HERBAL MEDICINE USE IN MURANG'A COUNTY AND ANTIFLEA ACTIVITY AND SAFETY OF *TITHONIA DIVERSIFOLIA* AND *SENNA DIDYMOBOTRYA* EXTRACTS

BY

GITHINJI, JAMES MAINA (B.PHARM)

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DECLARATION

I declare that this thesis is my original work and has not been submitted for the award of a degree in the University.

Signature ……. Date …….

Githinji, James Maina (B.Pharm)

Reg. No J56/75109/14

Department of Public Health, Pharmacology and Toxicology

University of Nairobi

This thesis is submitted for examination with our approval as University supervisors.

Signature ……Date …….

Prof. T. Maitho (BVM, MSc, PhD)

Department of Public Health, Pharmacology and Toxicology

University of Nairobi

Signature ……. Date …….

Prof. J. M. Mbaria (BVM, MSc, PhD)

Department of Public Health, Pharmacology and Toxicology

University of Nairobi

DEDICATION

This work is dedicated to my dear wife, best friend and soul mate Jane, our sons Nephat, Brian, Alfred and Teddy for their support and encouragement throughout the study period. They have been my anchors and inspiration.

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ABBREVIATIONS

ANOVA	Analysis of variance
AR	Analytical grade
^{O}C	Degrees Celsius
CI	Confidence intervals
CM	Centimetres
DCM	Dichloromethane
Df	degrees of freedom
DMSO	Dimethyl Sulfoxide
DMAP	4-dimethylaminopyridine
FAD	Flea Allergic Dermatitis
G	Grams
g/dl	grams per decilitre
Hb	Haemoglobin
Hct	Hematocrits
IACUC	Institutional Animal Care and Use Committee
IC_{50}	Median Inhibitory Concentration
IP	Intellectual Property
IPP	Isoprenoid Pyrophosphate
Kg	Kilograms
Km	Kilometres
Km^2	Kilometres square
LD_{50}	Median Lethal Dose
M	Metres

MCH	Mean Cell Haemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Cell Volume
ML	Milliliter
Mm	Millimetre
NACOSTI	National Commission for Science, Technology and Innovations
OECD	Organization of Economic Cooperation and Development
PBS	Phosphate Buffer Saline
Pg	Picograms
PFAE	Pyrethrum Flowers Aqueous Extracts.
PLTs	Platelets
RBCs	Red blood cells
RDW	Red Blood Cell Distribution Width
SAS	Statistical Analysis System
SD	Standard Deviation
SEM	Standard Error of The Means
SDLAE/SLAE	*Senna didymobotrya* Leaf Aqueous Extract
TDLA/ TLAE	*Tithonia diversifolia* Flower Aqueous Extract
µg	Microgram
µl	Microliter
WBCs	White Blood Cells
W.H.O	World Health Organization

ABSTRACT

Herbal medicines have been used for a long time to control various human and animal diseases in the world. There is limited published information on the use of herbal medicines in Murang'a County of Kenya, despite the County having large and diverse species of plants claimed to have medicinal value in the literature. *Tithonia diversifolia* and *Senna didymobotrya* have been reported to have medicinal value. While *T. diversifolia* is used to control jigger fleas in Kakamega County of Kenya; both plants are used in the management of constipation, abdominal pain, diarrhoea, malaria, diabetes mellitus and microbial ailments. The objectives of this study were to investigate and document the use of herbal medicines in Murang'a County, and also to investigate antiflea activity and safety of *Tithonia diversifolia* and *Senna didymobotrya*. Questionnaires were used to interview a total of 28 herbalists for ethno-medical uses of the plants. A total of 119 plant specimens were collected after feedback from the herbalists. The plants were identified, mounted, allocated voucher numbers and stored in the herbarium at East Africa Herbarium. Fifty nine (50%) plants were used by the herbalists for the treatment of more than one disease condition, and they were also used by two or more herbalists for the treatment of same condition. *Tithonia diversifolia* and *Senna didymobotrya* were selected for the purpose of this study. The two plants specimens were collected from Murang'a County. Each plant part claimed to have antiflea activity was extracted by maceration using methanol and water, for methanol and aqueous crude extracts respectively. The crude extracts were tested for the *in vitro* activity using fleas obtained from dogs as a model for the other types of fleas like *Tunga penetrans* and the activity compared with those of pyrethrum flowers crude extracts. A total of ten fleas were placed in each of the 15 ml polypropylene centrifuge tubes which were fitted with a split filter paper pre-coated with aqueous extract. The extract were thereafter investigated for *in vitro* activity and the extracts which were active were used for the study of acute toxicity, dermal irritation, sensitivity and eye irritation tests, using standard methods. Data was analyzed using Microsoft excel and *in vitro* antiflea activity data was analyzed using Student's t-test, R version 3.4.3(2017-11-30). The acute toxicity was evaluated using OECD guideline 425. *Tithonia diversifolia* flowers extract had antiflea activity (93.3%) which was similar to that of pyrethrum flowers (90%), and it was only slightly toxic with LD_{50} of above 10,000 mg/kg body weight. *Senna didymobotrya* had lower antiflea activity (66.3%) when compared to *Tithonia diversifolia* leaves which had antiflea activity of 86.7% and also had more toxic effects on blood profile. The findings of this study increases the knowledge of herbal medicines used in Murang'a County, and fleas control and the plants can be studied further for the control of *Tunga penetrans* infestation in humans.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Botanical pesticides have a proven track record and a long use as simple extracts for pest control and have spun off important groups of synthetic pesticides from phytochemical sources such as pyrethroids and neonicotinoids (Harborne, *et al.,* 1999). The advantages of botanical pesticides lie in their rapid degradation, lack of persistence and bioaccumulation in the environment, which have been major challenges with the synthetic use (Helson, *et al.,* 1996, and Mkindi, 2015).

The long safety history of some plants natural products provides some confidence about their low risk, although this cannot be assumed for new products. The diversity and redundancy of phytochemicals in botanical extracts is also useful. Redundancy, which is the presence of numerous analogs of one compound, is known to increase the efficacy of extracts through analog synergism, reduce the rate of metabolism of the compounds, and prevent the evolution of pesticide resistance when selection occurs over several generations. From a research discovery point of view, the numbers of insect deterrents derived from plants seem endless as co-adaptation appears to have produced a huge diversity of novel compounds across the plant kingdom and a remarkable redundancy of plant defenses within each plant species. Research activities have provided application for behaviour modifying anti-feedants, essential oils with repellant, fumigant and insecticidal action, and a large number of agents with novel modes of action. Plant pesticides are also reported to be more cost effective compared to synthetic pesticides, (Mkenda *et al.,* 2015). Despite the many advantages, the botanical pesticide market has a number of major challenges and although there has been growth, it is not comparable to the non-botanical medicine market in recent years. Some of these challenges have been reviewed. The major setback is the costly toxicology testing for new products

which may have limited Intellectual Property (IP) protection and a relatively small market size (Isman, 2006).

Other challenges include economical supply of plant products, quality control, and lack of stability. There is also competition from other bio-pesticides and bio-control agents. These and other drawbacks have discouraged many investigators from carrying out substantial studies on medicinal plants. The aim of this study was to evaluate the medicinal plants used in Murang'a County. The study investigated the antiflea activities of *Senna didymobotrya* (Plate 1) and *Tithonia diversifolia* (Plate 2) crude extracts as well as their safety and efficacy profiles. It provides a framework for further studies, on isolation of active compounds for management of fleas as well as validation of the study plants for use.

Plate 1: Aerial part of *Senna didymobotrya* **Plate 2: Aerial part of *Tithonia diversifolia***

1.2 Problem statement

There is limited information on herbal medicines used in Murang'a County and therefore, this study provides information on herbal plants used in the County. Although local herbalists claim they treat many diseases with herbal remedies, the information on this aspect remains scanty. For instance, no information was available on *Tithonia diversifolia* and *Senna*

didymobotrya anti-flea activity. There is also no information available on acute toxicity of the aqueous extracts of the two plants despite the fact that traditional herbalists use water as their vehicle for extraction.

In Murang'a County where jigger infestation is prevalent, control is mainly through mechanical removal usually with sharp objects which are shared by several people and this can lead to transmission of other infections (Mwangi *et al.,* 2015). Although there are claims of many available herbal remedies used in the management of jigger infestation in Murang'a County, their potential has not been explored by way of scientific research.

This study provides information on herbal medicine use in Murang'a County, the anti-flea activity and toxic effects of the aqueous extracts of *Tithonia diversifolia* and *Senna didymobotrya* in animals.

1.3 Justification of the study

Fleas are the most predominant ectoparasites of domesticated pets especially dogs and cats throughout the world (Beugnet and Franc, 2010; Farkas, *et al*., 2009). They feed on blood by sucking through the skin of their hosts. These pests are not restricted to animals only. They are passed from farm animals to humans and cause similar effects as in the animal hosts. Due to their exclusive ability to reproduce in households, fleas are present all year round, even during winter season, which explains why dogs living in cold areas are also infested. Flea bites may induce pruritus and the major pathogenic effect is flea allergic dermatitis (FAD) among other health effects. FAD prevention is based on the control of the flea infestation, involving regular and continued use of anti-flea drugs, which are usually topical formulations but can also be given orally (Beugnet and Franc, 2010; Farkas, *et al.,* 2009; Masuda *et al.*, 2002; Medleau, *et al.,*2003). Epidemiological surveys regarding flea infestations have been remarkably reported in the literature. A study in Murarandia Division of Murang'a County shows there is significant reduction in agricultural productivity among farmers infested by

jigger fleas, where about 67.35% of farmers are infected by jiggers (Muhoro, 2015). One of the Sub-Counties in Murang'a County; Kandara was reported to have 6,200 school going children infested by Tungiasis in 2014 (Zablon, 2017). In spite of such surveys, not much effort has been made in order to provide sufficient health care to those suffering from jigger infestation, and most have resulted to mechanical removal of the jiggers with sharp objects such as needles (Mwangi *et al.,* 2015). There is little information on the effects of crude plant extracts like *Tithonia diversifolia* and *Senna didymobotrya* on fleas which is reported in animals. Additionally, the toxicity and safety profiles of these plants have not been reported adequately in various animals. It is important to investigate the LD_{50} of these plants' extracts as insecticides have been associated with accidental poisoning. If the two plants' extracts are found to be effective, more investigations can be conducted in order to check whether the same extracts can be effective on the sand flea, which is a jigger causing flea and is reported as a menace to the poor residents of Murang'a County among the many regions of Kenya.

1.4 Objectives

1.4.1 General Objective

The general objective of the study was to evaluate and document the use of herbal medicines in Murang'a County, investigate anti-flea activity and safety of *Tithonia diversifolia* and *Senna didymobotrya* extracts in animals.

1.4.2 Specific objectives

The specific objectives of this study were;

(i) To assess herbal medicine use in Murang'a County.

(ii) To investigate *in vitro* anti-flea activity and efficacy of *Tithonia diversifolia* and *Senna didymobotrya* crude extracts.

(iii) To determine the effects of *Tithonia diversifolia* and *Senna didymobotrya* extracts on skin sensitization and irritation.

(iv) To determine the safety of *Tithonia diversifolia* and *Senna didymobotrya* crude extracts.

1.5 Assumptions of the study

The herbal medicines use is distributed uniformly in the area of study and there is uniform distribution of anti-flea activity in selected parts of the plants, in different climate areas.

It was also assumed that the herbalists gave accurate information.

CHAPTER TWO

LITERATURE REVIEW

2.1 Scope of the Literature Review

The literature review contains information on traditional botanical insecticides, ethno medical uses and activity of herbal medicine, fleas and insecticides used to control fleas.

2.2 Traditional botanical insecticides

The traditionally used botanical insecticide products include; nicotine, rotenone, ryania, sabadilla and pyrethrum. Although nicotine and tobacco have a long history of use and are effective contact and ingested insecticides, they also have extremely high mammalian toxicity and are candidates for phase out from the market. In various countries, tobacco is still used in some greenhouse applications. Rotenone is the trade name of the insecticide derived from extracts of the tropical legumes *Derris and Lonchocarpus*. The main active principle, is isoflavonoid rotenone, which is moderately toxic to mammals due to its poor absorption and rapid metabolism, but is highly toxic to insects and fish, due to its rapid uptake and inhibition of respiratory electron transport at site 1 (Isman, 2002). Sabadilla is the seed extract of the neo-tropical lily *Schoenocaulon officinale*, which contains veratridine alkaloids, which have a neurotoxic mode of action. The extract has low mammalian toxicity and is a useful contact insecticide against a number of agricultural insects such as lepidoptera, leafhoppers, and thrips. Ryania is an extract from young stems of the South American shrub (Ryaniaspeciosa) containing the diterpene alkaloid ryanodine, which is a contact and ingested insecticide against horticultural and ornamental crop pests as well as animal ectoparasites. It exerts its toxicity by blocking the Ca^{2+}channels (Isman, 2002).

Pyrethrum is now the most important traditional botanical insecticide in the market. It is derived from the African daisy. Chrysanthemum pyrethrum produces an insecticidal

oleoresin which can be extracted with organic solvents. Pyrethrum extract contains six major pyrethrin compounds: pyrethrin I, II, jasmolin I, II, cinerin I, and II (Jensen *et al.,* 2006). These compounds are monoterpenes derived from IPP and DMAP in which an unusual cyclopropane ring is formed, while the jasmolone functionality is derived from cyclization of fatty acid derivatives. Pyrethrin is valued for its quick effect on flying insects especially the mosquitoes and houseflies. It is also effective against a wide variety of home, garden and nuisance insects, due to its action on the insects' nervous system Na^+ channels (Isman, 2002; McCage, *et al.,* 2002). The natural sources mentioned to have anti-flea activity include, lemongrass, rosemary (*Rosmarinus officinalis*), lemon, eucalyptus oil, lavender and Mexican marigolds (*asteraceae).* (*www.herbalcare.com*). Plant pesticides were reported to be more cost effective than synthetic pesticides for plants pest control (Mkenda *et al.,* 2015). Water extracts of *Tithonia diversifolia* were confirmed to have known insecticides compounds known as sesquiterpene lactones and tagitinin A (Mkenda *et al.,* 2015). Pesticides from plant compounds are less persistent in the environment due to their easy natural degradation. They are less harmful to the environment (Mkindi *et al.,* 2015).

2.2.1*Tithonia diversifolia*

Tithonia diversifolia is a common shrub which grows in grasslands, and is distributed in different regions in East Africa. It was introduced as an ornamental plant in Kenya from Central America, and is now found in Western and Central Provinces, Coastal regions and in some parts of Rift Valley. It grows in regions of 550-1950 m altitude regions with a mean annual temperature of 15-31^{o}C and a mean annual rainfall of 100-2000 mm.

The plant is commonly known as the Mexican sunflower, Tithonia and tree marigold. The various communities in Kenya have different local names for the plant, for instance, the Abagusii call it *Amaua maroro,* the Kikuyu *Maruru,* the Akamba *Ilaa,* the Luo *Maua makech, akech, Maua madung* and the Luhya *Maua amalulu.*

2.2.1.1 Botanical description

Tithonia diversifolia (Plates 3 and 4) belongs to the Asteraceae (also called Compositae) family, and is a woody herb or succulent shrub, 1.2-3 m tall. The plant has sub-ovate opposite or alternate leaves with 3-7 lobes, attenuate base, acute apex, crenate margin. Leaf size is 5-17 x 5-12 cm, densely pubescent beneath, palmate venation. Occasionally, the upper leaves are unlobed. Its flowers are yellow; with ray size of 3-6 cm by 0.5-1.8 cm. The flower heads are solitary on a peduncle 6-13 cm long. A mature stem bears several flowers at the top of branches. The plant flowers and produces seeds throughout the year. The seeds are light weight and are dispersed by wind, water, and animals (Beentje, 1994, Gachathi, 2007).

Plate 3: *Tithonia diversifolia* plant leaves

Plate 4: ***Tithonia diversifolia* flowers**

2.2.1.2 Ethno-medical uses and biological activity

Tithonia diversifolia infusion has been mentioned and used in the treatment of constipation, stomach pains, indigestion, sore throat, 'liver pain' and malaria. The various biological activities such as anti-inflammatory, analgesic, antimalarial, antiviral, antidiabetic, antidiarrhoeal, antimicrobial, antispasmodic, vaso-relaxant and cancer-chemoprevention effects have been reported in the literature (Chagas-Paula *et al*., 2012; Olayinka *et al.*, 2015). *Tithonia species* contains sesquiterpene lactones and diterpenoids which have biological activities against insects (Adayo, *et al.*, 1997). Most of the bioassays have been conducted on the extracts without specifying which compounds are responsible for each effect (Adayo, *et al.*,, 1997). *Tithonia diversifolia* is used in Ikolomani Division of Kakamega County, Kenya,

in management and control of jigger fleas (Shisanya, 2011). *Tithonia diversifolia* is commonly known and used in Nigeria in the management of Malaria, where the leaves are macerated in alcohol and drunk to treat chronic Malaria (Elufioye *et al.,* 2009; Goffin *et al.,* 2002). The use is supported by a study which revealed the presence of a new sesquiterpene lactone and the efficacy of methanolic extracts demonstrated *in vivo* in mice (Elufioye and Agbedahunsi 2004). *Tithonia diversifolia* has analgesic, anti-inflammatory and anti- diabetic activity according to the herbalists as reported by Sijuade, *et al.,* (2016).

Studies by Selvahohan *et al.,(*2012) demonstrated antibacterial activity of the aqueous extracts and provided scientific basis for the traditional use of the plants in treatment of bacterial infections. A *Tithonia diversifolia* phytochemical screening demonstrated appreciable amounts of alkaloids, flavonoids, phenols, tannins, terpenoids and aromatic compounds. Leaves and roots were found to have more secondary metabolites which accounted for their activity as opposed to the stems. Tona *et al.,* (2008) reported that *Tithonia diversifolia* Sesquiterpene lactones and Tagitinins are responsible for the antimicrobial and pesticide effects. Ogunfolakan *et al.,* (2010) suggested a possibility of *Tithonia diversifolia* leaf extract having reasonable potential as a broad spectrum antibiotic against human pathogens, but the research findings by Taiwo *et al.,* (2007) had reported Tithonia *diversifolia* not showing much applicable antibacterial effect on Gram positive and Gram negative bacteria.

In another study, Orwa *et al*., (2009), reported that *Tithonia diversifolia* leaves have more active constituents compared to the other parts, but Olayinka *et al.,* (2015) found that the roots demonstrated likelihood of having significantly high amounts of the bio-active compounds with antimicrobial activity.

Tithonia diversifolia is used in Uganda for field and storage pest management, (Kandungu *et al.,* 2013 and Mwine *et al.,* 2011). Adoyo *et al.,* (1997) found *Tithonia diversifolia*

concoction to be a very effective termite control pesticide. *Tithonia diversifolia* leaf extract was found to exhibit adverse effects on mosquito larvae hence the need for further evaluation for mosquito control (Nkumah, 2015 and Uhuo *et al.,* 2015).

Tithonia diversifolia is among the plants listed by the World Agroforestry Centre (ICRAF)'s Tree Diversity as pesticides alongside; Kaffir Orange, *Aloe forex, Tephrosia vogelli, Vernonia amygdalina* (strychnos spinosa), *Solanium incanum* and *Tagetes minuta*. *Tithonia diversifolia* was reported to possess hematological and acute toxic effects on the liver and kidneys which are dose and time dependent. However the toxic effects on liver and kidneys are reversible. The LD_{50} of the ethanoic extracts was reported to be greater than 1600 mg/kg (Elufioye *et al.,* 2009).

2.2.2 *Senna didymobotrya*

Senna didymobotrya belongs to Fabaceae family which is also called Leguminosae or pea family. The family comprises of flowering plants (angiosperms), within the order Fabales. *Senna didymobotrya* was previously referred to as popcorn cassia. It is a hairy, aromatic, and a several-stemmed shrub or small tree, which is usually five meters tall, but can grow up to nine meters. The branches are smooth and cylindrical, striate, pubescent to villous, rarely hairy. The leaves are up to a metre long and are made up of elongated oval leaflets each up to 6.5 cm long. Naturally, *Senna didymobotrya* is often found growing on waste ground, in riparian or inhabiting mountainous wooded grassland or evergreen bush land. It tolerates light frost. The plant grows in altitudes of between 900-2400 metres.

2.2.2.1 Botanical description

Senna didymobotrya (Plate 5) leaves are pinnately compound, narrowly oblong-elliptical in outline, 10 to 50 cm long; stipules broadly ovate heart-shaped, 6 to 17 mm by 8 to10 mm, acuminate, palmately veined, reflexed, deciduous; petiole terete, 1 to 8 cm long, stem of up to 40 cm long, both pubescent and eglandular; petioles up to 3 mm long; leaflets in 8 to 18

pairs, chartaceous, elliptical oblong, 2 to 6.5 cm by 0.5 to 2.5 cm, two to three times longer than wide, base oblique, apex rounded but sharp, pubescent to hairless, marginal vein distinct. Inflorescence an erect, axillary, 20 to 30 flowered, spike-like raceme, 10 to 50 cm long; peduncle terete, 5 to 8 cm long, pubescent; bracts broadly ovate, 8 to 27 mm x 5 to15 mm, black green, at first imbricate and enclosing the flower buds; bracteoles absent; pedicel slender, 3 to10 mm long, densely pubescent; five sepals, sub equal, egg-shaped, 9 to 14 mm long, hairy, green; petals 5, slightly unequal, at first incurved, later on more spreading, ovate to obovate, 17 to 27 mm by 10 to 16 mm, with a slender, about 1 mm long claw, glabrous, bright yellow, delicately veined; stamens 10, filaments shorter than anthers, anthers of two lower stamens 9 to 11 mm long, three upper stamens staminodial, anthers of five median stamens about 5 mm long; ovary and stipe velvety pubescent; style slender, glabrous, recurved, about 10 mm long; stigma punctiform.

Fruits are flat, 9 to 16 seeded pod, linear-oblong, 7 to 12 cm by 1.5 by 2.5 cm, glabrescent, short beaked, dehiscent or indehiscent when dry, depressed between the seeds, sutures raised, blackish-brown. Seeds are flattened, oblongoid, apiculate, 8-9 mm x 4-5 mm x 2.5 mm, smooth, pale brown; areole elliptical, 3-4 mm x 0.7-1.5 mm (Orwa *et al.*,2009).

Plate 5: ***Senna didymobotrya*** **plant**

2.2.2.2. Ethno-medical uses and bioactivity

Senna didymobotrya is widely used as a purgative and as an anti-malaria medicine. A decoction of the leaves is used against abdominal disorders. Leaves and roots contain a number of anthraquinones, choline, and the tri-saccharide raffinose. It is also used as an ornamental plant due to its brightly coloured flowers (Orwa *et al.,* 2009). *Senna didymobotrya* is traditionally used by the Kipsigis community of Kenya in control of malaria (Korir *et al.,* 2012). It is also used in management of livestock diseases (Njoroge and Bussman, 2007). *Senna didymobotrya* is hardy and quite free from pests and diseases (Orwa *et al.,* 2009).

Senna didymobotrya is also used in the management of skin diseases in animals by application of leaves pulp and young stems that have been pounded on the animal skin (Njoroge and Bussman, 2007). Decoctions made from the leaves are used to control ticks (Njoroge and Bussman, 2006). Other uses of the leaves include treatment of diarrhoea, dysentery and as a diuretic agent, laxative and as an agent for inducing emesis (Tabuti, 2007). The ash of burnt twigs is used to coat the inside of guard for milk storage by the Kipsigis people, in Kenya. This milk can be kept in the guard for a long period of time even for a year (Tabuti 2007). In Kenya and Uganda, *Senna didymobotrya* roots infusion is used to treat diarrhoea (Tabuti, 2007). The powder of the roots or leaf decoction is taken to treat venereal diseases and abscesses. Other uses of the plant include, antihypertensive, diabetes, treatment of P.I.D (Pelvic Inflammatory Disease), fibroids, to stimulate lactation, procuring of abortion, treatment of fungal and bacterial infections (Tabuti 2007 and Kareru, *et al.,* 2008).

Senna didymobotrya extracts showed antimicrobial effects against Gram positive, Gram negative and fungal isolates. Its acute toxicity was found to be dose and concentration dependent with the LD_{50} of DCM extract being between 1000-5000 mg per kg body weight (Korir *et al.,* 2012).

2.3 Fleas' Life Cycle and Role as Disease Vectors

Fleas are small wingless bloodsucking insects of the order siphonaptera, with a characteristic jumping movement. They mainly feed on birds and mammals including humans. The most important fleas are those that feed on rats, human, dogs and cats. They are vectors for bubonic plague and typhus diseases. The dog and cat fleas closely resemble each other. These fleas feed on blood of the host animals, but sometimes bite human as well. They can live on a wide range of animals. They can live without food for several months, but female fleas must get a blood meal before producing eggs. Both dog (Plate 6) and Cat fleas (Plate 7) are associated with the transmission of tapeworms. Dog fleas spread *Dipylidium caninum* (flea tapeworm, double-pore tapeworm or cucumber tapeworm). T*apew*orms affect pets and human pet-owners, especially children (Garcia-Martos *et al.,* 2014). Fleas are also vectors for diseases such as plague, endemic typhus and *ricketssia typhi* according to Bitam, *et al.,* (2010).

The sand flea, which is also known as *Tunga penetrans*, Chigoe or jigger, burrow into the skin of humans causing jigger infestation (Awoke and Kassa, 2006).

Chicken fleas (*Ceratophyllus Gallinae*) or Sticktight flea (*Echidnophaga gallinacea*) are known to infest bird cages, poultry shelters and other building structures where animals and birds are housed. Host of adult flea include chicken, turkeys, pigeons, people and other mammals. Sticktight fleas are a common problem in dogs and have been reported on horses, pigs and humans (Philips, 2013).

According to Bitam, *et al.,* (2010), flea-borne infections are emerging or re-emerging throughout the world, and their incidence is on the rise. The past decades have seen a dramatic change in the geographic and host ranges of many vector-borne pathogens, and their diseases. This process is often enhanced by the climatic changes and the destruction of wild habitats. Fleas, as hosts for a wide range of largely under studied pathogens (except *Yersinia*

pestis) are no exception, and flea-borne diseases may re-emerge in epidemic form (Bitam, *et al.,* 2010). In Murang'a County, the prevalence of *Tunga penetrans* is associated with poor living conditions such as earthen, mud-walled houses and feet exposure to unhygienic conditions such as wearing open shoes (Mwangi *et al.,* 2015).

Plate 6: **Dog Flea (*Ctenocephalides canis*)**

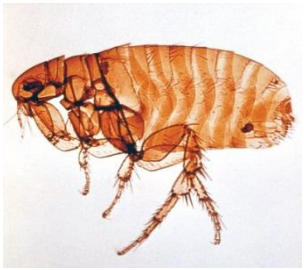

Plate7: **Cat fleas (*Ctenocephalides felis*)**

Photo obtained from *doyourownpestcontrol.com* (accessed on 3rd March, 2017)

The dog and cat fleas closely resemble each other.

2.3.1 Life cycle of fleas.

The life cycle of fleas has four stages, egg, larva, pupa, and adult (Plate 8 and Plate 9**)**.

Adult fleas are 1-4 mm long with a flat narrow body. They are in various colors ranging from light to brown. Though wingless, their well-developed legs are adopted for jumping.

The larvae are 4-10 mm long, are white, legless, but very mobile. The pupa stage is characterized by the cocoon which is sticky and becomes covered with dust, sand and other particles thus camouflaged. Both male and female take blood meals. They breed close to the resting or sleeping places of their host, in dust, dirt, rubbish, cracks on the floors or walls, carpets, animal burrows and birds' nests.

Fleas require high humidity for their development. Their larvae feed on organic matter like the feaces of the host, small dead insects and undigested blood expelled by the adult fleas. The larva then spins a loose whitish cocoon and develops into a pupa. Fleas may remain in cocoon for years in a vacant house but they emerge from the cocoons on receiving a

stimulus such as vibrations caused by their hosts, developing fully in to adults within 1-2 weeks. It takes two to three weeks for the fleas to develop from eggs to adults in optimal conducive conditions.

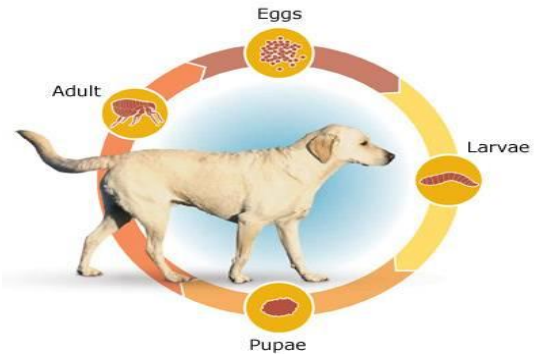

Plate 8: **life cycle of dog flea**

The entire flea cycle takes about 16 days, only 5% of the eggs mature to adult fleas (Plate 9)

Adopted from Novartis Animal Health Australia (2009)

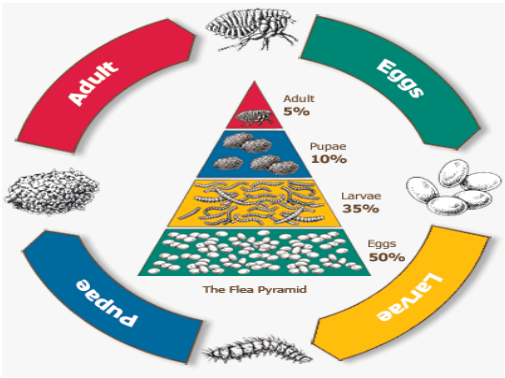

Plate 9: **Life cycle of the dog flea showing maturity proportions** (***Adopted from Julian, 2012)***

2.3.2 Behaviour of fleas

Fleas avoid light and are found among the hair and fur or feathers of the hosting animal. They are also found in peoples' clothing and bedding. They feed several times at night or during the daytime. Most species feed on one or two species of hosts in the normal situation but in the absence of their normal hosts, they feed on human and other animals.

Fleas move by jumping and can jump as high as 30 cm depending on the species.

2.3.3 Public Health importance

Fleas are pests which are a nuisance and they are vectors of diseases. Three types of plagues in human are associated with fleas. These are bubonic plague, pneumonic plague and septicemic plague. Rat and cat fleas are said to cause murine typhus fever caused by *rickettsia typhi*. This is mainly transmitted by the rat and cat fleas, where humans acquire the infection as result of contamination from the dried feaces and crushed bodies of the fleas. The sand fleas, chigoes or jigger fleas are only a millimeter long and are nuisance as the females burrow into the skin. They are common in Central America, South America, the West Indies and Africa.

Mostly, a person is usually affected by one or two jiggers at a time, but infestations with hundreds of jiggers also occurs. Under favourable conditions a complete cycle from eggs through larvae, pupa and finally adult, can be as short as 18 days (WHO, 1997, Awoke and Kassa 2006).

Arthropods (fleas and ticks) determine the community composition of bacteria (Hawlena *et al.,* 2013). Fleas are hosts for many vector- borne pathogens and their diseases (Bitam *et al.,* 2010). Among the many diseases causing bacteria hosted by fleas is *Yersinia pestis* which causes plague in man. No one type of flea is specific to humans and only a fraction of the fleas come into contact with human on regular basis. Fleas and ticks have economical rather than direct effects on human. Abundance of human associated fleas (*Pulex irritans, Ctenocephalides felis, and X.cheopis*) however, has been described in human dwellings in plague-endemic regions of Africa (Laudisoit *et al.,* 2007). Human (*P irritans*) fleas are associated with plague transmission from human to human, and Cat fleas (*C fellis)* too are suspect as in outbreaks for example in northwest Uganda (Bitam *et al.,* 2010). Clinical manifestations of plague are; bubonic plague that is most common, septic plague without bubo, and pneumonic plague, meningitis and pharyngitis (Prentice, 2007). Pneumonic plague

is rapidly fatal if untreated. Fleas and ticks must be controlled in order to reduce risk of disease transmission as well as to address the economic losses associated with parasitization of domesticated animals (Gage *et al*, 2008). *Rickettsia typhi* is associated with fleas, ticks, mites and body lice. The obligate intracellular gram negative bacteria are transferred from rodents' reservoir by an arthropod to humans (Traub *et al*., 1978).

Tunga penetrans fleas are associated with secondary bacterial infections in the lesions. Pathogenic bacteria which have been isolated from *tungiasis* lesions include *Clostridium tetani*, *Streptococcus pyogens*, *Staphylococcus aureus*, *Klebsiella aerogenes*, *Enterobacter agglomerans*, *Escherichia coli* as well as other enterobacteriaceae (Bitam *et al.,* 2010).

2.4 Summary of Common Insecticide Used to Control Fleas

A summary of common insecticides used to control fleas, types of formulations and associated hazards is given in Table 1.

Table 1: Common insecticides used to control fleas

Class /chemical	Example	formulation	Associated Hazards
Organophosphates	Malathion	Dust Spray	Insect resistance, CNS toxicant to animals and bees, Carcinogenic, Endocrine disruptor
Carbamates	Carbaryl (sevin®),	Dust , spray, collar Dip	Insect resistance, CNS toxicant to human, pets and bees, Reproductive and developmental ,toxicity, Carcinogenic
Essential oils	D-limonene, eugenol, clove, cinnamon, eucalyptus, geranium	Spot-on, Spray, and shampoo	Insect resistance, highly toxic to cats, skin irritants, can trigger Asthma,
Pyrethroids	Deltamethrin, cypermethrin, Cyhalothrin, Permetrin	Spot-on Dust Spray	Insect resistance, CNS toxicant, Highly toxic to bees, Highly toxic to cats
Pyrethrins	Pyrethrin 1 and 2	Spot-on, spray, Dip shampoo,	Highly toxic to pets Can trigger allergic reactions e.g. Asthma, CNS toxicant
Chlorinated hydrocarbons	DDT		Resistance develops
Nicotinoids	Imidacloprid	Spot-on	CNS toxicant to human and pets. Highly toxic to bees.
Insect growth regulators	Methoprene	Spot-on, spray, collar, Dip	
Amitraz	triatix® , Tacktic®	Collar, Spot-on	Possible carcinogenic Developmental toxicant, Endocrine disruption.

Source: Pesticide Research Institute. Powering Information Decisions (2017)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Area of Study

The study was carried out in Murang'a County, one of the five counties of the Central region of the Republic of Kenya. Murang'a County occupies a total area of 2,558.8Km^2 of which 11.2 km^2 is water mass and of the remaining land only 2,135 km^2 is arable. The County is bordered by Nyeri County to the North, Nyandarua County to the West, and Kirinyaga County to the East, Kiambu County to the South as well as Embu and Machakos Counties to the Southeast as shown in the map (Figure 10). The County lies between 914m above the sea level in the East and 3,353m above the sea level at the slopes of Aberdare ranges in the West. The County lies between latitudes 0^0 34' South 1^0 7' South and longitudes 36 ^{0} East and 37^0 27' East. The County is divided in to 7 Constituencies namely Kiharu, Kangema, Gatanga, Mathioya, Kigumo, Kandara and Maragwa. Murang'a County map is shown in Plate 10.

3.1.1 Population and Climatic Conditions

The population of Murang'a County was 942,581 persons who consisted of 457,860 males and 484,721 females with a growth rate of 0.4 % per annum as reported in Population and Housing Census (2009), KNBS (2017). The population was projected to rise to 966,672 persons in 2017. According to the Murang'a First County Integrated Development Plan 2013-2017 (2014), the County's labour force was 527,679 persons (age group 15-64 years) and was expected to increase to 538,339 persons by 2017. The rate of unemployment in the County was approximated at 17.7%, which translates to 93,241 persons, while 36.3 % of the County's population lived in abject poverty.

These levels of unemployment and poverty therefore call for the development and enactment of adequate policies and programme which improve the job opportunities in the County.

The County has 65 km of railway line (underutilized), 387.5 km bituminized, 1,313.1 km of graveled and 1,234.3 km of earth surface roads. Forty per cent of the population live in stone/brick walled houses, 24.3% mud and wood houses and 2.19% live in grass, straw and tin walled houses. Most of the houses in the County are roofed with corrugated iron sheets (94.38%); makuti and grass roofed constitute 0.18%. Most of the houses (60.04%) have earthen floors.

The County has three distinct climatic zones; equatorial type of climate in the west, sub-tropical climate at the Central and semi-arid conditions in the Eastern region. Long rain falls between March and May, and the highest rainfall is recorded in April. Short rains are received in October and November.

The major cash crops grown in the County include: tea, coffee, avocado, mangoes, macadamia and horticultural crops like tomatoes, cabbages, kales, spinach and French beans. Food crops which are grown in the County include maize, beans, bananas, sweet potatoes and cassava. The various types of schools in the County include: 1,080 pre-primary centres, 634 primary schools, 292 secondary schools, 48 youth polytechnics, one technical college, one medical college, one teachers' training college and two Universities. According to Murang'a First County 2013-2017 Development Plan (2014), there are 272 Health facilities of different levels wich include Hospitals (Public, Faith based and Private) and Nursing homes. There are 21 Health Centres (Public and Private), 114 Dispensaries and 137 Private clinics.

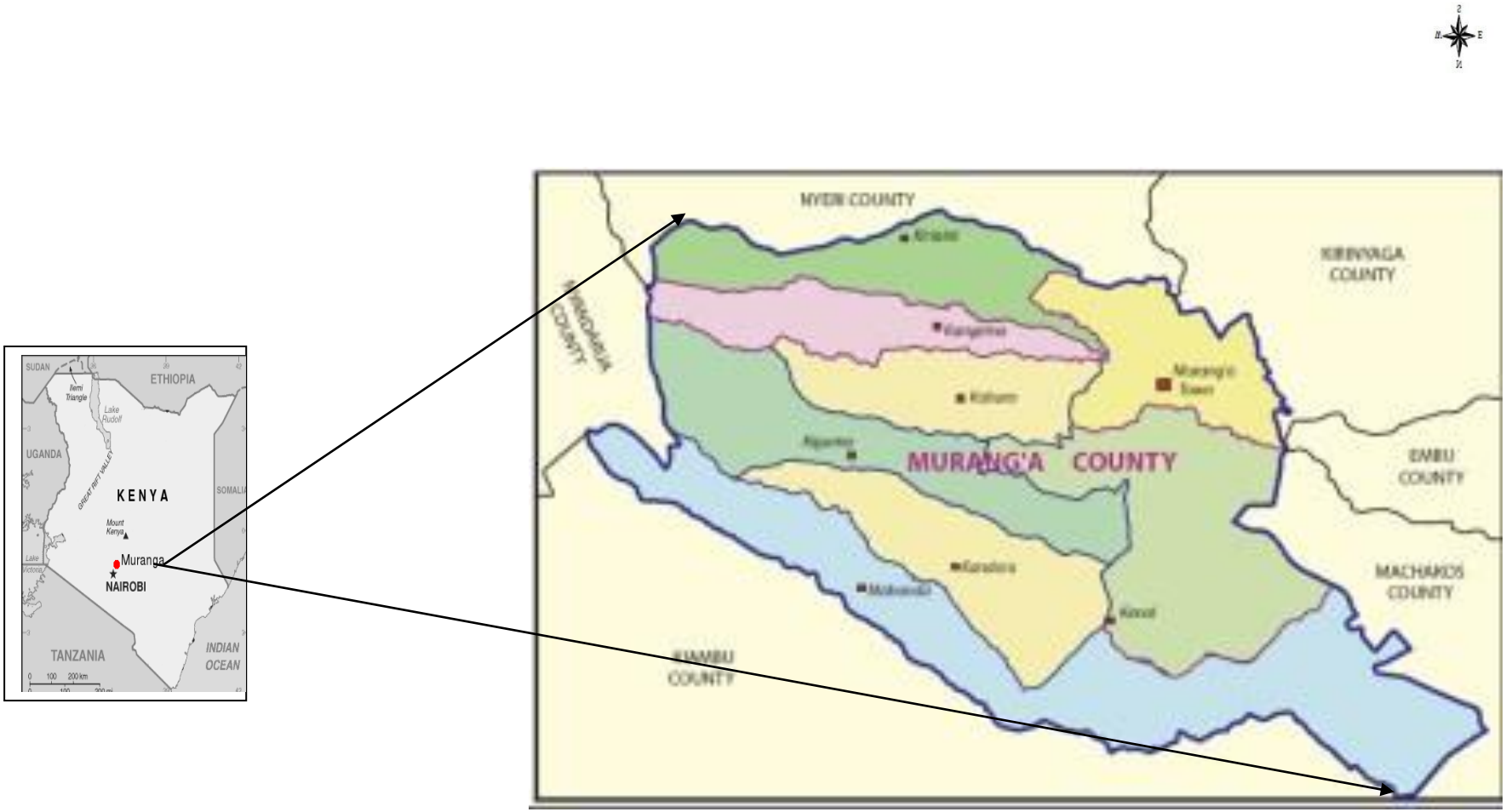

Plate 10: Map of Murang'a County

3. 2 Study design

Two study designs were used; Descriptive Survey design and Experimental. The study was carried out in order to determine the importance of the herbal medicines which were used in Murang'a County. Experimental laboratory study was carried out in order to investigate *in vitro* antiflea activity, determine the effects of the crude extracts on skin sensitization and irritation, and to determine safety of the selected plants

3. 3 Selection and recruitment of herbalists

Selection of the herbalists was done purposively from registered herbalists in the County.

The objective of the study was explained to the herbalists and only those willing to take part in the study were given the questionnaires to fill. The questionnaires had two main sections. The first section listed the various human body systems and diseases treated by the herbalists, where the herbalists filled the herbal plants they use for listed disorders. The other section had the other plants used on other illnesses and body systems not listed in the first section, as well as the plants herbalists used but not on specific body systems.

3. 3. 1 Ethno-botanical Survey

Different herbalists in Murang'a County were visited for the ethno-pharmacological survey after obtaining permission from the University of Nairobi, the National Commission for Science, Technology and Innovation (NACOSTI), the Murang'a County Commissioner and the Murang'a County Director of Education (Appendices 3, 5, 6 and 7). Prior to the visit, prior arrangements with the respective Intellectual Property offices and the local administration were made. Traditional herbalists of different locations within the County were interviewed on their knowledge about natural cures for various disease conditions and the medicinal plants they use to control fleas and jiggers. The structured interviews were performed with the aid of a questionnaire and the results obtained compared with different

Literature sources. Plates 11, 12, 13 and 14 show some of the meetings held with the herbalists.

Plate 11: **Muranga County herbalists at their meeting center at Maragua Jua Kali Sheds Murang'a**

Plate 12: **Murang'a Herbalists' Association Chairman addressing a meeting at Mount Kenya University hall**

Plate 13: Secretary of the Murang'a County herbalists' association addressing meeting at Mount Kenya University hall

Plate14: Herbalists displaying their certificates of registration

3. 3. 2 Plant Materials

The plants and the parts used by the herbalists in the management of the various disease conditions were collected based on their ethno-medical uses, and a follow-up of the existing literature leads. The voucher specimens of the plant species were submitted for authentication at the Herbarium of the National Museums of Kenya in Nairobi, where the voucher specimens were deposited. Among the plants mentioned to have pesticide effects, *Tithonia diversifolia and Senna didymobotrya* were selected for the study on their anti-fleas activity and their toxic effects.

The plant materials (roots, leaves, flowers and barks) of *Tithonia diversifolia* and *Senna didymobotrya* were collected from Maragua area (Murang'a South Sub-County) in Murang'a County. The collected specimens were photographed, identified and authenticated with the aid of a taxonomist from the East African Herbarium at the National Museums of Kenya where the voucher specimens were prepared and deposited. Plate 15 shows the mounting of the plant species in the field where the plants were collected.

Plate 15: Mounting of the voucher specimens of the plant species by a taxonomist from East African herbarium

3. 3. 3 Extraction of plant materials

Extractions of plant materials were done at the Kabete Campus, of the University of Nairobi using a previously reported method (Bibi *et al.*, 2012, Sasidharan *et al*, 2011).

The plant materials were transported to the Department of Public Health, Pharmacology and Toxicology laboratories and were washed thoroughly with running tap water, chopped into small pieces and then air-dried under the shade for a period of 14 days, then ground. Extraction was done by maceration using methanol and distilled water for the methanol and aqueous extracts respectively. A hundred grams of each part of the ground material from the plant samples were soaked separately with enough methanol in a one litre conical flasks and

then covered with a foil paper for 48 hours with constant shaking using a magnetic stirrer as shown in Plate 16. Thereafter each extract was filtered and reduced *in vacuo* at 60^{o}C and finally dried completely in the oven set at 35 ^{o}C. The aqueous extracts were prepared by boiling 100 g of each of the powdered crude extracts in water for five minutes. The extracts were cooled, filtered, and then freeze-dried. The dry and lyophilized extracts were weighed and stored in a freezer at – 20^{o}C while awaiting later use in the study.

Plate 16: Extraction of plants using either methanol or water

3. 3. 4 Preparation of stock extracts

The crude methanolic and aqueous extracts were dissolved separately in dimethyl sulfoxide (DMSO) and distilled water respectively at a concentration of 1000 mg/10ml. Required serial dilutions were prepared logarithmically under sterile conditions by adding calculated amounts of phosphate buffer saline (PBS) to obtain working concentration ranging from 100 mg/ml – 1mg/ml. Plate 17 shows the preparation of various concentration which were used in the study. All the prepared crude extract solutions were stored at 4^{o}C and retrieved only during use.

Plate17: **Preparation of the various concentrations of different crude extracts.**

3. 3. 5 Preparation for tubes of dilutions of the crude extracts

The polypropylene centrifuge tubes were prepared as reported by Stanneck *et al.,* (2012), but with minor modifications.

The 15 ml polypropylene centrifuge tubes for *in vitro* anti-flea activity were prepared by dissolving the crude extracts in distilled water and dilutions made in the same solvent as shown in Plate 18.

A filter paper strip 1.5 cm by 10 cm was soaked in the concentration of the extract for each extract concentration whose *in-vitro* activity was being tested as shown in Plates 19 and 20.

Water was allowed to dry off leaving a homogenous coating of the active compound on the filter paper which was fitted into the centrifuge tube; covering one side of the wall and leaving the uncovered side for observation.

Plate 18: The centrifuge tubes with various concentrations of extracts

Plate 19: Strips of the filter papers used to hold the extract

Plate 20: Filter papers being soaked in various concentrations of extracts

3. 3. 6 Experimental animals

All animals in these studies were individually housed and acclimatized for 5 days to laboratory conditions before beginning the study (OECD, 2001).Young mongrel dogs more than two months old were obtained locally from households surrounding the Kabete Campus of the University of Nairobi and after five days acclimatization, they were used to create colony for the fleas. Fleas used in the study were obtained from the dogs using the combs shown in Plate 21.

Plate 21: **The combs used to harvest fleas from dogs.**

Young adult female nulliparous and non-pregnant New Zealand albino rabbits were obtained from the University animal houses and were used for the determination of acute dermal irritation/corrosion (OECD, 404) and eye irritation test (OECD, 405).

Adult (8 to 12 weeks old), female, nulliparous and non-pregnant Wister rats weighing 90-130 g were obtained from the University of Nairobi animal house. They were thereafter used for acute toxicity tests of the extracts. The animals were housed under the standard laboratory conditions with temperatures of $25^{o}C \pm 3^{o}C$, natural light and relative humidity of 50-60%. They were fed on standard pellet diet and on unlimited supply of water (OECD, 2008).

3. 4 Bioassay Studies

3. 4. 1 Piloting of *In vitro* Anti-Fleas Activity

A total of 100 mg of each of the methanoic extract were weighed, wetted with 0.1 ml DMSO before dissolving them in 5.0 ml acetone. Whatman's Filter paper No.1 soaked in each of the extract dissolved in acetone to line each of 15 ml plastic tubes with lids. 100 mg of each of the aqueous extracts were dissolved in 5.0 ml acetone to coat the filter paper lining the tubes. Similarly, 100 mg of Sevin® were dissolved in 5.0 ml of acetone to coat the filter paper in the

tube and act as the positive control. All the acetone was allowed to evaporate leaving behind the coated filter.

Ten fleas which were obtained from the dogs were introduced into each tube and the lids which were previously perforated replaced. The effects of the drug extracts were observed on the 14^{th} and 36^{th} hour and findings were recorded. Results are shown in Table 2.

Table 2: Piloting of *in vitro* antiflea activity

Plant part	**Extraction method (Methanoic/ aqueous)**	**No. of viable flea after 14 hrs**	**Percentage of activity**	**No. of viable flea after 36 hrs**	**Percentage of activity**
Sevin (+ve control)	-	0	100	0	100
Tithonia flowers	Aqueous	0	100	0	100
Tithonia Leaves	Aqueous	10	0	4	60
Tithonia Stem	Aqueous	10	0	8	20
Tithonia Leaves	Methanoic	0	100	0	100
Senna leaves	Aqueous	10	0	10	0
Senna stem	Methanoic	0	100	0	100
Senna roots	Aqueous	N/a	N/a	N/a	N/a
Senna leaves	Methanoic	0	100	0	100
Senna roots	Methanoic	0	100	0	100
DMSO (-ve control)	-	10	0	10	0

3. 4. 2 Determination of *in vitro* anti-flea Activity of *Tithonia diversifolia* and *Senna didymobotrya*

15 ml Polypropylene centrifuge tubes were used in the contact assay against *Ctenocephalides felis* and *Ctenocephalides canis*.

Methanolic crude extracts were not tested though in the pilot study, they were found to be more active, because herbalists use water as the solvent for their concoctions.

Three concentrations of each crude aqueous extract (*Senna didymobotrya* leaves-SLAE, *Tithonia diversifolia* leaves-TLAE, *Tithonia diversifolia* flowers-TFAE) were prepared for testing *in-vitro* anti-flea activity. For each extract 1mg/ml, 10 mg/ml and 100 mg/ml concentrations were made. A Whatman filter paper no.1 strip measuring 10 cm by 1.5 cm was saturated with extract concentration for testing. It was later allowed to dry up leaving the paper coated evenly with extract at the concentration being tested. The coated stripe was fitted into 15 ml polypropylene centrifuge tube. A total of ten fleas held by the small loose cotton wool were picked randomly and transferred into these polypropylene tubes and screw cap with holes was replaced in order to hold the fleas inside, and avoid suffocation.

Similaly, a positive control using pyrethrum flower aqueous extract (PFAE) at similar concentrations were used for comparation. Viability of fleas in each tube was tested after 24 and 48 hours. *In vitro* anti-flea activity assay was done as described by Dryden *et al.,* (2015) but with modification. Adult *Ctenocephalides canis and felis* fleas from their natural habitat were obtained from the dogs obtained from Ndumboini households. The fleas were kept at a room temperature of 20^{O}C-24 ^{O}C and 80% relative humidity until sorting was complete and they were ready for use in the experiment. The fleas were held in a large container with small pieces of cotton wool which served as holding grounds for them. Each piece of cotton wool held an average of ten fleas and ten fleas were transferred randomly into each tube. The tubes were closed with an untreated screw cap with needle- punctured holes in the

center. These tubes were kept at ambient temperatures and humidity, and placed horizontally in order to ensure maximum contact between the fleas and the filter paper surface in the tube.

All original test data from the experiments were presented as means. All values and means were analyzed for statistical differences using the Student's t-test, R version 3.4.3 (2017-11-30) test after being tested for normality, at 95% Confidence Interval and p-value of significance being $p \leq 0.05$. Fleas' activities were monitored after 24 and 48 hours by gently tapping the tube on a hard surface. Tubes where all fleas showed normal occasional jumps and coordinated movements were evaluated as zero efficacies. Fleas with uncoordinated movements or fleas which lay on one side showing only weak leg movements were counted as alive despite the fact that they were not able to feed on the host. Fleas which were not moving at all after stimulation by tapping the vial were counted as dead. Untreated and solvent treated control vials served as controls.

3. 5 Acute Dermal Toxicity

The acute dermal toxicity of the crude aqueous extracts was tested using OECD, (2015) guidelines 404. Nine (three per test substance) healthy, adult female, nulliparous and non-pregnant New Zealand albino rabbits weighing 2.0-3.0 Kg were used for the study. The rabbits' fur was removed 24 hours before the test by closely clipping the dorsal area of their trunk. Care was taken to avoid abrasions on the skin. Only animals with healthy, intact skin were used. The test extract was applied onto skin of the test rabbits as a single application of 2000 mg/kg as shown in Plate 22. The test formulation covered a small area of approximately 6 cm^2 of the total body surface area of the rabbit and was covered with a gauze patch as shown in Plate 23, which was removed for observation after three minutes. If no dermal toxic effects were observed, a second patch was applied at a different site and removed after one hour. Since no toxic effects were observed, more exposure was found to be humanely allowable, and this was held in place with a non-irritating tape for up to four hours. Following

this period rabbits were washed with normal saline in order to remove the residue of the drug. Rabbits were observed daily for 14 days and the toxicity reactions evaluated included pruritus and local reaction. The dermal reactions were graded and recorded according to OECD grades and tabulated. Untreated skin areas of the test animal served as control.

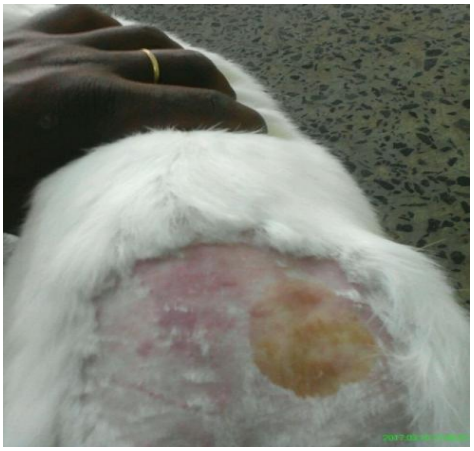

Plate 22: **Application of the test substance for dermal irritation test, after the initial three minutes**

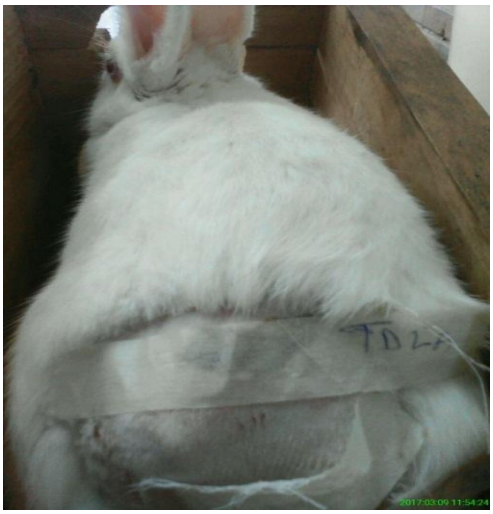

Plate 23: **A treated patch covered with gauze and held loosely using a masking tape.**

Eye irritation tests were also conducted using the New Zealand albino rabbits as described in OECD (2012).

3.6 Determination of Acute Toxicity Levels of the Active Crude Extract in Rats

Evaluation of acute toxicity and LD_{50} of the crude aqueous extracts was done using Wister rats and OECD (2001) method as described in guidelines 425. A total of 15 rats, four for *Senna didymobotrya* leave extracts, four (4) for *Tithonia diversifolia* leave extracts, four (4) for *Tithonia diversifolia* flower extracts and three (3) which served as controls, each per test group of adult female nulliparous and non-pregnant Wister rats weighing 90-130 g were used to investigate acute toxicity of active crude extract(s) as described in the limit test. Limit test was chosen because previous reports on methanol and ethanol crude extracts of the two plants indicated them as having low toxicity (Kamatenesi-Mugisha, *et al.,* 2013, Ezeonwumelu, *et al.,* 2012, Korir, et al., 2012, and Nyamwamu, et al., 2015).

The animals were selected randomly and identified by picric acid marks. They were housed individually in polycarbonate cages for five days prior to the administration of the extracts in order to allow for acclimatization to laboratory conditions. Prior to the administration of the extracts, single animals were fasted for four hours and weighed before the oral administration of single doses of 2000 mg/kg, to the groups of three rats as shown in Plates 24 and 25. Physiological saline was administered to the control group of animals. Observation for the signs of toxicity on the skin, eyes, mucus membranes and other parts of rats body such as salivation, lethargy, sleep, coma, convulsions, tremors, diarrhoea, body weight and mortality were monitored and recorded down at intervals of 30 minutes, 4 hours, 24 hours, 48 hours, one week, and two weeks. The data obtained was presented using Tables, and the LD_{50} values were determined as reported previously (OECD, 2008).

Plate 24: **Weighing of the Wister rats before dosing with the crude extracts.**

All the Wister rats were weighed before administration of test substance, 7 days and 14 days thereafter.

Plate 25: Administration of the test substance to Wister rats.

3. 7 Disposal of Experimental Animals

The laboratory animals were disposed in accordance with the guidelines set by the ethical committee of the University of Nairobi. The rabbits were euthanized using intravenous pentobarbital sodium at a dose rate of 150 mg/kg body weight or three times the anaesthetic dose (IACUC, 2016) and confirmation of euthanasia was performed by checking the reflexes.

The carcasses were disposed following the guidelines given by the Department of Veterinary Pathology, Microbiology and Parasitology of the University of Nairobi. The rats were euthanized using pentobarbital sodium 150 mg/kg by intraperitoneal route, confirmation done by checking reflexes and carcasses disposed in accordance to the standard guidelines.All the used fleas were euthanized using carbaryl *(Sevin) dust* shown in Plate 26, and disposed accordingly.

Plate 26: Sevin® pet dust that was used to kill all unused fleas

3. 8 Data Analysis

Data analysis was done as described below; using Student's t-test, R version 3.4.3 and graphs were drawn using Microsoft Excel for 2010 year.

The data obtained from *In vitro* anti-flea studies was expressed as a mean ± standard error of the mean (SEM) of the two independent experiments. Analysis was done by determining antiflea activity by Student's t-test, R version (3.4.3(2017-11-30). The data from acute toxicity studies was analyzed qualitatively and quantitatively using suitable statistical tools.

The LD_{50} values (Limit Test) were calculated using the Acute Oral Toxicity Guidelines (425) Statistical Program Version: 1.0) (OECD, 2008).

3. 9 Ethical Consideration

The experimental data which was obtained was handled with a lot of confidentiality and was used for research purposes only.

3. 10 Information Dissemination

The information obtained in the research was disseminated through thesis and by publication in the Journals.

CHAPTER FOUR

RESULTS

4.1 Findings on the Survey of herbal medicines use in Murang'a County.

4.1.1 Age of practitioners

A total of 27 herbal medicine practitioners were interviewed as described in the Materials and Methods in chapter Three.

Only 3.6 % or 1 person is classifiable as a youth and 18 % of herbalists are below the age of fifty years. A total of 64.3 % of all practitioners in this region are past the Kenyan retirement age of 60 years. The mean age of the herbalists being 62.4 years and standard deviation of 14.1, and these findings are shown in Table 3, and Figure 1.

Table 3: Age and Gender Distribution of the Herbalists

	GENDER		
Age of Herbalists in years	**Males**	**Females**	**Total**
31 - 40	1	1	2
41 -50	3	0	3
51-60	3	2	5
61-70	9	1	10
71-80	6	0	6
81-90	2	0	2
TOTAL	**24**	**4**	**28**

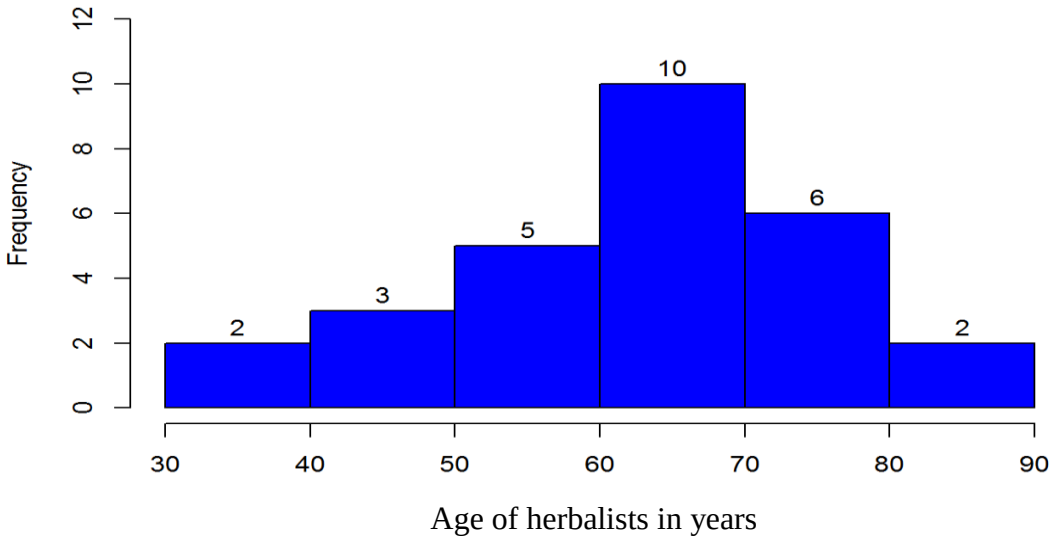

Figure 1: Distribution of practitioners by age

4.1.2 Gender of practitioners

A total of 24 (86 %) herbal medicine practitioners were males, while only 14% were females, and the findings are shown in Table 4.

Table 4: Gender of Herbal Practitioners.

Gender of the Practitioners	No. of Persons	Percentage
Males	24	86
Females	4	14
TOTAL	**28**	**100**

4.1.3 Source of ethno-medical knowledge

There are major deficiencies of adequate modern, scientific training among the herbalists as shown in Table 5. Majority of the herbalists, 39% acquired their knowledge from their family lineage, 35% of them learnt from seminars and the internet while 26% learned through observing the experienced and elder herbalists. Table 5 shows how the herbalists acquired knowledge.

Table 5: Knowledge Transfer among Herbalists in Murang'a

Category of Herbalist	No. of herbalists	Percentage
Inherited from a grandparent or parent	11	39
Learned from observing other herbalists	7	26
Taught in seminars or internet	10	35
TOTAL	**28**	**100**

4.1.4 Number of patients served by herbalists

The study revealed that a minimum of 450 patients seek medical services from herbal medicine practitioners in Murang'a County every month, and an estimated 5000 patients annually seek services from the herbalists, and this findings are shown in Table 6.

Table 6: Average number of patients treated per herbalist per month

No. of patients	No. of Herbalists	Monthly Average
Less than 10	6	60
Between 10 and 20	10	150
More than 20	12	240
TOTAL	**28**	**450**

N=28

4.1.5 Source of information about the herbalists in Murang'a County.

Despite evidence that the herbalists had minimal modern training on curative medicine, the study revealed that 31% of their patients were previously attended in the modern hospitals; probably because patients gave up on modern medicine for various reasons. The results are shown in Table 7. Other main sources of clients included social marketing and inter-herbalist referrals. Social gatherings like the market centres served as the best marketing opportunities as they attract 42% of the patients, as people interact with the herbalists alongside their usual market day routines. A significant proportion of the patients (27%), were as a result of inter-herbalist referrals.

Table 7: Source of patients attended by the herbalists

Source of clients	No. of herbalists	Percentage
Market day Promotions	12	42
From hospitals	9	31
From other herbalists	8	27
Total	**28**	**100**

4.1.6 Reasons why patients seek herbal medicine treatment.

The attitude towards herbal medicine in Murang'a County is that of the last option, as demonstrated by majority (79%) of the patients, who only visit the herbal medicine practitioners after conventional medicine failed to manage their ailments adequately. Patients also sought herbalists' services due to fear of side effects of modern medicines (14%), and also unaffordability of the conventional medical care (7%), and the findings are shown in Table 8.

Table 8: Reasons given by patients who seek herbal medicine care as reported by herbalists

Reasons for visit	No. of respondents	Percentage
Poor response of Conventional Medicines	22	79
Fear of side effects of conventional medicines	4	14
High cost of conventional medicines	2	7
Total	**28**	**100**

4.1.7 Follow-up of patient recovery by herbal medicine practitioners

Information on the efficacy of herbal medicine is scanty with only 8% of cases verified through laboratory analysis. Most (52%) of their clients came back for follow up and also to report on outcomes of the treatments as shown in Table 9.

Table 9: Follow-up of patients recovery by the herbalists

Feedbacks for herbalists	No. of respondents	Percentage
Patients who came back for review	15	52
Patient who did not come back are assumed healed	11	40
Hospital sources like laboratory reports	2	8
Total	**28**	**100**

4.1.8 Herbal remedies for Jigger fleas.

A total of 13 different herbs were used in the community for the management of jigger fleas. *Solanum incanum* was the most commonly used herb for control of jigger fleas in the region and it was used by more than 35% of the herbalists interviewed. Other plants used included; *Tithonia diversifolia*, *aloe* species, *Azadirachta indica*, *Senna didymobotrya and Venonia auricufera* as shown in Table 10. The local names of the plants are given in brackets in all Tables.

Table 10: Herbal remedies for Jigger fleas.

Plants in English/Scientific and local name	Frequency of use	Percentage
*Solanam incanum (*Mutongu)	18	35.3
Tithonia diversifolia (Maruru)	9	17.6
Aloe spp (Mugwanugu)	5	9.8
Azadirachta indica (Mwarubaine),	4	7.8
Senna didymobotrya (mwinu)	4	7.8
Venonia auricufera (Muchatha)	3	5.9
Solanum nigrum (Ndura)	2	3.9
Other (6) plants (one use each)	6	11.8
Total number of 13 plants	**51**	**100**

4.1.9 Herbal remedies for Pneumonia

A total of 33 different herbs were used in management of pneumonia. *Warburgia ugandensis* were the most (13.9%) commonly used alongside *Aloe* species, and Azadirachta *indica* (9.7%) each. Other plants with more than tree users were: E*rythrina abyssinica, Ficus*

sycomorus and Xanthoxylum usambarense. A total of 20 plants had only one user each as shown in Table 11.

Table 11: Herbal remedies for Pneumonia

Plants in English/Scientific and local name	Frequency of use	Percentages
Warburgia ugandensis (Muthiga)	10	13.9
Aloe species (Kiruma or Mugwanugu)	7	9.7
Azadirachta indica (Murumbaini)	7	9.7
Erythrina abyssinica (Muhuti)	4	5.6
Ficus sycomorus (Mukuyu),	4	5.6
Xanthoxylum usambarense (Mugucwa)	4	5.6
Ajuga remota (Wanjiru wa warurii)	3	4.2
Bridelia microntha (Mukoigo)	3	4.2
Lemon (Mutimu)	2	2.8
Garlic (kitunguu saumu),	2	2.8
Onion (gitunguru)	2	2.8
Ginger (Tangauthi),	2	2.8
Strychnos henningsii (Muteta)	2	2.8
Other (20) plants each one user	20	27.8
Total number of plants 33	**72**	**100**

4.1.10 Herbal remedies for Venereal diseases

The herbalists used 40 different herbs for treatments of venereal diseases. Only two plants were used by more than three herbalists. There were a lot of discrepancies in plants used. The most commonly used plants include *Aloe secundifolia* Engl, *Withania somnifera* (L), *Carica papaya* (L) and *Azadirachta indica* as shown in Table 12.

Table 12: Herbal remedies for Venereal diseases

Plants in English/Scientific and local name	Frequency of use	Percentages
Aloe secundifolia (Kiruma or Mugwanugu)	5	8.1
Withania somnifera (Murumbae)	4	6.5
Carica papaya (Mubabai),	3	4.8
Azadirachta indica (Murumbaini),	3	4.8
Oxygonum sinuatum (Conge),	3	4.8
Stem of banana (Kienja kia irigu),	3	4.8
Kigelia africana (Kiratina)	3	4.8
Vernonia auriculifera (Muchatha)	3	4.8
Albizia helminthica (Muguta),	2	3.2
Erythrina abyssinica (Muhuti),	2	3.2
Digitaria scalarum (Thangari)	2	3.2
Other 29 plants each one user	29	46.8
Total number of 40 plants	**62**	**100**

4.1.11 Herbal remedies for Malaria

Amongst the 17 different plants used in malaria treatment, *Ajuga remota* was the most commonly used, and was used by 16 (27.1 %) of the herbalists, followed closely by *Caesalpinia volkensii* 11 (18.6 %). Other major species include *Azadirachta indica* (11.9%), *Aloe secundifolia* Engl. (6.8%), *Strychnos henningsii* Gilg (6.8%) and *Senna didymobotrya* (6.8%) which were used by at least four herbalists each as shown in Table 13.

Table 13: Herbal remedies for Malaria

Names of the Plants	Frequency of use	Percentages
Ajuga remota (Wanjiru wa rurii)	16	27.1
Caesalpinia volkensii (Mubuthi)	11	18.6
Azadirachta indica (Murumbaini	7	11.9
Aloe species (Kiruma),	4	6.8
Strychnos henningsii (Muteta)	4	6.8
Senna didymobotrya (Mwinu)	4	6.8
Cuscuta kilimanjari (Thina)	2	3.4
Clausena anisata (Mutathi)	2	3.4
Other 9 different plants (each one use)	9	15.3
Total number of 17 plants	**59**	**100**

4.1.12 Herbal remedies for abdominal pain and diarrhoea.

Of the 22 plants used by the herbalists in the management of abdominal pains and diarrhoea, *Solanum incanum* was the most commonly used plant, and was mentioned by 17.6% of the herbalists. Only four plants were used by more than three herbalists. There is lack of uniformity as many other plants are used by one or two herbalists each as shown in Table 14.

Table 14: Herbal remedies for stomach ache and diarrhoea

Names of the Plants	Frequency of use	Percentages
Solanam incanum (Mutongu)	9	17.6
Plectranthus barbatus (Muigoya),	5	9.8
Vernonia auriculiferas (Muchatha),	4	7.8
Ficus Sycamorus (Mukuyu)	4	7.8
Warburgia ugandensis (Muthiga)	3	5.9
Caesalpinia volkensii (Muchuthi)	3	5.9
Mondia whytei (Muhukura)	2	3.9
Cuscuta kilimanjari (Gathina)	2	3.9
Erythrina abyssinica (Muhuti),	2	3.9
Ajuga remotes (Wanjiru wa rurie)	2	3.9

Acacia mallifera (Muthigira)	2	3.9
Prunus africana (Muiri),	2	3.9
Piliostigma thonningii (Murema)	2	3.9
Other 9 plants (one user each)	9	17.6
Total number of 22 plants	**51**	**100**

4.1.13: Herbal remedies used for typhoid.

A total of 30 plants were used by different herbalists in the management of typhoid. A total of 11 (20%) of the herbalist used *Croton megalocarpus* Del. in management of typhoid. Other plants which were used by more than three herbalists for management of the same include: *Physalis peruviana (5), Cassia spectabilis (5),* and *Bridelia micrantha (4).* A total of the 23 plants, were used by only one herbalist per plant showing lack of uniformity. Most of the herbalists combine more than one plant for the management of this condition as shown in Table 15

Table 15: Plants used in treatment of typhoid

Names of the Plants	Frequency of use	Percentages
Croton megalocarpus (Mukinduri)	11	20
Cassia spectabilis (Mwinu)	5	9.0
Physalis peruviana (Nathi)	5	9.0
Bridelia micrantha (Mukoigo)	4	7.3
Aloe vera (Kiruma)	3	5.5
Psidium guajava (Mubera)	2	3.6
Prunus Africana (Muiri)	2	3.6
Other 23 plants (one user each)	1	42
Total of 30 plants	**55**	**100**

4.1.14 Herbal remedies used for the management of back pain, bone and joint pains

A total 34 plants were used in the management of back pain, bone and joint pains, but only three plants were used by more than three herbalists. These were *Mystroxylon aethiopica* used by six herbalists, *Xanthoxylum usambarense* used by five herbalists, and *Cassia spectabilis* used by four herbalists. Most herbalists combine several plants. The widest range of herbal treatment was used in the management of this group of illnesses are shown in Table 16.

Table 16: Plants used in management of back pain, bone and joint pains

Names of the plants	Frequency of use of each	Percentages
Mystroxylon aethiopicum (Mukawa)	6	9.5
Xanthoxylum usambarense (Mugucwa)	5	7.9
Cassia spectabilis (Mwinu)	4	6.3
Croton megalocarpus (Mukinduri),	3	4.8
Urtica massaica (Hatha)	3	4.8
Warburgia ugandensis (Muthiga)	3	4.8
Solanum aculeastrum (Mutuura)	3	4.8
Azadirachta indica (Murumbaini),	3	4.8
Withania somnifera (Murumbae)	3	4.8
Prunus africana (Muiri),	2	3.2
Strychnos henningsii (Muteta)	2	3.2
Cloredendrum myriacoides (Munjuga Ira)	2	3.2
Hibiscus cuscus (Mugere)	2	3.2
Maytenus obscura (Muthuthi)	2	3.2
Other 20 plants (one user each)	20	31.7
Total of 34 plants	**63**	**100**

4.1.15 Herbal remedies used in Murang'a County for worms in children and adults

A total of 20 Plants were used in the management of helminths. *Senna didymobotrya* was the most used herb, and was used by **17** herbalists, followed by *Albizia helminthica (7), Euclea*

divinorum (**5**) and *Myrfine africana* (**5**), and *Carica papaya* which were used by four herbalists as shown in Table 17.

Table 17: Herbal remedies used in for worms in children and adult

Names of the Plants	Frequency of use	Percentages
Senna didymobotrya (Mwinu)	17	28.8
Albizia helminthica (Muguta)	7	11.9
Euclea divinorum (Mukinyai)	5	8.5
Myrfine africana (Mugaita)	5	8.5
Carica papaya (Mubabai)	4	6.8
Croton megalocarpus (Mukinduri)	3	5.1
Cucurbita maxima (Marenge),	2	3.4
Azadirachta indica (Murumbaini),	2	3.4
Croton Macrasthycus (Mutundu),	2	3.4
Caesalpina volkensii (Mubuthi)	2	3.4
Other 10 plants (one user each)	10	16.9
Total of 20 plants	**59**	**100**

4.1.16 Plants used by herbalists in Murang'a County to treat fleas and jiggers

A total of ten plants were used in fleas and jiggers control. *Solanum incanum* (40%) was used mostly followed by *Tithonia diversifolia* **(17%),** *Azadirachta indica* **(15%)** and *Tagetes minuta* **(12%)** *Senna didymobotrya (**8%**), Caesalpina volkensii (**8%** and Vernonia lasiopus* **(6%)** had more than one mention and are shown in Table 18.

Table 18: Plants used to control fleas and jiggers

Names of the Plants	Frequency of Use	Percentages
Solanum incanum (Mutongu)	21	36.2
Tithonia diversifolia (Maruru)	9	15.5
Azadirachta indica (Murumbaini)	8	13.8
Tagetes minuta (Mubangi)	6	10.3
Senna didymobotrya (Mwinu),	4	6.9
Caesalpinia Volkensii (Muchuthi).	4	6.9
Vernonia lasiopus (Muchatha)	3	5.2
Ximenia Americana	1	1.7
Zanthoxylum gilletii (Mucagatha),	1	1.7
Trichilia emetic (Mururi)	1	1.7
Total of 10 plants	**58**	**100**

4.2 *In Vitro* Antiflea Activity of *Tithonia diversifolia* and *Senna didymobotrya* Crude Extracts in Comparison with Pyrethrum Flowers Aqueous Crude Extract.

Various concentrations were used for all the four extracts being compared. All the extracts showed a concentration and time dependent fleas killing.

Means for *Tithonia diversifolia* leaves aqueous extract (TLAE) are shown in Table **19.**

Table 19: Means of fleas for *Tithonia diversifolia* leaves aqueous extract (TLAE)

Concentration	Means of dead fleas N_1	Means of live fleas N^*_2	Initial Number of fleas N_2	Efficacy percentage $(N_2- N_1)/N_2$ X 100
100 mg/ml	8.67	1.33	10	86.7
10 mg/ml	5.33	4.77	10	53.3
1 mg/ml	3.33	6.7	10	33.3

KEY N_2 = Initial number of fleas

N_1 = Mean Number of Dead Fleas

N^*_2 = Means of live fleas

The means for *Tithonia diversifolia* flowers aqueous extract are shown in Table 20.

Table 20: Means of fleas for *Tithonia diversifolia* flower aqueous extract (TFAE)

Concentration	**Mean of dead fleas N_1**	**Mean of live fleas N^*_2**	**Initial Number of fleas N_2**	**Efficacy percentage $(N_2- N_1)/N_2$ X 100**
100 mg/ml	9.33	0.77	10	93.3
10 mg/ml	8.0	2.0	10	80
1 mg/ml	3.67	6.33	10	36.7

The means for Senna didymobotrya leaves aqueous extracts are shown in Table 21.

Table 21: Means of fleas for *Senna didymobotrya* leave aqueous extract (SLAE)

Concentration of crude extract	**Mean of dead fleas N_1**	**Mean of live fleas N^*_2**	**Initial Number of fleas N_2**	**Efficacy percentage $(N_2- N_1)/N_2$ X 100**
100 mg/ml	6.63	3.37	10	66.3
10 mg/ml	4.33	5.77	10	43.3
1 mg/ml	4.0	6.0	10	40

The means for *Pyrethrum flower* aqueous extract (PFAE) are shown in Table 22.

Table 22: Means of fleas for *Pyrethrum flower* aqueous extract (PFAE)

Concentration of crude extract	Mean of dead fleas N_1	Mean of live fleas N^*_2	Initial Number of fleas N_2	Efficacy percentage $(N_2- N_1)/N_2$ X 100
100 mg/ml	9.0	1.0	10	90
10 mg/ml	7.33	2.67	10	73.3
1 mg/ml	6.0	4.0	10	60

Antiflea Result Analysis

Results of *In vitro* antiflea activity of all extracts in the study are compared and are shown in Figure 2

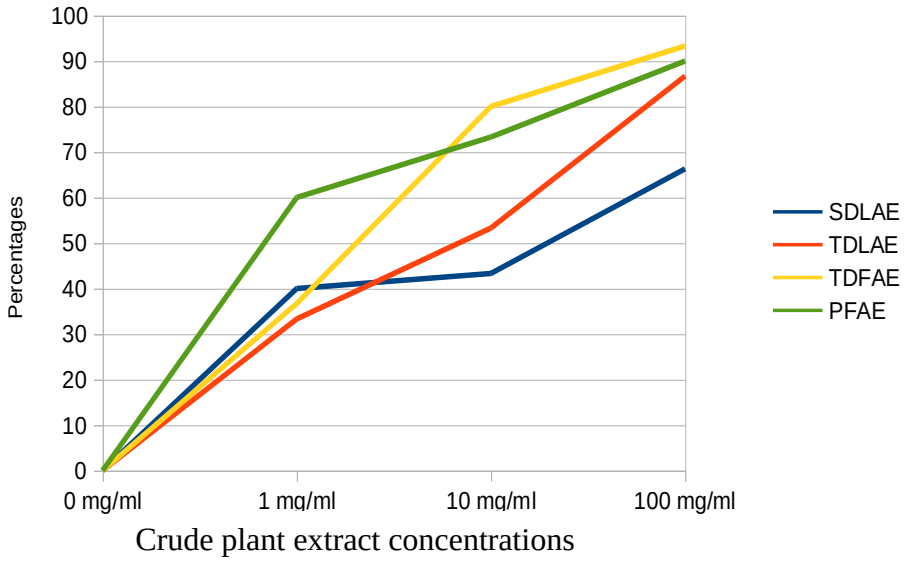

Figure 2: *In vitro* antiflea activity of SLAE, TDLAE, and TDFAE compared with PFAE extracts.

4. 3 Acute Dermal Toxicity Profile of *Tithonia diversifolia* and *Senna didymobotrya* Crude Extracts in New Zealand Albino Rabbits

4.3.1 Acute Dermal Irritation

The dermal irritation test was carried out since the extracts under study are expected to be used topically for the management of ectoparasites.

In *vivo* dermal irritation/corrosion test, young healthy New Zealand albino rabbits had a large portion of their dorsal region shaved 24 hours prior to the test. A dermal patch was made by soaking 6 cm^2 cotton gauze in the test solution. The patch was then applied on to the skin and was held loosely in position using a masking tape. The patched extract was held in position for four hours and there after the residue was washed off the skin using distilled water OECD (2015). The dose used on the rabbit was 0.5 ml of 500 mg/ml of the extract**.**

Test substance

A total of 500 mg of *Tithonia diversifolia* flowers aqueous extract were weighed on the scale and dissolved in 1 ml of distilled water in order to form the extract solution (TDFA) which was used in the test. A total of 500 mg of *Tithonia diversifolia* leaves aqueous extract were weighed on the scale and dissolved in 1 ml of distilled water in order to form the extract solution (TDLA) which was used in the test. Similarly, a total of 500 mg of *Senna didymobotrya* leaves aqueous extract were weighed on the scale and dissolved in 1 ml of distilled water in order to form the extract solution (SDLA) which was used in the test.

Test animals

Nine New Zealand albino rabbits, aged between 8-12 weeks, female and nulliparous were used in the study. The rabbits were weighed before and after the study. Effects of the crude extract on the weight of the New Zealand albino rabbits are shown in Tables 23, 24 and 25

Table 23: TDFA rabbit weight before and after application of the crude extract

Rabbits	Weight in Kg at the beginning	Weight in kg at the end
TDFA1	2.38	2.39
TDFA2	1.62	1.62
TDFA3	2.40	2.40

p = 0.423, no significant weight difference at 95% CI.

Table 24: TDLA rabbit weight before and after application of the crude extract

Rabbit	Weight in Kg at the beginning	Weight in Kg at the end
TDLA1	2.23	2.17
TDLA2	2.10	2.09
TDLA3	1.13	1.21

p = 0.943, no significant weight difference at 95% CI.

Table 25: SDLA rabbit weight before and after application of the crude extract

Rabbit	Weight in kg at the beginning	Weight in kg at the end
SDLA1	2.21	2.29
SDLA2	2.10	1.99
SDLA3	0.94	1.03

p = 0.788**,** meaning that there was no significant weight difference at 95% CI

Prior data available for dermal irritation showed no harmful effect. There was neither erythema nor irritation warranting discontinuation of the test**.**

TDFA- *Tithonia diversifolia* flowers aqueous extract

TDLA- *Tithonia diversifolia* leaves aqueous extract

SDLA- *Senna didymobotrya* leaves aqueous extract

The effects of the crude extracts on the skin of the New Zealand albino rabbits are as shown in Tables 26, 27 and 28.

Table 26: TDFA Dermal toxicity of *Tithonia diversifolia* flowers crude aqueous on New Zealand albino rabbit

Time after removal of the patch	**O minutes**			**60 minutes**			**24 hours**			**48 hours**			**72 hours**		
Rabbits	TDFA			TDFA			TDFA			TDFA			TDFA		
Series	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Erythema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oedema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 27: TDLA Dermal toxicity of *Tithonia diversifolia* leaves crude aqueous on New Zealand albino rabbit

Time after removal of the patch	**O minutes**			**60 minutes**			**24 hours**			**48 hours**			**72 hours**		
Rabbits	TDLA			TDLA			TDLA			TDLA			TDLA		
Series	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Erythema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oedema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 28: SDLA Dermal toxicity of *Senna didymobotrya leaves* crude aqueous on New Zealand albino rabbit grading as per OECD 204 guidelines

Time after removal of the patch	**O minutes**			**60 minutes**			**24 hours**			**48 hours**			**72 hours**		
Rabbits	SDLA			SDLA			SDLA			SDLA			SDLA		
Series	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Erythema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oedema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

KEY

TDFA- *Tithonia diversifolia* flowers aqueous extract

TDLA- *Tithonia diversifolia* leaves aqueous extract

SDLA- *Senna didymobotrya* leaves aqueous extract

ERYTHEMA

0…… No erythema

1…… Very slight erythema (barely perceptible)

2…… Well defined erythema

3……. Moderate to severe erythema

4……. Severe erythema (beef red)

OEDEMA

0…… No oedema

1…… Very slight oedema, (barely perceptible)

2…… Slight oedema, edges of the area well defined by definite rising

3……. Moderate oedema (raises approximately 1mm)

4……. Severe oedema (raised more than 1mm and extending beyond the area of exposure)

4.3.2 Eye irritation and Dermal test results for *Tithonia diversifolia* flowers aqueous crude extract, *Tithonia diversifolia* leaves aqueous crude extract, and *Senna didymobotrya* leave aqueous crude extract.

All the extracts were neither highly acidic nor highly alkaline.

The above *external* dermal corrosion and irritation tests proved the extracts were acceptable for eye irritation tests. A total of 500 mg of *Tithonia diversifolia* flowers aqueous extract were weighed on the scale and dissolved in 1 ml of distilled water to form the extract solution (TDFA) which was used in the test. A total of 500 mg of *Tithonia diversifolia* leaves aqueous extract were weighed on the scale and dissolved in 1 ml of distilled water to form the extract

solution (TDLA) which was used in the test. Similarly, 500 mg of *Senna didymobotrya* leaves aqueous extract were weighed on the scale and dissolved in 1 ml of distilled water to form the extract solution (SDLA) which was used in the test.

The test substances were applied on one of the rabbit's eye leaving the other eye to serve as the control. The degree of irritation was evaluated by scoring the lesions of the conjunctiva, cornea and iris at specific interval as per OECD (2012).

Preparation for the *in vivo* test

The test animals were obtained from a breeder in Jomo Kenyatta University and were acclimatized for two weeks before being introduced into the study.

The study animals included nine healthy, 9 weeks old New Zealand albino rabbits were used in the study. Both eyes were examined and found to be healthy 24 hours before the test.

The rabbits were housed individually in animal's room with a temperature of 20 to 23^0 C and relative humidity of about 60%. The room was artificially lit, 12 hours of lighting and 12 hours of darkness. The rabbits were fed on Unga rabbit pellets with coccidiostats and unrestricted supply of clean drinking water.

Test procedure: The test substance was applied on the conjunctiva sac in one eye of the test rabbit after gently retracting the lower eyelid. The eye lids were then held together for a second to prevent spillage of the extract. The other eye was left without the extract, in order to act as a control.

The test eyes were left unwashed for 24 hours after the instillation of the test substance.

Dose level

A volume of 0.1 ml of 500 mg/ml of the test substance was instilled in the eye using an eye dropper. The test was carried out in one rabbit initially, and having been found to be non-irritant and non-corrosive, the test was confirmed in two other rabbits. All the rabbits were observed for 3 days.

Clinical observation and grading of the eye reaction

The eyes were examined at specific interval as follows; 1 hour, 24 hours 48 hours and 72 hours. The observations show that no animal had severe pain, distress, corneal perforation or significant corneal laceration. There was also no animal which developed any ocular lesions hence the tests were terminated 3 days post instillation of the test substances.

Effects of the *Tithonia diversifolia* Leaves aqueous crude extract on the eye of the albino New Zealand rabbits are shown in Table 29.

Table 29: *Tithonia diversifolia* Flowers aqueous extract eye irritation test results

Rabbit	Cornea	Iris	Conjunctiva	Chemosis
TDFA1	0	0	0	0
TDFA2	0	0	0	0
TDFA3	0	0	0	0

No significant irritation was observed over the entire period.

Effects of the *Tithonia diversifolia* Leaves aqueous crude extract on the eye of the albino New Zealand rabbits are shown in Table 30.

Table 30: *Tithonia diversifolia* leaves aqueous extract eye irritation test results

Rabbit	Cornea	Iris	Conjunctiva	Chemosis
TDLA1	0	0	0	0
TDLA2	0	0	0	0
TDLA3	0	0	0	0

No significant irritation was observed over the entire period.

Effects of the *Senna didymobotrya* Leaves aqueous crude extract on the eye of the albino Newzealand rabbits are shown in Table 31.

Table 31: *Senna didymobotrya leaves* aqueous extract eye irritation test result

Rabbit	Cornea	Iris	Conjunctiva	Chemosis
SDLA1	0	0	0	0
SDLA2	0	0	0	0
SDLA3	0	0	0	0

No significant irritation was observed over the entire period.

KEY

CORNEA

0…No ulceration or opacity was observed

1…Scattered or diffuse areas of opacity

2…Easily discernible translucent area

3…Nacrous area (no details of iris visible)

4…Opaque cornea

IRIS

0…Normal

1…Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperraemia

2…Hemorrhage, gross destruction or no reaction to light.

CONJUNCTIVA

0…Normal

1…Some blood vessels hyperaemic

2…Diffuse crimson colour, individual vessels not easily discernible

3…Diffuse beefy red

CHEMOSIS

0…Normal

1…Some swelling above normal

2…Obvious swelling with partial eversion of the lids

3…Swelling with lids about half closed

4…Swelling with lids more than half closed.

4.4 Acute Oral Toxicity of *Tithonia Diversifolia* Flowers and Leaves, and *Senna Didymobotrya* Crude Extracts in Wister Albino Rats

Limit test was done using 2000 mg of *Tithonia diversifolia* crude leaf aqueous extract per kg body weight of Wister albino rats. Unique identity code was allocated to each animal as TDFLA 1, 2, 3, 4 or 5 for *Tithonia diversifolia* leaves aqueous extracts which were administered to the rats.

The extract was dissolved in distilled water**.**

Test animals: Healthy, female, nulliparous, adult (6-8 weeks), Wister albino rats, weighing from 90 to 130 grams were used. The rats were obtained from the University of Nairobi, Kabete campus, Department of Public Health, Pharmacology and Toxicology, experimental animal house. Each rat was housed in its own cage and was fed on standard mice pellets and allowed free access to water.

Previous studies by Elufioye, *et al.,* (2009) found the LD_{50} of *Tithonia diversifolia* ethanoic extract as more than 1600 mg per Kg body weight. Nyamwamu, *et al.,* (2015) found the LD_{50} of *Senna didymobotrya* methanoic and dichloromethane crude root extracts killed 80% of the mice at a dose of 5000 mg/kg body weight with an LD_{50} of 1927 mg/kg after a period of 14 days respectively. This background information helped to reduce the number of animals used since the intention was to investigate on possibility of the plants use as ecto-parasiticide.

The extracts were in granular form having been extracted using water, followed by freeze drying. A total of 2000 mg of the crude plant extract were dissolved in distilled water and the volume was adjusted to 5 ml. Concentration of the solution used was 400 mg of crude extract

per milliliters of the solution. The rats were weighed before administration of the extracts, on the 7^{th} and 14^{th} days after administration of the extracts to the rats.

4.4.1 LD $_{50}$ of *Tithonia diversifolia* flower aqueous crude extract

Limit test was done using 2000 mg of *Tithonia diversifolia* flower aqueous crude extract per kg body weight of Wister albino rats. Identification codes allocated were; TDFA 1, 2, 3, 4 and 5. The extract was dissolved in distilled water.

Test animals: Healthy, nulliparous, female, adult (6-8 weeks), Wister albino rats, weighing from 90 to 120 grams. A total of 2000 mg of the crude extract were dissolved in distilled water and the volume adjusted to 5 ml.

The concentration of solution used was 400 mg of crude extract per milliliters of the solution.

The rats were weighed before administration of the extracts, on the 7^{th} and 14^{th} days after administration of the extracts

Limit Test

Limit test was done using 2000 mg of *Senna didymobotrya* leaf aqueous crude extract per kg body weight of Wister albino rats. Identification codes allocated were; SDLA 1, 2, 3, 4, and 5.

Vehicle: The Plant extract was dissolved in distilled water**.**

Test animals: Healthy, female, nulliparous, adults (6-8 weeks), Wister albino rats, weighing from 90 to 130 grams. The rats were obtained from the University of Nairobi, Kabete campus, Department of Public Health, Pharmacology and Toxicology, experimental animal house. Each rat was housed in its own cage and rats were fed on standard mice pellets and allowed free access to water. The rats were selected randomly and grouped into three groups of five rats each.

Test conditions

Previous studies found that a dose of 2000 mg per kg body weight was nontoxic (Afolayan, *et al.*, 2016), 2000 mg of the crude extract were dissolved in distilled water and the volume adjusted to 5 ml.

The concentration of solution used was 400 mg of crude extract per milliliters of the solution.

The rats were weighed before administration of the extracts, on the 7^{th} and 14^{th} days after administration of the extracts.

4.4.1.1: Effects of the crude extracts on weight of the test animals.

The effects *Tithonia diversifolia* leaves aqueous crude extract on weight of the Wister rats are shown in Table 32.

Table 32: Effects of *Tithonia diversifolia* leaves aqueous on rat weights before and after oral administration of the crude extract to Wister rats

RAT	Weight in gram at the beginning (day 0)	Dose in mg	Volume in ml	Weight in gram on day7	Weight in gram on 14
TDLA1	99.58	199.16	0.50	107.16	136.10
TDLA2	110.11	220.22	0.55	124.36	133.27
TDLA3	104.20	208.40	0.52	116.45	135.57
TDLA4	107.52	215.04	0.54	119.59	121.61
TDLA5	98.22	Control	Control	101.67	108.03

P = 0.01049 at day 7. The value indicates that there is a significant weight difference at 95% CI at the beginning and on day 7.

p = 0.04384 at day 14. The value indicates that there is a significant weight difference on day 14 for *Tithonia diversifolia* Leaves Aqueous crude extract.

The effects of *Tithonia diversifolia* Flowers aqueous crude extracts on weight of the Wister rats are shown in Table 33.

Table 33: Effects of *Tithonia diversifolia* Flowers Aqueous on rat weights before and after oral administration of the crude extract to Wister rats

RAT	Weight in gram at the beginning (day 0)	Dose in mg	Volume in ml	Weight in gram on day 7	Weight in gram on day 14
TDFA1	100.00	200.00	0.50	122.07	134.44
TDFA2	100.27	200.54	0.51	115.07	118.34
TDFA3	98.90	197.80	0.49	113.54	124.03
TDFA4	102.29	204.58	0.511	115.92	126.13
TDFA5	97.34	Control	control	105.13	108.85

Weight beginning and after day 7, p = 0.02222 at day 7. The value indicates that there is a significant weight difference at 95% CI.

p = 0.02646 at day 14. The value indicates that there is a significant weight difference in the beginning and on day 14 for *Tithonia diversifolia* Flowers Aqueous crude extract.

The effects of *Senna didymobotrya* Leaves Aqueous extract on weights of the Wister rats are shown in Table 34**.**

Table 34: Effects of *Senna didymobotrya* Leaves Aqueous extract on rat weights before and after oral administration of the crude extract

RAT	Weight in gram at the beginning (day 0)	Dose in mg	Volume in ml	Weight in gram on day 7	Weight in gram on day 14
SDLA1	109.70	219.40	0.55	111.30	122.07
SDLA2	104.35	208.70	0.52	114.21	115.88
SDLA3	119.20	238.40	0.60	132.70	138.13
SDLA4	120.01	240.02	0.60	135.23	140.38
SDLA5	95.89	Control	Control	100.28	107.69

Weight at the beginning and after 7 days for *Senna didymobotrya* Leaves Aqueous crude extract p = 0.1586 at day 7. The value indicates that there is no significant weight difference at 95 CI in the beginning. p = 0.1733 on day 14. The value indicates that there is no significant difference at 95% CI.

4.4.1.2 Limit Test Results

The results of the limit test are summarized in Table 35. No rat died or was found in moribund condition. One of the rats in *Senna didymobotrya Leaves aqueous* liter showed distress signs on the neck, but was stable up to the 14th day after drug administration. Another one in the same liter was wet with urine. The findings are shown in Table 35.

Table 35: Limit Test Results of Wister Rats

Extract Sample	Means of initial weight (g)	Means of weight (g) on day 7	Means weight (g) on day 14	mortality or moribund	Toxicity signs
TDFA (H_2O)	100.37 ± 1.41	116.65 ± 3.75	125.74 ± 6.67	0/4	Nil
TDLA (H_2O)	105.35 ± 4.55	116.89 ± 7.23	131.64 ± 6.80	0/4	Nil
SDLA (H_2O)	113.32± 7.60	123.36 ± 12.35	129.12 ± 12.01	0/4	Nil
Control (H_2O)	97.15± 1.20	102.36 ± 2.50	108.19 ± 0.60	0/3	Nil

TDLA: *Tithonia diversifolia* Leaves Aqueous

TDFA: *Tithonia diversifolia* Flowers Aqueous

SDLA: *Senna didymobotrya* Leaves Aqueous

One –Way Analysis of Variance (ANOVA) at 95 CI and level of significant being $p \leq 0.05$

f = 1.9488

p= 0.2004 on day 7 and 14. The value indicates that there is no significant weight difference.

4.4.2 Effects of the Extracts on Blood Profile of the Wister Rats

The effects of the extracts on the blood profile are shown in the Tables 36 to 43.

Table 36: Effects of the crude extracts on Red Blood Cells 14 days after oral administration to Wister rats.

Crude extract type	TDLA	TDFA	SDLA	Control
No. of subjects	4	4	4	3
Mean RBCs (10^{12})	6.76	6.21	5.27	6.62
SD	1.26	2.15	3.56	0.75

TDLA: *Tithonia diversifolia* Leaves Aqueous

TDFA: *Tithonia diversifolia* Flowers Aqueous

SDLA: *Senna didymobotrya* Leaves Aqueous

Each extract was compared with the control with level of significance being $p \leq 0.05$ and 95% CI, TDLA $p = 0.261$ meaning that there is no significant difference from the control.

TDFA had a $p = 0.3604$ meaning that there is no significant difference from the control.

SDLA had a $p = 0.5037$ meaning that there is no significant difference from the control.

The effects of the extracts on blood Haemoglobin are shown in Table 37.

Table 37: Effects of the crude extracts on Haemoglobin levels 14 days after oral administration to Wister rats.

Crude Extract Type	TDLA	TDFA	SDLA	Control
No. of subjects	4	4	4	3
Mean Hb (g/dl)	15.83	14.63	13.68	14.27
SD	1.26	2.15	3.56	0.75

TDLA: *Tithonia diversifolia* Leaves Aqueous

TDFA: *Tithonia diversifolia* Flowers Aqueous

SDLA: *Senna didymobotrya* Leaves Aqueous

Each extract was compared with the control with level of significant being $p \leq 0.05$ and TDFA had a $p = 0.3958$ meaning that there was no significant difference from the control.

SDLA had a $p = 0.008943$ meaning that there was a significant difference from the control.

95% CI, TDLA $p = 0.1358$ meaning that there was no significant difference from the control.

TDFA had a $p = 0.3958$ meaning that there was no significant difference from the control.

SDLA had a $p = 0.008943$ meaning that there was a significant difference from the control.

Effects of the crude extracts on hematocrit are shown in Table 38.

Table 38: Effects of the crude extracts on hematocrit 14 days after oral administration to Wister rats

Crude extract type	TDLA	TDFA	SDLA	Control
No. of subjects	4	4	4	3
Mean Hct (%)	40.58	36.55	46.45	37.46
SD	9.13	13.20	4.67	4.76

TDLA: *Tithonia diversifolia* Leaves Aqueous

TDFA: *Tithonia diversifolia* Flowers Aqueous

SDLA: *Senna didymobotrya* Leaves Aqueous

Each extract was compared with the control with level of significant being $p \leq 0.05$ and 95% CI, TDLA $p = 0.1358$ meaning that there was no significant difference from the control.

TDFA had a $p = 0.1958$ meaning that there was no significant difference from the control.

SDLA had a $p = 0.02649$ meaning that there was a significant difference from the control.

Effects of crude extracts on the blood mean cell volume are shown in Table 39

Table 39: Effects of the crude extracts on Mean corpuscular volume 14 days after oral administration to Wister rats.

Crude extract type	TDLA	TDFA	SDLA	Control
No. of subjects	4	4	4	3
Mean MCV(Fl)	59.73	58.95	64.18	56.63
SD	4.36	3.99	3.17	1.29

TDLA: *Tithonia diversifolia* Leaves Aqueous

TDFA: *Tithonia diversifolia* Flowers Aqueous

SDLA: *Senna didymobotrya* Leaves Aqueous

Each extract was compared with the control with level of significant being $p \leq 0.05$ and 95% CI, TDLA had a $p = 0.1952$ meaning that there was no significant difference from the control.

TDFA had a $p = 0.6986$ meaning that there was no significant difference from the control.

SDLA had a $p = 0.01276$ meaning that there was a significant difference from the control.

Effects of the crude extracts on the Mean Cell Hemoglobin are shown in Table 40.

Table 40: Effects of the crude extracts on Mean Cell Hemoglobin 14 days after oral administration to Wister rats.

Crude extract type	TDLA	TDFA	SDLA	Control
No. of subjects	4	4	4	3
Mean MCH (pg)	21.73	21.95	21.98	21.50
SD	0.92	0.54	2.32	0.79

TDLA: *Tithonia diversifolia* Leaves Aqueous

TDFA: *Tithonia diversifolia* Flowers Aqueous

SDLA: *Senna didymobotrya* Leaves Aqueous

Each extract was compared with the control with level of significant being $p \leq 0.05$ and 95% CI, TDLA $p = 0.09236$ meaning that there was no significant difference from the control.

TDFA had a p = 0.6201 meaning that there was no significant difference from the control.

SDLA had a p = 0.9211 meaning that there was no significant difference from the control.

Effects of the crude extract on Mean Cell Hemoglobin Concentration are shown in Table 41.

Table 41: Effects of the crude extracts on mean cell hemoglobin concentration 14 days after oral administration to Wister rats.

Crude extract type	TDLA	TDFA	SDLA	Control
No. of subjects	4	4	4	3
Mean MCHC(g/dl)	36.50	37.40	34.33	37.97
SD	2.72	2.29	3.41	1.48

Each extract was compared with the control with level of significant being $p \leq 0.05$ and 95%

CI, TDLA p = 0.8924 meaning that there was no significant difference from the control.

TDFA had a p = 0.8311 meaning that there was no significant difference from the control.

SDLA had a p = 0.05371 meaning that there was a no significant difference from the control.

Effects of the crude extracts on Red Blood Cell Distribution Width are shown in Table 42.

Table 42: Effects of the crude extracts on RBC distribution width 14 days after oral administration to Wister rats.

Crude extract type	TDLA	TDFA	SDLA	Control
No. of subjects	4	4	4	3
Mean RDW (%)	20.20	19.08	21.35	20.70
SD	0.71	1.48	1.02	0.30

TDLA: *Tithonia diversifolia* Leaves Aqueous

TDFA: *Tithonia diversifolia* Flowers Aqueous

SDLA: *Senna didymobotrya* Leaves Aqueous

Each extract was compared with the control with level of significant being $p \leq 0.05$ and 95%

CI, TDLA p = 0.1087 meaning that there was no significant difference from the control.

TDFA had a p = 0.1152 meaning that there was no significant difference from the control.

SDLA had a p = 0.1608 meaning that there was no significant difference from the control.

Effects of the crude extracts on Platelets are shown in Table 43.

Table 43: Effects of the crude extracts on Platelets 14 days after oral administration to Wister rats.

Crude extract type	TDLA	TDFA	SDLA	Control
No. of subjects	4	4	4	3
Mean PLTs (K/µL)	475.50	471.00	648.00	381.67
SD	295.12	195.26	206.87	298.63

TDLA: *Tithonia diversifolia* Leaves Aqueous

TDFA: *Tithonia diversifolia* Flowers Aqueous

SDLA: *Senna didymobotrya* Leaves Aqueous

Each extract was compared with the control with level of significant being $p \leq 0.05$ and 95%

CI, TDLA p = 0.05899 meaning that there was no significant difference from the control.

TDFA had a p = 0.4155 meaning that there was no significant difference from the control.

SDLA had a p = 0.3927 meaning that there was no significant difference from the control.

Effects of crude extracts on white blood cells are shown in Table 44.

Table 44: Effects of the crude extracts on White Blood Cells 14 days after oral administration to Wister rats.

Crude extract type	TDLA	TDFA	SDLA	Control
No. of subjects	4	4	4	3
Mean WBCs (K/µL)	7.65	10.12	10.30	10.58
SD	3.84	5.24	2.11	3.00

TDLA: *Tithonia diversifolia* Leaves Aqueous

TDFA: *Tithonia diversifolia* Flowers Aqueous

SDLA: *Senna didymobotrya* Leaves Aqueous

Each extract was compared with the control with level of significant being $p \leq 0.05$ at 95% CI, TDLA p = 0. 8549 meaning that there was no significant difference from the control.

TDFA had a p = 0.9174 meaning that there was no significant difference from the control.

SDLA had a p = 0.04209 meaning that there was a significant difference from the control.

The Effects of crude extracts on neutrophils are shown in Table 45.

Table 45: Effects of the crude extracts on neutrophils 14 days after oral administration to Wister rats.

Crude extract type	TDLA	TDFA	SDLA	Control
No. of subjects	4	4	4	3
Mean N (%)	27.40	38.48	27.43	49.93
SD	19.20	8.69	18.04	12.32

TDLA: *Tithonia diversifolia* Leaves Aqueous

TDFA: *Tithonia diversifolia* Flowers Aqueous

SDLA: *Senna didymobotrya* Leaves Aqueous

Each extract was compared with the control with level of significant being $p \leq 0.05$ and 95% CI, TDLA p = 0.02652 meaning that there was a significant difference from the control.

TDFA had a p = 0.8429 meaning that there was no significant difference from the control.

SDLA had a p = 0.02043 meaning that there was a significant difference from the control.

The effects of crude extracts on lymphocytes are shown in Table 46.

Table 46: Effects of the crude extracts on lymphocytes 14 days after oral administration to Wister rats

Crude extract type	TDLA	TDFA	SDLA	Control
No. of subjects	4	4	4	3
Mean L($x10^9$)	5.61	6.03	6.74	4.78
SD	3.63	4.13	0.37	1.66

TDLA: *Tithonia diversifolia* Leaves Aqueous

TDFA: *Tithonia diversifolia* Flowers Aqueous

SDLA: *Senna didymobotrya* Leaves Aqueous

Each extract was compared with the control with level of significant being $p \leq 0.05$ and 95% CI, TDLA $p = 0.2278$ meaning that there was no significant difference from the control.

TDFA had a $p = 0.8623$ meaning that there was no significant difference from the control.

SDLA had a $p = 0.0161$ meaning that there was a significant difference from the control.

The effects of the crude extracts on monocytes are shown in Table 47.

Table 47: Effects of the crude extracts on monocytes 14 days after oral administration to Wister rats.

Crude extract type	TDLA	TDFA	SDLA	Control
No. of subjects	4	4	4	3
Mean M($x10^9$)	0.21	0.26	0.27	0.28
SD	0.13	0.23	0.03	0.03

TDLA: *Tithonia diversifolia* Leaves Aqueous

TDFA: *Tithonia diversifolia* Flowers Aqueous

SDLA: *Senna didymobotrya* Leaves Aqueous

Each extract was compared with the control with level of significant being $p \leq 0.05$ and 95% CI, TDLA $p = 0.3009$ meaning that there was no significant difference from the control.

TDFA had a $p = 0.7506$ meaning that there was no significant difference from the control.

SDLA had a $p = 0.2045$ meaning that there was no significant difference from the control.

Effects of the extracts on eosinophils, 14 days after a single oral dose of 2000 mg per kilogram body weight are shown in Table 48.

Table 48: Effects of the crude extracts on eosinophils 14 days after oral administration to Wister rats.

Crude extract type	TDLA	TDFA	SDLA	Control
No. of subjects	4	4	4	3
Mean E($x10^9$)	0.12	0.17	0.17	0.20
SD	0.05	0.12	0.07	0.14

TDLA: *Tithonia diversifolia* Leaves Aqueous

TDFA: *Tithonia diversifolia* Flowers Aqueous

SDLA: *Senna didymobotrya* Leaves Aqueous

Each extract was compared with the control with level of significant being $p \leq 0.05$ and 95% CI, TDLA $p = 0.1192$ meaning that there was no significant difference from the control.

TDFA had a $p = 0.05288$ meaning that there was no significant difference from the control.

SDLA had a $p = 0.08231$ meaning that there was no significant difference from the control.

The findings of effects of the crude aqueous extracts on the basophils, 14 days after single dose of oral administration of various crude aqueous extracts are shown in Table 49.

Table 49: Effects of the crude extracts on basophils 14 days after oral administration to Wister rats.

Crude extract type	TDLA	TDFA	SDLA	Control
No. of subjects	4	4	4	3
Mean B($x10^9$)	0.02	0.0I5	0.020	0.010
SD	0.01	0.017	0.010	0.000

TDLA: *Tithonia diversifolia* Leaves Aqueous

TDFA: *Tithonia diversifolia* Flowers Aqueous

SDLA: *Senna didymobotrya* Leaves Aqueous

Each extract was compared with the control, level of significant being $p \leq 0.05$ and 95% CI.

TDLA p = 0.391 meaning that there was no significant difference from the control.

TDFA had a p = 0.6042 meaning that there was no significant difference from the control.

SDLA had a p = 0.1817 meaning that there was no significant difference from the control.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

From the first study, it was found that males dominate the practice of herbal medicine constituting 89.3% of herbalists, and majority (63%) of the herbalists, were above 60 years of age. The mean age of the herbal practitioners was 62.4 years, pointing to the fact that they are above the Kenyan retirement age. This show that probably the herbalists were practicing herbal medicine due to lack of alternative sources of income, hence the likelihood of poor understanding of the medical conditions, chemical composition of the herbs, inability to learn new concepts as well as lack of interest in advanced herbal medicine. Majority of the herbalists were either illiterate or semi-illiterate as more than 50% had not been educated beyond the primary school. These herbalists (82.8%) had inherited the skills from their fore fathers or observed other herbalists practice hence the inability to advance their skills and knowledge. Other studies which have reported similar findings include; Deeb, *et a*l, (2013), Perinbam and Nirmalraj (2015) and Gedif and Hahn (2002). However, Tekle, (2015), has similar findings except that majority of the herbalists were youthful, 66.7 % were aged between 26-45 years, thus the younger people can also be motivated and empowered to practice herbal medicine. There is a high risk of losing the herbal medicine knowledge and practice since the knowledge and practice run in families, with minimal documentation of the same, Kipkore, *et al*, (2014).

A significant proportion of the patients who were treated by the herbalists came from the hospitals, 80% of the patients sought herbal care because they had lost hope on the conventional care, especially the ones diagnosed with terminal and chronic conditions, they were hopeful of finding a cure. The rest of the patients were either financially constrained and

could not meet the treatment costs or did not want to experience the side effects of conventional medication. A study by Kitonde, *et al,* (2014) had similar findings, where majority of the patients seeking herbalists care were hopeful of affordable, effective and safe medical attention.

The most commonly used plant parts were the leaves in form of a liquid preparations, this coincides with a study done by Erasto, et *al,* (2005). A study by Ahmad *et al.,* (2014) also found that liquid preparations in form of decoctions were the most preferred.

The herbalists in the study mostly (50%) used *Ajuga remota* and *Caesalpinia volkansii* (43%) for treatment of malaria, the aloe species for treatment of venereal diseases, *Senna didymobotrya* for treatment of helminthes infestations and *Myctroxylon aethiopicum* for treatment of back, bone and joint problems. A study by Muthaura *et al.,* (2007), revealed similar findings where *Ajuga remota* and *Caesalpina volkensii* were the most commonly used plants in treatment of malaria.

Another study by Kareru *et al*., (2008) agrees with the above findings since *Ajuga remota* was the most commonly used plant to treat malaria, *Aloe kendongensis* was used for the treatment of venereal diseases and *Senna didymobotrya* for the treatment of worms.

In the control of fleas and jiggers, herbalists in Murang'a County commonly used *Solanum campylacanthum, Tithonia diversifolia, Azadirachta indica* and *Targetes minuta*. A study by Biswas *et al.* (2002), concur with the study where the herbalists used *Azadirachta indica* in the control of parasites including fleas. In their study, Etewa and Abaza (2011) also found that *Azadirachta indica* was commonly used in the management of ectoparasites including lice, ticks and mites. In conclusion, it is evident that many people use different herbal plants in the control of fleas and other ecto-parasites , for instance, Soukand, *et al*., (2010) demonstrated that human fleas, bedbugs and clothes moths could be controlled using several, families of herbs.

Tithonia diversifolia flowers aqueous extract (TFAE) was the most effective in anti- flea activity. At 100 mg/ml, TFAE killed 93.3% and at 10 mg/ml it killed 80.0 % of fleas in 24 hours, while pyrethrum flowers aqueous extract (PFAE) at the same concentrations killed 90.0% and 73.3% of the fleas respectfully in 24 hours. The *Tithonia diversifolia* leaves aqueous extract (TLAE) killed 86.7% and 53.3%, while *Senna didymobotrya* leaves aqueous extract (SLAE) killed 66.3% and 43.3% of the fleas within 24 hours at the same concentration.

The study on *Tithonia diversifolia* aqueous flowers and leaves extracts were used at 2000 mg per kg during the acute oral toxicity test. The LD_{50} of the extracts was above the dose used since there was no significant oral acute toxicity based on its effect on the weight and hematological profile except for Neutrophils, and there was no mortality within 24 hours.

Kamatenesi-Mugisha, *et al.*, (2013) found that the LD_{50} of *Tithonia diversifolia* leaves ethanoic extract was 11,481 mg per kg body weight, while that of the aqueous extract was 12,302 mgs per kg body weight. Ezeonwumelu, *et al.*, (2012), found that the LD_{50} of the aqueous extract of *Tithonia diversifolia* leaves was above 10,000 mg per kg body weight. There was no major sign of acute toxicity or death of the rats, within 24 hours.

A study by Elufioye, *et al.*, (2009), revealed insignificant acute toxic effects as demonstrated by absence of hematological changes, when 400-1600 mg per kg of ethanoic extract was used. Funmilayo and Ayodele (2016) also found that there was no significant toxic effects, both hematological and biochemical when *Tithonia diversifolia* meal was fed on cockerels for 98 days.

However, Oyewole, *et al.*, (2007), found that the intra-peritoneal LD_{50} of *Tithonia diversifolia* aqueous extract was 120 mg per kg body weight, with the same dose (100 mg/ kg) repeated daily for 14 days. Unlike in this study, he reported severe acute toxic effects as portrayed by the significant weight changes, hematological changes and high mortality. This

could arise from accumulation as result of multiple doses and the intra-peritoneal route of administration.

The acute dermal irritation and eye irritation of the crude aqueous extracts of both *Tithonia diversifolia* leaves and flowers were insignificant.

2000 mg per kg body weight of *Senna didymobotrya* leaf aqueous extract was used in the study for acute oral toxicity test, the LD_{50} was found to be above 2000 mg per kg body weight. There was significant acute toxic effect as portrayed by the hematological changes in the significant changes on the hemoglobin, hematocrit, Mean Cell Volume, White Blood Cells and neutrophils; however there was no mortality reported or significant weight loss in the study animals. Moreover, there was no significant acute dermal toxicity/corrosion, or eye irritation/ corrosion. Korir, *et al*, (2012) found that the LD_{50} of the *Senna didymobotrya* DCM extract was between 1000 mg and 5000mg per kg body weight. There were no significant acute toxic effects at low doses, but the toxic effects increased significantly as the dose increased above 3000 mg per kg body weight and with continuous daily dosing as illustrated by the weight loss.

Nyamwamu, *et al*, (2015), found that the LD_{50} of the methanoic and Dichloromethane *Senna didymobotrya* crude root extracts was 1927 mg per kg body weight while that of the hexane and water extracts were more than 5000 mg per kg body weight, in mice, thus in line with the findings in this study. Aqueous extract is thus only slightly toxic if accidentally ingested.

Nyamwamu, *et al*, (2015), and Njoroge and Bussman, (2007), also reported topical use of *Senna didymobotrya* extracts in the management of both human and livestock dermal conditions and ectoparasites, thus agreeing with the safety of the extracts for topical use as in this study.

Tithonia diversifolia flowers aqueous (TDFA) extract was the safest as it did not affect the blood profile significantly. It was also found to be the most effective on fleas, with very close

resemblance with the positive control (pyrethrum flowers aqueous extract), in its antiflea activity.

Tithonia diversifolia (TDLA) leaves aqueous extract had better antiflea activity than *Senna didymobotrya* (SDLA) leave aqueous extract and had activity slightly below the flowers. It was also found that it was not very toxic to the blood profile as only neutrophils showed significant decline as compared with the control Wister rats.

Senna didymobotrya (SDLA) *aqueous* extract were the least effective of the four extracts tested. The LD_{50} of the extract was found to be above 2000 mg/Kg in Wister rats. However, the extract was found to lower important blood parameters like haemoglobin, hematocrits, mean cell volume, neutrophils and lymphocytes.

5.2 Conclusion

The following conclusions were made from the studies.

The study findings show that there is a high risk of losing the practice of herbal medicine, as majority of the people practicing herbal medicine are aged. The younger generation of people, who are educated and well versed in technology, need to be inspired in to Ethno-pharmacology, in order to promote integration of herbal medicine with modern science, medicine and technology. This would lead to more research and documentation, encouraging more young people to practice herbal medicine openly and also enabling the society to seek herbal medicine confidently and openly. Better research and integration with the modern science will encourage well educated persons to take up the practice, since they will understand science better, hence understand the human body physiology and biochemistry well, and understand diseases conditions better, as well as the effect of the herbal medicine on the human body and possible interactions with other conventional drugs. Science and Technology will also encourage gender equity hence encouraging many females to take up the practice. Most of the herbalists are either peasant farmers or retirees. Integration of herbal

medicine and its practice with modern science and technology would increase the practitioners' income hence becoming full time jobs, since many modern citizens will embrace it.

It was found that there was no significant difference in the *In vitro* anti-flea activity of pyrethrum flowers aqueous extract compared with *Tithonia diversifolia* aqueous leaves and flowers (*p-value = 0.8321*) extracts as well as *Senna didymobotrya* leaves aqueous extract.

5.3 Recommendations

The following recommendations were made from the studies**.** Further studies should be conducted in order to evaluate and document efficacy and safety of various herbs claimed to have medicinal activity by the Murang'a County herbalists.

Further studies should be conducted on *Tithonia diversifolia* and S*enna didymobotrya* extracts in order to improve on purification of usable pesticides, which are cost effective especially in management of the jigger menace in Kenya and other parts of the world. More emphasis should be on the flowers of *Tithonia diversifolia* having demonstrated both effectiveness and safety. LD_{50} of *Tithonia* flowers extracts also need to be established. However further studies are needed to purify the extracts especially for topical use in fleas and jiggers control as they will provide, safe, cost effective and efficient pesticides. The herbalists of Kakamega County who boil *Tithonia diversifolia* and use the concoction to clean the jigger infested areas of the body, should be encouraged to continue with the practice. The people of Murang'a County and the rest of the Country can also consider using the concoction since it was found to be safe and has antiflea activity.

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APPENDICES

APPENDIX 1: Herbalists Questionnaire

HERBALISTS QUESTIONNAIRE

Instructions

Kindly fill this questioner by ticking in the brackets and fill in the blank spaces.

All information provided on this questionnaire will be confidential and will only be used for the purpose of this study.

Name of Herbalist…….

(Optional)…….

Location of practice…….

Division of practice……

Please indicate your age in years……

Please tick your gender

Male [ ] Female [ ]

PART ONE

1. Are you a herbal medicine practitioner? (Mark only one option)

   Yes [ ] No [ ]

2. How many years have you practiced in traditional herbal medicines?

   Less than 5 years [ ] 5-10 years [ ] More than 10 years [ ]

3. How did you become a herbal practitioner?

   Inherited from a parent or grandparents [ ]

   Observed other herbalists [ ]

   Taught in seminars by botanists and internet [ ]

   Others [ ]

4. How many clients do you treat per month on average?

   Less than 10 [ ]

   Between 10 and 20 [ ]

   More than 20 [ ]

5. What is your source of clients?

   Referred by other herbalists [ ]

   From hospitals [ ]

   Promotions on market days [ ]

6. What would you say about the patient seeking your treatment?

   Their conditions are unmanageable with conventional medicines [ ]

   The patients fear side effects of conventional medicines [ ]

   The patients cannot afford the prescribed conventional drugs [ ]

7. How do you get your practice feed backs?

   Patients always come back for review [ ]

   Patient who do not come back are assumed [ ]

   To have recovered [ ]

   Hospital sources like laboratory reports [ ]

8. Do you treat both human and animals?

   Both human and animals [ ]

   Human only [ ]

   Animals only [ ]

PART TWO

Please list any two or three plants you use for the following conditions

Jiggers flea ……

…….

……

Pneumonia or chest infections …. …….

……..

……

Venereal diseases ……..

……..

…….

Malaria ……

……

……

Stomachaches and diarrhoea ……..

…….

……

Typhoid …….

……

……

Back pain, bones, and joint pains……..

……

……

Worms in children and adult ……..

…….

……..

Worms in animals ……..

…….

……..

Fleas and tick in animals including chicken ……..

…….

…….

East coast fever in cows …….

……

……..

List down any other plants used commonly and the conditions you use them for.

APPENDIX 2: List of plants used by Herbalists in Murang'a County

Family	Voucher No	Scientific Name	Local name	Part used	Disease/ symptom treated	No. of users
Solanaceae	15	*Solanum campylacanthum*	Mûtongu	Fruits	Jiggers Fleas	18
				Roots	Worms	1
					Fleas	3
					Abdominal pains and diarrhoea	9
Asteraceae	36	*Vernonia lasiopus*	Mûcatha	Leaves	Jigger flea	3
				Stem	Abdominal pains and diarrhoea	4
					Diabetes	1
					Typhoid	1
					Worms	1
					STIs	1
					Malaria	
					Skin Diseases	1
Meliaceae	18	*Azadirachta indica*	Mwarubaini		Jigger flea	4
					ECF	1
					Arthritis	3
					Malaria	7
					Worms	2
					Typhoid	2
					Pneumonia	7
					Ticks and fleas	4
					STDs	2
Verbenaceae	33	*Rotheca myricoides*	Munjuga Iria		Malaria	1
					Arthritis	2
					STDs	1
					Pain	1
					Typhoid	1
Caesalpiniaceae	122	*Caesalpinia volkensii*	Mûbûthi	Leaves,Seeds	Malaria	11
					Fleas	4

Family	Voucher No	Scientific Name	Local name	Part used	Disease/ symptom treated	No. of users
					Abdominal diseases	3
					Typhoid	1
					Worms	2
					STDs	2
					Arthritis	1
					ECF	1
Solanaceae	25	*Physalis peruviana*	Nathi	Leaves	Malaria	1
					Typhoid	5
					STDs	1
Myrtaceae	27	*Psidium guajava*	Mûbera	Leaves	Typhoid	2
					Tonsils	1
					ECF	1
Rutaceae	48	*Clausena anisata*	Mûtathi		Typhoid	1
					Malaria	2
Euphorbiaceae	45	*Croton megalocarpus*	Mûkindûri	Leaves	Typhoid	11
				Bark	Arthritis	3
				Roots	Worms	3
					Amoeba	1
					ECF	1
					Pneumonia/cough	1
Asteraceae	1	*Tithonia diversifolia*	Marûrû/ Kirurite	Latex		
				Leaves	Jigger fleas	9
Asteraceae	92	*Tagetes minuta*	Mûbangi	Leaves	Ticks and fleas	6
Bignoniaceae	4	*Kigelia africana*	Mûratina	Bark	STDs	5
				Fruit	Aphrodisiac, immune booster	1

Family	Voucher No	Scientific Name	Local name	Part used	Disease/ symptom treated	No. of users
Rutaceae	107	*Fagaropsis hildebrandtii*	Mûkaragati		Arthritis	1
Boraginaceae	91	*Cordia africana*	Mûringa	Bark	Epilepsy	1
					Tooth ache	1
Euphorbiaceae	44	*Ricinus communis*	Mwarîki/ Mubariki	Roots	STDs	1
Gramineae	54	*Sorghum versicolor*	Mûhîa	Roots	STDs	1
Euphorbiaceae	121	*Synadenium compactum*	Watha		ECF	11
Gramineae	20	*Pennisetum clandestinum*	Wîtima		Pneumonia	1
					STDs	2
Labiatae	22	*Ajuga remota*	Wanjirû wa rûrie	Stem	Malaria	16
					STDs	1
					Pneumonia	3
					Stomach ache	2
					Worms	1
Asteraceae	103	*Conyza newii*	Mûrûnga anake Horse Weed	Leaves	Malaria	1
					Typhoid	1
					Arthritis	1
Leguminosae	2	*Senna didymobotrya*	Mwînû	Leaves	Malaria	4
				Roots	Worms	17
					Typhoid	5
					Arthritis	4
Amaranthaceae	69	*Cyathula polycephala*	Maramata	Leaves	Back Pains	1
					Arthritis	1
Commelinaceae	10	*Commelina africana*	Mûkengeria	Leaves Stem	Nutritional	1
Euphorbiaceae	8	*Bridelia micrantha*	Mûkoigo	Bark	Typhoid	4
					Pneumonia	3

Family	Voucher No	Scientific Name	Local name	Part used	Disease/ symptom treated	No. of users
					Poly menorrhea	1
Apocynaceae	82	*Carissa bispinosa*	Mûkawa	Roots	Arthritis	6
					Typhoid	1
					STDs	1
Solanaceae	47	*Withania somnifera*	Murambae		STDs	3
					Arthritis	3
					Typhoid	
Meliaceae	64	*Trichilia emetica*	Mûrûri	Flowers	Jiggers	1
Rhamnaceae	106	*Rhamnus prinioides*	Mûkarakinga	Stem	Arthritis	1
Mimosaceae	87	*Acacia hock ii, acacia lanai, acacia seal*	Mûgaa		Pneumonia	1
					STDs	2
Flacourtiaceae	114	*Dovyalis abyssinica*	Mûkambura		Pneumonia	1
Verbenaceae	85	*Lantana camara*	Mûkenia		Malaria	1
					STDs	1
					Arthritis	1
Rosaceae	111	*Prunus africana*	Mûiri	Bark	BPH	1
					Typhoid	2
					Pneumonia	1
					Arthritis	3
					Stomach ache & diarrhoea	2
					Detoxification	1
					Worms in animals	1
Araceae	66	*Manihot esculenta*	Matûma	Leaves	Tonsils	1
Leguminosae	46	*Erythrina Abyssinica*	Mûhûtî		Pneumonia	4
					Typhoid	1
					Worms	1
					STDs	1
					Stomach ache	2
					Otitis media	1

Family	Voucher No	Scientific Name	Local name	Part used	Disease/ symptom treated	No. of users
Euphorbiaceae	9	*Croton macrostachyus*	Mûtûndû		Worms	2
					Bleeding	2
					Antibacterial	1
Ebenaceae	56	*Euclea divinorum*	Mûkinyai	Leaves	worms	5
Rutaceae	63	*Zanthoxylum gilletii*	Mûcagatha		Jiggers	1
					Malaria	1
Leguminosae	96	*Acacia mearnsii*	Mûthanduku		STDs	1
					Heavy Menses	1
Labiatae	6	*Plectranthus barbatus*	Mûigoya	Leaves	Worms in animals	1
					Stomachache and diarrhoea	5
Leguminosae	31	*Albizia coriara / Albizua gummifera*	Mûkûrwe		ECF	1
					Pneumonia, Typhoid	1
Rutaceae	107		Mûkaragati		Pneumonia	1
					Arthritis	1
Sterculiaceae	38	*Dombeya burgessiae/ Dombeya goetzenii*	Mûkeû		Antibacterial, abdominal pains	1
Olacaceae	81	*Ximenia americana*	Mûtûra (ndûra)	Roots	Tooth ache	1
					Jigger flee	1
					Arthritis	3
					Abdominal pains	1
					Typhoid	1
Combretaceae	34	*Combretum mole/ Piliostigma thonningii*	Mûrema		Abdominal pains and diarrhoea	2
Leguminosae	65	*Malvasia verticillata*	Mûkûra		Pneumonia	1
Combretaceae	110		Mûruruku		Pneumonia	1
Ochnaceae	98	Indigenous	Mungirima		Arthritis	1
Bignoniaceae	68		Mûriûmwe		Arthritis	1
		Cucurbita maxima	Marenge		Worms	2

Family	Voucher No	Scientific Name	Local name	Part used	Disease/ symptom treated	No. of users
Bignoniaceae	68		Mûcakaranda		Ticks and fleas	1
Flacourtiaceae	108	*Trimeria grandifolia*	Mûhîndîhîndî	Stem	Arthritis, Paralysis	1
Asteraceae	5	*Bidens pilosa*	Mûcege		Eye diseases	1
Malvaceae	11	*Microglossa pyrifolia*	Mûhinga	Whole plant	Typhoid	1
Moraceae	28	*Ficus thonningii*	Mûgumo		Typhoid	1
Labiatae	35	*Leonotis mollisima*	Mûchii		ECF	1
Labiatae	50	*Fuertia africana*	Gathîrîga		Stomach ache and diarhoea	1
Leguminosae	79		Mûbeca		Snake bite	1

APPENDIX 3: Proposal Approval by the Board of Postgraduate Studies

DEPT. OF PUBLIC HEALTH PHARMACOLOGY & TOXICOLOGY
2 – MAR 2016
RECEIVED
C.A.V.S.

Plse file
Hubu
2/3/2016

UNIVERSITY OF NAIROBI
BOARD OF POSTGRADUATE STUDIES

Telephone: 3318262 Ext. 28267
Fax Number: 243626
Telegrams: "Varsity of Nairobi
E-mail: bps@uonbi.ac.ke
YOUR REF:
OUR REF: J56/775109/2014

P.O. Box 30197, 00100
NAIROBI, KENYA

27th February 2016

Dr. James Maina Githinji
c/o Chairman,
Department of Public Health, Pharmacology
& Toxicology
Faculty of Veterinary Medicine
CAVS

Dear Dr. Githinji,

REF: RESEARCH PROPOSAL AND SUPERVISORS

This is to inform you that the Director, acting on behalf of the Board of Postgraduate Studies has approved your Master of Science research proposal titled:"**A study of herbal medicines use, antiflea activity, and toxic effects of *Tithonia diversifolia* and *Senna didymobotrya* extracts in animals".**

She has also approved, **Prof. T. Maitho** and **Prof. J.M. Mbaria** as supervisors of your thesis.

You should therefore begin consulting them and ensure that you submit your thesis for examination on or before 30th August 2016. The Guidelines on Postgraduate Supervision can be accessed on our website (www.bps.uonbi.ac.ke) while the Research Notebook is available at the University Bookstore.

Yours sincerely,

SUSAN DARYA (MS)
FOR: DIRECTOR, BOARD OF POSTGRADUATE STUDIES

Cc: Dean, Faculty of Veterinary Medicine
Chairman, Department of Public Health, Pharmacology & Toxicology
Prof. T. Maitho, (Supervisor) – Department of PHPT
Prof. J.M. Mbaria, (Supervisor) – Department of PHPT

SD/bwg

APPENDIX 4: Application For By NACOSTI Research Permit

UNIVERSITY OF NAIROBI
COLLEGE OF AGRICULTURE AND VETERINARY SCIENCES
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF PUBLIC HEALTH, PHARMACOLOGY AND TOXICOLOGY

Department of Public Health
Pharmacology and Toxicology
P.O. Box 29053,
Kabete, KENYA

Telephone:Nairobi 020-2453621, 020-3592734; 0203592735
Telegraph: Univet, Nairobi
Telex: 22095 VARSITYK
Fax: +254 2 631325

26th April, 2016

National Council for Science and Technology
P.O. Box 30623-00100,
NAIROBI, KENYA

Dear Sir/Madam,

RE: APPLICATION FOR RESERCH PERMIT

Dr. James Maina Githinji is a postgraduate student at the University of Nairobi, Faculty of Veterinary Medicine, Department of Public Health, Pharmacology and Toxicology.

He is undertaking a course on MSc Pharmacology and Toxicology and is applying for a permit to enable him conduct his research and complete his studies. The academic research is entitled "A Study of Herbal Medicines Use, Antiflea Activity, and Toxic Effects of *Tithonia diversifolia* and *Senna didymobotrya* Extracts".

Attached please find an approved research proposal.

We shall be grateful for your assistance.

Yours faithfully,

DEPT. OF PUBLIC HEALTH,
PHARMACOLOGY & TOXICOLOGY
P. O. Box 29053
KABETE - NAIROBI
KENYA.

Prof. T. Maitho
Dept. of Public Health, Pharmacology & Toxicology

APPENDIX 5: NACOSTI Research Permit

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone:+254-20-2213471,
2241349,3310571,2219420
Fax:+254-20-318245,318249
Email:dg@nacosti.go.ke
Website: www.nacosti.go.ke
when replying please quote

9th Floor, Utalii House
Uhuru Highway
P.O. Box 30623-00100
NAIROBI-KENYA

Ref. No. **NACOSTI/P/16/08795/11153**

Date: **16th June, 2016**

Dr. James Maina Githinji
University of Nairobi
P.O. Box 30197-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"A study of herbal medicines use antiflea activity and toxic effects of tithonia diversifolia and senna didymobotrya extracts in animals,"*** I am pleased to inform you that you have been authorized to undertake research in **Murang'a County** for the period ending **13th June, 2017.**

You are advised to report to **the County Commissioner and the County Director of Education, Murang'a County** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.

DR. STEPHEN K. KIBIRU, PhD.
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Murang'a County.

The County Director of Education
Murang'a County.

THIS IS TO CERTIFY THAT:
DR. JAMES MAINA GITHINJI
of UNIVERSITY OF NAIROBI, 0-10208
SABASABA,has been permitted to
conduct research in *Muranga County*

Permit No : NACOSTI/P/16/08795/11153
Date Of Issue : 16th June,2016
Fee Recieved :Ksh 1000

on the topic: *A STUDY OF HERBAL MEDICINES USE ANTIFLEA ACTIVITY AND TOXIC EFFECTS OF TITHONIA DIVERSIFOLIA AND SENNA DIDYMOBOTRYA EXTRACTS IN ANIMALS*

for the period ending:
13th June,2017

.....
Applicant's Signature

.....
Director General
National Commission for Science, Technology & Innovation

CONDITIONS

1. You must report to the County Commissioner and the County Education Officer of the area before embarking on your research. Failure to do that may lead to the cancellation of your permit
2. Government Officers will not be interviewed without prior appointment.
3. No questionnaire will be used unless it has been approved.
4. Excavation, filming and collection of biological specimens are subject to further permission from the relevant Government Ministries.
5. You are required to submit at least two(2) hard copies and one(1) soft copy of your final report.
6. The Government of Kenya reserves the right to modify the conditions of this permit including its cancellation without notice

REPUBLIC OF KENYA

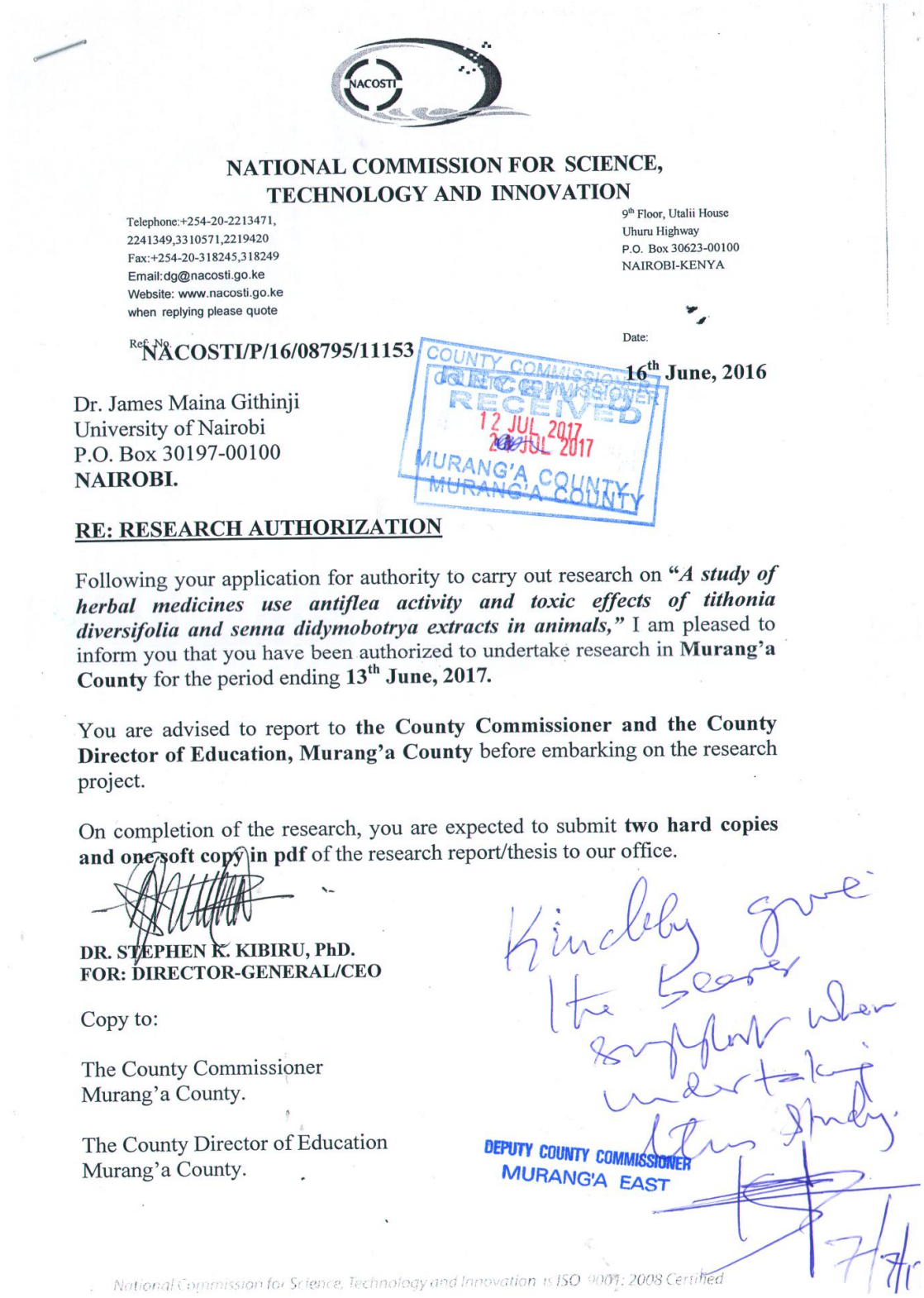
National Commission for Science, Technology and Innovation

RESEARCH CLEARANCE PERMIT

Serial No. A 9583

CONDITIONS: see back page

APPENDIX 6:Murang'a County Commissioner Authorization.



NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone:+254-20-2213471, 2241349,3310571,2219420
Fax:+254-20-318245,318249
Email:dg@nacosti.go.ke
Website: www.nacosti.go.ke
when replying please quote

9th Floor, Utalii House
Uhuru Highway
P.O. Box 30623-00100
NAIROBI-KENYA

Ref. No. NACOSTI/P/16/08795/11153

Date: 16th June, 2016

COUNTY COMMISSIONER RECEIVED 12 JUL 2017 MURANG'A COUNTY

Dr. James Maina Githinji
University of Nairobi
P.O. Box 30197-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"A study of herbal medicines use antiflea activity and toxic effects of tithonia diversifolia and senna didymobotrya extracts in animals,"*** I am pleased to inform you that you have been authorized to undertake research in **Murang'a County** for the period ending **13th June, 2017.**

You are advised to report to **the County Commissioner and the County Director of Education, Murang'a County** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.

DR. STEPHEN K. KIBIRU, PhD.
FOR: DIRECTOR-GENERAL/CEO

Kindly give the bearer support when undertaking the study.

DEPUTY COUNTY COMMISSIONER MURANG'A EAST

Copy to:

The County Commissioner
Murang'a County.

The County Director of Education
Murang'a County.

National Commission for Science, Technology and Innovation is ISO 9001: 2008 Certified

APPENDIX 7:Murang'a County Director of Education Authorization.

MINISTRY OF EDUCATION
STATE DEPARTMENT OF BASIC EDUCATION

Email: cdemuranga@gmail.com
Telephone: 060 2030227
When replying please quote

COUNTY DIRECTOR OF EDUCATION
P.O BOX 118 – 10200
MURANG'A

REF: MGA/CTY/GEN./64/VOL.II/37 **30th March, 2017**

Dr. James Maina Githinji
University of Nairobi
P.O.Box 30197-00100
NAIROBI

RE: RESEARCH AUTHORIZATION

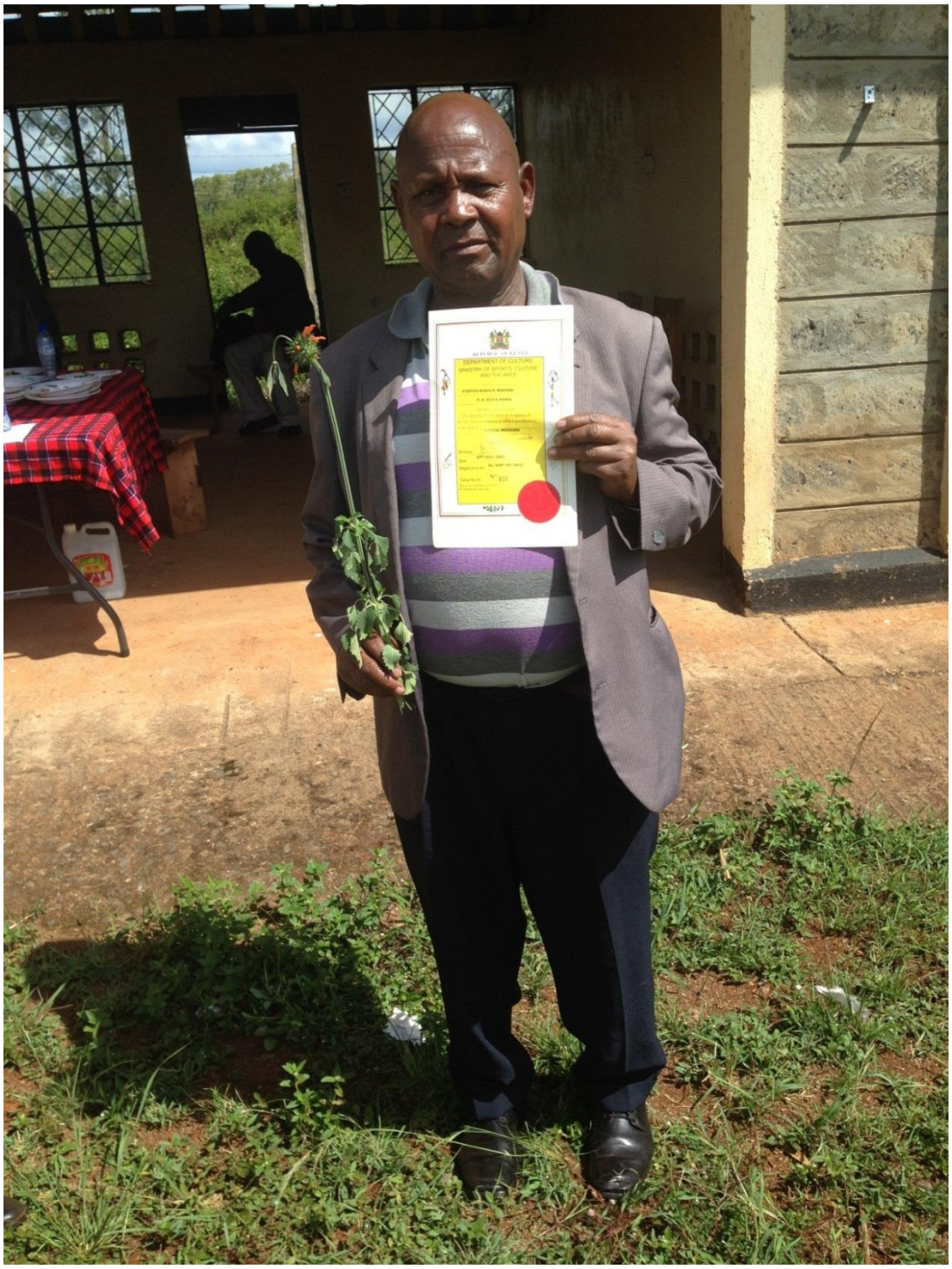
The County Education office is in receipt of your request and authority letter from the National Commission for Science, Technology and Innovation, reference No. NACOSTI/P/16/08795/11153 dated 16th June, 2017 to carry research on **"A study of herbal medicines use antiflea activity and toxic effects of *Tithonia diversifolia* and *Senna didymobotrya* extracts in animals".**

Authority is hereby granted to carry out research in **Murang'a County** for a period ending **13th June, 2017.**

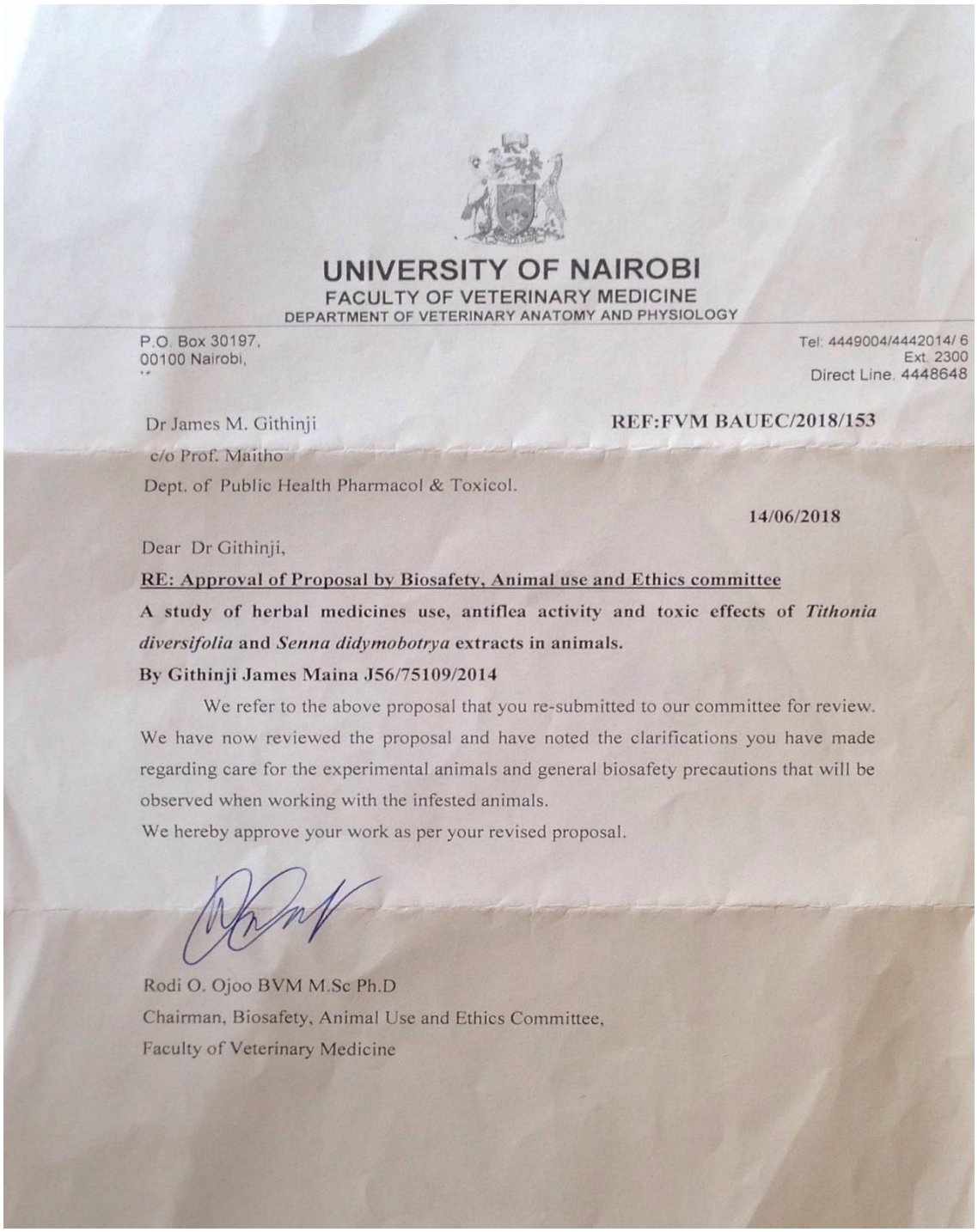
COUNTY DIRECTOR OF EDUCATION MURANG'A 30 MAR 2017 P.O. Box 118-10200, MURANG'A

Charles Khayira
County Director of Education
MURANG'A

APPENDIX 8: A Herbalist Holding the Certificate Issued by the Kenyan Government, Department of Culture, Ministry of Sports Culture and Arts



APPENDIX 9: Biosafety, Animal Use and Ethics Committee Approval



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

P.O. Box 30197,
00100 Nairobi,

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

Dr James M. Githinji
c/o Prof. Maitho
Dept. of Public Health Pharmacol & Toxicol.

REF:FVM BAUEC/2018/153

14/06/2018

Dear Dr Githinji,

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

A study of herbal medicines use, antiflea activity and toxic effects of *Tithonia diversifolia* and *Senna didymobotrya* extracts in animals.

By Githinji James Maina J56/75109/2014

We refer to the above proposal that you re-submitted to our committee for review. We have now reviewed the proposal and have noted the clarifications you have made regarding care for the experimental animals and general biosafety precautions that will be observed when working with the infested animals.

We hereby approve your work as per your revised proposal.

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

APPENDIX 10: Plagiarism Report

turnitin Turnitin Originality Report

A STUDY OF HERBAL MEDICINES USE, ANTIFLEA ACTIVITY, AND TOXIC EFFECTS OF TITHONIA DIVERSIFOLIA AND SENNA DIDYMOBOTRYA EXTRACTS by Githinji James Maina

For Master of Science in Pharmacology& Toxicology

Processed on 13-Jun-2018 19:55 EAT

- ID: 975377035
- Word Count: 15461

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APPENDIX11. Papers Published from the Study

Attached herewith are papers which were published in the following journals:

1. Githinji, J. M., Maitho, T., and Mbaria, J. M. (2018). Ethno botanical Study of Plants Used in the control of Diseases and Pests in Murang'a County, Kenya. IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) ***Vol*** *.13*: (4):*Ver.II.* 56-62. www.iosrjournals.org. Doi: 10.9790/3008-1304025662
2. Githinji, J. M., Maitho, T., and Mbaria, J. M. (2018). Antifleas Activity and Safety of Tithonia diversifolia and Senna didymobotya Extracts. Journal of Pharmacy and Pharmacology Research; Vol.2 (3): pp 078-092. www.fortunejournals.com Doi: 10.26502/fjppr.0012.

Iosr Journal of Pharmacy and Biological Sciences (iosr-jpbs) e-ISSN-2278-3008, 2319-7676. Volume 13, Issue 4 Ver.II (july-Aug 2018), 56-62 www.iosrjournals.org

Ethno-botanical Study of Plants Used in Control of Ectoparasites in Murang'a County, Kenya

Githinji, J. M[1*], Maitho, T.[1], and Mbaria, J. M[1]

[1]*Department of Public Health, Pharmacology & Toxicology, University of Nairobi, P.O. Box 29053-00625, Nairobi, Kenya*

** Corresponding Author: Githinji, J. M[1]*

Abstract: *Herbal medicine has been practiced in the world since ancient times, yet limited research data has been documented on their use in Murang'a County, Kenya. The aim of this study was to identify different herbs of medicinal value in Murang'a County, their uses, and effect on ectoparasites with emphasis on fleas and jiggers control. A cross-sectional study was carried out using a questionnaire and a total of 28 herbalists were interviewed. The herbalists identified 122 herbs which were used for the treatment of various illnesses and ectoparasites. A total of 18 plants were used to control jiggers and fleas and Solanum campylacanthum was the most commonly used plant. Despite the common use of Solanum campylacanthum in the control of jiggers and fleas, there is still a high burden of jiggers in Murang'a County and hence there is a need to evaluate other plants, which grow in large numbers in the County where their use have been documented in other areas. In this study Tithonia diversifolia and Senna didymobotyra were studied. The two plants were found to be moderately safe with LD_{50} of above 2000 mg/kg.*

Keywords*: Herbal medicines, control, pests and jiggers.*

Date of Submission: 10-07-2018 Date of acceptance: 27-07-2018

I. Introduction

Plants have been used by human beings for maintenance of life and as sources of shelter, clothing, food, and medicine for man and livestock. Studies on medicinal use of plants by different communities in various cultural set-ups are reliable sources of knowledge for sustainable use and conservation of plants. WHO (1993) stipulated guidelines on the conservation of medicinal plants and documentation is an impressive key to providing basis for decision making and policy design on conservation of the plants. Like the rest of the world, various communities in Kenya have encouraged ethno-pharmacological documentation.

Fleas are the most predominant ectoparasites of domesticated animals and pets especially dogs and cats throughout the world [1]. They feed on blood by sucking through the skin of their hosts and these pests are not restricted to animals only.

Jigger (*Tunga penetrans*) is a small flea that inhabits sandy beaches and soil and is commonly found in human dwellings. According to the Ministry of Health [2], an estimated 1.4million Kenyans or 4% of the population was infested by jiggers. Jigger infestation cause morbidity and inability of victims to work and leads to low productivity and is a burden to both economy and families. A study carried out in Murarandia area of Murang'a County indicated that there was a significant reduction in agricultural productivity when people infested by jiggers were used as labourers, as compared to labourers who were free of jigger infestation [3]. Another study carried out among primary school pupils in Murang'a County revealed that the prevalence of jigger infestation was as high as 21.5% across all age-groups, with the most common method of control being mechanical removal of jiggers using sharp objects [4]. This not only creates a multiplicity of risks in terms of spread of other infectious diseases amongst the population, but also increases chances of infection through broken skin and may lead to painful and septic wounds, eventually limiting the victims' mobility. This therefore calls for alternative methods of treating and preventing jigger and flea infestation.

The Kenyan Ministry of Health in its National Policy Guidelines for Prevention Control of Jiggers [2] acknowledges that use of chemicals such as pyrethrins and pyrethroids, organophosphates, carbamates, and fumigants to control jiggers exhibit varying levels of efficacy, and safety to humans, environment and non-target organisms such as pets. In addition, usage of chemical control requires technical understanding of usage,

precautions and disposal of empty containers, which is a challenge to semi-literate and illiterate household heads. Availability and accessibility of the chemical compounds and implementation therefore is very challenging.

In addition, common control of jiggers using potassium permanganate and hydrogen peroxide which are used commonly is not sustainable. These popular chemicals lack sufficient supportive research on efficacy and safety of their supply may not be guaranteed. Potassium permanganate is listed by the United States and the International Narcotics Control Board as a controlled precursor chemical used in the production of cocaine. More Countries are doing extensive monitoring of supply of these chemical and this lead to their unavailability for control of jiggers. Murang'a County is ranked among the Counties which are worst hit by the jigger menace, together with Kiambu, Kilifi, Nyeri, Kwale, Baringo, Busia, Kakamega, Siaya, and Marsabit Counties. Jigger infestation is associated with low socio-economic status amongst the fifteen affected Counties. The children and the elderly are among the worst affected by jiggers, with infestations occurring in Schools, homes and other Public places. Kenya has over 1.8million children affected by Jiggers. Over 10% of the children with the jigger infestation drop out of school, and they are unable to concentrate in education and work due to feet infestation. There is also a decreased agricultural productivity of infested adults and loss of social capital due to stigma associated with this problem. The major contributor of the stigma is the lack of the political will to fight the menace by the politicians. Among projects identified by the Murang'a County Government in the Strategic Planning for 2013-2017 was public participation in establishing a treatment Centre for Jiggers

The naming of plants has been documented in Central region of Kenya [5]. Gachathi developed a guide to plant names, their uses and cultural values in the Kikuyu community.

The current study was conducted in order to document medicinal plants used to control fleas and jiggers in Murang'a County.

II. Materials And Methods

Study design

A cross-sectional survey using semi structured questionnaires was carried out in order to study the herbal medicines used by traditional medicine practitioners in order to manage jiggers, fleas and ticks, as well as other disease conditions in Murang'a County

.

Study Area

The study was carried out in Murang'a County, which lies in Central region of Kenya. Murang'a County occupies a total area of 2,558.8 Km^2 of which 11.2 km^2 is a water mass and the remaining land of 2,135km^2 is arable. The County lies between 914m above the sea level in the East and 3,353 m above the sea level along the slopes of Aberdare ranges in the West. The County lies between latitudes 0^0 34' South 1^0 7' South and longitudes 36 ^{0} East and 37^0 27' East. Long rain falls between March and May, with April recording the highest rainfall and short rains are received in October and November.

The County had a total population 936,228 persons who consist of 451,751 males and 484,477 females, and a growth rate of 0.4 % per annum as reported in 2009 Census. The rate of unemployment in the County was about 17.67%, which translates to 93,241 persons, while 36.3 % of the County population lives in abject poverty. A total of 40% of the population live in stone/brick walled houses, 24.3% mud and wood houses and 2.19% live in grass, straw and tin walled houses. Most of the houses in the County are roofed with corrugated iron sheets (94.38%); while makuti and grass roofed houses constitute 0.18%. Most of the houses (60.04%) have earth floor.

Plant collection and Identification

Selection and recruitment of herbalists

A reconnaissance study was done in January 2016 and thereafter it was followed by actual studies between May and December 2016. Simple sampling of the herbalists that were recognized by the Ministry of culture and social services in Murang'a County was done. Herbalists who were willing to participate in the study were recruited and interviewed using a semi structured questionnaire.

Ethno-botanical Study

A total of twenty-eight herbalists were interviewed in consultative meetings which were held at Maragua Jua Kali herbalists' social hall. Field collection of plant specimens was done with the help of the herbalists and voucher specimens were prepared, and authenticated by a Botanist from the East Africa herbarium, of the National Museum of Kenya where they were deposited. The data collected included the respondents' demographics, names of plants and the parts of the plants used, methods of preparation and administration of the extracts.

Analysis of data

The data collected was analyzed descriptively using Student's t-test, R version 3.4.3 and presented using tables and familiarity indices. Familiarity index was used as an indicator of popularity of a plant species and was determined using a method reported previously [7].

III. Results

Demographic data of the herbalists

Demographic data of 28 herbalists is given below. Majority of the herbalists 86%) are males, and majority (89.3%) were married, 7.1% are divorced and 3.6% are widowed. Majority of herbalists (50%) had attained Primary level of education, 32.1% Secondary level and only 17.9% had Post-Secondary level of education. All the herbalists in the study are religious, majority (89.3%) being Protestants, and (7.1%) being Catholics. Majority (64.3%) of the herbalists treated humans only and 25% of herbalists treated animals as well. The demographic data of the herbalists is shown in Tables 1.

Table 1: Demographic data of Murang'a herbalists

Variables		No of herbalists	Percentage
Age	31-40	2	7.1
	41-50	3	10.7
	51-60	5	17.9
	61-70	10	35.7
	71-80	6	21.4
	81-90	2	7.1
Gender	Males	24	86
	Females	4	14
Occupation	Full time herbalists	9	33.3
	Farmers	17	63.0
	Employed	1	3.7
Source of knowledge	Hereditary	12	42.9
	Observation	8	28.6
	Seminars and internet	11	39.3
Level of Education	Primary school	14	50
	Secondary school	9	32.1
	Post-secondary	5	17.9
Religion	Catholics	2	7.1
	Protestants	25	89.3
	Other religions	1	3.6
Marital status	Married	25	89.3
	Divorced	2	7.1
	Widowed	1	3.6

NB: N=28

Monthly workload, sources of clients, feedback methods and nature of practice of the herbalists are given in Table 2.

Table 2: A Summary of the Murang'a herbalists' activities

Variables		No of herbalists	Percentage
Monthly workload	Less 10 clients	7	25
	Between 10 and 20	14	50
	More than 20	7	25
Source of clients	Inter-herbalist referral	7	26.9
	Market day promotions	11	42.3
	Hospitals	8	30.8
Clients reason for visit	Failure of hospital drugs	20	71.4
	Side of hospital medicines	3	10.7
	Cost of hospital medicine	2	7.1
	Other reasons	3	10.8
Clients feedback	Patients come for review	13	46.4
	Patients not coming back	10	35.7
	Patients laboratory results	2	7.1
	Other feedbacks	3	10.8
Nature of practice	Treats humans only	21	75
	Treats human and animals	7	25

NB: N=28

Medicinal plants use

A summary of medicinal plants used in Murang'a County is given in Tables 8.

Table 8: ***Plants used for the management of jiggers and fleas in Murang'a County***

Botanical names	Types of plants	Part of plants used	Familiarity Index	Other major medicinal use of the plants
Solanum campylancanthum[a,b]	Shrub	Fruits, roots	0.75	Colds, stomachache and treatment of anthrax in sheep [5]
Vernonia lasiopus	Shrub	Leaves, stems	0.11	Antitrypanosomal, antihelmintic, venereal and skin diseases, [5],[6],[7]aphrodisiac
Caesalpinia volkensii[b]	Climber/ Liana	Leaves, seeds	0.14	Antibioterial, antifungal,pesticidal, malaria, venereal diseases, aphrodisiac[5,8]
Azadirachta indica[a,b]	Tree	Leaves	0.29	Antifungal, antimalarial, analgesic, antiviral, antibacterial, antipyretic, hypoglycaemic, contraceptive, antitumor, anthelmintic [9]
Tagetes minuta[a,b,c]	Herb	Leaves	0.21	Antifungal, antibacterial, allopathic and insecticide[8]
Aloe vera[a,b]	Shrub	Stems,and leaves	0.11	Laxative, anti-malaria, ECF in animals,[5],[8],[11] antioxidant,antimicrobial,immunomoderator,hypoglycemic, wounds[10]
Nicotiana tabacum[b,c]	Shrub	Leaves		Hypoglycemic, antifungal, antibacterial, insecticidal [11]
Senna didymobotrya[a,b]	Shrub	Leaves, roots	0.14	Antimicrobial, antifungal, pesticide [12]
Trichilia emetica[a]	Tree	Flowers	0.04	Stomachache , leprosy, pneumonia,[5,8]
Albizia antihelminthica[b]	Tree	Leaves	0.04	antioxidant, antihelminic, Anti-inflamatory, analgesic,[13]
Tithonia diversifolia	Shrub	Leaves	0.32	Analgesic, anti-inflamatory, anti-malarial, antiviral , antiplasmodic, hypogycemic, antimicrobial, cancer chemopreventive, biopesticide[14]
Dicrostachys cineria	Shrub	Leaves , stem and barks	0.04	
Xanthoxylum gilletii[a]	Tree	Barks of stem and roots Leaf	0.04	Antimicrobial, cytotoxic and analgesic, antiplasmosis in cattle, coughs and cold [5],[15]
Solanum aculeastrum[a,b]	Shrub	Berries Roots.	0.07	Bronchitis, gonorrhoea, antioxidant, antibacterial, [8,16]
Jacaranda mimosifolia	Tree	Barks of stems- Leaf.	0.04	Venereal diseases,Molluscicidal,tripanocidal, hypoglycimic, anti-inflammatory, immuno-stimulant, anti-snake venorm, anti-cancer [17],[18]
Jatropha curcas	Shrub	leaves and stalk	0.04	Analgesic, Antibacterial, antioxidant, antifungal, antinflammatory[18]

Key: a (Jiggers), b (fleas)

III. Discussion

The findings of the study show that males 89.3%dominates the practice of herbal medicine. The findings are similar to those reported [20]. Majority (82.9%) of the herbalists are above50 years. These findings concur with a study done in Lebanon [21], and unlike the findings of the study [20] in which 66 % of herbalists

were younger and were aged between 26 and 45 years. The findings also show that majority of the herbalists are semi-literate, with 17.9% having tertiary level education, 32.1% Secondary level and 50% with primary school level of education. The findings are in agreement with results [21]. Majority (89.3%) of the herbalists are married, and are mainly (64.3%) part time herbal practitioners. Most of the herbalists (89.1 %.) were Protestants and were either peasant farmers or retirees.

The main source of herbal medicine knowledge is hereditary as 42.9% of herbalists acquired their knowledge from their grandparents, parents or elder relatives, 28.6% of the herbalists learned practice by observing other experienced herbalists while 39.3% of the herbalists searched the internet in order to get the knowledge. Majority (42.9%) of the herbalists attended between 10 and 20 clients per month, while only 28% of the herbalists attended more than 20 clients which is not in line with other findings [22]. The major source of herbalists' clients was from promotions on market days which attracted 39.3% of the patients, who were eager to have their problems solved especially when the herbalists were available to attend them as they went about their usual daily activities. Patients' self-referral from the hospitals constituted 28.6%, of a group of patients who have lost hope due to their low socioeconomic status, making conventional medicine out of reach, had advanced stages of diseases or had chronic illnesses which they thought modern medicine was taking too long to cure, and hoped herbalists would offer a quicker and safer options which is in line with earlier [23] findings. The inter-herbalists referrals made up 25% of the clients being attended.

The herbalists used one plant to treat more than one condition, and used a combination of many plants to treat a specific condition. In most cases, liquid preparations were obtained by boiling the plants in water and the findings are in line with findings of [20]. The findings have shown that there is a high risk of completely losing the practice of herbal medicine, as majority of practitioners are aged, and are not documenting their work due to their low levels of literacy. The younger generation who are educated and well versed with technology need to be inspired in to Ethno Pharmacology, in order to promote integration of herbal medicine with modern science, medicine and technology, leading to more research and documentation, encouraging more young people to practice herbal medicine openly as well as raising the societal confidence in seeking herbal treatment.

Modernization of the herbal medicine would increase the practitioners' income, and also make practice a full time job, since many modern citizens will embrace it. This can lead to the conservation of biodiversity as it can be a source of raw materials for medicines. Better research and with integration of modern science will encourage well educated persons to take up the practice with better understanding of science behind it, human Physiology, Biochemistry, and diseases conditions, as well as effects of the herbal medicine on the human body and possible interactions with the conventional drugs. The Science and Technology will also encourage gender equity in the practice.

Fleas host many vector- borne pathogens and their diseases [24]. Among the many diseases causing bacteria hosted by fleas is *Yersinia pestis* which causes plague in man. Fleas and ticks have economical and health effects on humans. Both Human (*P. irritans*) and cat (*C. felis*) fleas are associated with plague transmission from human to human, and *C. felis* is also associated with the outbreaks of diseases for example fatal pneumonic plague in northwest Uganda [24]. There is a need to reduce the risks of diseases transmissions, as well as economic losses associated with fleas in domestic animals. *Tunga penetrans* fleas are associated with secondary bacterial infections lesions in the body. The Pathogenic bacteria which have been isolated from *tungiasis* lesions includes*Clostridium tetani*, *Streptococcus pyogens*, *Staphylococcus aureus*, *Klebsiella aerogenes*, *Enterobacter agglomerans*, *Escherichia coli* as well as other enterobacteriaceae [24].

Jiggers continue to be a menace in Murang'a County, since it affects people of low socio-economic status. The ethno-medical plants in this area are growing freely on roadsides and on the uncultivated land. The medicinal plants are a possible solution for the poor to kill these parasites using the available medicinal plants. A total of 144 plants were collected from the area of study, 16 of which were mentioned by different herbalists as having been used for fleas, jiggers or ticks. There were no toxic effects reported following use of the plants in humans. *Azadirachtaindica* was one of the plants used by the herbalists in the region. The plant however is not readily available in most areas of the County.

IV. Conclusions

More studies need to be conducted on the specific plants mentioned in the study and also on *Tithonia diversifolia* which is used in Kakamega region for the anti-parasitic activity especially for jiggers and fleas. There are many plants which are used by herbalists in Murang'a County to treat various ailments and parasites. There is need to do more research in order to facilitate their use.

Conflict Of Interest

The authors declare that there are no conflicts of interest.

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Research Article

Antifleas Activity and Safety of Tithonia diversifolia and Senna Didymobotrya Extracts

Githinji James Maina*, Maitho Timothy, Mbaria James Muchunu

Department of Public Health Pharmacology and Toxicology, Nairobi, Kenya

***Corresponding Author:** Githinji JM, Department of Public Health Pharmacology and Toxicology, University

Of Nairobi, P.O. BOX 29053-00625, Nairobi, Kenya, E-mail: githinjijames27@gmail.com

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Abstract

Background: *Tithonia diversifolia* and *Senna didymobotrya* have been used traditionally as bio-pesticides. Their use in the control of fleas, and also their aqueous extract safety is not adequately documented.

Methods: Antifleas activity of *Tithonia diversifolia* and *Senna didymobotrya* were compared with *Chrysanthemum cinerariifolium*, and also their acute toxicity in Wister rats and dermal and eye irritation in Newzealand albino rabbit were studied using a method reported previously. Crude aqueous extracts of flowers and leaves for *T. diversifolia*, leaves of *S. didymobotrya* and flowers of *C. cinerariifolium* were prepared and serial dilutions of the crude extracts and control drugs were prepared. Whatman's filter paper no.1 stripes were coated with plant extracts which were used to investigate antifleas activity, using fleas obtained from mongrel dogs. The activities were observed after 24 and 48 hours by counting the number of life fleas in the polypylene tubes.

Results: *T. diversifolia* showed most (93%) antifleas activity, *C. cinerariifolium* (90%) and *S. didymobotrya* 66.3%. The three plants extracts studied did not show any signs of eye or dermal toxicity and had LD_{50} of above 2000 mg/kg.

Conclusions: Further studies should be conducted in order to find out if *T. diversifolia* flowers can be used to control jigger flea.

Keywords: T diversifolia flowers; Fleas control; Jiggers

1. Introduction

Fleas are ecto-parasite which are passed from domestic animals to humans and cause similar effects as in the animal hosts. Fleas have exclusive ability to reproduce in households, are present all year round, regardless of the seasons, which means that dogs living in cold areas are also infested. Flea bites can cause pruritus and their key pathogenic effect is flea allergic dermatitis (FAD) as well as other health effects. FAD prevention relies on the control of the flea infestation, involving regular and continued use of anti-flea chemical agents, which are normally topical formulations but occasionally can be given orally [1].

Fleas are pests which are a nuisance and they are vectors of diseases. Three types of plagues in human are associated with fleas. These are bubonic plague, pneumonic plague and septicemic plague. Rat and cat fleas are associated with murine typhus fever which is caused by *rickettsia typhi* which is mainly transmitted by the rat and cat fleas, where humans acquire the infection as result of contamination from the dried feaces and crushed bodies of the fleas. The sand fleas, chigoes or jigger fleas are only a millimeter long and are nuisance as the females burrow into the skin. Mostly, a person may be affected by one or two jiggers at a time, but Infestations with hundreds of jiggers also occurs. Under favourable conditions a complete cycle from eggs through larvae, pupa and finally adult, can take 18 days [2].

Arthropods like fleas and ticks determine the community composition of bacterial [3]. Fleas host many vector-borne pathogens and their diseases [4]. The common disease causing bacterium which is *Yersinia pestis* causes plague in man. None of the flea types is specific to humans and only a fraction of the fleas come into contact with human on regular basis. Abundance of human associated fleas (*Pulex irritans, Ctenocephalides felis, and X.cheopis*) however, has been described in human dwellings in plague-endemic regions of Africa [5]. Human (*P irritans*) fleas are associated with plague transmission from human to human, and Cat fleas (*C fellis*) are suspected in the plague outbreaks for example in Northwest Uganda [4]. Clinical manifestations of plague are; bubonic plague which is most common, septic plague without bubo, and pneumonic plague, meningitis and pharyngitis [6]. Pneumonic plague is rapidly fatal if untreated. Fleas and ticks should be controlled in order to reduce risk of disease transmission and also reduce economic losses associated with parasitization of domesticated animals [7]. *Rickettsia typhi* is associated with fleas, ticks mites and body lice. The obligate intracellular gram negative bacteria are transferred from rodents' reservoir by an arthropod to humans [8].

Bitam et al., [4] associated *Tunga penetrans* fleas with secondary bacterial infections in the lesions among them; *Clostridium tetani, Streptococcus pyogens, Staphylococcus aureus, Klebsiella aerogenes, Enterobacter agglomerans, Escherichia coli* as well as other enterobacteriaceae. A study in Kandara Sub-Counties in Murang'a County Kenya reported that 6,200 school going children are infested by Tungiasis in 2014 [9]. Most of the affected patients use mechanical means to remove jiggers with sharp objects such as needles [10]. Tithonia species contains sesquiterpene lactones and diterpenoids which have biological activities against insects [11]. *Tithonia diversifolia* is used in Ikolomani Division of Kakamega County, Kenya, in management and control of jigger fleas [12]. There is limited

information on the effects of crude plant extracts like *Tithonia diversifolia* and *Senna didymobotrya* on fleas which are hosted in animals. Additionally, the toxicity and safety profiles of these plants have not been reported adequately in various animals. It is therefore important to investigate the LD_{50} of these plants' extracts as insecticides have been associated with accidental poisoning. If the two plants' extracts are found to be effective on fleas, more investigations should be conducted in order to check if the same extracts can be effective on the sand flea, which is a jigger causing flea and is reported as a menace to the poor residents of Murang'a County and other regions of Kenya.

The current study was conducted in order to investigate in vitro antiflea activity of *Tithonia diversifolia* flowers and leave crude extracts and also *Senna didymobotrya* leaves crude extracts. The other objective of the study was to determine skin sensitization and irritation as well as determine the acute toxicity of these extracts.

2. Materials and Methods

2.1 Approvals of the study

The study was approved by National Commission for Science Technology and Innovations (NACOSTI) and also Biosafety, Animal Use and Ethics Committee (BAUEC) of University of Nairobi.

2.2 Experimental animals

Young adult female nulliparous and non-pregnant Newzealand albino rabbits were obtained from the University of Nairobi animal house and were used for the determination of acute dermal irritation/corrosion (OECD, 404) and eye irritation test (OECD, 405). Adult (8 to 12 weeks old), female, nulliparous and non-pregnant Wister rats weighing 90-130 g were obtained from the University of Nairobi animal house and were used for acute toxicity tests of the extracts. All animals in these studies were individually housed and acclimatized for 5 days to laboratory conditions before beginning the study [13]. Fleas used in the study were obtained from the local mongrel dogs. The animals were housed under the standard laboratory conditions (temperatures of 25°C ± 3°C, natural light and relative humidity of 50 - 60%). They were fed on standard pellet diet and on unlimited supply of water [14].

2.3 Plant materials

Tithonia diversifolia and *Senna didymobotrya* plants specimens which included aerial parts and roots were collected from Maragua area of Murang'a County. The collected specimens were photographed, identified and authenticated with the aid of a taxonomist at the National Museums of East Africa where the voucher specimens were prepared and deposited. The plant materials were transported to the Department of Public Health, Pharmacology and Toxicology laboratories and were washed thoroughly with running tap water, chopped into small pieces and then air-dried under the shade for a period of 14 days and then grounded. Extractions of plant materials were done at the Kabete campus, of the University of Nairobi using a previous reported method [15-16]. The crude methanolic and aqueous extracts were

dissolved separately in dimethyl sulfoxide (DMSO) and distilled water respectively at a concentration of 1000 mg/10 ml. Serial dilutions were prepared logarithmically under sterile conditions by adding calculated amounts of distilled water in order to obtain working concentration ranging from 100 mg/ml – 1 mg/ml. All the prepared crude extract solutions were stored at 40°C and retrieved only during use.

2.4 Determination of in vitro anti-flea activity of *Tithonia diversifolia* and *Senna didymobotrya*

Polypropylene (15 ml) centrifuge tubes contact assay against *Ctenocephalides felis* and *Ctenocephalides canis* were used in the study as previously reported [17], but with minor modifications. Methanolic crude extracts were not tested although in piloting study they were found to be more active, reason being the fact that herbalists use water as the solvent for their concoctions. Three concentrations of each crude aqueous extract (*Senna didymobotrya* leaves-SLAE, *Tithonia diversifolia* leaves-TLAE, *Tithonia diversifolia* flowers-TFAE) were prepared for testing in-vitro anti-flea activity. For each extract, 1 mg/ml, 10 mg/ml and 100 mg/ml concentrations were made. A Whatman filter paper no.1 strip measuring 10 cm by 1.5cm was saturated with the extract concentration to be tested and was later allowed drying up, thus leaving the paper evenly coated with the extract at the concentration being tested. The coated stripe was fitted into the 15 ml polypropylene centrifuge tube. A total of ten fleas held by the small loose cotton wool were randomly picked and transferred into these polypropylene tubes and screw cap with holes replaced to hold the fleas inside, avoiding suffocation.

Similarly, a positive control using pyrethrum flower aqueous extract (PFAE) at similar concentrations were included for comparison. Viability of fleas in each tube was tested as previously described Dryden et al., [18]. Adult fleas *Ctenocephalides canis* and *felis* from the natural habitat were obtained from the mongrel dogs obtained from Ndumboini households. The fleas were held in a large container with small pieces of cotton wool which served as holding grounds for them. Each piece of cotton wool held an average of ten fleas. A total of 10 fleas were picked randomly and were transferred into each tube. The tubes were closed with an untreated screw cap with needle- punctured holes in the center in order to hold the fleas inside, avoiding suffocation. These tubes were kept at ambient temperatures and humidity, horizontally in order to ensure maximum contact between the fleas and the filter paper surface in the tube.

2.5 Acute dermal toxicity

The acute dermal toxicity of the crude aqueous extracts was tested using OECD [19] guidelines 404. Nine (three rabbits per test substance) healthy, adult female, nulliparous and non-pregnant Newzealand albino rabbits weighing 2.0-3.0 Kg were used. The test extract was applied onto the skin of the test rabbits as a single application of 2000 mg/kg. Eye irritation tests were also conducted using the Newzealand albino rabbits as described in the OECD the guidelines 405.

2.6 Determination of acute toxicity levels of the active crude extract in rats

Evaluation of acute toxicity and LD_{50} of the crude aqueous extracts was done using Wister rats and OECD [13] method as described in guidelines 425. A total of 15 rats, four for *Senna didymobotrya* leave extracts, four for *Tithonia diversifolia* leave extracts, four for *Tithonia diversifolia* flower extracts and three which served as controls, each per test group of adult female nulliparous and non-pregnant Wister rats weighing 90-130 g were used to investigate acute toxicity of active crude extracts as described in the limit test.

2.7 Data Analysis

Data analysis was done using Student's t-test, R version 3.4.3 and graphs were drawn using Microsoft Excel for 2010 year. The data obtained from In vitro anti-flea studies was expressed as a mean ± standard error of the mean (SEM) of the two independent experiments. The data from acute toxicity studies was analyzed qualitatively and quantitatively using suitable statistical tools. The LD_{50} values were calculated using the Acute Oral Toxicity Guidelines (425) Statistical Program Version: 1.0) [14].

3. Results

The findings of the study are presented below

3.1 In Vitro Antifleas Activity of *Tithonia diversifolia* and *Senna didymobotrya* Crude Extracts in Comparison with Pyrethrum Flowers Aqueous Crude Extract.

Means of the aqueous extracts are shown in Table 1.

Table 1: Means of fleas for all aqueous extract (TLAE, TFAE, SDLAE, and PFAE).

Extract	Concentration	Means of dead fleas N_1	Means of life fleas N_2*	Initial Number of fleas N_2	Efficacy percentage $(N_2 - N_1)/ N_2 \times 100$
TLAE	100 mg/ml	8.67	1.33	10	86.7
	10 mg/ml	5.33	4.77	10	53.3
	1 mg/ml	3.67	6.33	10	36.7
TFAE	100 mg/ml	9.33	0.77	10	93.3
	10 mg/ml	8.0	2.0	10	80
	1 mg/ml	3.67	6.33	10	36.7
SDLAE	100 mg/ml	6.63	3.37	10	66.3
	10 mg/ml	4.33	5.77	10	43.3
	1 mg/ml	4.0	6.0	10	40.0
PFAE	100 mg/ml	9.0	1.0	10	90
	10 mg/ml 1 mg/ml	7.33 6.0	2.67 4.0	10 10	73.3 60.0

KEY:

N2- Initial number of fleas;

N1-Mean Number of Dead Fleas;

N2*-Means of life fleas;

TDFA-Tithonia diversifolia flowers aqueous extract;

TDLA- *Tithonia diversifolia* leaves aqueous extract;

SDLA-Senna didymobotrya leaves aqueous extract;

PFAE-Pyrethrum Flowers aqueous extract

Results of in vitro antiflea activity of all extracts in the study are compared and are shown in Figure 1.

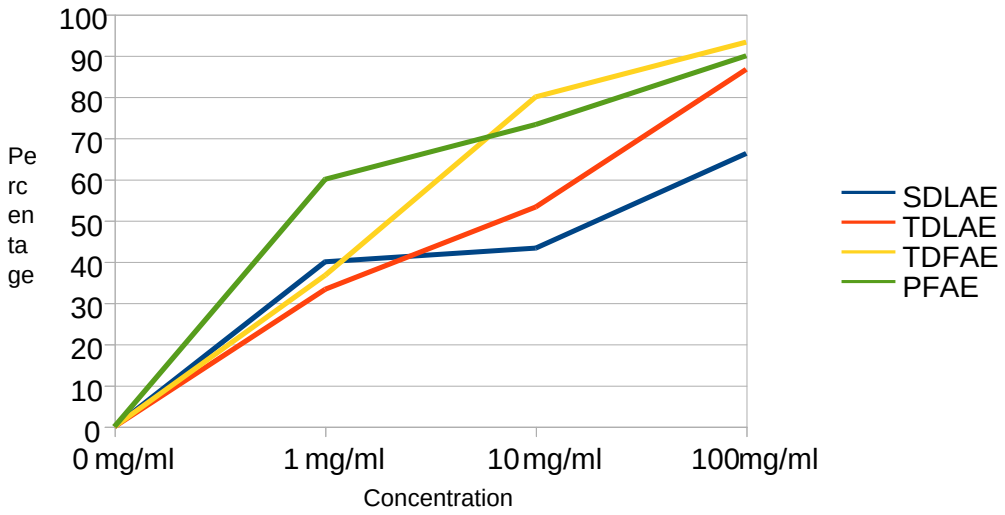

Figure 1: *In vitro* activity of SLAE, TDLAE, and TDFAE compared with PFAE extracts.

3.2 Acute Dermal Toxicity Profile of *Tithonia diversifolia* and *Senna didymobotrya* Crude Extracts in Newzealand Albino Rabbits

3.2.1 Acute dermal irritation: Effects of the extracts on the weight of the rabbits are shown in Table 2.

Table 2: Rabbits mean weights before and 14 days after application of the extract.

Rabbits	means weightin Kg at the beginning	means weight in kg at the end	p Value	Significant not significant
TDFA	2.13	2.14	0.424	Not significant
TDLA	1.82	1.82	0.943	Not significant
SDLA	1.75	1.77	0.94	Not significant

Significant weight difference at 95% CI and $p \leq 0.05$

The effects of the crude extracts on the skin of the Newzealand albino rabbits are shown Table 3.

Table 3: Dermal toxicity of crude aqueous extracts on Newzealand albino rabbit grading as per OECD 204

Crude Extract	Time after removal of the patch	0 minutes	60 minutes	24 hours	48 hours	72 hours
TDLA	Erythema	0	0	0	0	0
	Oedema	0	0	0	0	0
TDFA	Erythema	0	0	0	0	0
	Oedema	0	0	0	0	0
SDLA	Erythema	0	0	0	0	0
	Oedema	0	0	0	0	0

ERYTHEMA:

0-No erythema;

1-Very slight erythema (barely perceptible);

2-Well defined erythema;

3-Moderate to severe erythema;

4-Severe erythema (beef red);

OEDEMA:

0-No oedema;

1-Very slight oedema, (barely perceptible);

2-Slight oedema, edges of the area well defined by definite rising;

3-Moderate oedema (raises approximately 1mm);

4-Severe oedema (raised more than 1mm and extending beyond the area of exposure).

3.3 Eye irritation test results for *Tithonia diversifolia* flowers aqueous crude extract, *Tithonia diversifolia* leaves aqueous crude extract, and *Senna didymobotrya* leave aqueous crude extract Results of eye irritation test are shown in Table 4.

Table 4: *T. diversifolia* Flowers aqueous extract eye irritation test results

Crude Extracts	Cornea	Iris	Conjunctiva	Chemosis
TDFA	0	0	0	0
TDLA	0	0	0	0
SDLA	0	0	0	0

CORNEA:

0-No ulceration or opacity was observed;

1-Scattered or diffuse areas of opacity;

2-Easily discernible translucent area;

3-Nacreous area (no details of iris visible);

4-Opaque cornea;

IRIS:

0-Normal;

1-Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia;

2-Hemorrhage, gross destruction or no reaction to light;

CONJUNCTIVA:

0-Normal;

1-Some blood vessels hyperaemic;

2-Diffuse crimson color, individual vessels not easily discernible;

3-Diffuse beefy red;

CHEMOSIS:

0-Normal;

1-Some swelling above normal;

2-Obvious swelling with partial eversion of the lids;

3-Swelling with lids about half closed;

4-Swelling with lids more than half closed.

NB: No significant irritation was observed over the entire period of the study.

3.4 Acute Oral Toxicity of *Tithonia diversifolia* Flowers and Leaves, and *Senna Didymobotrya* Crude Extracts in Wister Albino Rats

3.4.1 Effects of the crude extract on weight of the test animals: The effects aqueous crude extract on weight of the Wister rats are shown in Tables 5, 6 and 7. The effects T. diversifolia leaves aqueous (TDLA) extract on weight of the Wister rats are shown in Tables 5.

Table 5: Effects of T. diversifolia leaves aqueous (TDLA) on rat weights before and after oral administration of the crude extract to Wister rats.

RAT	Weight in gram at the beginning	Dose in mg	Volume in ml	Weight in gram on day7	Weight in gram on 14
TDLA1	99.58	199.16	0.50	107.16	136.10
TDLA2	110.11	220.22	0.55	124.36	133.27
TDLA3	104.20	208.40	0.52	116.45	135.57
TDLA4	107.52	215.04	0.54	119.59	121.61
TDLA5	98.22	Control	Control	101.67	108.03

p = 0.01049 at day 7. The value indicates that there is a significant weight difference at 95% CI at the beginning and on day 7; *p = 0.04384* at day 14. The value indicates that there is a significant weight difference on day 14 for *Tithonia diversifolia* Leaves Aqueous crude extract.

The effects of *Tithonia diversifolia* Flowers aqueous crude extracts on weight of the Wister rats are shown in Table 6.

Table 6: Effects of T. diversifolia Flowers aqueous (TDFA) on rat weights before and after oral administration of the crude extract to Wister rats.

RAT	Weight in gram at the beginning	Dose in mg	Volume in ml	Weight in gram on day 7	Weight in gram on day 14
TDFA1	100.00	200.00	0.50	122.07	134.44
TDFA2	100.27	200.54	0.51	115.07	118.34
TDFA3	98.90	197.80	0.49	113.54	124.03
TDFA4	102.29	204.58	0.511	115.92	126.13
TDFA5	97.34	Control	Control	105.13	108.85

Weight beginning and after day 7, p = 0.02222 at day 7.

The value indicates that there is a significant weight difference at 95% CI.

p = 0.02646 at day 14. The value indicates that there is a significant weight difference in the beginning and on day 14 for *Tithonia diversifolia* Flowers Aqueous crude extract.

The effects of *Senna didymobotrya* Leaves Aqueous extract (SDLA) on weights of the Wister rats are shown in Table 7.

Table 7: Effects of *Senna didymobotrya* Leaves Aqueous extract (SDLA) on rat weights before and after oral administration of the crude extract.

RAT	Weight in gram at (day 0)	Dose in mg	Volume in ml	Weight in gram on day 7	Weight in gram on day 14
SDLA1	109.70	219.40	0.55	111.30	122.07
SDLA2	104.35	208.70	0.52	114.21	115.88
SDLA3	119.20	238.40	0.60	132.70	138.13
SDLA4	120.01	240.02	0.60	135.23	140.38
SDLA5	95.89	Control	Control	100.28	107.69

Weight at the beginning and after 7 days for Senna didymobotrya Leaves Aqueous crude extract p = 0.1586 at day 7. The value indicates that there is no significant weight difference at 95 CI in the beginning. p = 0.1733 on day 14. The value indicates that there is no significant weight difference at 95% CI.

3.4.2 Acute LD_{50} results: The results of the limit test are summarized in Table 8. No rat died or was found moribund condition. One of the rats in *Senna didymobotrya* Leaves aqueous liter shown distress signs on the neck, but was stable up to the 14th day after drug administration. Another one in the same liter was wet with urine. The findings are shown in Table 8.

Table 8: Acute Toxicity Results of Wister Rats

Extract Sample	**Means of initial weight (g)**	**Means of weight (g) on day 7**	**Means weight (g) on day 14**	**mortality or moribund**	**Toxicity signs**
TDFA	100.37 ± 1.41	116.65 ± 3.75	125.74 ± 6.67	0/4	Nil
TDLA	105.35 ± 4.55	116.89 ± 7.23	131.64 ± 6.80	0/4	Nil
SDLA	113.32 ± 7.60	123.36 ± 12.35	129.12 ± 12.01	0/4	Nil
Control (H_2O)	97.15 ± 1.20	102.36 ± 2.50	108.19 ± 0.60	0/3	Nil

TDLA- *Tithonia diversifolia* Leaves Aqueous; TDFA- *Tithonia diversifolia* Flowers Aqueous; SDLA-*Senna didymobotrya* Leaves Aqueous.

One-Way Analysis of Variance (ANOVA) at 95 CI and level of significant being $p \leq 0.05$, $p = 0.2004$ the value indicates that there is no significant weight difference at long run.

3.4.2.1 Effects of the extracts on blood profile of the Wister rats:

The effects of the extracts on the blood profile are shown in the Tables 9.

Table 9: Effects of the crude extracts on Red Blood Cells (RBCs) 14 days after oral administration to Wister rats.

Blood profile	**Crude extract**	**TDLA (4 rats)**	**TDFA (4 rats)**	**SDLA (4 rats)**	**Control (3 rats)**
RBCs (10^{12})	Means	6.76 ± 1.26	6.21 ± 2.15	5.27 ± 3.56	6.62 ± 0.75
	p Value	0.261	0.3604	0.504	
Hb (g/dl)	Means	15.83 ± 1.26	14.63 ± 2.15	13.68 ± 3.56	14.27 ± 0.75
	p Value	0.136	0.396	0.009	
Hct (Percentage)	Means	40.58 ± 9.13	36.55 ± 13.2	46.45 ± 4.67	37.46 ± 4.76
	p Value	0.136	0.196	0.026	
MCV(Fl)	Means	59.73 ± 4.36	58.95 ± 3.99	64.18 ± 3.17	56.63 ± 1.29
	p Value	0.195	0.699	0.013	
MCH (pg)	Means	21.73 ± 0.92	21.95 ± 0.54	21.98 ± 2.32	21.50 ± 0.79
	p Value	0.092	0.620	0.921	

MCHC (g/dl)	Means	36.50 ± 2.72	37.40 ± 2.29	34.33 ± 3.41	37.97 ± 1.48
	p Value	0.892	0.831	0.054	
RDW	Means	20.20 ± 0.71	19.08 ± 1.48	21.35 ± 1.02	20.70 ± 0.30
	p Value	0.109	0.115	0.161	
PLTs (K/ µL)	Means	475.50 ± 295	471.00 ± 195	648.00 ± 299	381.67 ± 298.63
	p Value	0.059	0.416	0.393	
WBCs (K/ µL)	Means	7.65 ± 3.84	10.12 ± 5.24	10.30 ± 2.11	10.58 ± 3.00
	p Value	0.855	0.917	0.042	
N(Percentages)	Means	27.40 ± 19.20	38.48 ± 8.69	27.43 ± 18.04	49.93 ± 12.3
	p Value	0.027	0.843	0.020	
L(×10^9)	Means	5.61 ± 3.63	6.03 ± 4.13	6.74 ± 0.37	4.78 ± 1.66
	p Value	0.228	0.862	0.016	
M(×10^9)	Means	0.21 ± 0.13	0.26 ± 0.23	0.27 ± 0.03	0.28 ± 0.03
	p Value	0.301	0.751	0.205	
E(×10^9)	Means	0.12 ± 0.05	0.17 ± 0.12	0.17 ± 0.07	0.20 ± 0.14
	p Value	0.119	0.053	0.082	
B(×10^9)	Means	0.02 ± 0.01	0.015 ± 0.017	0.020 ± 0.01	0.10 ± 0.00
	p Value	0.391	0.604	0.18	

The extracts were compared with the control with level of significant being $p \leq 0.05$ at 95% CI,

RBCs-Red Blood Cells; Hb (g/dl)-Haemoglobin; Hct - Hematocrit; MCV (FI)-Mean Corpuscular Volume; MCH-Mean

Cell Hemoglobin; MCHC-Mean Cell Hemoglobin Concentration; RDW-Red blood cells distribution width; PLT-

Platelets; WBC-White Blood Cells; N-Neutrophils; L-Lymphocytes; M-Monocytes; E-Eosinophil; B-Basophils.

4. Discussion

A discussion of antifleas efficacy and safety of the two plants is given below.

Tithonia diversifolia flowers aqueous extract (TFAE) was the most effective in anti- flea activity. At 100 mg/ml,

TFAE killed 93.3% and at 10 mg/ml it killed 80.0 % of fleas in 24 hours, while pyrethrum flowers aqueous extract (PFAE) at the same concentrations killed 90.0% and 73.3% of the fleas respectfully in 24 hours. The *Tithonia diversifolia* leaves aqueous extract (TLAE) killed 86.7% and 53.3%, while *Senna didymobotrya* leaves aqueous extract (SLAE) killed 66.3% and 43.3% of the fleas within 24 hours at the same concentration. These findings agree with findings of Adayo *et al.*, [11] and also its use for control of jiggers in Ikolomani Division of

Kakamega County, Kenya [12]. However the findings reported were on the *Tithonia diversifolia* leaves only. The current study found flowers had more antiflea activity.

During the acute oral toxicity testing, *Tithonia diversifolia* flowers and leaves aqueous extracts were used at 2000 mg per kg. The LD_{50} of the extracts was above the dose used since there was no significant oral acute toxicity based on its effect on the weight and hematological profile except for Neutrophils, and there was no mortality within 24 hours. These findings are in agreement with reports of Ezeonwumelu *et al.,* [20], and Kamatenesi-Mugisha *et al.,* [21] who found that *Tithonia diversifolia* leaves aqueous extract had LD_{50} of above 10000 mg/kg.

A study by Elufioye *et al.,* [22], reported insignificant acute toxic effects as demonstrated by absence of hematological changes, when 400-1600 mg per kg of ethanoic extract was used. Funmilayo *et al.,* [23], also found that there were no significant toxic effects, on hematological and biochemical parameters when *Tithonia diversifolia* meal was fed on cockerels for 98 days.

The current study found leaves of *Tithonia diversifolia* had a value of $p = 0.0104$ at day 7, and $p = 0.044$ at day 14, while flowers had a value of $p = 0.0222$ at day 7 and $p = 0.0265$ at day 14 and had an effect on weight gain in the Wister rats unlike S. didymobotrya. These findings support the use of T. diversifolia in animal feeds as reported previously by Mauricio *et al.,* [24].

However, Oyewole *et al*., [25] found that the intra-peritoneal LD_{50} of *Tithonia diversifolia* aqueous extract was 120 mg per kg body weight, with the same dose (100 mg/ kg) repeated daily for 14 days. Unlike in this study, he reported severe acute toxic effects as portrayed by the significant weight changes, hematological changes and high mortality. This difference is likely due to multiple dosing and use of intra-peritoneal route of administration. The acute dermal irritation and eye irritation of the crude aqueous extracts of both *Tithonia diversifolia* leaves and flowers were insignificant.

A total of 2000 mg per kg body weight of *Senna didymobotrya* leaf aqueous extract was used in the study for acute oral toxicity test, the LD_{50} was found to be above 2000 mg per kg body weight. However, significant acute toxic effects were observed being indicated by the hematological changes on the Hemoglobin ($p = 0.009$), Hematocrit ($p = 0.026$), Mean Cell Volume ($p = 0.013$), White Blood Cells ($p = 0.042$), Neutrophils ($p = 0.0204$) and Lymphocytes ($p = 0.016$) although there were no mortalities reported or significant weight loss in the animals. Moreover, there was no significant acute dermal toxicity/corrosion, or eye irritation/ corrosion. Korir *et al*., [26] found that the LD_{50} of the *Senna didymobotrya* DCM extract was between 1000 mg and 5000 mg per kg body weight. There were no significant acute toxic effects at low doses, but the toxic effects increased significantly as the dose increased above 3000 mg per kg body weight and with continuous daily dosing as illustrated by the weight loss. Nyamwamu *et al*., [27], found that the LD_{50} of the methanoic and Dichloromethane *Senna didymobotrya* crude root extracts was 1927 mg per kg body weight while that of the hexane and water extracts were more than 5000 mg per kg body weight in

mice, and this is in agreement with the findings of the present study. Aqueous extract is thus only slightly toxic if accidentally ingested. Nyamwamu *et al*., [27] and Njoroge *et al*., [28] also reported topical use of *Senna didymobotrya* extracts in the management of both human and livestock dermal conditions and ectoparasites, and this is in agreement with results on the safety of extracts for topical use as in this study.

The study found that *Tithonia diversifolia* flowers aqueous (TDFA) were safer than the other extracts used in the study, as it did not affect the blood profile significantly. It was also found to be the most effective on fleas, with very close resemblance with the positive control (pyrethrum flowers aqueous extract), in its antiflea activity. *Tithonia diversifolia* (TDLA) leaves aqueous extract had better activity than *Senna didymobotrya* (SDLA) leave aqueous extract but had activity level slightly below that which was observed in the flowers. It was also found that it was not very toxic to the blood cells as only neutrophils ($p = 0.027$) showed significantly decline as compared with the control Wister rats.

5. Conclusion

The following conclusions were made from the studies. The study findings show that there was no significant difference in the *In vitro* anti-flea activity of *C. cinerariifolium* (pyrethrum) flowers aqueous extract compared with *Tithonia diversifolia* aqueous leaves and flowers (*p-value of 0.8321*) extracts as well as *Senna didymobotrya* leaves aqueous extract. Flowers of *Tithonia diversifolia* needs to be investigated further for pesticide activities.

6. Recommendations

The following recommendations were made from the studies**.**

Further studies should be conducted on *Tithonia diversifolia* and S*enna didymobotrya* extracts in order to improve on purification of usable pesticides, which are cost effective especially in control of the jigger menace in Kenya and other parts of the world. More emphasis should be placed on the flowers of *Tithonia diversifolia* having demonstrated both effectiveness and safety. However, further studies should be conducted in order to purify the extracts of the flowers in order to provide safe, cost effective and efficient pesticides. Lastly, Kakamega herbalists who use boiled *Tithonia diversifolia* concoction to clean jigger infested areas of the body should be encouraged and the people of Murang'a and the rest of the country can consider using the concoction.

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