. This was contrary to the findings of (Ismaeel et al., 2014) where they found out that rats which received *Catha edulis* hydro-ethanol extracts developed a reduction in RBC count as compared to the control rats which received normal saline. This variation could be due to the dose given and also the type of extracts used. A similar finding was reported by Alele and colleagues who suggested that the polycythaemia could be due to the decrease in plasma volume without a change in RBC mass in which the erythrocytes becomes more concentrated (Alele et al., 2013).

Increase in the levels of liver enzymes is usually indicative of liver damage (Chibuogwu et al., 2021; Otieno, 2016). In the present study, there was no substantial difference in the average levels of ALP, ALT, and AST, Albumin, Creatinine, Direct Bilirubin and Total protein in male and female rats which received different doses of the Aqueous leaf extracts of *Catha edulis* (Vahl.) Endl. This indicates that the extracts are unlikely to be harmful to the liver. However, the average levels of TB and Urea in female rats which were given 1000 mg/kg *Muguka* Aqueous extracts were substantially greater ($p<0.05$) than the average levels of rats in the control group. Bilirubin is a product of heme catabolism. It is conjugated with glucuronic acid in the liver making it soluble before it is excreted in the bile (Barañano et al., 2002). The levels of Bilirubin may be elevated in various disease conditions therefore making it a good indicator of liver damage (Barañano et al., 2002). The observed increase in serum Total Bilirubin and Urea levels could be suggestive of liver and kidney damage respectively but this is inconclusive as the other biomarkers were normal (Alsalahi et al., 2012).

Histopathological examination of the liver, lung, kidney, heart and testes of the male and female *Sprague Dawley* rats revealed that high, intermediate and low doses of the Aqueous leaf extracts of *Catha edulis* (Vahl.) Forssk. ex Endl., resulted in local congestion of hepatic and renal vessels. Furthermore, a low dose of *Muguka* Aqueous extracts resulted in localized interstitial connective tissue proliferation, multifocal kidney interstitial haemorrhage and localized tubular epithelium necrosis in the kidney in some female rats. The lungs and testes, on the other hand, showed no negative effects. The histoarchitecture of liver of female and male *Sprague Dawley* rats which were given a high dose (2000mg/kg bwt) of crude *Catha edulis* extracts showed a degenerative vacuolation, coagulative necrosis in pericentral region, haemorrhage and congestion (Alsalahi et al., 2012). Such changes were minimal in the medium dose (1000mg/kg bwt) group (Alsalahi et al., 2012). Moderate and severe necrotic lesions in the renal parenchyma were observed in the medium and high dose respectively of

the female *Sprague Dawley* rats (Alsalahi et al., 2012). The histopathological findings in this study indicate that there was chronic renal injury in male *Sprague Dawley* rats which were given a high dose and female *Sprague Dawley* rats which were given an intermediate and low dose of Catha *edulis* (Vahl.) Forssk. ex Endl.

5.2 CONCLUSION

The conclusions drawn from the results of this study were;

i. *Catha edulis* (Vahl.) Forssk. ex Endl., is a potential medicinal plant which is used to treat a wide variety of ailments both in humans and animals.

ii. The Aqueous, Methanol and Acetone extracts of *Catha edulis* (Vahl.) Forssk. ex Endl., have antibacterial action against gram positive bacteria *Staphylococcus aureus* and *Bacillus cereus.*

iii. Short-term administration of *Catha edulis* (Vahl.) Forssk. ex Endl., Aqueous extracts causes chronic kidney damage in *Sprague Dawley* rats, as determined by histological findings.

5.3 RECOMMENDATION

The following recommendations were made from the study;

i. There is a need in training and registering the Traditional Herbalists in order to harmonize the dosage of *Catha edulis* (Vahl.) Forssk. ex Endl., which is used in treating different ailments.

ii. Future studies on bio-screening *Catha edulis* (Vahl.) Forssk. ex Endl., should be done in order to identify and isolate the specific compounds with anti-microbial properties and by doing this, new compounds may be discovered.

REFERENCES

Abdul-Mughni, A. S., El-Nahla, S. M., Hassan, S. A., & Ali, A. A. D. (2018). Teratogenic effects of Khat (Catha edulis) in New Zealand rabbit. *Journal of Advanced Veterinary and Animal Research*, *5*(1), 25–36. https://doi.org/10.5455/javar.2018.e242

Ageely, H. M. (2009). Prevalence of Khat chewing in college and secondary (high) school students of Jazan region, Saudi Arabia. *Harm Reduction Journal*, *6*, 5–11. https://doi.org/10.1186/1477-7517-6-11

Al-hebshi, N., Al-haroni, M., & Skaug, N. (2006). In vitro antimicrobial and resistance-modifying activities of aqueous crude khat extracts against oral microorganisms. *Archives of Oral Biology*, *51*(3), 183–188. https://doi.org/10.1016/J.ARCHORALBIO.2005.08.001

Al-hebshi, N. N., & Al-ak'hali, M. S. (2010). Experimental gingivitis in male khat (Catha edulis) chewers. *Journal of the International Academy of Periodontology*, *12*(2), 56–62.

Al-Juhaishi, T., Al-Kindi, S., & Gehani, A. (2012). Khat: A widely used drug of abuse in the Horn of Africa and the Arabian Peninsula: Review of literature. *Qatar Medical Journal*, *2012*(2), 1–6. https://doi.org/10.5339/qmj.2012.2.5

Al-Meshal, I. A., Ageel, A. M., Parmar, N. S., & Tariq, M. (1985). Catha edulis (khat): use, abuse and current status of scientific knowledge. *Fitoterapia*.

Al-Motarreb, A., Baker, K., & Broadley, K. J. (2002). Khat: Pharmacological and medical aspects and its social use in Yemen. *Phytotherapy Research*, *16*(5), 403–413. https://doi.org/10.1002/ptr.1106

Al-thobhani, M.A.H., Sathyanarayana, N.B., Simon, L., Sondur, S. . (2008). First Comparative Genotypic Study on Khat (Catha edulis Forsk .) Genotypes from Yemen. *Middle Eastern and Russian Journal of Plant Science and Biotechnology*, *2*(1), 1–8. http://www.globalsciencebooks.info/Online/GSBOnline/images/0812/MERJPSB_2(1&2

)/MERJPSB_2(1)1-8o.pdf

Al-Mamary, M., Al-Habori, M., Al-Aghbari, A. M., & Baker, M. M. (2002). Investigation into the toxicological effects of Catha edulis leaves: a short term study in animals. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *16*(2), 127–132.

Al-Maweri, S. A., Warnakulasuriya, S., & Samran, A. (2018). Khat (Catha edulis) and its oral health effects: An updated review. *Journal of Investigative and Clinical Dentistry*, *9*(1), e12288.

Al'Absi, M., & Grabowski, J. (2012). Concurrent use of tobacco and khat: added burden on chronic disease epidemic. *Addiction*, *107*(2), 451–452.

Al'Absi, M., Nakajima, M., Dokam, A., Sameai, A., Alsoofi, M., Saem Khalil, N., & Al Habori, M. (2014). Concurrent tobacco and khat use is associated with blunted cardiovascular stress response and enhanced negative mood: a cross-sectional investigation. *Human Psychopharmacology: Clinical and Experimental*, *29*(4), 307–315.

Alele, P., Ajayi, & Imanirampa. (2013). Chronic khat (Catha edulis) and alcohol marginally alter complete blood counts, clinical chemistry, and testosterone in male rats. *Journal of Experimental Pharmacology*, 33. https://doi.org/10.2147/jep.s46635

Alfaifi, H., Abdelwahab, S. I., Mohan, S., Taha, M. M. E., Syame, S. M., Shaala, L. A., & Alsanosy, R. (2017). Catha edulis Forsk.(Khat): evaluation of its antidepressant-like activity. *Pharmacognosy Magazine*, *13*(Suppl 2), S354.

Ali, W. M., Al Habib, ; K F, Al-Motarreb, A., Singh, R., Hersi, A., Hussam, ;, Faleh, A., Asaad, N., Shukri, M. ;, Saif, A., Almahmeed, W., Sulaiman, K., Amin, H., Al-Lawati, J., Nizar, ;, Bustani, A., Al-Sagheer, N. Q., Al-Qahtani, A., Jassim, M. ;, & Suwaidi, A. (2011). *Coronary Heart Disease Acute Coronary Syndrome and Khat*

Herbal Amphetamine Use An Observational Report. https://doi.org/10.1161/CIRCULATIONAHA.111.039768

Alsalahi, A., Abdulla, M. A., Al-Mamary, M., Noordin, M. I., Abdelwahab, S. I., Alabsi, A. M., Shwter, A., & Alshawsh, M. A. (2012). Toxicological features of Catha edulis (Khat) on livers and kidneys of male and female Sprague-Dawley rats: A subchronic study. *Evidence-Based Complementary and Alternative Medicine*, *2012*. https://doi.org/10.1155/2012/829401

Anderson, D., & Carrier, N. (2009). Khat in colonial Kenya: A history of prohibition and control. *The Journal of African History*, *50*(3), 377–397.

Banjaw, M. Y., Miczek, K., & Schmidt, W. J. (2006). Repeated Catha edulis oral administration enhances the baseline aggressive behavior in isolated rats. *Journal of Neural Transmission*, *113*(5), 543–556. https://doi.org/10.1007/s00702-005-0356-7

Barañano, D. E., Rao, M., Ferris, C. D., & Snyder, S. H. (2002). Biliverdin reductase: A major physiologic cytoprotectant. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(25), 16093–16098. https://doi.org/10.1073/pnas.252626999

Berihu, B. A., Asfeha, G. G., Welderufael, A. L., Debeb, Y. G., Zelelow, Y. B., & Beyene, H. A. (2017). Toxic effect of khat (Catha edulis) on memory: Systematic review and meta-analysis. *Journal of Neurosciences in Rural Practice*, *8*(1), 30–37. https://doi.org/10.4103/0976-3147.193524

Bogale, T., Engidawork, E., & Yisma, E. (2016). Subchronic oral administration of crude khat extract (Catha edulis forsk) induces schizophernic-like symptoms in mice. *BMC Complementary and Alternative Medicine*, *16*(1), 1–11. https://doi.org/10.1186/s12906-016-1145-6

Chibuogwu, C. C., Njoku, U. O., Nwodo, F. C. O., Ozougwu, E. O. V., & Nweze, N. V.

(2021). Toxicity assessment of the methanol extract of Jatropha tanjorensis (Euphorbiaceae) leaves. *Future Journal of Pharmaceutical Sciences*, *7*(1), 4–11. https://doi.org/10.1186/s43094-021-00294-4

Corkery, J. M., Schifano, F., Oyefeso, A., Ghodse, A. H., Tonia, T., Naidoo, V., & Button, J. (2011). 'Bundle of fun'or 'bunch of problems'? Case series of khat-related deaths in the UK. *Drugs: Education, Prevention and Policy*, *18*(6), 408–425.

Fan, D. P., Beckman, B. E., & Gardner Eckstrom, H. L. (1975). Mode of cell wall synthesis in gram positive bacilli. *Journal of Bacteriology*, *123*(3), 1157–1162. https://doi.org/10.1128/jb.123.3.1157-1162.1975

Farkas, V. (1979). Biosynthesis of cell walls of fungi. *Microbiological Reviews*, *43*(2), 117–144. https://doi.org/10.1128/mmbr.43.2.117-144.1979

Fatima, N., Rizwan, M., Hobani, Y. H., Sayed, A. El, Kumar, B. V., Sunosi, R. Al, Abdulwahab, S. I., Areeshi, M. Y., Alvi, A., & Oriaby, M. E. (2017). *Gas chromatography / Mass spectroscopy analysis of Catha edulis Forsk , A psycho stimulant revealed potent solvent dependent antimicrobial activity*. *6*(2), 197–204.

Gambaro, V., Arnoldi, S., Colombo, M. L., Dell'Acqua, L., Guerrini, K., & Roda, G. (2012). Determination of the active principles of Catha Edulis: Quali–quantitative analysis of cathinone, cathine, and phenylpropanolamine. *Forensic Science International*, *217*(1–3), 87–92. https://doi.org/10.1016/J.FORSCIINT.2011.09.028

Geisshüsler, S., & Brenneisen, R. (1987). The content of psychoactive phenylpropyl and phenylpentenyl khatamines in Catha edulis Forsk. of different origin. *Journal of Ethnopharmacology*, *19*(3), 269–277. https://doi.org/10.1016/0378-8741(87)90004-3

Getahun, W., Gedif, T., & Tesfaye, F. (2010). Regular Khat (Catha edulis) chewing is associated with elevated diastolic blood pressure among adults in Butajira, Ethiopia: A comparative study. *BMC Public Health*, *10*, 4–11. https://doi.org/10.1186/1471-2458-

10-390

Guidelines, O., The, F. O. R., & Of, T. (2013). *Oecd guidelines for the testing of chemicals*. *July*, 1–22.

Hadi M. Mujlli,. (2005). *Neurosciences*, *86*(October 2004), 219–222.

Ismaeel, B.-J., Mohammad, A. D., Fahaid, H. A.-H., Luke, O. N., Hussein, F. S., Abdul-Moneim, J., & Mahmoud, A.-K. (2014). Derangement of hemopoiesis and hematological indices in Khat (Catha edulis) - treated rats. *African Journal of Biotechnology*, *13*(2), 349–355. https://doi.org/10.5897/ajb2013.13373

JAN NEXSON PARHUSIP, A., & BOING SITANGGANG, A. (2011). Antimicrobial Activity of Melinjo Seed and Peel Extract (Gnetum gnemon) Against Selected Pathogenic Bacteria. *Microbiology Indonesia*, *5*(2), 103–112. https://doi.org/10.5454/mi.5.3.2

Karambu, S., Matiru, V., Kiptoo, M., & Oundo, J. (2013). Characterization and factors associated with diarrhoeal diseases caused by enteric bacterial pathogens among children aged five years and below attending Igembe District Hospital, Kenya. *Pan African Medical Journal*, *16*, 1–8. https://doi.org/10.11604/pamj.2013.16.37.2947

Kithinji, D., Maina, S., Ndwigah, S., Mugo, H., & Oyugi, J. (2021). *Antimicrobial Properties of Catha edulis (Miraa) against Select Bacterial and Fungal Pathogens, an in-vitro Experimental Study*.

Kiunga, J. K., Lukhoba, C. W., Dossaji, S. F., & Yenesew, A. (2016). A Survey Of Traditional Medicinal Uses Of Catha Edulis (Celastraceae) In Meru And Embu Counties Of Kenya. *International Journal of Ethnobiology & Ethnomedicine*, *3*(1), 1–12.

Kiunga, J., Lukhoba, C., Dossaji, S. F., & Yenesew, A. (2016). *A SURVEY OF TRADITIONAL MEDICINAL USES OF CATHA EDULIS (CELASTRACEAE) IN MERU*

AND EMBU COUNTIES OF KENYA Phytochemical investigation of erythrina species for antimalarial principles View project Phytochemistry View project.

Kokwaro, J. O. (2009). *Medicinal plants of east Africa*. University of Nairobi press.

Lee Ventola, C. (2015). *The Antibiotic Resistance Crisis Part 1: Causes and Threats* (Vol. 40, Issue 4).

Lesage, G., & Bussey, H. (2006). Cell Wall Assembly in Saccharomyces cerevisiae . *Microbiology and Molecular Biology Reviews*, *70*(2), 317–343. https://doi.org/10.1128/mmbr.00038-05

Manghi, R. A., Broers, B., Khan, R., Benguettat, D., Khazaal, Y., & Zullino, D. F. (2009). Khat use: lifestyle or addiction? *Journal of Psychoactive Drugs*, *41*(1), 1–10.

Michuki, G., & Kivuva, J. (2013). Supply and demand dynamics of Miraa in selected production and onsumption regions of Kenya. *Nairobi: NACADA*.

Muema, E., Mbaria, J., Nguta, J., Kinyanjui, P., Kamau, J., Nyachieo, A., Chepkwony, S., Kamau, J., Onkoba, N., & Nyachieo, A. (2016). Toxicity and Safety of Khat (Catha edulis) Consumption during Pregnancy using Olive Baboons (Papio anubis) as Experimental Models: A Prospective Randomised Study. *Greener Journal of Epidemiology and Public Health*, *4*(3), 061–070. https://doi.org/10.15580/gjeph.2016.3.102116188

Mwenda, J M, Arimi, M. M., Kyama, M. C., & Langat, D. K. (2003). Effects of khat (Catha edulis) consumption on reproductive functions: a review. *East African Medical Journal*, *80*(6), 318–323.

Mwenda, Jason M., Owuor, R. A., Kyama, C. M., Wango, E. O., M'Arimi, M., & Langat, D. K. (2006). Khat (Catha edulis) up-regulates testosterone and decreases prolactin and cortisol levels in the baboon. *Journal of Ethnopharmacology*, *103*(3), 379–384. https://doi.org/10.1016/J.JEP.2005.08.016

Mwita, A. C., Maina, M., Nyairo, N. M., & Ramasawmy, B. (2016). Assessment of profit efficiency among sweet yellow passion fruit farmers in Mbeere south Embu county, Kenya. *Journal of Animal and Plant Sciences (JAPS)*, *27*(3), 4305–4314.

Nyongesa, A. W., Patel, N. B., Onyango, D. W., Odongo, H. O., & Wango, E. O. (2008). Khat (Catha edulis) lowers plasma luteinizing hormone (LH) and testosterone secretion, but increases cortisol levels in male rabbits. *Journal of Ethnopharmacology*, *116*(2), 245–250. https://doi.org/10.1016/J.JEP.2007.11.022

Odenwald, M., Neuner, F., Schauer, M., Elbert, T., Catani, C., Lingenfelder, B., Hinkel, H., Häfner, H., & Rockstroh, B. (2005). Khat use as risk factor for psychotic disorders: A cross-sectional and case-control study in Somalia. *BMC Medicine*, *3*, 1–10. https://doi.org/10.1186/1741-7015-3-5

Okumu, M. O., Ochola, F. O., Mbaria, J. M., Kanja, L. W., Gakuya, D. W., Kinyua, A. W., Okumu, P. O., & Kiama, S. G. (2017). Mitigative effects of Moringa oleifera against liver injury induced by artesunate-amodiaquine antimalarial combination in wistar rats. *Clinical Phytoscience*, *3*(1). https://doi.org/10.1186/s40816-017-0052-9

Ongeri, L., Kirui, F., Muniu, E., Manduku, V., Kirumbi, L., Atwoli, L., Agure, S., Wanzala, P., Kaduka, L., Karimi, M., Mutisya, R., Echoka, E., Mutai, J., Mathu, D., & Mbakaya, C. (2019). Khat use and psychotic symptoms in a rural Khat growing population in Kenya: A household survey. *BMC Psychiatry*, *19*(1), 1–10. https://doi.org/10.1186/s12888-019-2118-3

Organization, W. H. (2016). *World health statistics 2016: monitoring health for the SDGs sustainable development goals*. World Health Organization.

Osman, F. A., & Söderbäck, M. (2011). Perceptions of the use of khat among Somali immigrants living in Swedish society. *Scandinavian Journal of Public Health*, *39*(2), 212–219.

Otieno, O. M. (2016). *Prophylactic Efficacy of Moringa Oleifera Leaf Extracts Against Liver Injury Induced by Artesunate-amodiaquine Antimalarial Combination*. https://pdfs.semanticscholar.org/a43a/de746a9e094c5b0b8bde5dfe6bfe8cc6d6c1.pdf

Pennings, E. J. M., Opperhuizen, A., & van Amsterdam, J. G. C. (2008). Risk assessment of khat use in the Netherlands: A review based on adverse health effects, prevalence, criminal involvement and public order. *Regulatory Toxicology and Pharmacology*, *52*(3), 199–207. https://doi.org/10.1016/J.YRTPH.2008.08.005

Riyaz, S., Imran, M., Gleeson, D., & Karajeh, M. A. (2014a). Khat (Catha edulis) as a possible cause of autoimmune hepatitis. *World Journal of Hepatology*, *6*(3), 150.

Riyaz, S., Imran, M., Gleeson, D., & Karajeh, M. A. (2014b). Khat (Catha Edulis) as a possible cause of autoimmune hepatitis. *World J Hepa-Tol*, *6*(3). https://doi.org/10.4254/wjh.v6.i3.150

Sheikh, K. A., El-Setouhy, M., Yagoub, U., Alsanosy, R., & Ahmed, Z. (2014). *Khat chewing and health related quality of life: cross-sectional study in Jazan region, Kingdom of Saudi Arabia*. https://doi.org/10.1186/1477-7525-12-44

Siddiqui, R. (2012). Antibacterial and Anti-Acanthamoebic Properties of Catha Edulis (Khat). *Journal of Bacteriology & Parasitology*, *03*(07). https://doi.org/10.4172/2155-9597.1000152

Stefan, J., & Mathew, B. (2005). Khat chewing: an emerging drug concern in Australia? *Australian and New Zealand Journal of Psychiatry*.

Van Wyk, B.-E., & Wink, M. (2018). *Medicinal plants of the world*. CABI.

Wabe, N. T. (2011). Chemistry, Pharmacology, and Toxicology of Khat (Catha Edulis Forsk): A Review. In *Addict & Health* (Vol. 3, Issue 4).

Wayne, P. (2014). *Clinical and Laboratory Standards Institute, M100-S24 Performance Standards for Antimicrobial Susceptibipity Testing; 24th informational supplement*

(Issue January).

Yokoi, H., Aratake, T., Nishio, S., Hirose, J., Hayashi, S., & Takasaki, Y. (1998). Chitosan production from shochu distillery wastewater by funguses. *Journal of Fermentation and Bioengineering*, *85*(2), 246–249.

APPENDICES

APPENDIX 1: RESEARCH PROPOSAL APPROVAL

UNIVERSITY OF NAIROBI
GRADUATE SCHOOL

Telephone: 020 491 8033
E-mail: gs@uonbi.ac.ke

P. O. Box 30197 - 00100
NAIROBI, KENYA

Our Ref: J56/11246/2018

26th January, 2021

Kevin Kariuki Githua
C/o Chairman, Department of PHPT
Faculty of Veterinary Medicine
CAVS

Dear Dr. Githua,

RESEARCH PROPOSAL AND SUPERVISORS

This is to inform you that the Director, Graduate School has approved your MSc. research proposal titled **"Studies on Ethnopharmacology, Antimicrobial Activity and Toxicity of Catha Edulis Forssk,"** with effect from 5th March, 2020.

She has also approved **Prof. T. E. Maitho** and **Dr. Joseph M. Nguta** as the supervisors of your thesis.

You should therefore begin consulting them and ensure that you submit your thesis for examination. The Guidelines on Postgraduate Supervision can be accessed on our website (www.gs.uonbi.ac.ke) while the Research Notebook is available at the University Bookstore.

Yours sincerely,

CATHERINE NJUE (MS.)
FOR: DIRECTOR, GRADUATE SCHOOL

cc Dean, Faculty of Veterinary Medicine
Prof. T. E. Maitho – Department of PHPT
Dr. Joseph M. Nguta – Department of PHPT

CN/lwk

APPENDIX 2: APPROVAL BY BIOSAFETY, ANIMAL USE AND ETHICS COMMITTEE

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

P.O. Box 30197,
00100 Nairobi,
Kenya.

Tel: 4449004/4442014/ 6
Ext. 2300
Direct Line. 4448648

REF: FVM BAUEC/2020/256

Dr. Kevin Kariuki Githua
University of Nairobi
Dept. PHP & T
08/01/2020

Dear Dr. Githua

RE: Approval of proposal by Faculty of Veterinary Medicine, Biosafety, Animal use and Ethics committee

Studies on ethnopharmacology, antimicrobial activity and toxicity of Catha edulis forssk.

Dr. Kevin Githua J56/ 11246/2018.

We refer to your MS.c proposal submitted to our committee for review and your application letter dated December 2019. We have reviewed your application for ethical clearance for the study.

The number of rats to be used and the subacute toxicity protocol guideline meets minimum standards of the Faculty of Veterinary medicine ethical regulation guidelines.

We have also noted that registered veterinary surgeons will supervise the work.

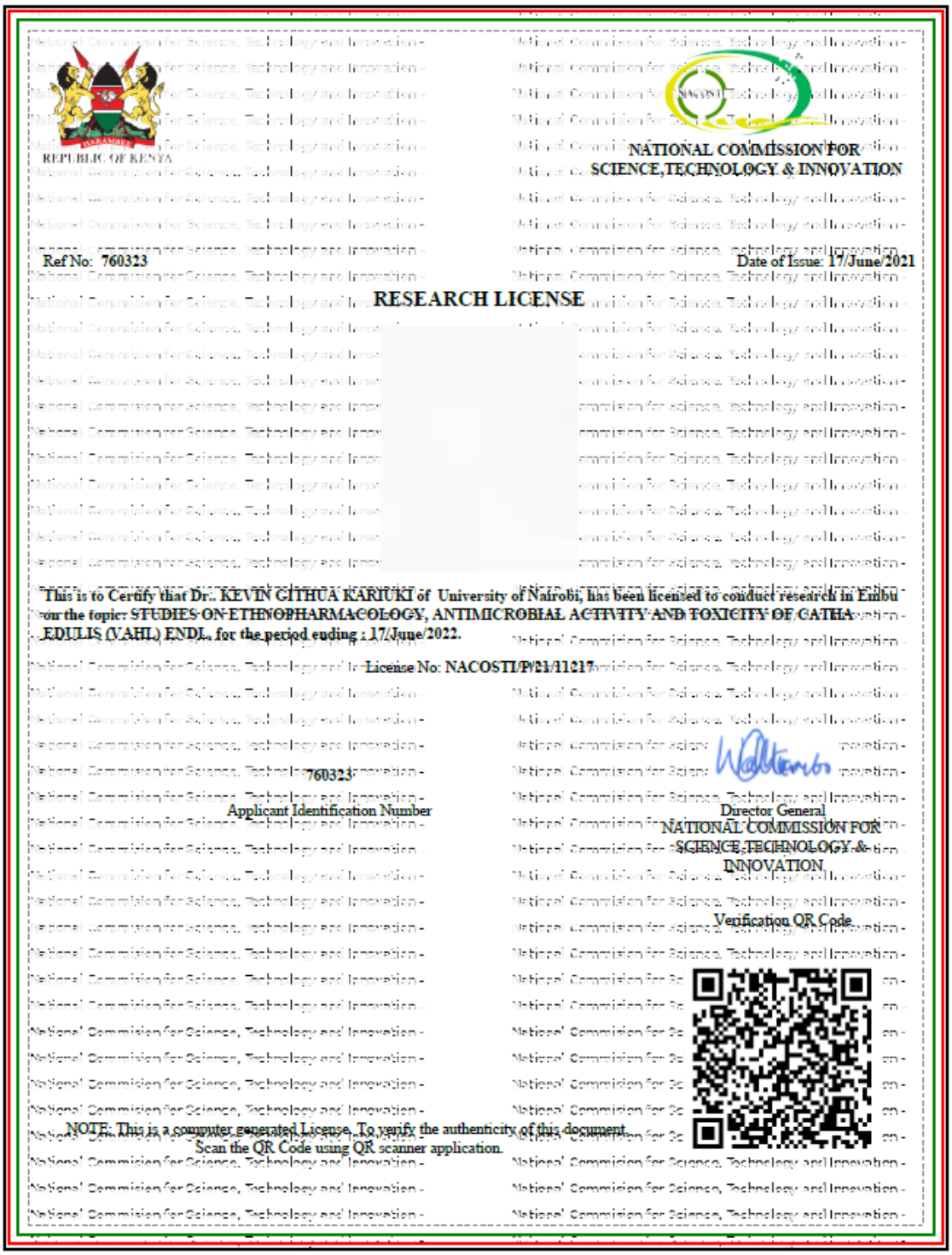
We hereby give approval for you to proceed with the project as outlined in the submitted proposal.

Yours sincerely,

Kaluwa

Dr. Catherine Kaluwa, BVM, MSc, Ph.D
Chairperson,
Biosafety, Animal Use and Ethics Committee,
Faculty of Veterinary Medicine,
University of Nairobi.

APPENDIX 3: RESEARCH APPROVAL BY NACOSTI



REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 760323

Date of Issue: 17/June/2021

RESEARCH LICENSE

This is to Certify that Dr.. KEVIN GITHUA KARIUKI of University of Nairobi, has been licensed to conduct research in Embu on the topic: STUDIES ON ETHNOPHARMACOLOGY, ANTIMICROBIAL ACTIVITY AND TOXICITY OF CATHA EDULIS (VAHL) ENDL. for the period ending : 17/June/2022.

License No: NACOSTI/P/21/11217

760323

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

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APPENDIX 4: PLANT IDENTIFICATION BY THE NATIONAL MUSEUMS OF KENYA

NATIONAL MUSEUMS OF KENYA

WHERE HERITAGE LIVES ON

NATIONAL MUSEUMS OF KENYA

WHERE HERITAGE LIVES ON

09/12/2019

REF: NMK/BOT/CTX/1/2

Mr. Kevin Kariuki

Tel.0721 983555

Nairobi.

Dear Sir,

PLANT IDENTIFICATION

The plant specimen you brought to us for identification has been determined as follows:

Catha edulis (Vahl.) Endl.

(**Family: Celastraceae**)

Thank you for consulting the EAH for plant identification and confirmation.

Yours Sincerely,

Peris Kamau

For: Head, Botany Department.

APPENDIX 5: PLAGIARISM

STUDIES ON ETHNOPHARMACOLOGY, ANTIMICROBIAL ACTIVITY AND TOXICITY OF CATHA EDULIS (VAHL.) FORSSK.EX ENDL. (CELESTRACEAE) IN SPRAGUE DAWLEY RATS

ORIGINALITY REPORT

12%	%	12%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Sook-Shien Lee, Nget-Hong Tan, Jayalakshmi Pailoor, Shin-Yee Fung. "Safety Evaluation of Sclerotium from a Medicinal Mushroom, Lignosus cameronensis (Cultivar): Preclinical Toxicology Studies", Frontiers in Pharmacology, 2017 Publication	1%
2	Luke R. Tembrock, Mark P. Simmons, Christopher M. Richards, Patrick A. Reeves et al. "Clonal Diversity, Cultivar Traits, Geographic Dispersal, and the Ethnotaxonomy of Cultivated Qat (Catha edulis, Celastraceae)", Economic Botany, 2020 Publication	1%
3	"Final Report on the Safety Assessment of BHT", International Journal of Toxicology, 10/7/2002 Publication	1%

APPENDIX 6: PUBLISHED MANUSCRIPT

F1000Research F1000Research 2022, 11:286 Last updated: 20 OCT 2022

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RESEARCH ARTICLE

REVISED Studies on the ethnopharmacology, antimicrobial activity, and toxicity of *Catha edulis* (Vahl.) Endl., in *Sprague Dawley* rats [version 2; peer review: 2 approved]

Kevin Kariuki Githua[1,2], Timothy Elias Maitho[1], Joseph Mwanzia Nguta[1], Mitchel Otieno Okumu[1,3]

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v2 **First published:** 07 Mar 2022, **11**:286
https://doi.org/10.12688/f1000research.109243.1
Latest published: 18 Aug 2022, **11**:286
https://doi.org/10.12688/f1000research.109243.2

Abstract

Background: The Mbeere South community of Embu County consume leaves of *Catha edulis* for its stimulant and euphoretic actions. Other indigenous uses of the plant are undocumented. Information on the pharmacology and safety of this plant is also scanty. This study aimed to document the ethnopharmacology, antimicrobial properties, and toxicity of *C. edulis* leaves collected from the Mbeere South community in Kenya.

Methods: Ethnopharmacological data was collected from 35 informants using semi-structured questionnaires. Leaf extracts of *C. edulis* were prepared using acetone, water, and methanol. The antimicrobial properties of these extracts were evaluated against *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Candida albicans*. The toxicity of the aqueous extract was determined using hematological, biochemical, and histopathological parameters in male and female *Sprague Dawley* rats at 250 mg/kg, 500 mg/kg, and 1000 mg/kg doses over 28 days. $p<0.05$ was considered significant.

Results: All informants were male, most were married, >50 years old, with >10 years of experience. The sources, local names, preparation, storage conditions, indications, frequency of use, dosage, and side effects of *C. edulis* were documented. All extracts were ineffective against *E. coli*, *P. aeruginosa,* and *C. albicans.* They had limited efficacy against *B. cereus* and *S. aureus.* Significant differences were observed in the hematological and biochemical parameters of rats at the tested doses. Low, intermediate, and high doses of the aqueous extract of *C. edulis* produced local congestion of the cardiac and hepatic vessels. Localized interstitial connective tissue proliferation, multifocal kidney interstitial hemorrhage, and localized tubular epithelium necrosis were also observed in female rats.

Open Peer Review

Approval Status ✓ ✓

	1	2
version 2 (revision) 18 Aug 2022		
version 1 07 Mar 2022	✓ view	✓ view

1. **Kishwar Ali**, College of the North Atlantic-Qatar, Doha, Qatar
University of Doha for Science and Technology, Doha, Qatar

2. **Julia Kimondo**, Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya

Any reports and responses or comments on the article can be found at the end of the article.