LARVICIDAL ACTIVITY OF EXTRACTS FROM *Lippia kituiensis, Lippia javanica, Phytolacca dodecandra, Pittosphorum viridiflorum* AND *Synadenium compactum* AGAINST *Rhipicephalus appendiculatus*

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A Thesis Submitted to the Graduate School in Partial Fulfillment for the Requirements for the Award of Master of Science Degree in Biochemistry of Egerton University

EGERTON UNIVERSIRTY

OCTOBER, 2014

DECLARATION AND RECOMMENDATIONS

DECLARATION

I, Caroline Jepchirchir Kosgei, declare that this thesis is my original work and has not been submitted wholly or in part for any award in any institution.

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Ms. Caroline J. Kosgei

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RECOMMENDATION

We wish to confirm that this research thesis has been prepared under our supervision and have our approval to be presented for examination as per the Egerton University regulations.

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DEDICATION

I dedicate this work to my family, parents and siblings for their moral, emotional and financial support.

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ABSTRACT

Rhipicephalus appendiculatus is a tick that transmit East Coast Fever to livestock. Use of synthetic acaricides for tick control, is limited by high costs, resistance, presence of chemical residues in animal products and environmental pollution. The use of plant-based products as a control strategy is regarded safe, and is widespread among pastoralists in Kenya and other parts of Africa. This study aimed at screening extracts of selected plants against *Rhipicephalus appendiculatus* larvae. These plants were *Phytolacca dodecandra*, *Synadenium compactum*, *Pittosphorum viridiflorum*, *Lippia kituiensis* and *Lippia javanica*. Methanol extraction were done on the selected plants and the resultant methanol crude extracts subjected to preliminary bioassay screening against *R. appendiculatus* larvae using contact toxicity. Sequential extractions were done on bioactive methanol extracts using water, hexane and ethyl acetate. The resultant extracts were subjected to bioassay against the larvae. From the start of the experiment, mortality data was obtained at 6, 12, 24 and 48 hrs. LC_{50} and LC_{90} in mg/ml were determined for each extract using SPSS. Methanol extracts of *L. kituiensis, P. viridiflorum,* and *P. dodecandra* had LC_{50} of 21.3 (18.1-24.5), 30.5 (25.7- 35.1) and 39.1 (31.1-46.9) while LC_{90} were 38.6 (32.5-51.6), 63.1 (52.7-83.9), 84.6 (67.7-124.2) at 48 hrs respectively. Hexane extract of *L. kituiensis* had LC_{50} of 12.6 (11.0-14.1), 10.6 (9.0-12.0), 6.7 (5.2-7.9), and 4.8 (2.2-5.9) and LC_{90} of 19.5 (17.0-24.4), 17.4 (15.0-22.0), 10.8 (9.1-14.3) and 7.7 (6.3-13.0) at 6, 12, 24 and 48 hrs respectively. At 48 hrs, hexane extract of *P. viridiflorum* had LC_{50} of 22.5 (18.3-26.6) and LC_{90} of 45.5 (36.9-66.8) while aqueous extract of *P. dodecandra* had LC_{50} of 17.3 (15.2-19.4) and LC_{90} of 26.8 (23.3-34.4). Hydro-distillation of fresh leaves of *L. kituiensis* and *L. javanica* resulted in essential oil production, which was also subjected to the larvae and LC_{50} and LC_{90} in mg/ml determined. The LC_{50} were 3.3 (3.1-3.3), 3.2 (3.1-3.3), 3.1 (3.0-3.2) and 3.1 (3.0-3.2) for *L. kituiensis* oil and 3.1 (3.0-3.2), 3.1 (3.0-3.2), 3.0 (2.9-3.1), 2.9 (2.8-3.1) for *L. javanica* oil at 6, 12, 24 and 48 hrs respectively. LC_{90} were 4.1 (3.9-4.4), 4.0 (3.8-4.3), 3.9 (3.8-4.2), 3.9(3.7-4.1) for *L. kituiensis* oil and 3.9 (3.7-4.2), 3.9 (3.7-4.2), 3.8 (3.6-4.1), 3.7 (3.6-4.0) for *L. javanica* at 6, 12, 24 and 48 hrs respectively. Cytotoxicity analysis using vero cells were done on extracts that showed bioactivity against the larvae and none of the extracts was cytotoxic hence were considered safe for practical use. Due to potency observed in the selected plants against the larvae, they could be used as lead compounds for the development of plant based acaricides.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

LIST OF ABREVIATIONS AND ACRONYMS

AIDS	Aquired Immunodeficiciency Syndrome
ANOVA	Analysis of Variance
DMSO	Dimethly Sulfoxide
ECF	East Coast Fever
ELISA	Enzyme-linked immunosorbent Assay
Eos	Essential oils
FAO	Food and Agricultural Organization
FBS	Fetal Bovine Serum
GC-MS	Gas Chromatography- Mass Spectrometry
GDP	Gross Domestic Products
GPR	General Purpose Reagent
HIV	Human Immuno-deficiency Virus
IC	Inhibitory Concentration
ICIPE	International Center for Insect Physiology and Ecology
IIRR	International Institute of Rural Reconstruction
ITDG-EA	Intermediate Traditional Development Group- East Africa
KARI	Kenya Agricultural Research Institute
KEMRI	Kenya Medical Research Institute
LC	Lethal Concentration
LD	Lethal Dose
MEM	Minimum Essential Medium
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazoliumbromide
MTBE	methyl-t-butyl ether
NCI	National Cancer Institute
OD	Optical Density
SPSS	Statistical Package for Social Sciences

CHAPTER ONE

INTRODUCTION

1.1 Background information

Vector borne diseases are a global problem that hinders productivity of animals. These vectors are ectoparasites which comprise ticks, insects and mites. Ectoparasites particularly ticks are responsible for severe losses in the livestock industry. Studies by Wall (2007) found that 80% of 1200 million cattle were at risk of getting tick-borne diseases causing a global annual loss of US $ 7000 million. In Kenya, annual loss due to East Coast Fever alone which is transmitted by *R. appendiculatus* tick was US$ 54.4 million in 2003 (Minjauw and Mcleod, 2003).

Ticks belong to phylum *Arthropoda*, class *Arachnida* and subclass *Acari*. It is a large and diverse group with an estimated 50,000 described tick species (Halliday *et al.,* 2000). Ticks have two body segments namely the capitulum comprising the mouthparts and sensory palps, and the idiosoma containing most of the organs including the anus and genital aperture (Oliver, 1989). Ticks have three families namely *Ixodidae*, *Argasidae* and *Nuttalliedae*. *Ixodidae* (hard ticks) consists of about 701 species and 14 genera, *Argasidae* (soft ticks) consists of 191 species. The number of genera in the latter group is controversial and currently under discussion. *Nuttalliellidae* consists of one species namely *Nuttalliella namaqua* (Guglielmone *et al.,* 2010).

Ticks are vectors of diseases affecting livestock, humans and wildlife (Parola and Raoult, 2001; Piesman and Eisen, 2008). They transmit protozoan pathogens that cause diseases such as *theileriosis* and *babesiosis* in livestock (Peter *et al.,* 2005), rickettsial diseases such as *anaplasmosis* which affects cattle and *ehrlichiosis* affecting humans and dogs (Dumler *et al.,* 2001). Viral diseases that are tick-borne include meningoencephalitis and Colorado tick fever, affecting both humans and animals (Lane and Crosskey, 1996). In addition, they lead to weight loss in animals due to disturbances while feeding and anemia due to blood sucking (Rajput *et al.,* 2006). They also cause tick paralysis since they inject toxins that are neurotoxic (Norval and Horak, 2004).

The use of synthetic acaricides has been the main method of controlling ticks. However, synthetic acaricide use has many shortcomings, which include development of resistance by ticks, environmental pollution, high cost, and presence of residues in meat and milk products

(Silva-Aguayo, 2006). This therefore necessitates a search for alternative molecules with acaricidal activity and which are safer than the current products of tick control.

Plants produce novel compounds of medicinal importance. About 28% of all new chemical entities launched on to the market between 1981 and 2002 had plant origin (Newman *et al.,* 2003), while 24% were artificial products that mimic natural products (Newmann *et al.,* 2000). According to Habeeb (2010), there are huge prospects for the use of plants from tropical and subtropical regions of Africa, Asia and South America to come up with acaricides that will control ticks effectively. In Kenya, studies to screen indigenous plants used by pastoralists in controlling ticks have not been fully exhausted. This study therefore aimed at determining larvicidal activity against *R. appendiculatus* of plants used by pastoralists in Uasin Gishu and Baringo counties, and those obtained from previous literature reported to have acaricidal properties

1.2 Statement of the problem

Livestock farming in East Africa is constrained by ectoparasites particularly ticks. Ticks diminish the productivity of domestic animals through transmission of pathogens that cause diseases such as *theileriosis* (ECF) and anemia due to blood sucking. Synthetic acaricides used today are progressively becoming harmless as the ticks develop resistance. They are expensive to livestock keepers and causes environmental pollution. Thus there is need to identify and develop novel, safe, cheap, effective and biodegradable acaricides.

1.3 Objectives

1.3.1 General objective

To determine larvicidal activity of extracts from *Lippia kituiensis, Lippia javanica, Phytolacca dodecanadra, Pittosphorum viridiflorum* and *Synadenium compactum* against *Rhipicephalus appendiculatus* larvae.

1.3.2 Specific objectives

i. To determine larvicidal activity of methanol crude extracts of *L. kituiensis, L. javanica, P. dodecanadra, P. viridiflorum* and *S. compactum* against *R. appendiculatus* larvae.

ii. To determine larvicidal activity of hexane, ethyl acetate and aqueous crude extracts of *L. kituiensis, L. javanica, P. dodecanadra, P. viridiflorum* and *S. compactum* against *R. appendiculatus* larvae.

iii. To determine larvicidal activity of essential oils of *L. kituiensis*, *L. javanica* and *P. viridiflorum* against *R. appendiculatus* larvae.

iv. To identify the phytochemicals present in methanol, hexane, ethyl acetate and aqueous crude extracts of plants used in the study.

v. To determine the chemical composition of the essential oils present in *L. kituiensis, L. javanica and P. viridiflorum.*

vi. To perform cytotoxicity tests on bio-active methanol, hexane, ethyl acetate and aqueous crude extracts of plants used in the study.

1.4 Hypotheses

i. Methanol crude extracts of *L. kituiensis, L. javanica, P. dodecanadra, P. viridiflorum* and *S. compactum* do not have larvicidal activity against *R. appendiculatus.*

ii. Hexane, ethyl acetate and aqueous crude extracts of *L. kituiensis, L. javanica, P. dodecanadra, P. viridiflorum* and *S. compactum* do not have larvicidal activity against *R. appendiculatus.*

iii. Essential oils *L. kituiensis, L. javanica* and *P. viridiflorum* do not have larvicidal activity against *R. appendiculatus.*

iv. There are no phytochemicals in methanol, hexane, ethyl acetate and aqueous crude extracts of the plants used.

v. Chemical composition of the essential oils of *L. kituiensis, L. javanica* and *P. viridiflorum* cannot be identified.

vi. Bio-active methanol, hexane, ethyl acetate and aqueous crude extracts of the plants used in the study are cytotoxic.

1.5 Justification

Livestock farming is a pillar in the economy of a country since livestock products such as milk and meat are foreign exchange eaners. Nutritionaly, these animal products are a source of dietary proteins which enhance health in the population. Tick borne diseases that have developed resistance against synthetic acaricides continually diminish productivity of animals. Ethno-veterinary knowledge and practices play an important role in complementing modern approaches

in the management of diseases and their vectors. However, scientific research to rationalize and validate the potency of ethno-veterinary based products against ticks hasn't been exhausted. This study therefore looks for alternative drugs such as plant extracts that will improve tick control.

CHAPTER TWO

LITERATURE REVIEW

2.1 Importance of livestock

Livestock industry is important to the country's economy and contributes to the livelihoods of a large proportion of rural as well as urban households in some developing countries. An estimated 1.3 billion people living in third world countries depend directly or indirectly on livestock for their livelihoods (FAO, 2009). Worldwide, livestock contributes about 40% to the agricultural gross domestic product (GDP) and constitutes about 30% of the agricultural GDP in developing world (World Bank, 2009). In Kenya the sector contributes 12% of the country's GDP and 42 % of agricultural GDP (SNV, 2008). In addition, livestock farming contributes to the nutritional status of the world's population (Bwibo *et al.,* 2003). It reduces the risks associated with crop failure beside acting as a form of insurance (Freeman *et al.,* 2007). These estimates highlight the important contribution of livestock to sustainable agricultural development and if not managed, ticks will continue to cripple this very important industry (Wall, 2007).

2.2 Economic importance of ticks

In Kenya the estimated annual cost of *theileriosis* which is transmitted by *R. appendiculatus* in the smallholder dairy system was US$ 54.4 million and in the traditional system was US$ 34.1 million in 2003 (Minjauw and Mcleod, 2003). In Zimbabwe approximately US$ 5.6 million was lost annually due to *cowdriosis* (Mukhebi *et al.,* 1999). Other cost incurred due to tick infestation by famers include building and maintenance of dipping tanks and sprays, labour needed for mustering stock, purchase of acaricides for tick control, and purchase of therapeutic drugs against diseases. In United States, it was estimated that US$ 2.5 billion was the total 5-year expenditure for lyme disease in the human population in the late 1990s (Maes *et al.,* 1998). In Germany, *borreliosis* treatment was estimated to cost US$ 12,000 per case in 2002 (Talaska, 2002).

2.3 Taxonomy of ticks

Ticks are classified into three families which are *Ixodidae, Argasidae* and *Nuttalliellidae*. The first family *Ixodidae,* are hard ticks since they have a hard sclerotized shield covering the

anterior part of the idiosomal dorsum in females and the entire dorsal surface, in males. Their mouthparts points forward and are visible from above during all life cycle. The genera of medical and veterinary importance in this family include *Amblyomma, Dermacentor, Haemaphysalis, Hyalomma, Ixodes, Rhipicephalus* and *Boophilus* (Horak *et al.,* 2002).

Soft ticks (*Argasidae*) lack a dorsal shield and their integument is leathery and can be wrinkled, granulated, mammillate or tuberculate. Argasid mouthparts are on the ventral surface of the body and are not visible from above except during the larval stage. Their cuticle enlarges to contain the large volume of blood ingested, which may be between 5 to 10 times their unfed body weighs (Rajput *et al.,* 2006). Argasid ticks of medical and veterinary importance belong to the genera *Argas, Ornithodoros* and *Otobius*. About 10 % of the species in *Ixodidae* and *Argasidae* are of medical importance (Jongejan and Uienberg, 2004).

The last family of ticks is *Nuttalliellidae*, which is rare and is almost currently unknown, nearly 80 years after its finding. Few specimens have however been collected from Southern and Eastern Africa (Keirans, 2009).

2.4 *Rhipicephalus appendiculatus*

R. appendiculatus belong to *Ixodidae* family. It is brown in colour and likes feeding on the ear of cattle hence commonly referred to as a brown ear tick. Cattle, dogs, goats and sheep are the main host although buffaloes, elands and waterbucks do serve as non-domestic hosts (Wanzala, 2009). *R. appendiculatus* prefer cool, shaded shrubby or woody savannas with at least 24 inches of annual rainfall. It is prevalent in Southern Sudan, Eastern Zaire, Eastern and South Africa. It inhabits areas 2300 meters above sea level (Norval *et al.,* 1992).

It is a three-host ixodid tick and is a vector of major economic importance in Africa since it transmits *Theileria parva*, which cause *theileriosis* a threatening disease in the livestock industry in Eastern, Southeastern and Central Africa. It also transmits corridor disease in cattle, Nairobi sheep disease virus and Thogoto virus (Walker *et al.,* 2000). On cattle, the immature stages of *R. appendiculatus* attach on the neck and dewlap, eyelids, cheeks, ears and muzzle. The adult *R. appendiculatus* likes feeding on the ear pinna of bovid while in heavy infestations, adults can also be found also around the eyelids, base of horns, upper neck even in the tail-brush and anus (Wanzala, 2009). Morphologically, male and female *R. appendiculatus* differs

in appearance. Figure 1 below shows the morphological appearances of both male and female adults.

Figure 1: *Rhipicephalus appendicualtus* tick.

Left male; right female. The male has a capitulum covering the entire dorsal surface while the capitulum covers the anterior part of the idiosomal dorsum in female. Adopted from (Sonenshine, 1993; Walker *et al.*, 2000).

2.5 Lifecycle of ticks

Ticks have different life cycles depending on which family they belong. The two common families are hard ticks (*ixodidae*) and soft ticks (*argasidae*).

a) Ixodid (Hard) ticks

Ixodid ticks have four developmental stages mainly eggs, six legged larvae, eight-legged nymph and eight-legged adult. The female lay around 2000 to 20,000 eggs in one batch (Horak *et al.*, 2002). They may feed several times on the host during their life cycle and thus may be one, two, or three host ticks. One-host tick moult twice on its host an example is *Rhipicephalus annulatus*. Two-host ticks moult once on its host, from larval to nymph and then the engorged nymph drops off, moults on the ground and the resulting adult has to find a second host animal to feed on, examples are *Rhipicephalus evertsi* and *Hyalomma marginatum* (Walker *et al.*, 2003).

Three-host ticks do not moult on the host. Eggs are laid under a shade and incubate for 40 to 60 days, before they hatch to larvae. Larvae then quest for the first host and engorge for 4 to

6 days before dropping down to moult to nymph. The nymph then quest for the second host and engorges for 4 to 8 days before dropping and moulting to an adult. The adult males and females quest for a third host with different reasons, the female for a blood meal, while the males in search of female ticks in order to mate and sometimes feed on them. The females feed for about 10 days and just before fully engorged they mate with males and dropping down once fully engorged carrying enough sperms to fertilize the eggs. The adult female then goes to a shaded environment and hatches the eggs and the cycle continues. This type of lifecycle is evident in *R. appendiculatus* described in the Figure 2 below (Speybroeck *et al.,* 2004).

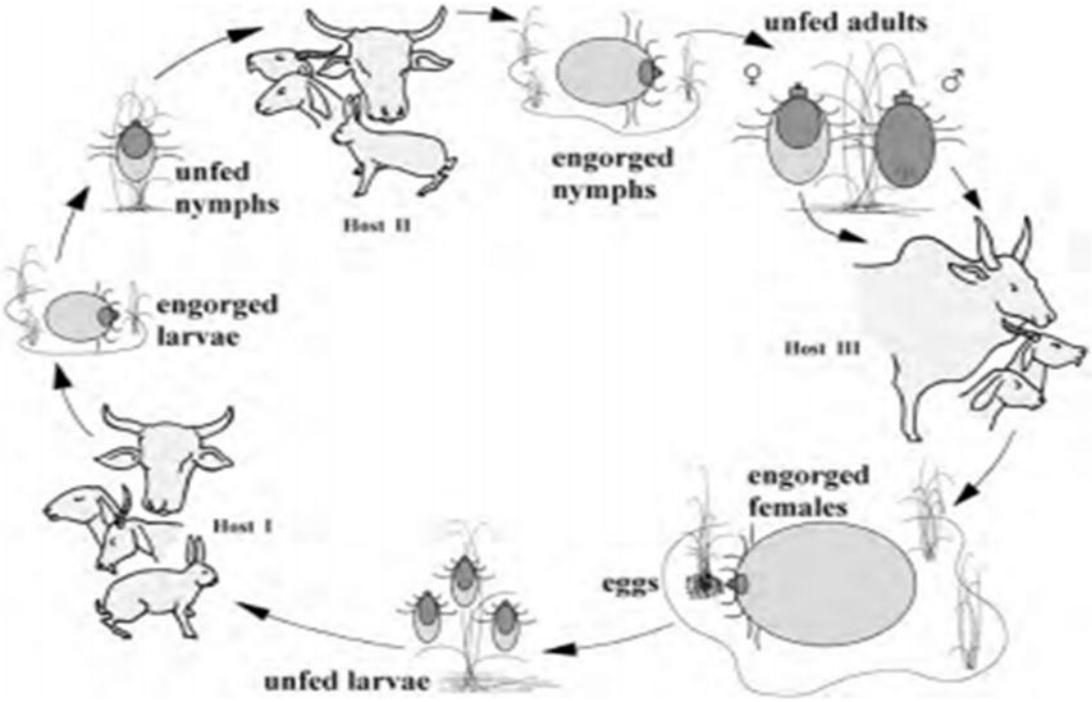

Figure 2: Lifecycle of *R. appendiculatus* tick (Speybroeck *et al.*, 2004)

b) Argasid ticks (Soft ticks)

Argasid tick lay between 100 to 500 eggs under a shade. These eggs then hatch to larval stages. Depending on the species, 2–8 nymphal stages can occur following moulting of the larval stages. Each stage detaches before feeding again on a fresh host. The number of nymphal stages varies both within and between species (Oliver, 1989). The duration of feeding in nymphal and adult argasids is much shorter than in ixodid ticks. It usually takes from few

minutes to a few hrs. The adults can feed more than once, with numerous egg batches being laid (Hoogstraal, 1985).

2.6 Host seeking behavior of ticks

Ticks have variety of peripheral sensory organs that are hair-like structures on the body, legs, and mouthparts. They also have sensilla on the dorsal surface of tartus leg for detectability. Ticks use sensilla to locate their host and interact with other ticks (Parola and Raoult, 2001). They have an instant response to chemical stimuli such as CO_2, NH_3, air vibrations and body temperature that signify the presence of a host (Parola and Raoult, 2001). On receiving the stimuli, they become prepared to attack. They have two host-seeking behavior patterns namely the ambush strategy, which involves ticks climbing up the vegetation with their front legs held out when questing for a host and the hunter's strategy involving ticks emerging from their habitat and running towards their hosts when they appear. *Dermacentor variabilis* is an example of a tick, which uses ambush strategy while *Amblyomma hebraeum* and *R. appendiculatus* are example of ticks using hunter's strategy (Sonenshine, 1993). Ambush strategy by *Dermacentor variabilis* as it quest for the host is shown in Figure 3 below (Myrmecos, 2012). On attachment to the host, ticks have two intervals of feeding namely a slow phase for several days followed by quick phase in the last 12–24 hrs before detachment. During feeding, there may be a tenfold enlargement in tick weights by the end of the slow phase, and an additional tenfold increase at the conclusion of the last rapid phase (Parola and Raoult, 2001).

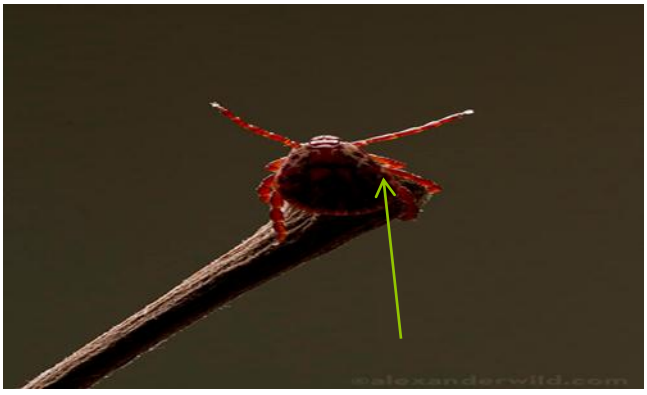

Figure 3: *Dermacentor variabilis* tick. It is raising its front legs as it quest for the host. Adopted from (Myrmecos, 2012).

2.7 Geographical distribution of ticks

The distribution and abundance of ticks in the world is dependent on the distribution of their hosts and climatic conditions. Climatic changes resulting in increasing temperatures has resulted in the shortening of the lifecycle of ticks, thus increased reproduction rate hence abundance of ticks (www.icttd.nl).

Ixodid ticks of medical importance include *Rhipicephalus* species, *Amblyomma variegatum, Boophilus microplus*, *Haemaphysalis* and *Dermacentor* species. *Rhipicephalus* species are found on mammals on the African continent (Walker *et al.*, 2000). *Amblyomma variegatum* is widely spread in tropical Sub-Saharan Africa and feeds on domestic livestock (Pegram and Eddy, 2002). *Boophilus microplus* of the genus *Boophilus* is distributed in South-East Asia and the tropics and feeds on livestock. *Dermacentor* species is present in all the continents except Australia and it feeds on livestock. Genus *Haemaphysalis* is present in Asia, Europe and to some extent Australia, where it feeds on livestock. *Ixodes* is widely distributed throughout wooded or grassy environments of the world and attack a wide range of host hence responsible for many zoonotic diseases (Camicas *et al.*, 1998).

Argasid ticks of medical importance are *Argas, Ornithodoros* and *Otobius*. These ticks have varying distribution globally. *Argas miniatus* is disseminated in the neotropical region and both *A. persicus* and *A. reflexus* are located in southern Europe and central Asia. The three *Argas* species usually feed on birds. *Argas monolakensis*is found in Western USA and it feeds on man (Schwan *et al.*, 1992). *Otobius megnini* is found in western parts of the USA and parts of South America deep in the external ear canals of livestock, companion animals and occasionally man. Among the *Ornithodoros* species, *O. savignyi* is found in semi-desert areas and feeds on the legs of cattle (Hoogstraal, 1985). *Nuttalliella namaqua* is restricted to South Africa (Parola and Raoult, 2001).

2.8 Ticks as vectors

Tick-borne protozoan diseases affecting livestock include *theileriosis* caused by *Theileria parva* and transmitted by *R. appendiculatus* and *babesiosis* which is transmitted by *Boophilus microplus* and caused by *Babesia bovis* and *Babesia bigemina* (Peter *et al.*, 2005). *Ehrlichia ruminantium* also causes heartwater disease in livestock, transmitted by *Amblyomma hebraeum* (Bekker *et al.*, 2001). These tick borne diseases in livestock lead to protein-energy malnutrition

in many African communities (Muller and Krawinkel, 2005). Tick-borne rickettsioses of humans include monocytic and granulocytic *ehrlichiosis*, caused by *Ehrlichia chaffeensis* and *Ehrlichia phagocytophila. E. chaffeensis* is transmitted by *Amblyomma americanum* and *E. phagocytophila* by *Ixodes scapularis* in the USA and by *Ixodes ricinus* in Europe (*Dumler et al.,* 2001).

2.9 Toxicity and paralysis of ticks

Strains of *Hyalomma truncatum* have toxins in their saliva that cause sweating sickness and acute dermatitis in cattle (Norval and Horak, 2004). *I. rubicundus* found in South Africa also contain toxins in saliva which when injected on cattle it leads to death since it cause tick-induced paralysis (Walker *et al.,* 2003). Ticks are of limited medical importance in humans since they are detached immediately from the site of the bite once noticed. Nonetheless, they can cause paralysis or induce allergic reactions for example *Ixodes holocyclus* injects a potent neurotoxin into the site of its bite. It affects children under the ages of one in eastern Australia. The symptoms develop slowly as a raised flaccid paralysis, which can result in death due to respiratory failure if treatment is not sought quickly (Grattan-Smith *et al.,* 1997). Allergies due to tick bite are more common than tick-paralysis, and may be dangerous (Stone *et al.,* 1989). *Argas* bite many city residents in the absence of its host which is domestic pigeon, *Columba livia* causing severe allergic reactions (Dautel *et al.,* 2009).

2.10 Medicinal Plant extracts used in controlling of ticks

Medicinal plants have been in use for a long time as a remedy to several ailments affecting man and animals. According to Kaaya, (2000) pasteur grasses are capable of repelling, trapping and killing ticks, since they possess hairs called trichomes that retard ticks from climbing to the top of the grasses in order to attach themselves on passing animals. These grasses therefore represent whole plants that have anti-tick effects. Other plants reported to have anti-tick effect are *Stylosanthes* plants producing certain fluid active against ticks (Kaaya, 2000).

Leaf extracts of *Tephorosia vogelii* have been shown to be highly toxic to one, two, and three host ticks (Kaposhi, 1992). Cattle sprayed with the extracts, got residual protection period from re-infestation by ticks for 10 days. At the same time extracts of *Calpurnia aurea* leaves used by the Borana people of Northern Kenya and Southern Ethiopia to treat louse infestations in humans and calves have been reported to have anti-tick properties (Zorloni *et al.,* 2010). The activity observed in the plant extracts is due to presence of phytochemicals (Ahn *et al.,* 1998).

Among the phytochemicals known to possess insecticidal activities include essential oil, terpenoids, tannins and saponins (Morrissey and Osbourn, 1999; Plaza *et al.,* 2004; Ribeiro *et al.,* 2007; Fernandez-Salas *et al.,* 2011).

2.11 Plant Phytochemicals

Plant phytochemicals aid in protecting plants against predators and pathogens besides attracting pollinating insects (Weiss, 1997). Common phytochemicals present in plants include; saponins, tannins, flavonoids, cardiac glycosides and essential oils (Ahn *et al.,* 1998).

a) Saponins

Saponins are naturally occurring surface-active glycosides produced mostly by plants, lower marine animals and some bacteria (Yoshiki *et al.,* 1998). They are glycosides with distinctive foaming characteristic and they get their name from the soapwort plant *Saponaria* (Sen *et al.,* 1998). The structure of saponins consist of a sugar moiety (glucose, galactose, glucuronic acid and xylose) linked by a glycoside linkage and attached to a hydrophobic aglycon (sapogenin) which can either be a triterpenoid or a steroid (Fenwick *et al.,* 1991). The complexity of saponins structure is due to variability of the aglycone structure and position of attachment of the moieties in the aglycone (Fenwick *et al.,* 1991). The ability of a saponin to foam is due to combination of the nonpolar sapogenin and the water-soluble side chain (Francis *et al.,* 2002). Many saponins are known to be possess molluscicidal, (Escalante *et al.,* 2002), insecticidal (Morrissey and Osbourn, 1999) and antifungal (Delmas *et al.,* 2000) properties.

b) Cardiac glycosides

They are present in many plants and historically served as medicinal and poison to the heart hence their name (Majak, 2001). Cardiac glycosides are characterized by a steroidal aglycone that can be either cardenolides or bufadienolides. Na^+-K^+-adenosinetriphosphatase in cardiac muscle is the major pharmacological receptor of cardiac glycosides in the heart. Inhibition of this receptor by cardenolides and bufadienolides affects intracellular electrolyte concentrations.This results in forceful contractions of the myocardium (Schoner and Scheiner-Bobis, 2007). Therapeutically, cardiac glycosides are used for the treatment of congestive heart failure in humans, although in domestic herbivores they have been reported to be toxic when consumed at the natural concentrations in plants (Majak and Benn, 2000).

c) Flavanoids

Flavonoids are polyphenols of plant origin widely distributed in foods and beverages. They occur both in the free form (aglycones) and as glycosides. The most common classes are the flavones, flavonols, flavanones, catechins, isoflavones and anthocyanidins (Nijveldt *et al.*, 2001). All flavonoids share a basic C6-C3-C6 phenyl-benzopyran backbone (Harnafi and Amrani, 2007). The position of the phenyl ring relative to the benzopyran moiety allows a broad separation of these compounds into various classes. Flavanoids are involved in scavenging of oxygen derived free radicals due to presence of hydroxyl groups (Nijveldt *et al.,* 2001). They have also been reported to enhance vaso-relaxant process (Bernatova *et al.,* 2002).

d) Tannins

Tannin is a word that generally describes a group of polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution, a property known as astringency (Cowan, 1999). Tannins are present virtually in every plant part (Scalbert, 1991). The two classes of tannins are hydrolysable and condensed. Hydrolysable tannins contain gallic acid usually as multiple esters with D-glucose while condensed tannins (proanthocyanidins) are derived from flavonoid monomers (Serafini *et al.,* 1994). Gymnosperms and monocots produce only condensed tannins while dicots can produce either condensed tannins, hydrolysable tannins or a mixture of both types (Haslam, 1988). Pharmacological importance of tannins includes possession of antimicrobial properties shown to be toxic against filamentous fungi, yeasts, and bacteria (Scalbert, 1991).

e) Essential oils

Essential oils (EOs) are volatile compounds, characterized by a strong odour and produced by aromatic plants, which represents 10% of the plant kingdom (Adorjan and Buchbauer, 2010). They are present in various plant parts such as flowers, leaves, stem, fruits, seeds and roots and stored in special brittle secretory structures, such as glands, secretory hairs and trichomes (Sangwan *et al.,* 2001; Combrinck *et al.,* 2006). The total essential oil composition of plants is generally very low and rarely exceeds 1% (Bowles, 2003). Chemical composition of essential oils does vary depending on genetic composition of the plant, climatic conditions and period when plant material was collected for oil extraction (Sangwan *et al.,* 2001). These three factors influence the biochemical synthesis of essential oils in a given plant,

resulting in same species of plants producing similar essential oils but different chemical composition. Hydro-distillation using a Clevenger type apparatus is the common method for extraction of essential oils (Abad *et al.*, 2012).

Essential oils have a broad spectrum of bioactivity owing to the presence of several active ingredients, which are the volatile components that work through various modes of action (Abad *et al.*, 2012). The volatile molecules present in the oil are classified as terpenes (monoterpenes: C10, sesquiterpenes: C15, and diterpenes: C20), phenolic-derived aromatic and aliphatic components (Abad *et al.*, 2012). Due to various components present, essential oils have various bio efficacies which include antibacterial, antifungal, antiviral, antiparasitic and insecticidal (Plaza *et al.*, 2004).

2.12 Criteria for the selection of medicinal plants for drug discovery

According to Fabricant and Farnsworth (2001) there are four standard approaches for selecting plants with potential bioactivity: (1) random selection followed by chemical screening, (2) random selection followed by a bioassay on the test organism, (3) follow-up of bioactivity reports of the plant and (4) follow-up of ethno medicinal uses of plants against infectious agent. The first method also called phytochemical approach searches for classes of phytochemicals with potential bioactivity against the test organism (e.g. saponins, tannins). In the second approach, all available plant parts are collected, irrespective of prior knowledge and experience. However, the method is costly and laborious since it totally relies on type of test organism and activity criteria used (Fabricant and Farnsworth, 2001). The third approach exploits availability of published reports on bioactivity of different plants (Cos *et al.*, 2006). The fourth approach is also known as ethno medicinal approach based on, oral or written information obtained from organized traditional medical systems, herbalism and folklore. Ethno medicinal knowledge can also be acquired from various sources, such as books, review articles and computer databases (Cos *et al.*, 2006).

2.13 Medicinal plants used in this study

The following medicinal plants were selected for use in this study: *P. dodecandra*, *L. kituiensis, L. javanica*, *S. compactum* and *P. viridiflorum*. Selection of *S. compactum var compactum*, *P. viridiflorum* and *P. dodecandra* were based on ethnobotany information

obtained from interviewing herbalist in Uasin gishu and Baringo counties. *L. javanica* and *L. kituiensis* were selected based on previous reports on their uses.

2.13.1 *Lippia kituiensis* and *Lippia javanica*

L. kituiensis synonym *L. ukambensis* and *L. javanica* belong to *verbaceace* family. Genus *Lippia* has about 200 species that are often aromatic in nature and distributed throughout Central and South Americas as well as in tropical Africa (Arthur *et al,* 2011). Both plants are erect, small, and woody. It grows to a height of 2 m. Their images are shown in Figure 4 below.

Figure 4: *L. kituiensis* (left) and *L. javanica* plants (right)

According to a review on *Lippia* plants, the predominant use of various species of *Lippia* is for the treatment of respiratory diseases (Pascual *et al.,* 2001). Tea infusions of the leaves of *L. javanica* are used against common symptoms of HIV and AIDS, treatment of lung infections, dysentery and diarrhea (Palgrave *et al.,* 2003). The acetone and methanol extracts of *L. javanica* have been found to have antimicrobial properties against *B. subtilis, P. mirabilis* and *S. aureus* (Samie *et al.,* 2005) while its essential oil have very strong and lasting repellent activity against adults cattle ticks of *H. marginatum* (Magano *et al.,* 2011).

Volatile oil of *L. javanica* has been reported to have antimicrobial properties (Shikanga *et al.,* 2010). *L. javanica* aqueous leaf extracts at 10% and 20% w/v were found to be effective at controlling *R. appendiculatus, R. evertsi, B. decoloratus* and *Hyalomma* species (bont-legged ticks) found at study site in Zimbabwe (Madzimure *et al.,* 2011).

On the other hand, previous studies on *L. kituiensis* showed chemotype camphor to be repellent against maize weevils (*Sitophilus Zeamais*) (Mwangi *et al.,* 1992). Essential oil of *L. kituiensis* has also been shown to be repellent against *Anopheles gambiae* (Omolo *et al.,* 2004).

Evaluation of intact potted plant of *L. kituiensis* reduced mosquito bite of *A. gambie* in semi field experimental huts by 30-40% (Seyoum *et al.,* 2002). *L. kituiensis* is used by Kipsigis people for milk treatment and preservation (Mureithi, 2000). The most common phytochemicals present in *Lippia* species include terpenes (sesquiterpenes, di and triterpenes), flavonoids, phenylpropanoids and naphthoquinones (Catalan and de Lampasona, 2002).

2.13.2 *Pittosphorum viridiflorum*

P. viridiflorum synonym *P. mannii*, *P. floribundum* and *P. dalzielii* belong to *pittosphoraceae* family (Momeni *et al.,* 2010). It grows as a large shrub or small tree with scented, pale green, firmly clustered leaves. The leaves are 2-3 inches long and rolled inward along the edges (Wagner *et al.,* 1999). Figure 5 below shows image of *P. viridiflorum*.

Figure 5: *P. viridiflorum* plant

It is native to South Africa and is widely distributed in the Eastern half of the country (Matshinyalo and Reynolds, 2002). *P. viridiflorum* is used as a medicine to treat fever, malaria, inflammation and stomach ache and as an antidote for insect bites (Seo *et al.,* 2002). The leaves of this plant possess antimicrobial properties (Ramanandraibe *et al.,* 2000). *P. viridiflorum* hexane extract has been found to have low toxicity against brine shrimp with LD_{50} value>1mg/ml (Otang *et al.,* 2013). Investigation of its toxicity on Cheng cell line showed that acetone extracts of the plant is weakly cytotoxic with IC_{50} (ug/ml) of 246.95 ± 25.19 (Mbeng, 2013). Saponins, alkaloids, phenolics proanthocyanidins and Flavonoid have been reported to be present in *P. viridiforum* (Mbeng, 2013).

2.13.3 *Phytolacca dodecandra*

P. dodecandra belongs to *Phytolaccaceae* family commonly called endod in some parts of the world (Misganaw *et al.,* 2012). This family consists of 16 genera and 100 species with genus *Phytolacca* having about 35 species in tropical and subtropical regions (Hedberg *et al.,* 2000). It is native to sub-Saharan Africa (Schemelzer and Gurib-Fakim, 2008). It is a perennial climbing plant and grows rapidly in highlands that lie at (1600-3000 m above sea level). It produces berries which are commonly used in Ethiopia for washing cloths because when mixed with water it produces foaming detergent solution (Lemma *et al.,* 1979). Figure 6, below shows image of *P. dodecandra.*

Figure 6: *P. dodecandra* plant

Previous studies on *P. dodecandra* have shown the leaves of this plant is used for treating ringworms, while roots and stem are used to alleviate dysentery and other stomach disorders (Kisangau, 2007). The seeds are used as molluscicides (Allen-Gil and Aldea, 2003). Phytochemical analysis of *P. dodecandra* has shown the plant contains flavonoids and saponin (Mekonnen *et al.,* 2012). while other studies has shown extracts of hexane, ethyl acetate, methanol and dichloromethane of this plant contained phenolic compounds, while terpenoids was present in hexane, ethyl acetate, and dichloromethane (Ogutu *et al.,* 2012). Toxicity studies on *P. dodecandra* showed that both human and guinea pigs do tolerate skin irritation (Mekonnen *et al.,* 2012).

2.13. 4 *Synadenium compactum*

It belongs to *Synadenium* genus, the smallest genus of the *Euphorbiaceae* family (Kinghorn, 1980). It is a succulent shrub reaching a height of 12 feet and commonly called African milk bush since it secretes poisonous milky-white latex on breaking the twigs. It is native in East Africa, while in in other parts of the world it grows as an ornmemtal plant (Olivier *et al.,* 1992). There are two varieties of *S. compactum* namely: *S. compactum var rubrum* that has purplish red leaves and *S. compactum var compuctum* that has dark green leaves (Olivier *et al.,* 1992). Figure 7 below, show the images of *S. compactum var compuctum.*

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Figure 7: *S. compactum* plant

Synadenium genera, has been reported to have various classes of phytochemicals which include flavonoids, saponins, diterpenes, phorbol esters (Jassbi, 2006). In Kenya, decoction of *S. compactum* leaves and stem bark is given to cattle to drink as a way of controlling ticks (ITDG and IIRR, 1996). Due to the presence of the latex, majority of the species in this genus have been pharmacologically evaluated against anti-inflammatory, antitumor, analgesic, immune-regulatory and fibrinolytic experimental models (Jager, 1996; Rajesh *et al.,* 2006; Nogueira *et al.,* 2008). Other studies on *S. compactum* include isolation of two esters of Synadenol from the Latex (Olivier *et al.,* 1992).

2.14 Mode of application of extracts used in controlling ticks

Spraying is the common method of administering plant extracts that possess acaricidal activity on animals. Examples are plant extracts of *Salanecto manii* leaves, which are crushed and mixed with water then sprayed on the areas infested by ticks on the animal. *Ajuga remota*

root and leaf extracts are also crushed then mixed with little water, filtered and then sprayed directly on tick infested areas (Opiro *et al.,* 2010). However spraying is less efficient in controlling ticks since not all parts of the animal body come in contact with the acaricide (Drummond, 1983).

Dipping involves immersion of the animal in a dip-tank containing chemical solutions. Cotton seed oil and tobacco extract are examples of plant extracts with acaricidal activity that have been applied on animals by dipping (Angus, 1996). Synthetic acaricides such as organophosphate are also applied on the animal by dipping (Bram *et al.,* 2002). Dipping is highly effective since all body parts come in contact with the acaricide, however its costly in terms of construction of dip-tanks and the facility must be managed carefully so that the dips are maintained at the proper concentration and the cattle are dipped properly (Drummond, 1983).

Smearing is also used for drug administration and it involves topical application. The sap of *Euphorbia hirta* is extracted then smeared directly on tick-infested parts (Opiro *et al.,* 2010). Other methods of application include ear tags, neckbands, tail bands and pour-on. These methods are particularly useful for the pyrethroids since they have long residual activity (Rajput *et al.,* 2006).

2.14.1 Mode of action of plant extracts used in controlling ticks

A study conducted by Williams (1991), demonstrated that ethanol extracts of marine plant of *Laurencia obtusa, Padina vickerisiae, Liagora farinosa,* and *L. elongate* inhibited oviposition and embryogenesis of adult female *B. microphilus* tick on topical application. *Commiphora molmol* extract of *Myrrh* can quickly infiltrate the cuticle to body cavity, destroy the epithelial gut cells and cause death of fowl tick *Argas persicus* (Massoud *et al.,* 2005). Some plant extracts act as repellents since they prevent the parasite from coming in contact with the host. Examples of these plants include plant extract of *Stylosanthes humilis* and *Stylosanthes hamata* tropical legumes, which are repellent against *B. microplus* larvae (Castrejon *et al.,* 2003). Inhibition of oviposition by *Annona squamosa* in female ticks of the species *Boophilus microplus*, *Hyalomma anatolicum* and *Rhipicephalus haemaphysalis* has been reported Kalakumar *et al.,* (2000).

2.14. 2 Mode of action of synthetic acaricides

Pyrethroids quickly interfere with sodium and potassium ion transport in nerve membranes, resulting in impulsive depolarization, increased neurotransmitter secretion, and neuromuscular obstruction causing paralysis while insufficient exposure of the pyrethroids can cause the knock-down effect on insect. Kalakumar *et al.,* (2000) showed that Annona squamosa inhibited oviposition in female ticks of the species Boophilus microplus, Hyalomma anatolicum and Rhipicephalus haemaphysalis. Synthetic acaricides such as Organophosphates works by inhibiting acetylcholinesterase enzyme which is responsible for acetylcholine destruction hence applications of this drug on ticks causes spontaneous muscular contractions followed by paralysis (Merck manuals, 2012). Formamidines such as amitraz causes paralytic effects due to blockage of octopamine receptors, which lead to over-excitation and consequently paralysis and death in the larvae (Chena *et al.,* 2007).

2.16 Toxicity evaluation of plant extracts

Recent scientific findings have shown that plant extracts contain phytochemicals that are potentially cytotoxic, genotoxic, mutagenic and carcinogenic (Ernst, 2004). Toxicity usually results from adverse effects due to interaction between toxicants and cells (Syahmi *et al.,* 2010). Among the adverse effects of plant extracts, are interference of renal tubular function and induction of acute renal failure. There is therefore a need to further investigate safety of ethno medicinal preparations. It is necessary that pharmacological studies should always be accompanied by toxicological screening (Cos *et al.,* 2006).

There are several *in vitro* studies for determination of toxicity of plant extracts. Among them is the crustacean *Artemia salina* Leach (brine shrimp) which is an invertebrate and has been widely used for studies of ecotoxicology, as well as of broad toxicology of chemicals (Cleuvers, 2003) and natural compounds (Caldwell *et al.,* 2003). Vero cell line from African green monkey (Sassi *et al.,* 2008), ovarian cells, human cancer cell lines (Ukiya *et al.,* 2002) and Chang liver cells (Chang, 1954), are frequently used for determination of cytotoxicity. This is because these cells provide an acceptable model to explore general cellular cytotoxicity since many of the known drug-induced cytotoxic mechanisms are inhibition of mitochondrial function, disruption of intracellular calcium homeostasis, activation of apoptosis, oxidative

stress, inhibition of specific enzymes and formation of reactive metabolites, which are common to most cells (Mbeng, 2013) .

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study areas were Uasin Gishu and Baringo counties in Kenya, together with botanical garden of Egerton University. Baringo county borders Turkana county to the North, Samburu and Laikipia counties to the East, Keiyo Marakwet and West Pokot to the West. The county covers an area of 8,655 km^2. It lies between Latitudes 00° 13″ South and 1° 40″ north and Longitudes 35° 36″ and 36° 30″ east. Rainfall ranges from 500 to 1500 mm annually while the mean annual maximum temperature ranges between 25°C and 30°C in the Southern part and about 30°C in the Northern part. Figure 8 shows the map of Baringo county.

Figure 8: A map of Baringo county

adopted from http://www.emosociety.org/society/Countypage/Baringo

Uasin Gishu county is situated in mid west of the Rift valley Province and borders six counties, Elgeyo Markwet to the East, Trans Nzoia to the North, Kericho to the South, Baringo to the South East, Nandi to the South West and Bungoma to the West. It lies between longitude 34°50″ to 35°37″ East, and on latitude 0°03″south to 0°05″ North. It has a total area of 3, 218 km^2. Temperatures range from a minimum of 8.4 °C to a maximum of 27 °C while average rainfall range from 900 mm to 1,200 mm per annum. Figure 9 shows the map of Uasin gishu county.

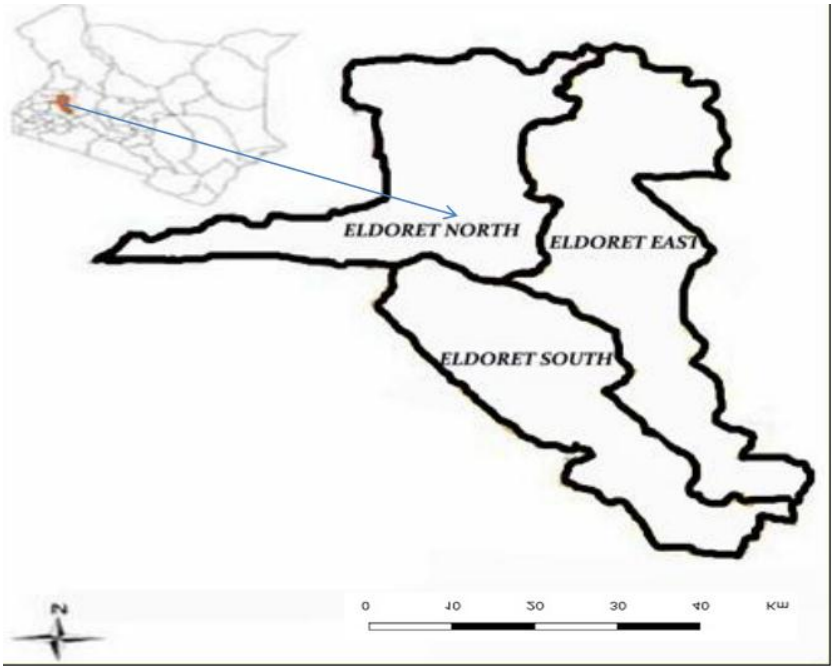

Figure 9: A map of Uasin gishu county

(http://www.kenyampya.com)

Botanical garden of Egerton university is a 300 acre pieace of land, that was established in 2003 as a centre for teaching, research, environmental conservation and recreational facility. It is at an altitude of 2127 m. In line with teaching, research and environmental conservation, the garden harbors rare and threatened plant species collected from all over the country. It has a wide range of medicinal plants that are common in various parts of the country (http://nakurupostnews.com).

3.2 Survey and selection of the plants used in the current study

Selection of plants that were used in this study was based on ethnobotany information obtained through interviewing herbalist found within Uasin gishu and Baringo counties using a questionnaire (ITDG-EA, 2000). The herbalists were interviewed using a questionnaire shown in Appendix 1. Previous literature on plants used for management of ticks was used to select *L. javanica* and *L. kituiensis*. Previous studies of *L. javanica* have shown the leaves of this plant to be widely used for management of ticks by pastoral communities in Zimbabwe (Madzimure *et al.*, 2011; Magano *et al.*, 2011). A vourcher specimen of this plant was deposited in Botanical garden of Egerton University. Selection of *L. kituiensis* was because it belonged to the same family as *L. javanica.* Plants of the same family produce similar chemical profiles thus sometimes exhibit same biological activity. *L. kituiensis* was also obtained from Botanical garden of Egerton University after identification by the taxonomist.

In Baringo county, eight herbalist were interviewed and they suggested *Maerua subcordata* (roots), *Tagetes minuta* (flowers and leaves), *Hypoestis verticillaris* (leaves), and *P. viridiflorum* (leaves). Only *P. viridiflorum* was selected because in terms of response from those interviewed it rated highest (5 out of 8). In Uasin gishu county, 10 herbalist were interviewed and they suggested *Solanum incanum* (fruits), *Euphorbia resinfera* (stem), *S. compactum* (leaves) and *P. dodecandra* (leaves). *S. compactum* belonging to *Eurphorbiaceae* family and *P. dodecandra* belonging to *Phytolacceae* family were selected from Uasin gishu county, because in terms of response of those interviewed they were also ranged highest (7 out of 10) for *S. compactum* and (8 out of 10) for *P. dodecandra.*

3.3 Collection of plant materials

Leaves of the selected plants in both counties were collected in a sack after being identified by the taxonomist. In Uasin gishu county, *P. dodecandra* was found on the roadside while *S. compactum* was found on cleared farms. In baringo county *P. viridiflorum* was found on bushes. Leaves of both *L. kituiensis* and *L. javanica* obtained from botanical garden of Egerton University were also collected in a sack. Plant leaves were used since it was the plant part suggested by herbalists, besides they are abundant material which can regenerate in a short period. Leaves are more exposed to insect than other parts of the plants and thus produce wide variety of secondary metabolites reported to have potential insecticidal effect (Valladares *et al.*,

2003). In a study conducted by Chungsamarnyart *et al.*, 1991 using 151 plants to determine larvicidal effect of plant crude-extracts on the tropical cattle tick (*B. microplus*), he found that leaves were the more active component.

3.4 Preparation of plant material for extraction

Both leaves of *L. kituiensis* and *P. viridiflorum* collected were devided into two; those to be extracted crude extracts (non-volatile compounds) and the remaining to be extracted essential oils (volatile compounds). Collected leaves of *L. javanica* were only used for extraction of essential oils (volatile compounds). This is because the plant material obtained was not adequate for extracting crude extracts. Extraction of essential oils from *L. kituiensis*, *P. viridiflorum* and *L. javanica* were because these plants were aromatic and previously reported in literature to contain essential oils. The remaining leaves belonging to *P. dodecandra*, and *S. compactum* were kept aside for extraction of non-volatile compounds since they lacked the oil.

3.5 Reagents for extraction

Solvents used for extraction of nonvolatile compounds (crude extracts), were obtained from a commercial supplier. The solvents used were methanol, hexane, and ethyl acetate. They were General Purpose Reagents (GPR) which required prior distillation before use. The solvents were selected because they had varying polarities i.e methanol polar, hexane non-polar and ethyl acetate medium polar (Lekgari, 2010). This is important for extraction of wide a variety of phytochemicals.

3.6 Extraction of crude extracts

Leaves of *L. kituiensis* and *P. viridiflorum* kept aside for extraction of crude extracts (non-volatile compounds) and collected leaves of *P. dodecandra*, *S. compactum* were air dried under a shade. Air-drying was done by spreading leaves of each plant on top of a sack laid on the floor, for a period of 7-14 days while turning them periodically to expose all the leaves to air. The leaves were dried under a shade to retain their active components, and were weighed frequently until a constant weight was obtained and thus considered dry. Drying of the leaves was important before extraction of crude extracts because this allowed the leave cuticles to open up enhancing easy extraction of the phytochemicals. Dried leaves were grounded using a blending machine (Thomas-Wiley Laboratory Mill Model 4) at Kenya Agricultural Research Institute (KARI), Njoro. The powdered materials were weighed with each 500 g being extracted

with 1.8 liters of 95% methanol at room temperature for 72 hrs. It was then filtered a through a Buchner funnel and the filtrate was concentrated to dryness under reduced pressure using rota-vapor machine (BUCHI – R 205).

The concentrated crude methanol extract was divided into two, one part approximately a quarter of the yield was stored at 8ºC until qualitative determination of phytochemicals and evaluation of the larvicidal properties against *R. appendiculatus* according to Siviral, (2011). The other remaining part was placed in a separating funnel then suspended in distilled water to remove available sugars. It was then followed by addition of hexane which was added repeatedly until hexane was colourless meaning no more compounds present in the methanol extract could dissolve in hexane. The hexane extract was then concentrated to dryness under reduced pressure using rota-vapor machine mentioned above resulting in hexane crude extract. Hexane crude extract was then stored at similar conditions as methanol extract until qualitative determination of phytochemicals and evaluation of the acaricidal properties against *R. appendiculatus* larvae.

Once hexane had removed all its compounds from methanol extracts, ethyl acetate was then added to the separating funnel repeatedly until the ethyl acetate was colourless meaning no more compounds present in the methanol extracts could dissolve in ethyl acetate. The ethyl acetate extracts was then concentrated to dryness under reduced pressure using rota-vapor mentioned above, hence resulting in ethyl acetate crude extract. The crude extract was stored according to Siviral, (2011) until qualitative determination of phytochemicals, and evaluation of the larvicidal properties against *R. appendiculatus*. The remaining extract in the separating funnel after hexane and ethyl acetate removed their compounds was the aqueous extract. This extract was also stored according to Siviral, (2011) until qualitative determination of phytochemicals and evaluation of the acaricidal properties against *R. appendiculatus* larvae.

3.7 Extraction of volatile compounds

Fresh leaves of *L. javanica* approximately 10 kg and leaves of *L. kituiensis* and *P. viridiflorum* kept aside for extraction of essential oils, approximately 15 kg and 25 kg respectively, were subjected to hydro-distillation. This was done using a modified clevenger-type apparatus as shown in the Figure 10 for at least four hrs according to Papachristos and Stamopoulos, (2004) with slight modification. The essential oils obtained were dried over

anhydrous sodium sulphate and their yield ascertained. The oils were then stored in sealed glass vials at 4^{o}C until chemical composition analysis using GC-MS and larvicidal bioassay against *R. appendiculatus* done.

Figure 10: Modified clevenger-type apparatus

3.8 Rearing of *R. appendiculatus* larvae

The larvae used for the bioassay were reared according to (Bailey, 1960) at ICIPE. A circumference of about 22 cm of hair at the back of the rabbit was firstly shaved. This allowed porcelain cloth that was folded cylindrically to be attached at the area shaved using conta glue. Male and female adult ticks of *R. appendiculatus* were placed inside the folded porcelain cloth, at the back of the rabbit as shown in Figure 11a. The rabbits were then placed inside cages and were fed with rabbit pellets and water. A collar was also placed on the neck of the rabbit to prevent the rabbit from rubbing the back with its head due to irritation caused by tick bites as shown in Figure 11a. After feeding for at least 6 days, adult male and female *R. appendiculatus* mate. Complete engorgement of females followed mating and this occurred after feeding for another 4 days. Once fully engorged the female dropped from the rabbit skin and were collected on the glass vial as shown in Figure 11b.

After staying for 2-5 days, the engorded female laid eggs as shown in Figure 11c. The eggs were then incubated at 25-27^{o}C and 80 percent relative humidity, for 21-30 days followed by hatching to larvae as shown in Figure 11d. Generally, larvae can stay for up to six months without food and they only moult to the next stage (nymph) once they feed. In this experiment,

the larvae were allowed to stay for 5 days to harden before being allowed to feed on the rabbit again. They fed for 6 days before detaching and were incubated for 12-16 days followed by moulting to nymphs as shown in Figure 11e. Nymphs can also stay for 7 months without food but in this experiment they were allowed to harden for 5-10 days then placed on a rabbit to feed. After feeding for 3-6 days, they dropped from the rabbit and were collected in glass vials. They were incubated for 12-16 followed by moulting to adults as shown in Figure11f. The adults were then placed on the rabbit again and the cycle was repeated.

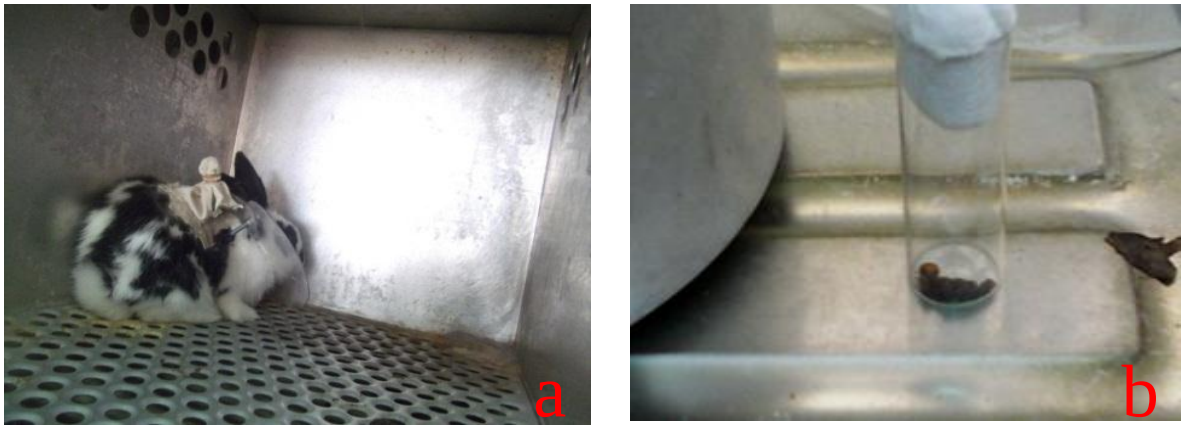

Figure 11: (a) A rabbit inside a cage containing containing adult male and female *R. appendiculatus* inside porcelain cloth that is attached at the back and (b) Engorged females collected in a glass vial

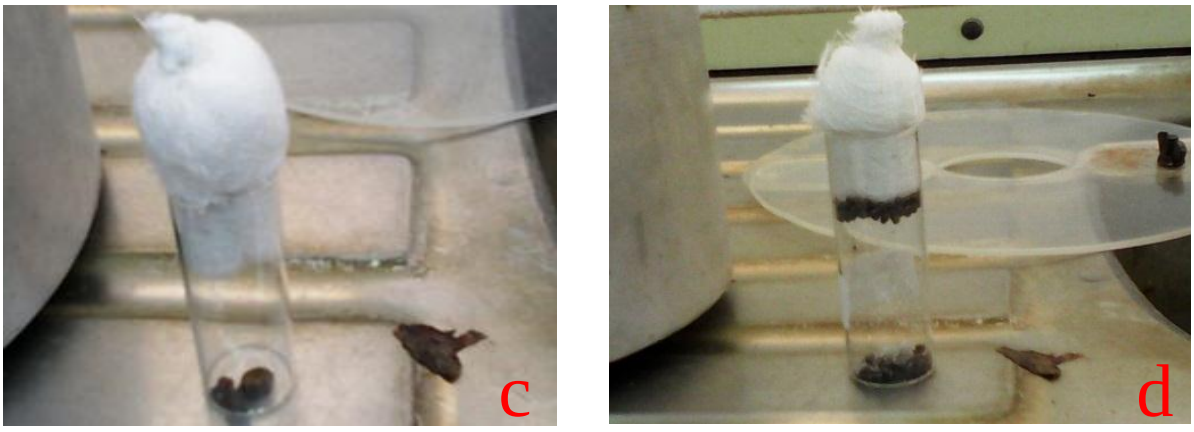

Figure 12: (c) Engorged female lying eggs inside the glass vial and (d) larvae larvae collected in a glass vial

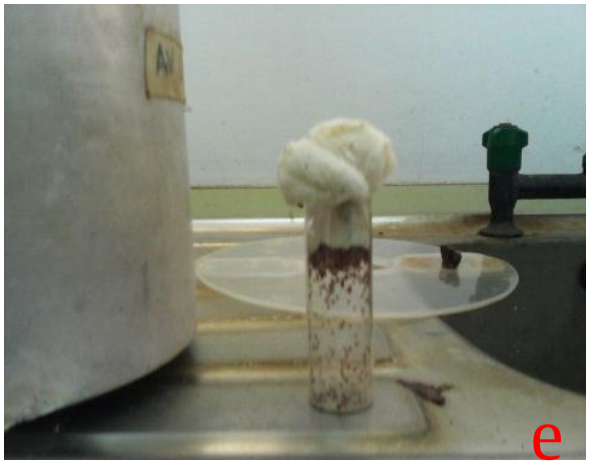

Figure 13: (e) Larvae and (f) Nymphs

3.9 Larvicidal bioassay

3.9.1 Larvicidal bioassay of the crude extracts

a) Methanol crude extracts

The larval bioassay were done according to (FAO, 2004) with slight modification. Methanol extracts of the four plants (*P. dodecandra, P. viridiflorum, S. compactum, L. kituiensis*) were first subjected to preliminary bioassay to determine the plants that were active and their concentrations. The initial concentration used during preliminary bioassay was 25 mg/ml and since most plants could not kill at this concentration, the concentration was doubled to 50 mg/ml. Preliminary screening was done by attaching Whatman No. 1 filter paper to the bottom of petri dish (15 cm) using double sided cellophane tape and 20 larvae were placed inside. A concentration of 50 mg/ml of methanol extract of each plant was prepared. It was then sprinkled on the petri dish containing the larvae using a pasteur pipette, making sure that the filter papers were wet, and the larvae came in contact with the sprinkled extract. The experiment was replicated three times and petri dishes were held at 75% relative humidity and 25°C. From the start of the experiment, mortality data was obtained at 6, 12, 24 and 48 hrs. The concentrations that caused over 60% mean larval mortality in preliminary screening were selected for actual bioassay. Methanol extract of *P. dodecandra*, *P. viridiflorum* and *L. kituiensis* were selected and their stock solutions were 100 mg/ml, 75 mg/ml, 50 mg/ml respectively. The stock solutions were prepared by weighing 10 g, 7.5 g, and 5 g of *P. dodecandra, P. viridiflorum* and *L. kituiensis* respectively, in 100 ml of distilled water containing 2% DMSO. Serial dilutions of the stock solutions were prepared and the

concentrations were 50 mg/ml, 45 mg/ml, 40 mg/ml, 35 mg/ml, 30 mg/ml, 26 mg/ml, 22 mg/ml, 18 mg/ml, 14 mg/ml, 10 mg/ml and 6 mg/ml for methanol extract of *L. kituiensis*, while those of methanol extract of *P. viridiflorum* were 75 mg/ml 70 mg/ml, 65 mg/ml, 60 mg/ml, 55 mg/ml, 50 mg/ml, 45 mg/ml, 40 mg/ml, 35 mg/ml, 30 mg/ml, 25 mg/ml, 20 mg/ml, 15 mg/ml, 10 mg/ml and 5 mg/ml. The concentrations of methanol extract of *P. dodecandra* were 100 mg/ml, 90 mg/ml, 80 mg/ml, 70 mg/ml, 60 mg/ml, 50 mg/ml, 40 mg/ml, 30 mg/ml, 20 mg/ml, 10 mg/ml and 5 mg/ml. The concentrations obtained were then subjected to bioassay against the larvae as shown in Figure 12 and 13 below. Negative control set was 2% DMSO obtained after experimental determination. Positive control used was amitraz (0.2% v/v) which is commercially available. The larvae were considered dead when they could not respond by moving their appendages when prodded with a pin.

Figure 14: Larval bioassay of methanol extract of *L. kituiensis* left; methanol extract of *P. viridiflorum* right

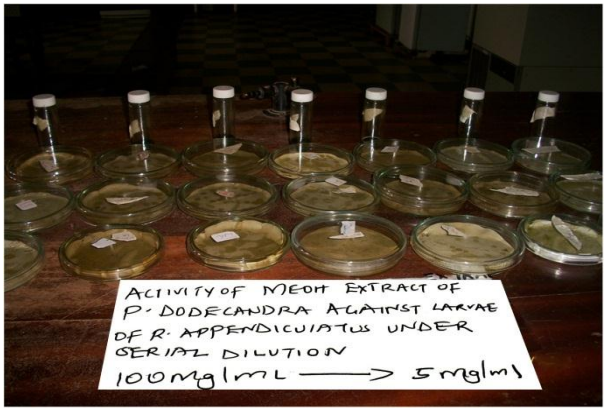

Figure 15: Larval bioassay of methanol extract of *P. dodecandra*

b) Hexane, ethyl acetate and aqueous crude extracts

Hexane, ethyl acetate and aqueous extracts of bioactive methanol extracts were subjected to preliminary bioassay against the larvae of *R. appendiculatus*, to determine the extracts and the concentrations that were active. Bioassay were done according to FAO, (2004) with slight modification. A concentration of 25 mg/ml of hexane, ethyl acetate and aqueous extracts of each plant was prepared. It was then sprinkled on the petri dish containing 20 larvae each using a pasteur pipette. During sprinkling, the filter papers was ensured wet, and the larvae were exposed to the sprinkled extracts. The experiment was replicated three times and petri dishes held at 75 % relative humidity at 25°C. From the start of the experiment, mortality data was collected at 6, 12, 24 and 48 hrs. Those extracts that caused above 60% mean larval mortality were selected. Hexane extract of both *L. kituiensis* and *P. viridiflorum* and aqueous extract of *P. dodecandra* were selected and their stock solutions were 25 mg/ml, 50 mg/ml and 30 mg/ml respectively. Stock solutions were prepared by weighing 1.25 g, 1.5 g and 2.5 g of *L. kituiensis, P. dodecandra* and *P. viridiflorum* respectively then dissolving in 2% DMSO. Serial dilution of the stock solutions resulted in 10 concentrations ranging from 25 mg/ml to 7.5 mg/ml for hexane extract of *L. kituiensis,* 10 concentrations ranging from 50 to 5 mg/ml for hexane extract of *P. viridiflorum* and 9 concentrations ranging from 30 to 7 for aqueous extract of *P. dodecandra*. The concentrations obtained were subjected to bioassay against the larvae as shown in Figure 14 and 15 below. Negative control was set which was 2% DMSO, while the positive control was amitraz® 0.2% v/v. The larvae were considered dead when they could not respond by moving their appendages when prodded with a pin.

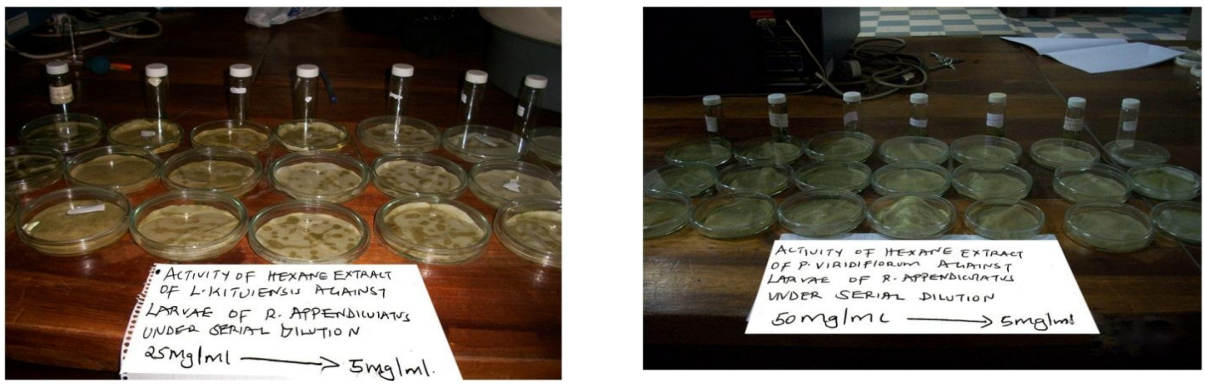

Figure 16: Larval bioassay of hexane extract of *L. kituiensis* left ; hexane extract of *P. viridiflorum* right

Figure 17: Larval bioassay of aqueous extract of *P. dodecandra*

3.9.2 Larvicidal bioassay of essential oils

The yield of essential oil of *P. viridiflorum* was not enough for larval bioassay. Only essential oils of *L. javanica* and *L. Kituiensis* were subjected to a bioassay. Essential oils of *L. javanica* and *L. kituiensis* were both weighed and their density obtained. *L. javanica* had a density of 0.99 mg/ml while *L. kituiensis* was 0.92 mg/ml. Activity of the essential oils at different concentrations against *R. appendiculatus* larvae were preliminary determined. The bioassay were done according to FAO, (2004) with slight modification. This was done by attaching whatman No. 1 filter paper to the bottom of petri dish (15 cm) using double sided cellophane tape and 20 larvae placed inside. Different concentrations of the essential oils were sprinkled on the petri dish containing the larvae. From the start of the experiment, mortality data were obtained at 6, 12, 24 and 48 hrs. The petri dishes were held at 75% relative humidity at 25°C. The concentration that was found to be active was 4 mg/ml for *L. javanica* and 4.5 mg/ml for *L. kituiensis* and these concentrations were the stock solutions. The stock solutions were approximately 0.4 ml of *L. javanica* oil and 0.49 ml of *L. kituiensis* oil solubilized in 2% DMSO.

Serial dilutions of stock solutions resulted in different concentrations, which sprinkled on petri dishes containing 20 larvae each, and whatman no. 1 filter papers (15 cm) attached at the bottom. The concentrations for *L. javanica* were 12 and it ranged from 4 to 1.5 mg/ml while for *L. kituiensis* it ranged from 4.5 to 1.5 mg/ml being 13 in number. The treatment was replicated three times and the experiment repeated once for each oil. Time was recorded immediately the larvae were exposed to the oil extracts. Figure 16 below shows larval bioassay of essential oils of both *L. javanica* and *L. kituiensis*. Negative control was set which was 2% DMSO, while the

positive control was amitraz® 0.2% v/v. The larvae were considered death when they could not respond by moving their appendages when prodded with a pin.

Figure 18: Larval bioassay of essential oils, left *L. javanica*; right *L. kituiensis*

3.10 Phytochemical analysis of crude plant extracts

Chemical tests to identify phytochemical constituents of methanol, hexane, ethyl acetae and aqueous extracts of each plant were carried out. It was done qualitatively, using standard procedures according to (Edeoga *et al.*, 2005; Khan *et al.*, 2011). Test for tannins was done by adding 0.1% ferric chloride to approximately 2 ml of the test solution of each extract. Formation of a blue black colouration indicated presence of tannins. Test for phlobatanins was done by boiling about 2 ml of the test solution of each extract with 0.5 ml of 1% HCL, deposition of red precipitated confirmed presence of phlobatanins. Test for saponins was done by adding 5 ml of distilled water to approximately 10 ml of the test solution. This was followed by adding a few drops of olive oil and shaking vigorously. Formation of an emulsion confirmed presence of saponins. Test for flavonoids was done by adding 2 ml of dilute aqueous ammonia solution to 5 ml portion of the test solution of each extract. Addition of concentrated H_2SO_4 latter followed and a yellow colouration that disappeared after standing confirmed presence of flavonoids. Test for steroids was done by adding 2 ml of both acetic anhydride and H_2SO_4 to 3 ml of each test solution of each extract. Change of colour from violet to blue or green confirmed presence of steroids. Test for terpenoids (Salkowski's test), was done by mixing 5 ml of each extract with 2 ml of $CHCL_3$ and 3 ml of concentrated H_2SO_4 to form a layer. Positive test was indicated by formation of red colouration at the interface. Test for cardiac glycosides was done by mixing 5

ml of each extract with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was followed by underlying 1 ml of concentrated H_2SO_4. Formation of a brown ring at the interface indicated presence of cardiac glycosides.

3.11 GC-MS analysis of *Lippia kituiensis* essential oil

Samples of essential oils were diluted in methyl-t-butyl ether (MTBE) (1:100) and analyzed on an Agilent GC-MS apparatus equipped with an Rtx-5SIL MS ('Restek') (30 m x 0.25 mm, 0.25 µm film thickness) fused-silica capillary column. Helium (at 0.8 mL/min) was used as a carrier gas. Samples were injected in the split mode at a ratio of 1:10 – 1:100. The injector was kept at 250 ºc and the transfer line at 280 ºC. The column was maintained at 50º C for 2 min and then programmed to 260º C at 5º C /min and held for 10 min at 260 ºc. The MS was operated in the electron impact ionization (EI) mode at 70 eV, in m/z range 42-350. The identification of the compounds was performed by comparing their retention indices and mass spectra with those found in literature (Adams, 2007) and supplemented by Wiley 7N.l, HPCH 1607.L and FLAVORS.L GC-MS libraries. The relative proportions of the essential oil constituents were expressed as percentages obtained by peak area normalization, all relative response factors being taken as one.

3.12 Cytotoxicity assay

Extracts of the plants that showed acaricidal properties against *R. appendiculatus* larvae were tested for *in vitro* cytotoxicity using MTT calorimetric assay (Mosmann, 1983). Vero cells (ATCC CCL-81) established from the kidney of a normal African green monkey (*Cercopithecus aethiops*), were used to determine the cytotoxicity of the plant extracts. These cells were obtained from KEMRI Nairobi. The Cells were first grown in Minimum Essential Medium (MEM) Eagle's Base supplemented with 15% Fetal Bovine Serum (FBS), 2.62 g/L $NaHCO_3$, 20 mM L-glutamine, 10 ml/L Penstrep and 0.5 mg Fungizoid using T-75 culture flask.

Culturing of the cells were done at 37ºC in 5% CO_2 for 24 hrs and once they attained confluence they were harvested by trypsinization and pooled into 50 ml vial. Cell suspension (1 x 10^5 cell/ml) approximately 100 µl were seeded into the 96-well flat-bottomed micro-titer plate containing100 µl of MEM (growth media) and incubated at 37ºC in 5% CO_2 for 48 hrs, to attain confluence. Once confluency was attained, the growth media was aspirated and replaced with

100 µl of maintance media which were placed in all the wells except the first two wells of row A which were replaced with 150 µl of 500 ug/ml of plant extracts. Three fold serial dilution was then carried out from row A to row H, resulting in exposure of the cells to increased concentrations of the plant extracts ranging from (500 µg/ml-0.23µg/ml). The contents in the plate were further incubated at 37 ºC for 48 hrs.

Generally, the 96-microtiter plate carried four different samples with every first two wells from row A to H carrying one sample while the third wells from row A to H contained the cells and the maintance media only. This third well of the 96-well plate served as the negative control. After the incubation period, MTT (10 µL of 5 mg/mL) was added into each well and the cells incubated for another 4 hrs until purple precipitates (formazan) were clearly visible under a microscope. Subsequently, the supernatant was removed and replaced with acid-isopropanol (0.04N HCl in isopropanol). The well plate was gently shaken for 15 minutes to dissolve the formazan, followed by measurement of optical density (OD) using ELISA scanning multiwell spectrophotometer (Multiskan Ex labssystems) at 562 nm and 690 nm. The 690 nm was the absorbance of background reference filter while the 562 nm was the absorbance of formazan. Percentage growth inhibition at each concentration was automatically calculated using a graphic program Ms excel, 2003 using the formular below (Ngeny *et al.,* 2013).

$$\% \text{ growth inhibition} = 100 - \left[\frac{OD_{\text{sample } 562} - OD_{690}}{OD \text{ control}_{562} - OD_{690}} \right] \times 100$$

The IC_{50}, which is the concentration of the extracts, that reduced viable cell by 50%, was automatically calculated from graphs generated by the graphic program. Extracts were considered cytotoxicy if there $IC_{50} < 20$ µg/ml according to guidelines set by the National Cancer Institute (NCI) (Geran *et al.,* 1972).

The methodology of the current study is summarized in Figure 17.

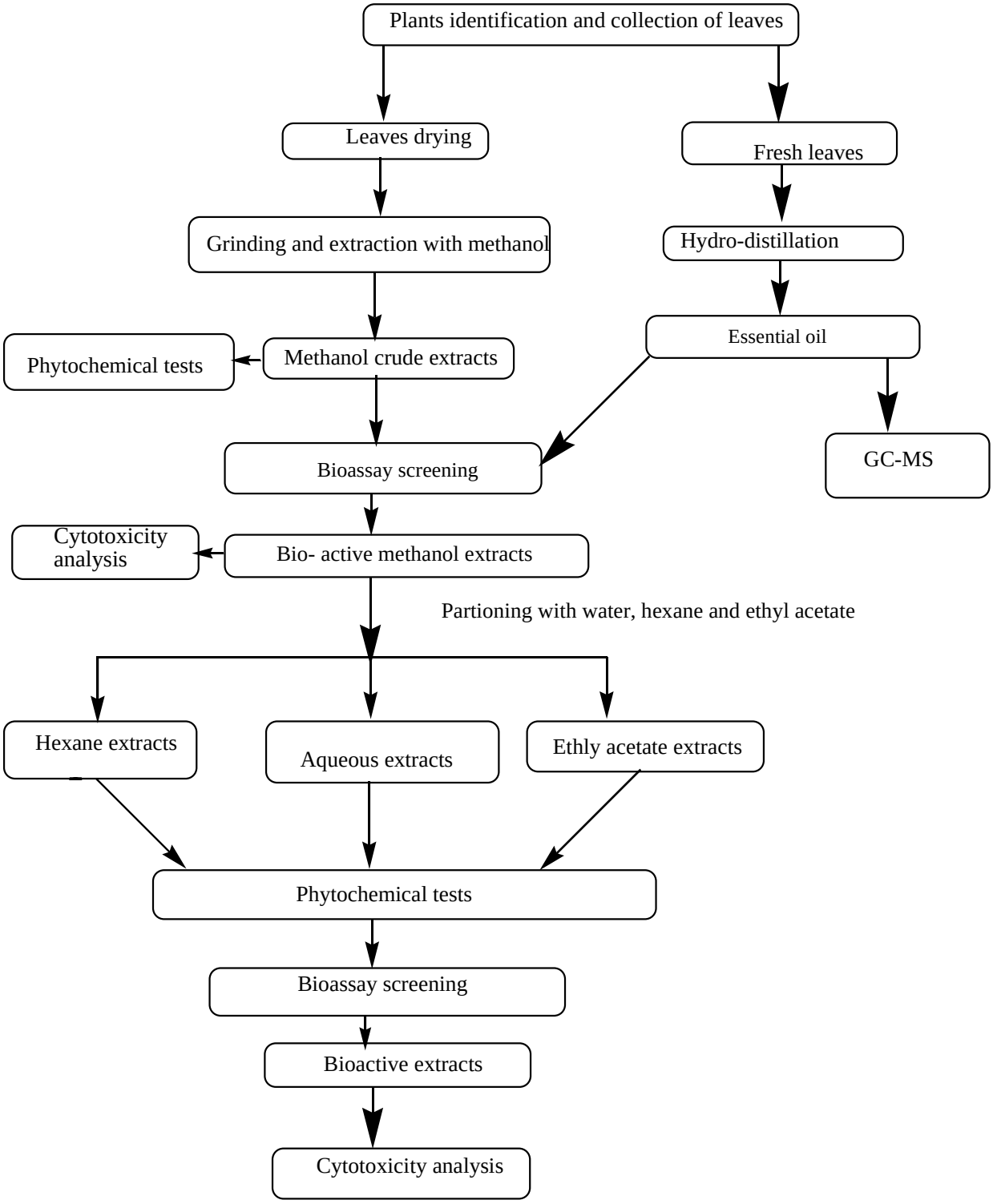

Figure 19: Summary of the methodology

3.13 Statistical analysis

Those extracts that were bioactive against the larvae were subjected to probit regression analysis to calculate concentration dependent mortality for the LC_{50} and LC_{90} values. SPSS 20 statistical software was used to determine the associated 95% confidence interval. Graphs were plotted to show the trend of the various response variables. The significant difference in activity of extracts against the larvae at 6, 12, 24 and 48 hrs were analyzed using one way ANOVA. The GC-MS spectra data were analyzed by GC–MS data base to obtain the chemical composition of the oil. Ms excel, 2003 was used to determine percentage growth inhibition of vero cells by extracts that showed bio-activity against the larvae.

CHAPTER FOUR

RESULTS

4.1 Preliminary screening of methanol crude extracts

Preliminary screening of methanol crude extracts of *L. kituiensis, S. compactum, P. dodecandra* and *P. viridiflorum* against *R. appendiculatus* larvae resulted in mean larval mortalities (%) shown in Table 1 below. Appendix 2 shows detailed preliminary larval bioassay results at 50 mg/ml. The mean larval mortalities (%) observed were 100, 60, 73.3 and 13.3 for methanol extracts of *L. kituiensis*, *P. dodecandra*, *P. viridiflorum* and *S. compactum* respectively at 48 hrs. No mortality was observed within 12 hrs in all the extracts. Only *L. kituiensis* extract demonstrated 16.7% mortality at 24 hrs. Of the four plant methanol extracts, that of *S. compactum* was dropped from the study due to very low larval mortality, which was less than 60%. No mortality was observed in the negative control within 48 hrs while in the positive control (Amitraz®) 100% mortality was observed at 48 hrs.

Table 1: Preliminary screening results of methanol extracts

Methanol extracts at 50 mg/ml	**Mean larval mortalities (%) at the hrs shown below**			
	6 hrs	**12 hrs**	**24 hrs**	**48 hrs**
L. kituiensis	0±0	0±0	16.7±15.3	100±0
S. compactum	0±0	0±0	0±0	13.3±15.3
P. dodecandra	0±0	0±0	0±0	60±10
P. viridiflorum	0±0	0±0	0±0	73.3±11.6
Amitraz ®(0.2 % v/v) [P]	0±0	56.7±11.8	90±17.3	100±0
(2% DMSO)[Q]	0±0	0±0	0±0	0±0

[P] Positive control; [Q] Negative control

4.2 Larvicidal bioassay of the selected methanol extracts

4.2.1 Methanol extract of *L. kituiensis*

The study observed that when the tick larvae came into contact with methanol extract of *L. kituiensis*, they became docile compared to those in the negative within the first 12 hrs. No mortality was observed within the 12 hrs, while at 24 hrs only a mortality of 16.7% was observed at the highest concentration (50 mg/ml). At 48 hrs, significant mortality was observed which varied depending on the concentration. Table 2 below shows the mean percentage larval mortalities between 0-48 hrs while larval bioassay results are shown in Appendix 3. Those alive within the 48 hrs were unable to move their appendages, unless prodded with a pin a sign that they might had been knocked down. At 48 hrs where there was significant mortality, the LC_{50}

was 21.3 (18.1-24.5) mg/ml and LC_{90} was 38.6 (32.5-51.6) mg/ml. Appendix 4 shows the LC values which were generated from probit regression analysis of the bioassay at 48 hrs, while Figure 18 shows the mean larval mortalities (%) and standard deviation of the bioassay data at 48 hrs. The larvae in the positive control displayed tremors and convulsion for 6 hrs followed by 56.7% mortality occurring at 12 hrs and 100% mortality occurring at 48 hrs. No mortality was observed in the negative control within the 48 hrs.

Table 2: Mean larval mortalities caused by methanol extract of *L. kituiensis*

Concentration in mg/ml	**Mean larval mortalities (%) at the hrs shown below**			
	6 hrs	**12 hrs**	**24 hrs**	**48 hrs**
6	0±0	0±0	0±0	0±0
10	0±0	0±0	0±0	10±10
14	0±0	0±0	0±0	16.7±15.3
18	0±0	0±0	0±0	36.7±11.5
22	0±0	0±0	0±0	46.7±5.8
26	0±0	0±0	0±0	60±10
30	0±0	0±0	0±0	70±17.3
35	0±0	0±0	0±0	83.3±5.8
40	0±0	0±0	0±0	93.3±11.5
45	0±0	0±0	6.7±5.8	100±0
50	0±0	0±0	16.7±15.3	100±0
Amitraz ® (0.2% v/v)[P]	0±0	56.7±11.8	90±17.3	100±0
(2% DMSO)[Q]	0±0	0±0	0±0	0±0

[P] positive control;[Q] Negative control

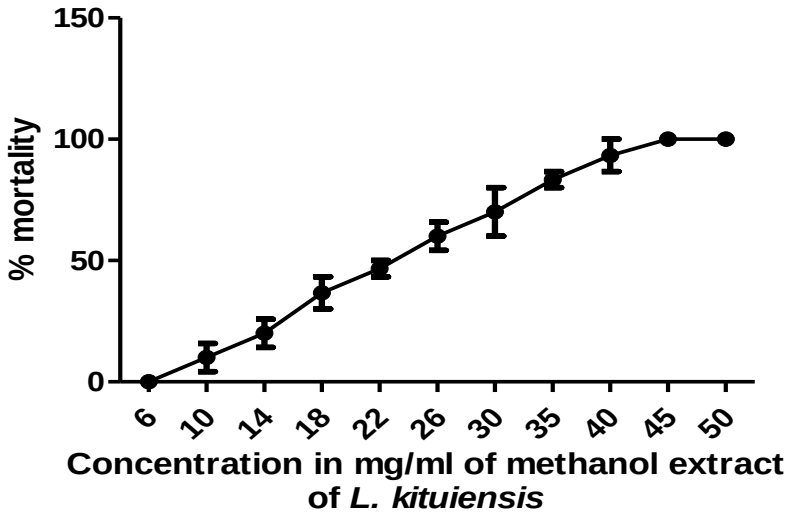

Figure 20: Mean larval mortalities (%) ± sd of methanol extract of *L. kituiensis* at 48 hrs

4.2.2 Methanol extract of *P. viridiflorum*

When the larvae were introduced to the extract, they became inactive as compared to those in negative control. The activity of the larvae declined as time progressed depending on the concentration. Table 3 below shows the mean percentage larval mortalities between 0-48 hrs while larval bioassay results are shown in Appendix 5. Within 24 hrs, no mortality was observed. A common phenomena at 24 hrs in about 50% of the larvae at the highest concentration of 75 mg/ml was desiccation. Varying mortality depending on concentration was observed at 48 hrs with most of the larvae that were dead looking desiccated. The LC_{50} was 30.5 (25.7-35.1) mg/ml and LC_{90} was 63.1 (52.7-83.9) mg/ml at 48 hrs. Appendix 6 shows the LC values which were obtained from probit regression analysis of the bioassay data at 48 hrs. Figure 19 below shows mean larval mortalities (%) and standard deviation of the bioassay data at 48 hrs. No mortality was observed in the negative control within the 48 hrs. Mortality of 100% was observed at 48 hrs in the positive control (Amitraz®). Larvae in the positive control displayed tremors and convulsion, which was different from what was observed in larvae subjected to the methanol extract of *P. viridiflorum*.

Table 3: Mean larval mortalities caused by methanol extract of *P. viridiflorum*

Concentration in mg/ml	**Mean larval mortalities (%) at the hrs shown below**			
	6 hrs	**12 hrs**	**24 hrs**	**48 hrs**
5	0±0	0±0	0±0	0±0
10	0±0	0±0	0±0	6.7±5.8
15	0±0	0±0	0±0	13.3±11.5
20	0±0	0±0	0±0	26.7±5.8
25	0±0	0±0	0±0	30±10
30	0±0	0±0	0±0	46.7±5.8
35	0±0	0±0	0±0	56.7±11.5
40	0±0	0±0	0±0	60±15.3
45	0±0	0±0	0±0	66.7±15.3
50	0±0	0±0	0±0	73.3±11.5
55	0±0	0±0	0±0	83.3±5.8
60	0±0	0±0	0±0	90±10
65	0±0	0±0	0±0	96.7±5.8
70	0±0	0±0	3.3±5.8	100±0
75	0±0	0±0	10±17.3	100±0
Amitraz® (0.2% v/v)[P]	0±0	56.7±11.8	90±17.3	100±0
(2% DMSO)[Q]	0±0	0±0	0±0	0±0

[P] positive control: [Q] Negative control

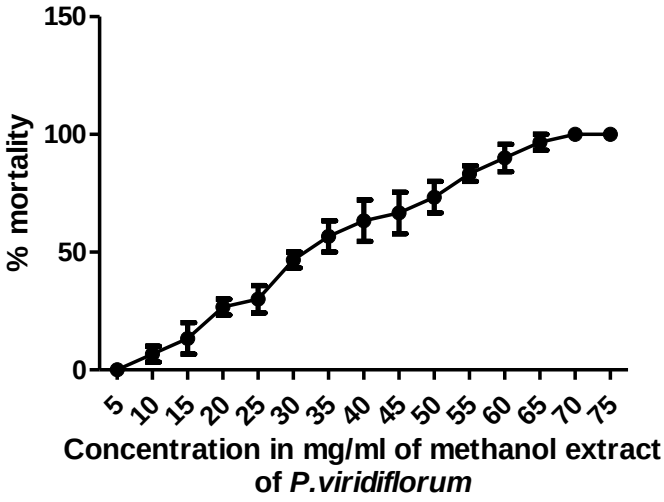

Figure 21: Mean larval mortalities (%) ± sd of methanol extract of *P. viridiflorum* at 48 hrs

4.2.3 Methanol extract of *P. dodecandra*

Toxic symptoms caused by *P. dodecandra* on the larvae were not observable under the microscope (X 4.5). However, there was a general reduction in movement in larvae exposed to the extracts compared to those in the negative control. Table 4 below shows the mean larval mortalities between 0-48 hrs while larval bioassay results are shown in Appendix 7. No mortality was observed within 24 hrs while at 48 hrs significant mortality was observed which varied with concentration. Mortality at 48 hrs resulted in LC_{50} of 39.1(31.1-46.9) mg/ml and LC_{90} of 84.6(67.7-124.2) mg/ml. Appendix 8 shows the LC values which were obtained from probit regression analysis of the bioassay data at 48 hrs. Figure 20 shows mean percentage larval mortalities and standard deviation of the bioassay data at 48 hrs. No mortality was observed in the negative control within the 48 hrs, while 100% mortality was observed at 48 hrs in the positive control.

Table 4: Mean larval mortalities caused by methanol extract of *P. dodecandra*

Concentration in mg/ml	Mean larval mortalities (%) at the hrs shown below			
	6 hrs	**12 hrs**	**24hrs**	**48 hrs**
5	0±0	0±0	0±0	0±0
10	0±0	0±0	0±0	3.3±5.8
20	0±0	0±0	0±0	20±11.5
30	0±0	0±0	0±0	33.3±15.3
40	0±0	0±0	0±0	40±10
50	0±0	0±0	0±0	56.7±5.8
60	0±0	0±0	0±0	70±20
70	0±0	0±0	0±0	80±10
80	0±0	0±0	0±0	93.3±5.8
90	0±0	0±0	0±0	96.7±5.8
100	0±0	0±0	6.7±5.8	100±0
Amitraz ® (0.2% v/v)[P]	0±0	56.7±11.8	90±17.3	100±0
(2% DMSO) [Q]	0±0	0±0	0±0	0±0

[P] positive control; [Q] Negative control

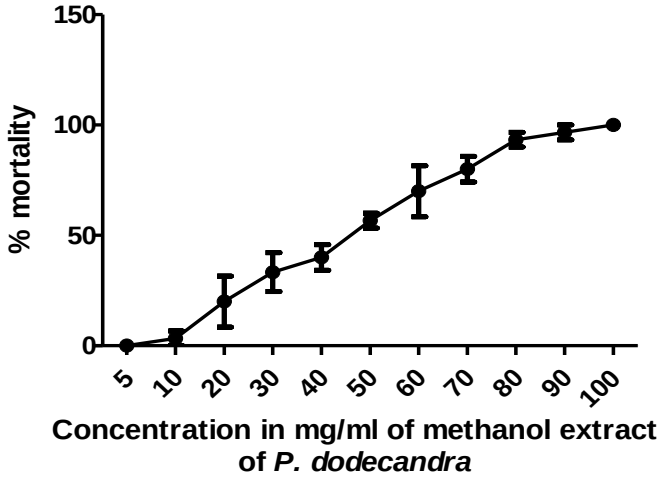

Figure 22: Mean larval mortalities (%) ± sd of methanol extract of *P. dodecandra* at 48 hrs

4.3 Effect of hexane, ethyl acetate and aqueous extracts on *R. appendiculatus* larvae

Hexane, ethyl acetae and aqueous extracts obtained from portioning the methanol extracts of *L. kituiensis*, *P. viridiflorum* and *P. dodecandra* with the three solvents, resulted in mean larval mortalities (%) displayed in Table 5 below. At 48 hrs, hexane extract of *L. kituiensis* and *P. viridiflorum* caused 100% and 60% mean larval mortalities while aqueous extract of *P.*

dodecandra caused 90% mortality. Appendix 9 shows the larval bioassay results. Those extracts which caused above 60% mean larval mortalites at 25 mg/ml were selected.

Table 5: Mean larval mortalities caused by hexane, ethlyacetate and aqueous extracts

Extracts at 25 mg/ml	**Mean larval mortalities (%)at the hrs shown below**			
	6hrs	**12 hrs**	**24 hrs**	**48 hrs**
Aqueous extract of *L. kituiensis*	0±0	0±0	0±0	0±0
Hexane extract of *L. kituiensis*	100±0	100±0	100±0	100±0
Ethly acetate extract of *L. kituiensis*	0±0	0±0	0±0	10±10
Aqueous extract of *P. viridiflorum*	0±0	0±0	0±0	6.7 ±5.8
Hexane extract of *P. viridiflorum*	0±0	0±0	0±0	60±20
Ethly acetate extract *P. viridiflorum*	0±0	0±0	0±0	16.7±15.3
Aqueous extract of *P. dodecandra*	0±0	0±0	0±0	90±5.8
Hexane extract of *P. dodecandra*	0±0	0±0	0±0	3.3±5.8
Ethly acetate extract of *P. dodecandra*	0±0	0±0	0±0	6.7±5.8
Amitraz ® (0.2% v/v)[P]	0±0	56.7±11.8	90±17.3	100±0
(2% DMSO)[Q]	0±0	0±0	0±0	0±0

[P] Positive control; [Q] Negative control

4.3.1 Larvicidal bioassay of *L. kituiensis* hexane extract

Hexane extract of *L. kituiensis* gave 100% mean larval mortality at a concentration of 25 mg/ml within 6 hrs a phenomena that was not possible in the positive control. At 48 hrs, the lowest concentration of hexane extract had killed more than 50% of the larvae. Mean larval mortalities (%) at different concentrations between 0-48 hrs is shown in Table 6. Detailed larvicidal bioassay results are presented in Appendix 10. The behavioural observation in larvae on coming in contact with the extract was similar to what was observed in methanol extract of *L. kituiensis* however there was difference in time at which behavioral changes was observed. The larvae became docile almost immediately they came in contact with the extract and within the first one hour all the larvae in the highest concentration were unable to move their appendages unless they were prodded with a pin. This demonstrated a knockdown effect within the first 1 hour and within 3 hrs, 100% mortality mean larval mortality was observed in the highest concentration. Although mortality data was collected at 6 hrs, mortality had initially started within the first 3 hrs. The LC_{50} in mg/ml were 12.6 (11.0-14.1), 10.6 (9.0-12.0), 6.7 (5.2-7.9), and 4.8 (2.2-5.9) while the LC_{90} in mg/ml were 19.5 (17.0-24.4), 17.4 (15.0-22.0), 10.8 (9.1-14.3) and 7.7 (6.2-13.0) at 6, 12, 24 and 48 hrs respectively. The LC values generated from

probit regression analysis of bioassay data between 0-48 hrs are shown in Appendix 11 to 14. Results of one way ANOVA showed significant difference (P= 0.03, 95%) in activity of hexane extract of *L. kituiensis* at 6, 12, 24 and 48 hrs. Figure 21 shows mean larval mortalities (%) and standard deviation between 0-48 hrs.

Table 6: Mean larval mortalities caused by hexane extract of *L. kituiensis*

Concentration in mg/ml	**Mean larval % mortalities at the hrs shown below**			
	6	**12**	**24**	**48**
5	0±0	6.7±5.8	10±10	60±10
7.5	13.3±11.5	20±10	50±17.3	80±20
10	26.7±5.8	43.3±15.3	80±10	100±0
12	40±17.3	56.7±28.9	96.7±5.8	100±0
14	50±10	60±20	100±0	100±0
16	70±17.3	83.3±15.3	100±0	100±0
18	86.7±11.5	96.7±5.8	100±0	100±0
20	93.3±10	100±0	100±0	100±0
23	100±0	100±0	100±0	100±0
25	100±0	100±0	100±0	100±0
Amitraz ® (0.2% v/v)[P]	0±0	56.7±11.8	90±17.3	100±0
(2% DMSO)[Q]	0±0	0±0	0±0	0±0

[P]positive control, [Q]Negative control P=0.03

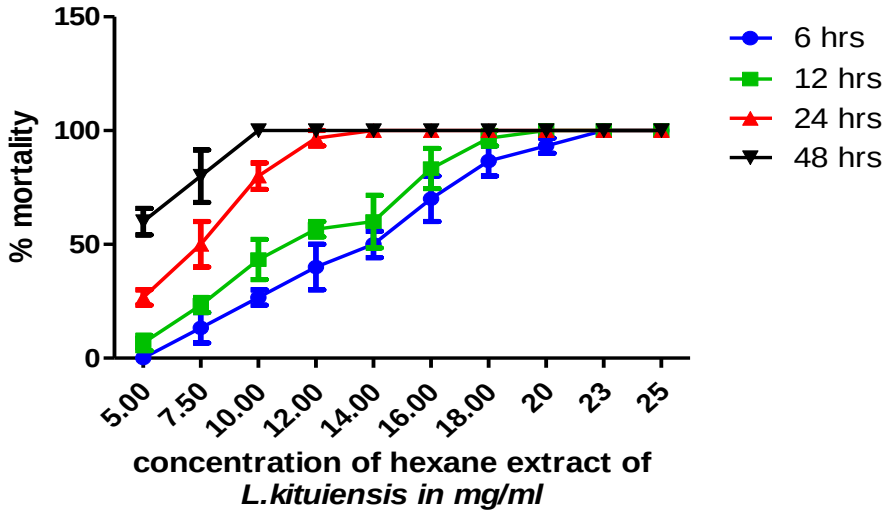

Figure 23: Mean larval mortalities (%) ± sd of hexane extract of *L. kituiensis* between 0-48 hrs

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4.3.2 Larvicidal bioassay of *P. viridiflorum* hexane extract

No mortality was observed within 24 hrs while varying mortality was observed depending on the concentrations used at 48 hrs. Table 7 shows the mean larval mortalities (%) between 0-48 hrs while larval bioassay results are shown in Appendix 15. Those observed dead were dessicated. LC_{50} and LC_{90} were 22.5 (18.3-26.6) mg/ml and 45.5 (36.9-66.8) mg/ml respectively at 48 hrs. Appendix 16 shows the LC values which were obtained from regression analysis of the bioassay data at 48 hrs. Figure 22 shows mean larval mortalities (%) and standard deviation at 48 hrs.

Table 7: Mean larval mortalities caused by hexane extract of *P. viridiflorum.*

Concentration in mg/ml	**Mean larval mortalities (%) at the hrs shown below.**			
	6hrs	**12 hrs**	**24 hrs**	**48 hrs**
5	0±0	0±0	0±0	0±0
10	0±0	0±0	0±0	10±10
15	0±0	0±0	0±0	26.7±5.8
20	0±0	0±0	0±0	40±17.3
25	0±0	0±0	0±0	56.7±5.8
30	0±0	0±0	0±0	63.3±15.3
35	0±0	0±0	0±0	70±17.3
40	0±0	0±0	0±0	80±10
45	0±0	0±0	3.3±5.8	96.7±5.8
50	0±0	0±0	6.7±11.5	100±9
Amitraz ® (0.2% v/v)[P]	0±0	56.7±11.76	90±17.3	100±0
(2% DMSO)[Q]	0±0	0±0	0±0	0±0

[P]Positive control, [Q] Negative control.

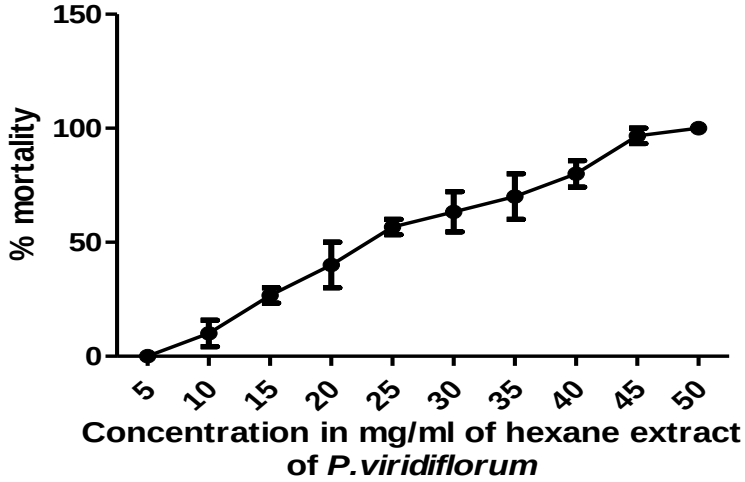

Figure 24: Mean larval mortalities (%) ± sd of hexane extract of *P. viridiflorum* at 48 hrs

4.3.3 Larvicidal bioassay of *P. dodecandra* aqueous extract of extract

The larvae exposed to the extracts remained active for about 6 hrs. As time progressed, they became weaker compared to those in the negative control, though no mortality was observed within 12 hrs. Insignificant mortality of 3.3% was observed at 30 mg/ml at 24 hrs. Varying mortalities depending on the concentration was observed at 48 hrs. Those dead had their appendages folded. Table 8 shows mean larval mortalities (%) between 0-48 hrs while larval bioassay results are shown in Appendix 17. Varying mortality observed at 48 hrs resulted in LC_{50} of 17.3 (15.2-19.4) mg/ml and LC_{90} of 26.8 (23.3-34.4) mg/ml. The LC values were obtained from probit regression analysis of the bioassay data between at 48 hrs and are shown in appendix 18. Figure 23 shows mean larval mortalities (%) and standard deviation at 48 hrs.

Table 8: Mean larval mortalities caused by aqueous extract of *P. dodecandra*.

Concentration in mg/ml	**Mean % larval mortality at the hrs shown below**			
	6 hrs	**12 hrs**	**24 hrs**	**48 hrs**
7	0±0	0±0	0±0	0±0
10	0±0	0±0	0±0	13.3±5.8
13	0±0	0±0	0±0	20±17.3
16	0±0	0±0	0±0	30±10
19	0±0	0±0	0±0	56.7±11.5
22	0±0	0±0	0±0	70±17.3
25	0±0	0±0	0±0	86.7±11.5
27	0±0	0±0	0±0	93.3±5.8
30	0±0	0±0	3.3±5.8	100±0
Amitraz® (0.2% v/v)[P]	0±0	56.7±11.8	90±17.3	100±0
(2% DMSO)[Q]	0±0	0±0	0±0	0±0

[P] positive control;[Q] negative control

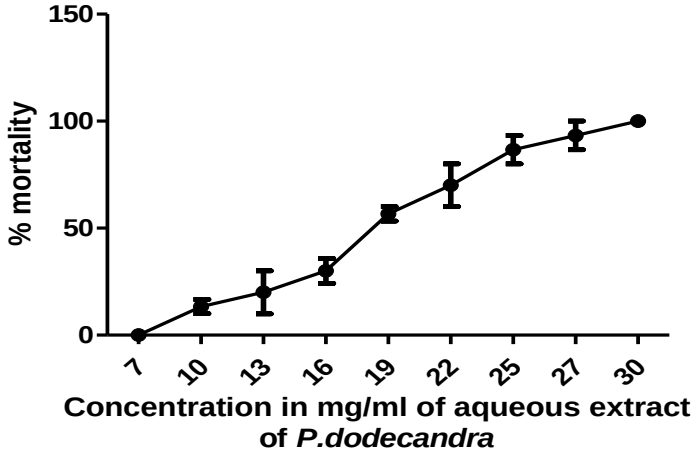

Figure 25: Mean larval mortalities (%) ± sd of aqueous extract of *P. dodecandra* at 48 hrs

4.4 Larvicidal bioassay of essential oils

4.4.1 Essential oil of *L. kituiensis* and *L. javanica*

Immediately the larvae were exposed to the oil extracts, they displayed tremors and convulsions and it persisted for 10 minutes at the highest concentrations (4.5 mg/ml for *L. kituiensis* and 4.0 mg/ml for *L. javanica*). This shows that sign they were paralysed. It was followed by 100% mortality occuring within 30 minutes in the highest concentrations of both oils. The larvae that were dead had their appendages extended side by side. No mortality was observed in the positive control within 6 hrs as compared to 100% mortality observed in both oil extracts, within the same period. Table 9 and 10 shows the mean larval mortalities (%) between 0-48 hrs for *L. kituiensis* and *L. javanica* respectively. Larval bioassay results for the two oil extracts are shown in appendix 19 and 24 respectively. Figure 24 and 25 shows the mean larval mortalities and standard deviations between 0-48 hrs for *L. kituiensis* and *L. javanica* respectively.

The LC_{50} in mg/ml were 3.3 (3.1-3.3), 3.2 (3.1-3.3), 3.1 (3.0-3.2) and 3.1 (3.0-3.2) while LC_{90} were 4.1 (3.9-4.4), 4.0 (3.8-4.3), 3.9 (3.8-4.2), 3.9 (3.7-4.1) at 6, 12, 24 and 48 hrs respectively for *L. kituiensis*. For *L. javanica*, LC_{50} were 3.1 (3.0-3.2), 3.1 (3.0-3.2) 3.0 (2.9-3.1), 2.9 (2.8-3.1) and LC_{90} were 3.9 (3.7-4.2), 3.9 (3.7-4.2), 3.8 (3.6-4.1) and 3.7 (3.6-4.0) at 6, 12, 24, and 48 hrs respectively. The LC values generated from probit regression analysis of bioassay data of both oils at 6, 12, 24 and 48 hrs are presented in appendix 20 to 23 for *L.*

kituiensis and 25 to 28 for *L. javanica.* Results of one way ANOVA showed no significant difference in activity of both oils at 6, 12, 24 and 48 hrs. For *L. kituiensis* oil, (P= 0.98, 95%) and P= (0.97, 95%) for *L. javanica* oil.

Table 9: Mean larval mortalities caused by *L. kituiensis* essential oil

Concentration in mg/ml	**Mean larval mortalities (%) at the hrs shown below**			
	6	12	24	48
1.5	0±0	0±0	0±0	0±0
2.0	1.7±2.9	1.7±2.9	1.7±2.9	1.7±2.9
2.2	3.3±5.8	3.3±5.8	3.3±5.8	3.3±5.8
2.5	11.7±2.9	11.7±2.9	11.7±2.9	13.3±2.9
2.8	20±5	20±5	23.3±5.8	28.3±2.9
3.0	35±5	38.3±5.8	45±5	46.7±2.9
3.3	43.3±7.6	50±5	51.7±7.6	56.7±5
3.5	51.7±7.6	56.7±10	61.7±2.9	65±36.7
3.7	63.3±2.9	66.7±5.8	70±5	75±5.8
3.9	81.7±5	85±2.9	88.3±2.9	91.7±2.9
4.0	90±2.9	96.7±2.9	100±0	100±0
4.2	98.3±2.9	100±0	100±0	100±0
4.5	100±0	100±0	100±0	100±0
Amitraz ® (0.2% v/v)P	0±0	56.7±11.8	90±17.3	100±0
(2% DMSO) Q	0±0	0±0	0±0	0±0
P Positive control; Q Negative control				P= 0.98

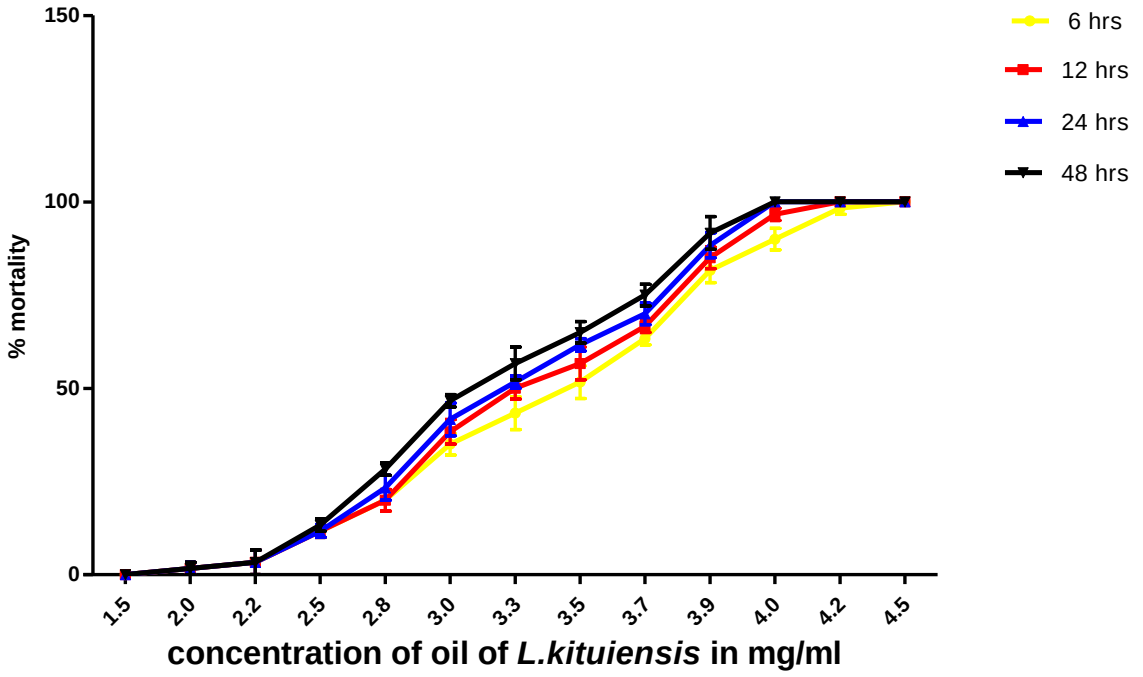

Figure 26: Mean larval mortalities (%) ± sd of oil extract of *L. kituiensis* oil between 0-48 hrs

Table 10: Mean larval mortalities caused by *L. javanica* essential oil

Concentration in mg/ml	**Mean % larval mortality at the hrs shown below**			
mg/ml	**6 hrs**	**12 hrs**	**24 hrs**	**48 hrs**
1.5	0±0	0±0	0±0	0±0
2.0	0±0	0±0	1.7±2.9	5±5
2.2	8.3±7.6	8.3±7.6	8.3±7.6	10±5
2.5	13.3±5.8	15±5	15±5	16.7±2.9
2.8	28.3±5.8	33.3±2.9	36.7±2.9	38.3±2.9
3.0	38.3±2.9	38.3±2.9	43.3±2.9	48.3±2.9
3.2	50±8.7	51.7±5.8	56.7±7.6	60±5
3.4	61.7±7.6	63.3±7.6	66.7±10	73.3±5.8
3.6	70±7.6	71.7±10.4	76.7±2.9	80±10.4
3.7	85±5	86.7±2.9	90±5	96.7±5.8
3.9	96.7±2.9	100±0	100±0	100±0
4.0	100±0	100±0	100±0	100±0
Amitraz ®(0.2 % v/v) [P]	0±0	56.7±11.8	90±17.3	100±0
(2.0% DMSO)[Q]	0±0	0±0	0±0	0±0

[P]Positive control,[Q]Negative control P=0.97

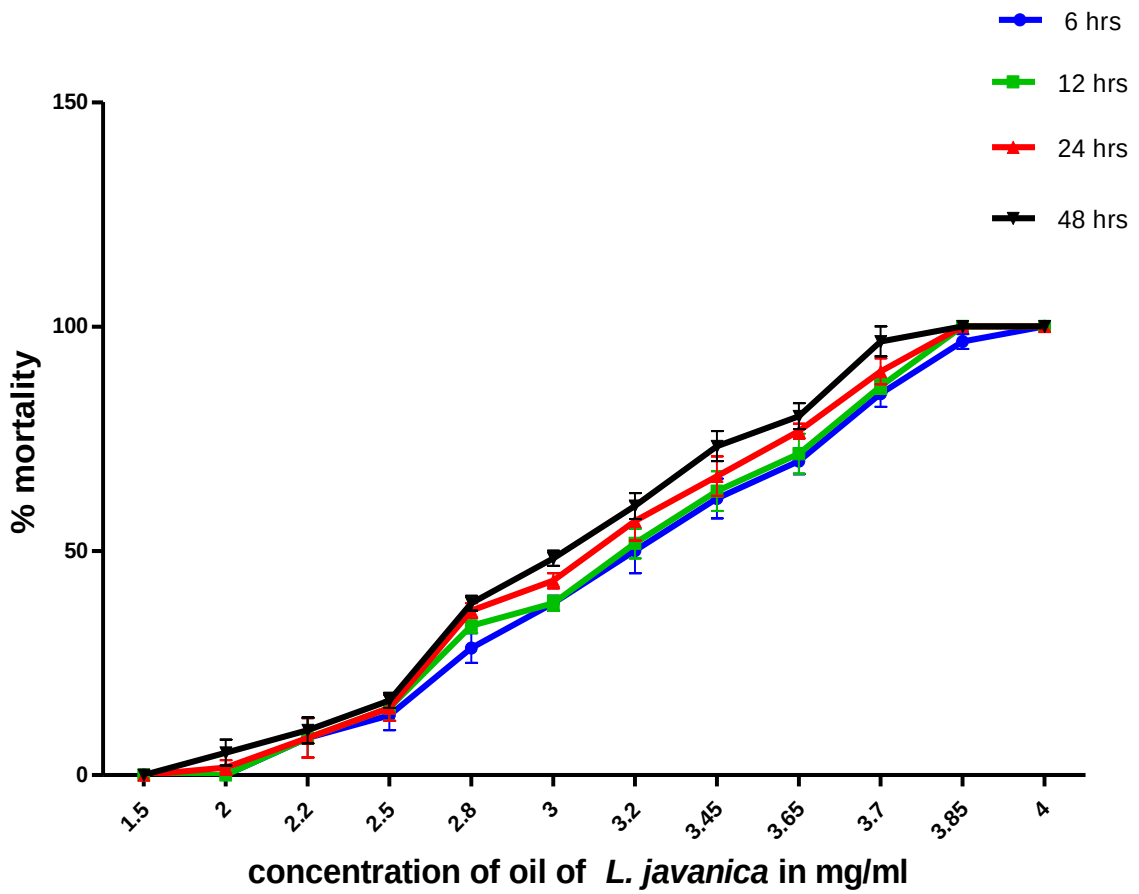

Figure 27: Mean larval mortalities (%) ± sd of oil extract of *L. javanica* between 0-48 hrs

4.5 Phytochemical composition of the plant crude extracts

The phytochemical composition of the 16 plant crude extracts used for determination of the larvicidal activity against *R. appendiculatus* are shown in Table 11 below. Those with a (+) indicate presence of the phytochemical tested and those with (-) shows absence of the tested phytochemical. Steroids, terpenoids and saponins were present in all the plant extracts used. Flavonoids were not present in aqueous extract of *L. kituiensis* and *S. compactum*. Cardiac glycosides were not present in aqueous extract of *P. dodecandra*. Tannins were not present in all the extracts of *P. dodecandra*, ethly acetate and hexane extracts of *L. kituiensis*, methanol and hexane extracts of *S. compactum* and hexane extract of *P. viridiflorum.*

Table 11: Results of phytochemical tests done on the selected plant extract

Extracts	**Results of Phytochemicals tested**						
	Saponin	**Tannins**	**Flavanoids**	**Phlobatanins**	**Steroids**	**Terpenoids**	**Cardiac glycosides**
Methanol extract of *L. kituiensis*	+	+	+	+	+	+	+
Ethly acetate extract of *L. kituiensis*	+	-	+	-	+	+	+
Hexane extract of *L.kituiensis*	+	-	+	-	+	+	+
Aqueous extract of *L.kituiensis*	+	+	-	-	+	+	+
Methanol of *P. dodecandra*	+	-	+	+	+	+	+
Ethly acetate extract of *P. dodecandra*	+	-	+	-	+	+	+
Hexane extract of *P. dodecandra*	+	-	+	-	+	+	+
Aqueous extract of *P.dodecandra*	+	-	+	-	+	+	-
Methanol extract of *S. compactum*	+	-	+	+	+	+	+
Ethly acetate extract of *S. compactum*	+	+	+	-	+	+	+
Hexane extract of *S. compactum*	+	-	+	-	+	+	+
Aqueous extract of *S. compactum*	+	+	-	-	+	+	+
Methanol extract of *P. viridiflorum*	+	+	+	+	+	+	+
Ethly acetate extract of *P. viridiflorum*	+	+	+	-	+	+	+
Hexane extract of *P. viridiflorum*	+	-	+	-	+	+	+
Aqueous extract of of *P. viridiflorum*	+	+	+	-	+	+	+

4.6 GC-MS analysis of Essential oil of *L. kituiensis*

The composition of oil was dominated by sequiterpenes (56.57%) followed by monoterpenes (36.36%), diterpenes 2.59% and others (5.19%). Major sequiterpenes which had composition above 1% were germacrene D, β-bourbonene, gamma-cadinene and 2-isopropyl-5-methyl-9-methylene- bicycle(4.4.0)dec-1-ene while major monoterpenes with composition above 1% include (1S,4S)-(-)- camphor, trans-sabinene hydrate, gamma-Terpinene, dl-limonene, alpha-terpinolene, l-Phellandrene, beta-myrcene, sabinene, camphene, alpha.-pinene, (-)-,4-terpineol, 4-methyl-1-(1-methylethyl)- 3-cyclohexen-1-ol, 14.29 borneol (=endo-borneol), camphore, and neo-allo-ocimene. Results of the GC-MS analysis of essential oil components of *L. kituiensis* are presented in appendix 29. Table 12 below shows oil components with area above 1%.

Table 12: Essential oil of *L. kituiensis* major components

Essential oil of *L. kituiensis* major components

Components	**Retention time**	**Area % concentration**
Monoterpenes		
Alpha-pinene (-)-	6.77	2.04
Camphene	7.23	7.26
Sabinene	7.91	2.15
beta-myrcene	8.36	1.49
l-Phellandrene	8.72	1.11
Dl-limonene	9.52	6.52
Gamma-terpinene	10.31	1.22
Trans-sabinene hydrate	10.71	4.45
Alpha-terpinolene	11.20	1.44
Neo-allo-ocimene	12.45	2.39
Camphor (1S,4S)-(-)-	13.26	18.29
Camphore	13.35	3.49
14.29 borneol (=endo-borneol)	13.87	1.77
4-methyl-1-(1-methylethyl)- 3-Cyclohexen-1-ol	13.97	1.43
4-terpineol	14.10	3.03
Sesquiterpenes		
Beta-bourbonene	19.43	1.36
2-isopropyl-5-methyl-9-methylene- Bicyclo[4.4.0]dec-1-ene	22.26	1.05
Germacrene D	20.35	3.20
Gamma-Cadinene	21.17	1.00
2-isopropyl-5-methyl-9-methylene- Bicyclo[4.4.0]dec-1-ene	22.26	1.05

4.7 Cytotoxicity assay

Growth inhibition of vero cells by methanol extracts of *L. kituiensis, P.dodecandra*, and *P.viridiflorm*; hexane extracts of *L. kituiensis* and *P. viridiflorm* and aqueous extract of *P. dodecandra* are presented in figure 21-23. All the extracts demonstrated no cytotoxic activity against vero cells at 500 µg/ml, as the IC_{50} could not be calculated at this concentration. For IC_{50} to be calculated the concentration need to be higher than 500 µg/ml. The absorbance values at 562 nm and 690 nm of the extracts and the negative controls are shown in appendix 30.

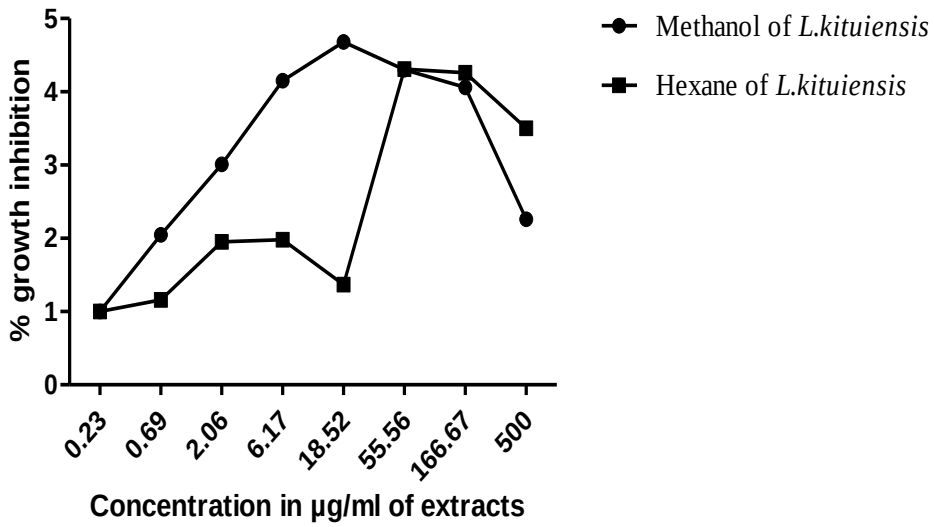

Figure 28: Growth inhibition (%) of vero cells against concentration in µg/ml of methanol and hexane extract of *L. kituiensis*

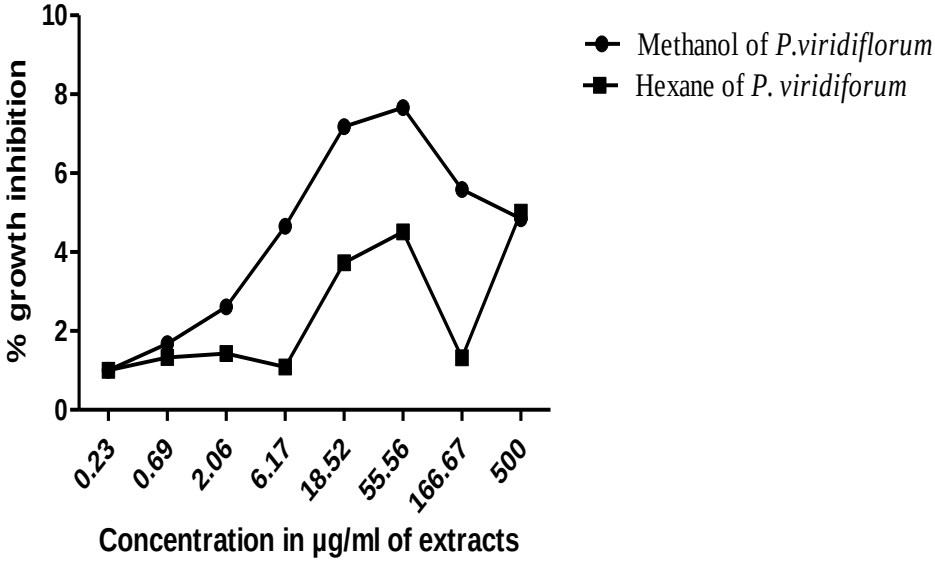

Figure 29: Growth inhibition (%) of vero against concentration in µg/ml of methanol and hexane extract of *P. viridiflorum.*

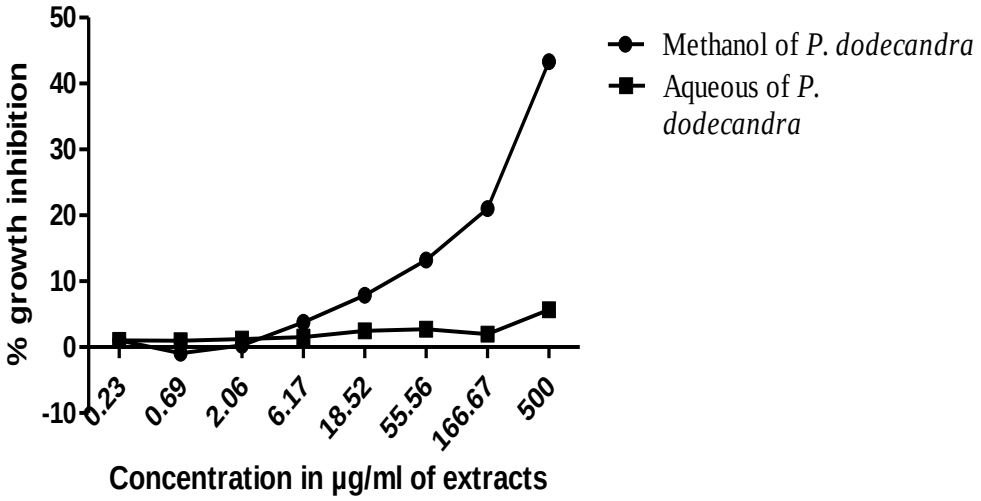

Figure 30: Growth inhibition (%) of vero cells against concentration in µg/ml of methanol and hexane extract of *P. dodecandra*

CHAPTER FIVE

DISCUSSION

5.1 Larvicidal activity of methanol crude extracts

Methanol extract of *L. kituiensis* was most active in this study. This is because it had the lowest LC_{50} and LC_{90} values at 48 hrs compared to other methanol extracts at the same period. At 48 hrs, methanol extract of *L. kituiensis* had LC_{50} of 21.3 (18.1-24.5) mg/ml and LC_{90} of 38.6 (32.5-51.6) mg/ml, followed by that of *P. viridiflorum* with LC_{50} of 30.5 (25.7-35.1) mg/ml and LC_{90} of 63.1 (52.7-83.9) mg/ml. Methanol extract of *P. dodecandra* showed least larvicidal activity with LC_{50} of 39.1 (31.1-46.9) mg/ml and LC_{90} of 84.6 (67.7-124.2) mg/ml.

Methanol extracts of different plants have been studied previously for their acaricidal activity. Previous study by Bagavan *et al.,* (2009) on methanol extracts of *G. superba* and *P. emblica* showed acaricidal activity against *H. bispinosa* tick with an LC_{50} of 225.57 and 256.08 ppm respectively. Leaf methanol extract of *R. communis* demonstrated acaricidal activity against the larvae of *R. microplus* tick with LC_{50} of 181.49 ppm and LC_{90} of 1,829.94 ppm respectively (Zahir *et al.,* 2009). All the phytochemical tested in the current study were present in methanol extracts of both *L. kituiensis* and *P. viridiforum* as shown in Table 11. These were saponins, flavonoids, terpenoids, steroids, cardiac glycosides, tannins, and phlobatanins. While methanol extracts of both *P. dodecandra* and *S. compactum* contained saponins, flavonoids, terpenoids, steroids, cardiac glycosides and phlobatanins with absence of tannins in both.

Terpenoids and flavonoids were among the secondary metabolites identified in *L. kituiensis* methanol extract which is similar to observation by Catalan and de Lampasona, (2002) on *Lippia* species. Presence of flavonoids and saponins in the methanol extract of *P. viridiflorum* compares with previous phytochemical analysis of acetone extract of the same plant (Mbeng, 2013). The results of the phytochemical tests done on methanol extract of *P. dodecandra* are in agreement with previous phytochemical analysis of the methanol leaf extract of this plant which showed that the plant contained flavonoids and saponin (Mekonnen *et al.,* 2012). Phytochemical analysis on methanolic leaf extract of *P. dodecandra* collected from Siaya and Migori districts in Kenya were found not to have terpenoids and this differs with the results of this study which showed presence of this compound (Ogutu *et al.,* 2012). This could

be due to difference in environmental conditions that evokes phytochemical production as an adaptive strategy (Zhao *et al.,* 2005).

Majority of these phytochemicals identified in both *L. kituiensis* and *P. viridiflorum* have been reported to have acaricidal activity. Shang *et al.,* (2013) proposed that flavonoids were the active compounds of acetic ether extract and contributed to the acaricidal activity of *A. coerulea* against *Psoroptes cuniculi*. Terpenoids which include eugenol, isoeugenol and methyl eugenol and butylide nephthalide present in many plant extracts have been shown to possess acaricidal activity against *T. putrescentiae* mite (Kwon and Ahn, 2002). Acaricidal properties of extracts from the aerial parts of *Hipericum polyanthemum* on the cattle tick *Boophilus microplus* were attributed to terpenoids (Ribeiro *et al.,* 2007). Tannins have been reported to have acaricidal effects against cattle tick, *R. microplus* (Fernandez-Salas, 2011). Cardiac glycosides isolated from *Calotropies procera* have been shown to be potent against camel tick *Hyalomma drometarii* as indicated by its lower LC_{95} value of 2539 (2207-2922) mg/l compared to Azadirachtin and neem oil which both had LC_{95} value of over 5000 mg/l (Al-Rajhy *et al.,* 2003). Acaricidal activity of root extracts of *P. decandra* against *Tetranychus cinnabarinus* spider mite was attributed to isolated Esculentoside which was the dominant active triterpene saponin (Ding *et al.,* 2013).

Although *L. kituiensis* and *P. viridiflorum* had similar phytochemicals, variation in killing the larvae by the two methanol extracts could be due to difference in quantity of the tested phytochemicals (Fennel and Staden, 2001). Presence of all the tested phytochemicals in both methanol extracts of *L. kituiensis* and *P. viridiflorum* could have contributed to synergistic effects, (Akın, 2010) causing 100% larval mortality at lower concentration of 50 mg/ml and 75 mg/ml respectively compared to 100 mg/ml in *P. dodecandra* which contained all the tested phytochemicals except tannins. Though methanol extract of *S. compactum* had similar phytochemicals as methanol extract of *P. dodecandra*, its inability to cause above 60% mortality of the larvae needed for selection could be attributed to difference in quantity of the tested phytochemicals (Fennel and Staden, 2001).

Previous studies on acaricidal properties of ethanolic extract of *Tagetes patula* showed that 100% mortality of larvae of *R. sangunes* was observed at 48 hrs at 50 mg/ml (Politi *et al.,* 2012). This was similar to the toxic dose that caused 100% mortality of *R. appendiculatus*

larvae in the current study by methanol extract of *Lippia kituiensis*. Over 90% mortality (95.7±2.9 %) was observed at a concentration of 100 mg/ml in *Petiveria alliacea* methanolic extract within 48 hrs against larvae of *R. microplus* (Rosado-Auilar *et al.*, 2010). The results are comparable to those of the methanolic extract of *P. dodecandra* in the present study at similar duration. The similarity in activity could be because plants of the same family (*phytolacceae*) often show similar chemical profiles (Hutchings *et al.*, 1996), although the amounts of specific compounds produced may differ (Fennel and Staden, 2001).

Beside being polar, methanol is a broad spectrum solvent and though it extracted the phytochemicals observed in Table 11, it also extracted sugars and essential oils from plant materials. All these molecules present in the methanol extracts could have worked synergistically (Akın, 2010) or individually in causing larval mortalities and the observed poisoning symptoms, which were paralytic effects in methanol extract of *L. kituiensis* and desiccation in methanol extract of *P. viridiflorum*. As a result, methanol extracts of all the three plants were portioned with solvents of different polarities i.e hexane nonpolar, ethyl acetate medium polar, and water highly polar. Hexane extracted nonpolar phytochemicals and essential oils, water extracted highly polar phytochemicals such as glycosides while ethyl acetate extracted medium polar phytochemicals (Lekgari, 2010). The portioning of the methanol extract with solvents of different polarities helped in classifying molecules present in the methanol crude extract as either polar, non polar or medium polar and thus aided in determining the extract which carried molecules responsible for activity observed in the methanol extract. The activity present in the methanol extract was hence attributed to bio-active compounds in either the aqueous extracts, hexane extracts or ethyl acetate extracts or synergistic effect of bioactive molecules present in all the three extracts.

5.2 Larvicidal activity of hexane, ethyl acetate and aqueous crude extracts

Determination of larvicidal activity of hexane, ethyl acetate, and aqueous extracts of *L. kituiensis, P. dodecandra* and *P. viridifllorum* against *R. appendiuclatus* resulted in selection of hexane extracts of both *L. kituiensis* and *P. viridiflorum* and only aqueous extract of *P. dodecandra*.

Ethyl acetate and hexane extract of *L. kituiensis* had similar phytochemicals as shown in Table 11. Both extracts showed presence of saponins, flavonoids, phlobatanins, steroids,

terpenoids, and cardiac glycosides while tannins and phlobatanins were absent in both. All these phytochemicals present in these two extracts have been reported to be acaricidal (Kwon and Ahn 2002; Ribeiro *et al.,* 2007; Fernandez-Salas, 2011; Shang *et al.,* 2013). A variation of 100% mortality in hexane extract and 30% mortality in ethyl acetate extract of both *L. kituiensis* during preliminary screening (25 mg/ml) at 48 hrs, may be attributed to presence of the essential oils in the hexane extract. Essential oil in hexane extract of *L. kituiensis* could have worked synergistically with other phytochemicals detected in Table 11, thus causing 100% larval mortality (Akın *et al.,* 2010) at 25 mg/ml. This is because essential oils are non-polar and since hexane is a non-polar solvent, the hexane crude extract was rich in essential oil beside the phytochemicals detected (Burt, 2004). Essential oils are volatile molecules which are very active due to their low molecular weight hence they can penetrate easily into various target sites. As a result the activity of several plants has been attributed to the presence of these molecules (Gutierrez *et al.,* 2009).

Ethyl acetate extract is more polar than hexane extract hence did not contain the oils. Essential oils are produced by aromatic plants only (Adorjan and Buchbauer 2010) and is obtained by hydro-distillation of fresh plant leaves. Hydro-distillation of *L. kituiensis* and *P. viridiflorum* leaves resulted in oil production while leaves of *P. dodecandra* lacked the oil. Presence of essential oil in the leaves of *L. kituiensis* and *P. viridiflorum* could have contributed to 100% mortality of larvae occurring at lower concentrations of 50 mg/ml and 75 mg/ml respectively in the methanol extracts compared to *P. dodecandra* which occurred at 100 mg/ml.

Although the methanol extract of *L. kituiensis* showed presence of all the tested phytochemicals as shown in Table 11, and also contained essential oils, hexane extract of this plant which lacked tannins and phlobatanins was still more active than the methanol extract. This is shown by lower LC_{50} of 4.8 (2.2-5.9) mg/ml and LC_{90} of 7.7 (6.2-13.0) mg/ml in hexane extract at 48 hrs, compared to LC_{50} of 21.3 (18.1-24.5) mg/ml and LC_{90} of 38.6 (32.5-51.6) mg/ml in methanol extract of *L. kituiensis* within the same period of time. The ability of the hexane extract of *L. kituiensis* to cause 100% mortality within the first 6 hrs compared to 48 hrs in methanol extract, and a decrease in the knock down times from 12 hrs in methanol extract to 3 hrs in hexane extract is a proof that hexane extract of *L. kituiensis* was more active. This could be due to other phytochemicals presents in methanol extract of *L. kituiensis* antagonizing the

action of essential oils present in the methanol extract making it less active compared to hexane extract. Hexane being nonpolar means its major constituents were essential oils due to similar nonpolar nature with the oil making it more active than the methanol extract.

Due to the volatile nature of compounds present in the essential oils, they are prone to rapid aerial oxidation and rearrangement hence quick loss of activity. However, the crude extracts has been reported to provide antioxidant protection of compounds present in the oil, and acts as a natural slow release formulation (Birkett *et al.,* 2008). This explains why there was significant difference in activity in hexane extract of *L. kituiensis* against the larvae at 6, 12, 24 and 48 hrs (P=0.03, 95%). At 48 hrs, 7 out of 9 concentrations of hexane extract of *L. kituiensis* used had killed 100% of the larvae as shown in the Table 6, while the lowest concentration 5 mg/ml had killed 60% of the larvae. This compares with that of hexane extract of *C. serrate* which was found to cause 100% mortality of *B. microplus* tick larvae at concentrations as low as 6.25 mg/ml obtained from serial dilution in 48 hrs (Ribeiro *et al.,* 2011).

The paralytic effects observed in both methanol and hexane extract of *L. kituiensis* could be attributed to presence of essential oils in both extracts. Pure essential oil without crude extracts of *L. kituiensis* was thus obtained by hydro-distillation of fresh leaves and subjected to a bioassay against the larvae. Aqueous extract of *L. kituiensis* did not show any acaricidal activity against the larvae within 48 hrs despite showing presence of acaricidal compounds which were saponins (Ding *et al.,* 2013), tannins (Fernandez-Salas, 2011), terpenoids (Kwon and Ahn, 2002) cardiac glycosides Al-Rajhy *et al.,* (2003). Water is a polar solvents and the phytochemicals present in this extract were polar. Lack of activity in this extract, could be due to lack of synergestic effects with non polar phytochemicals in causing larval mortality.

Although hexane extract of *P. viridiflorum* lacked tannins and phlobatanins its larvicidal activity was still greater than ethyl acetate and aqueous extracts of the same plant, which both lacked only phlobatanins as shown in Table 11. This is indicated by hexane extract of *P. viridiflorum* having LC_{50} of 22.5 (18.3-26.6) mg/ml and LC_{90} of 45.5 (36.9-66.8) mg/ml at 48 hrs while aqueous extract and ethyl acetate extract of *P. viridiflorum* were not selected for the actual bioassay due to low larval % mortlity of 6.7% and 16.7% respectively during preliminary screening. Failure of both ethyl acetate and aqueous extract of *P. viridiflorum* to cause significant larvicidal activity could be attributed to digestive enzymes on the extracts that could

have made the active compounds unavailable (Nalule *et al.*, 2011). Dessication was a prominent characteristic of the dead larvae subjected to the hexane extract of *P. viridiflorum* and the same characteristic was observed in dead larvae in methanol extract of the same plant, although the LC values were lower in hexane extract compared to methanol extract. This is a confirmation that activity observed in the methanol extract of *P. viridiflorum* could be attributed to compounds that were extracted by the hexane.

Flavanoids and saponins present in the hexane extract of *P. vridiflorum* could have worked synergistically (Akın, 2010) and caused the desiccation. This is because flavanoids have been reported to inhibit membrane tyrosine kinase which is involved in a variety of biological funtions (Formica and Regelson, 1995) including maintance of cell-cell adhesion. As a result there was disruption of the outer epicuticular waxy layer of larvae which prevents water loss resulting in death due dessication. Saponins on the other hand have been reported to form complexes with membrane sterols and produce membrane disintegration responsible for the water loss hence dessication (Montes-Belmont, 2009). Previous study that justify traditional use of *P. viridiflorum* in the treatment of mycotic infections associated with HIV/AIDS has attributed the activity observed in this plant to presence of flavonoids and saponins (Otang, 2012).

Hexane and ethyl acetate extract of *P. dodecandra* were not selected for the actual bioassay due to low mortality of 3.3% and 6.7%. Aqueous extract of *P. dodecandra* caused 90% mortality during preliminary screening and was thus subjected for actual larvicidal bioassay against *R. appenidculatus* larvae resulting in an LC_{50} of 17.3 (15.2-19.4) mg/ml and LC_{90} of 26.8 (23.3-34.4) mg/ml at 48 hrs. Both hexane and ethyl acetate extracts of *P. dodecandra* showed presence of similar phytochemicals namely flavonoids, saponins, steroids, terpenoids, and cardiac glycosides while aqueous extract showed presence of saponins, flavaniods, steroids and terpenoids as shown in table 11. All these phytochemicals present in this three extracts of *P. dodecandra* have been reported to have acaricidal activity (Kwon and Ahn, 2000; Al-Rajhy, *et al.*, 2003; Shang *et al.*, 2013; Ding *et al.*, 2013). Both ethyl acetate and hexane extract of *P. dodecandra* in the current study showed presence of terpenoids which is similar with what was obtained in previous phytochemical analysis of both extracts (Ogutu *et al.*, 2012). Although both hexane and ethyl acetate extract of *P. dodecandra* had more acaricidal phytochemicals

than the aqueous extract as shown in Table 11, their larvicidal activity was low and this could be due to lack of active compound refinement (Nalule *et al.,* 2011). However, compound refinement may render plant medicinal value inactive as plants impart their medicinal value through synergism, additive effect or antagonism to lessen toxicity on animal tissues (Nalule *et al.,* 2011). Other factors include effects of the digestive enzymes on bioavailability of active phytochemicals (Nalule, *et al.,* 2011), and presence of the active compound in low amounts (Fennel and Staden, 2001).

The larvicidal activity observed in the aqueous extract of *P. dodecandra* is in agreement with previous studies on several species of genus *phytolaccaeae*, which have shown that aqueous extract of fruits and leaves of these plants carry active compounds. These compounds in aqueous extracts have analgesic, antiinflammatory, bactericidal, fungicidal, mitogenic and molluscicide action (Quiroga *et al.,* 2001; Farias Magalhães *et al.,* 2003; Delporte *et al.,* 2009). Triterpene saponins are the primary toxic constituents of *phytolaccaeae* (Armstrong, 2009) and has been isolated in many aqueous plant extracts of genus *phytolaccaeae* (Hernández1 *et al.,* 2013), thus giving a chemotaxonomic significance to the subfamily *phytolaccaeae* (Gattuso, 1996). Systemic screening of some 600 wild types of Endod plants indicated that berries of a *Phytolacca* species, type 44, in Ethiopia contained as much as 25% by weight of saponins, from which the molluscicides, lemmatoxins, have been isolated and purified with organic solvents (Lemma *et al.,* 1972).

Acaricidal activity of root extracts of *Phytolacca decandra* against *Tetranychus cinnabarinus* spider mite was attributed to isolated Esculentoside P which was the dominant active triterpene saponin (Ding *et al.,* 2013). Saponin isolated from *Phytolacca tetramera* fruits have been reported to cause inhibitory effect against human pathogenic fungi (Escalante *et al*., 2002). Oleanoglycotoxin-A a triterpene saponin isolated from aqueous berry extracts of *P. decandra* has been attributed to the molluscicidal properties of this plant. Other triterpene saponins present in *P. dodecandra* are lemmatoxin A, B and C which have also been attributed for the molluscicidal activity of this plant (Harold *et al.,* 1993). Biological activities such as molluscicidal effect, antifertility, induction of immune interferon (INF-γ); enhancement of leukocyte phagocytosis and promotion of DNA transformation present in *Phytolacca acinosa* has been attributed to triterpene saponins, (Ma *et al.,* 2010).

In this study, larvae subjected to the aqueous extracts of *P. dodecandra* remained active for about 6 hrs before becoming weak and 100% mortality observed at 48 hrs. When examined under the microscope, all those larvae which were dead had there appendages folded and morphologically they were similar with those in the negative control. Previous studies on molluscidal properties of a compound isolated from the rhizorpers of *P. acinosa* have shown that the compound affected glycogen metabolism and protein synthesis in snails. Effect on glycogen metabolism and protein synthesis by the compound, was due to induction of partial liver cell necrosis thus affecting hepatic function. This leads to a direct impairment on glycogen synthesis and protein synthesis. It also affected the digestive tract function by causing reduction of glucose uptake, thus inhibiting glycogen synthesis.

In the present study, death of larvae could be due to a triterpene saponin based on previous findings of its bioactivity and abundance in the genus *phytolaccaeae*. Lethal effects of these saponin on tick larvae may be more related to energy metabolism of cells similar to that of molluscidal compound isolated from *P. acinosa*. Aqueous extract usually contain glycosides and sugars. Saponin being a glycoside is further confirmation that the active compound could be a tritepene saponin. The fact that active compounds were extractable by water is advantageous since the famer is able to manage tick infestation without extra cost of buying organic solvents and laboratory equipment's for extraction of compounds.

5.3 Larvcidal activity of essential oils

Essential oils have been reported to have various bio-efficacies, which include; antibacterial, antifungal, antiviral, antiparasitic and insecticidal (Plaza *et al.,* 2004). The enormous bioactivity of the essential oils have been attributed to low molecular weight of the volatile components present in the oil enabling them to diffuse rapidly through the skin membranes and thus reaching target sites quickly (Gutierrez *et al.,* 2009). Low molecular weight of the volatile components have also made the essential oils to be highly concentrated with one drop of the oil being reported to contain 40 million–trillion molecules hence another reason for their amazing bioactivity (Stewart, 2005).

GC-MS analysis of essential oil of *L. kituiensis* showed sequiterpenes being dominant (56.57%), followed by monoterpenes (36.36%), diterpenes 2.59% and others (5.19%). Monoterpenoids and sesquiterpenoids isolated from many essential oils have been reported to

show repellent, chemosterilant, antifeeding, and biocidal activities against different acarus (Erdal *et al.,* 2009). The domination of sesquiterpenes in the present study compares with previous acaricidal studies on *Drimys brasiliensis* essential oil, which contained predominantly sesquiterpenes (66%) and it caused 100% mortality on the larvae of *R. microplus*, at concentrations of 2.5%, 1.25% and 0.625% (Ribeiro *et al.,* 2008). Camphore, limonene` and alpha pinene were among the major oil constituent of *L. kituiensis* essential oil. This is in agreement with previous review on *Lippia* species which have shown these compounds to be present in large amounts in this species (Pauscal *et al.,* 2001).

GC-MS analysis of oil of *L. kituiensis* in Tanzania differed with current study with camphor having 36.5% and 4-thujanol having 18.5% (Chogo and crank, 1982). This is because essential oils of the same *Lippia* species growing in different geographical areas have been found to vary substantially in composition because of climatic conditions, large genetic diversity within the species and stable genetic traits of individual plants (Catalan and de Lampasona, 2002). The larvicidal properties of both *L. kituiensis* and *L. javanica* essential oils are attributed to major components present in the oil which have shown insecticidal properties and (Park *et al.,* 2008) the resulting synergistic action with minor components identified in the oil (Iacobellis *et al.,* 2005). Among the major monoterpenes and sesquiterpenes identified in *L. kituiensis* oil in the current study, whose bioefficacies have been ascertained are alpha pinene (2.04%), camphor (3.49%), germacrene D (3.20%), camphene (7.26%), dl limonene (6.52%) and 4-terpeniol(3.03%).

Isolation of alpha pinene from synthetic mixture that simulated natural rosemary oil caused a decline in the acaricidal activity of the synthetic mixture against *Tetranychus urticae* mite by 80% hence a conclusion that alpha pinene is the major contributer to the acaricidal activity of *Rosmarinus officinalis* against the mite (Miresmailli *et al.,* 2006). Oil of *Laurus novocanariensis* leaf has been identified to be dorminated by alpha pinene (10.4%) and has been reported to have acaricidal activity of 100% against *Psoroptes cuniculi* mites at concentrations of 10% and 5% within 24 hrs (Macchioni *et al.,* 2006). Other studies have shown alpha pinene rich plant *Eucalyptus globulus* (9.93%), and dl-limonene rich plant *Eucalyptus staigeriana* have caused 100% mortality on gravid female ticks at concentrations of 15% and 12.5%, respectively in five different concentrations (Chagas *et al.,* 2002). Dl limonene obtained

from citrus peel oil has been shown to be toxic on all life stages of *Ctenocephalides felis* (Hink and Feel, 1986).

Camphor (56.07%) has been identified as a chief essential oil component from *Ocimum kilimandscharicum* (Runyoro *et al.,* 2010) thus contribute to a wide range of therapeutic importance such as antimicrobial, antispasmodic, bactericide, carminative, hepatoprotective, antiviral and larvicidal properties present in the oil (Nagai *et al.,* 2011). Commercial camphor and terpinen-4-ol were found to be 7 to 48 times more toxic against both male and female *A. obtectus* adults mite than the less active monoterpenes tested (Papachristos *et al.,* 2004). Besides being active these monoterpenes and sesquiterpenes identified in this study have been reported to have repellent effects on insects. Previous repellancy studies on essential oil constituents of *L. kituiensis* showed chemotype camphor to be the most repellent against maize weevils (*Sitophilus zeamais*) (Mwangi *et al.,* 1992). 4-terpinenol, Camphor and α-pinene have been reported to be repellent against nymphs of the sheep tick *I. ricinus* (Thorsell *et al.,* 2006). Germacrene-D, has been reported to be an effective arthropod repellent (Bruce *et al.,* 2005). α-Terpinelene have been reported to show high repellency against the brown ear tick *R. appendiculatus* Neumann (Lwande *et al.,* 1998) .

GC-MS analysis was not performed on oil of *L. javanica* beacause the yield of the oil was inadequate although previous studies has shown the oil is rich in linalool, myrecene, limonene germacrene d, alpha pinene and camphor (Viljoen *et al.,* 2005). Most of the essential oil constituents of *L. javanica* previously identified in past studies were also present in *L. kituiensis* in the current study, and this could be because they both belong to the same family. Among these constituents present in *L. javanica* whose bioactivity has been ascertained include myrcene, linalool, germacrene D and limonene. Myrcene has been found to be repellent against *R. appendiculatus* and *Sitophilus zeamais* Motschulsky (Ndungu *et al.,* 1995). Linalool obtained from *ocimum canum* has been reported to be toxic against *Zabrates subfasciatus* a coleopteran (Weaver *et al.,* 1991). Linalool isolated from molasses grass *Melinis minutiflora* was found to be lethal against *R. microplus* tick larvae causing 100% mortality in 15 min (Prates *et al.,* 1998). The antimicrobial activities of *L. javanica* have been attributed to presence of limonene, germaceren D and myrcene (Terblanche and Kornelius, 1996).

In the present study, the oil of *L. kitueinsis* caused 100% mortality at a low concentration of 4.5 mg/ml compared to the hexane extract which caused the same % mortality at higher concentrations of 25 mg/ml after the same duration of time which was 6 hrs. This is similar to aforementioned study in which the LC_{50} of the hexane extract of *Piper aduncum* was 9.30 mg/ml while 0.1 mg/ml of the essential oil of the same plant caused 100% mortality after the same duration of time (Silva *et al.,* 2009). The knockdown effects caused by both essential oils of *L. kituiensis* and *L. javanica* was faster than both positive controls. A shorter knockdown time by *L. multiflora* oil preparations has been observed compared to benzyl benzoate and Delvap Super® a brand of dichlorvos which were the positive controls against the body lice and head lice (Oladimeji *et al.,* 2000).

From the results of the bioassay, there was no significant difference in activity at 6, 12, 24 and 48 hrs in both oils of *L. javanica* and *L. kituiensis* $P>0.05$. This explains the fact that essential oils are volatile compounds, which are likely to evaporate, and are prone to rapid aerial oxidation and chemical re-arrangement hence loss of activity quickly. Therefore, further increase in time could not increase larval mortality (Birkett *et al.,* 2008). From Figure 25 and 26, increasing the concentration of the oil extracts caused an increase in the larval mortality and this could be because a more concentrated oil contain more of the essential oil components in large quantities compared to a less concentrated oil.

The essential oil of both *L. kituiensis* and *L. javanica* caused 100 % mortality at concentrations of 4.5 mg/ml and 4.0 mg/ml respectively within the first 6 hrs, while the positive control amitraz (0.2%v/v) reported the same mortality at 48 hrs. This revealed that the oil extracts were more active compared to the positive control and this can be explained by the fact that the positive control is made of a single active ingredient, compared to the oil extracts that had several ingredients which have been reported to have acaricidal effects in previous studies. This factor explains why there is development of resistance on synthetic acaricides compared to natural acaricides. It is in agreement with previous studies on development of resistance by green peach aphids (*Myzus persicae*) to pure azadirachtin (the major ingredient of neem insecticide), but not to a refined neem seed extract containing equivalent amount of azadirachtin but with other many constituents (Feng and Isman, 1995).

The exact mode of action of essential oils is not well understood, due to numerous active components present. Because of their volatility (Adorjan and Buchbauer, 2010) they could have been inhaled easily through the respiratory tract and lungs of the larvae which were then distributed in the bloodstream to various target sites. Since essential oil are fat soluble (Moss *et al.*, 2003) they might have permeated the membranes of the skin before being captured by the micro-circulation and drained into the systemic circulation thus reaching all targets organs. According to the poisoning symptoms observed in both oils, they both caused paralysis before dying (knockdown effects) which was observed immediately the oils came in contact with the larvae. This paralysis was characterized by tremors and convulsion followed by 100% mortality occurring within 30 minutes in both oils, at the highest concentrations which were 4.5 mg/ml for *L. kituiensis* and 4.0 mg/ml for *L. javanica*. Even though the first data was collected at 6 hrs, mortality had started way earlier. The same behavioral observation was observed in the positive control although convulsion and tremors persisted for a longer duration of approximately 12 hrs before being knockdown and 56.7% of the larvae dying at 24 hrs.

The observed paralytic effect of the oils could be due to synergistic action of alpha pinene and camphor on the nervous system, since both have been reported to have anti-acetylcholinesterase activity (Picollo *et al.*, 2008). The mode of action is believed to be reversible competitive inhibition of acetylcholinesterase by the occupation of hydrophobic site of the enzyme active site (Tapondjou *et al.*, 2005). Acetylcholinesterase is responsible for termination of cholinergic impulses by hydrolysis of acetylcholine released during synaptic transmission. Inhibition of acetylcholinenesterase leads to accumulation of acetylcholine at the synapses which causes it to rise several folds in comparison to the normal levels which leads to paralysis then death (Koelle, 1975). Linalool present in both *L. javanica* and *L. kituiensis* could have facilitated penetration of other essential oil constituents through skin and membrane of the larvae. This is because previous studies have shown this monoterpene as a substance which aid penetration of drugs through the cuticle of acari, hence has been proposed as an adjuvant in an acaricide production to help carry the active ingredient through the outer membrane of insect (Letizia *et al.*, 2003). Paralytic effects of amitraz were due to blockage of octopamine receptors, which lead to over-excitation and consequently paralysis and death in the larvae (Chena *et al.*, 2007).

5. 4 Cytotoxicity assays

Studies on the cytotoxicity of plant extracts are useful in evaluating the toxicological risks associated with the use of plant extracts. Cytotoxicity tests are vital before a plant extracts can be considered as a lead compound in drug discovery. Vero cells were used to asses cytotoxicity because livestock are made of cells which are prone to toxicity due to plant extracts in the process of tick control. Plants extracts which showed activity against tick larvae were subjected to cytotoxicity analysis. These plant extracts were methanol extracts of *L. kituiensis*, *P. dodecandra* and *P. viridiflorm*; hexane extracts of *L. kituiensis*, *P. viridiflorm* and aqueous extract of *P. dodecandra*. Cytotoxicity results indicated that all the extracts were considered noncytotoxic. This is because the IC_{50} values could not be calculated in all the extracts at concentrations used and for cytotoxicity to be observed, the extracts ought to have a concentrations more than 500 µg/ml.

The results of the current cytotoxicity study are in agreement with previous toxicity studies, which reported that extracts of these plants were nontoxic. A 28-day oral administration of extracts of unripe berries of *P. dodecandra* to rats in an acute mammalian test, showed the extract being nontoxic (Lambert *et al.*, 1991). Lemma and Ames, (1975) reported that extracts of *P. dodecandra* were neither mutagenic nor carcinogenic. Both human and guinea pigs have been reported to tolerate skin irritation by *P. dodecandra* (Mekonnen *et al.*, 2012). Investigation of cytotoxicity on Cheng cell line showed that *P. viridiflorum* extract were weakly cytotoxic with IC_{50} (µg/ml) of 246.95 ± 25.19 (Mbeng, 2012). Both hexane and acetone extracts of *P. viridiflorum*, were considered nontoxic against brine shrimp with LD_{50} values > 1 mg/ml (Otang, 2013). Cytotoxicity results of *L. kituiensis* are comparable to previous cytotoxicity studies on phylogenetic related plant *L. multiflora* which showed *L. multiflora* tea infusion were not toxic to vero cells and fibroblast cells (Terblanché, 2000). Generally, all the extracts exhibited selective toxicity, by being toxic to the larvae of *R. appendiculatus* and not to vero cells. These extracts are thus potential lead compounds for development of plant based acaricides because of their safety.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From this study, methanol, hexane, ethyl acetate and aqueous crude extracts of plants obtained from Baringo and Uasin Gishu counties and those obtained from previous literature possessed larvicidal activity against *R. appendiculatus* larvae. Essential oils obtained from *L. kituiensis* and *L. javanica* also possessed larvicidal activity against *R. appendiculatus* larvae. Methanol extracts containing all the phytochemicals tested were more active than methanol extracts which lacked some of the tested phytochemicals. This implies there was a synergistic effect of the tested phytochemicals in causing larval mortality. Synergistic action of major and minor components present in the essential oils was responsible for the larval mortality observed in the essential oils. No cytotoxicity was observed in all the crude extracts that had shown acaricidal activity against *R. appendiculatus* larvae, hence they were considered safe for practical use.

6.2 Recommendations

i. The fractionation, purification and identification of the active compound should be carried out on the crude extracts that showed activity against *R. appendiculatus* larvae.
ii. Although the essential oils were more active than the positive control, its activity was lost quickly hence, formulations to improve its potency and stability should be developed.
iii. Further studies should be done to determine the amount of each tested phytochemical
iv. Studies are needed to evaluate effects of these plant extracts on other life stages of the tick including adults and in *in vivo* conditions.

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APPENDICES

Appendix 1: Ectoparasite project questionnaire

A) General Information

Name……..Gender……

Telephone number…….Age……

County……..Location……

Source of this practice (parents, herbalists, and others)

B) Specific Information

Plants used in tick control

Plant name	Part used	Preparation	Dose
1)			
2)			
3)			
4)			

C) Source of plants named above (forests, roadsides, bushes, farms)

Plant name	Source	Approximate distance from home
1)		
2)		
3)		
4)		

D) Conservation status of each plant named (decreasing, increasing, no change)

Plant name	Status
1)	
2)	
3)	
4)	

Recommend on what need to be done on these plants so that they do not decrease in status……

……

Appendix 2: Preliminary screening results of methanol extracts

Methanol extracts At 50mg/ml	**No of tests**	**No of larvae per petri dish**	**No of larvae dead at the hrs shown below mortality**			
			6 hrs	**12 hrs**	**24 hrs**	**48 hrs**
Lippia kituiensis	1	20	0	0	4	20
	2	20	0	0	0	20
	3	20	0	0	6	20
Synadenium compactum	1	20	0	0	0	0
	2	20	0	0	0	6
	3	20	0	0	0	2
Phytolacca dodecandra	1	20	0	0	0	14
	2	20	0	0	0	10
	3	20	0	0	0	12
Pittosphorum viridiflorum	1	20	0	0	0	12
	2	20	0	0	0	16
	3	20	0	0	0	16
Amitraz (0.2 % v/v)[P]	1	20	0	10	20	20
	2	20	0	14	20	20
	3	20	0	10	14	20
(2% DMSO)[Q]	1	20	0	0	0	0
	2	20	0	0	0	0
	3	20	0	0	0	0

[P] Positive control; [Q] Negative control

Appendix 3: Bioassay results of the methanol extract of *L. kituiensis*

Concentration in mg/ml	**No of test**	**No of larvae per petri dish**	**No of larvae dead at the hrs shown below**			
			6 hrs	**12 hrs**	**24 hrs**	**48 hrs**
6	1	20	0	0	0	0
	2	20	0	0	0	0
	3	20	0	0	0	0
10	1	20	0	0	0	2
	2	20	0	0	0	4
	3	20	0	0	0	0
14	1	20	0	0	0	6
	2	20	0	0	0	0
	3	20	0	0	0	4
18	1	20	0	0	0	6
	2	20	0	0	0	10
	3	20	0	0	0	6
22	1	20	0	0	0	10
	2	20	0	0	0	8
	3	20	0	0	0	10
26	1	20	0	0	0	12
	2	20	0	0	0	14
	3	20	0	0	0	10
30	1	20	0	0	0	12
	2	20	0	0	0	18
	3	20	0	0	0	12
35	1	20	0	0	0	16
	2	20	0	0	0	16
	3	20	0	0	0	18
40	1	20	0	0	0	20
	2	20	0	0	0	20
	3	20	0	0	0	16
45	1	20	0	0	2	20
	2	20	0	0	2	20
	3	20	0	0	0	20
50	1	20	0	0	4	20
	2	20	0	0	0	20
	3	20	0	0	6	20
Amitraz (0.2 % v/v) [P]	1	20	0	10	20	20
	2	20	0	14	20	20
	3	20	0	10	14	20
(2% DMSO)[Q]	1	20	0	0	0	0
	2	20	0	0	0	0
	3	20	0	0	0	0

[P] Positive control; [Q] Negative control

Appendix 4: Generated LC values of methanol extract of *L. kituiensis* at 48 hrs

Confidence Limits

	Probability	95% Confidence Limits for con			95% Confidence Limits for log(con)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
	.010	7.250	3.967	9.961	.860	.598	.998
	.020	8.227	4.774	10.987	.915	.679	1.041
	.030	8.915	5.368	11.695	.950	.730	1.068
	.040	9.469	5.861	12.261	.976	.768	1.089
	.050	9.945	6.295	12.743	.998	.799	1.105
	.060	10.370	6.688	13.170	1.016	.825	1.120
	.070	10.757	7.053	13.557	1.032	.848	1.132
	.080	11.115	7.395	13.915	1.046	.869	1.143
	.090	11.452	7.720	14.250	1.059	.888	1.154
	.100	11.771	8.032	14.567	1.071	.905	1.163
	.150	13.188	9.450	15.970	1.120	.975	1.203
	.200	14.435	10.739	17.207	1.159	1.031	1.236
	.250	15.599	11.966	18.370	1.193	1.078	1.264
	.300	16.723	13.167	19.511	1.223	1.119	1.290
	.350	17.838	14.363	20.666	1.251	1.157	1.315
	.400	18.964	15.570	21.867	1.278	1.192	1.340
	.450	20.121	16.797	23.143	1.304	1.225	1.364
PROBIT	**.500**	**21.328**	**18.056**	**24.532**	**1.329**	**1.257**	**1.390**
	.550	22.608	19.356	26.076	1.354	1.287	1.416
	.600	23.988	20.709	27.830	1.380	1.316	1.445
	.650	25.502	22.132	29.866	1.407	1.345	1.475
	.700	27.201	23.656	32.286	1.435	1.374	1.509
	.750	29.162	25.326	35.245	1.465	1.404	1.547
	.800	31.513	27.225	39.004	1.498	1.435	1.591
	.850	34.493	29.506	44.061	1.538	1.470	1.644
	.900	**38.646**	**32.515**	**51.578**	**1.587**	**1.512**	**1.712**
	.910	39.722	33.270	53.606	1.599	1.522	1.729
	.920	40.925	34.103	55.910	1.612	1.533	1.747
	.930	42.289	35.036	58.569	1.626	1.545	1.768
	.940	43.867	36.101	61.702	1.642	1.558	1.790
	.950	45.739	37.346	65.497	1.660	1.572	1.816
	.960	48.040	38.851	70.276	1.682	1.589	1.847
	.970	51.029	40.771	76.659	1.708	1.610	1.885
	.980	55.291	43.447	86.095	1.743	1.638	1.935
	.990	62.743	47.983	103.477	1.798	1.681	2.015

a. Logarithm base = 10.

Appendix 5: Bioassay results of methanol extract of *Pittosphorum viridiflorum*

Concentration in mg/ml	No of test	No of larvae per petri dish	No of larvae dead at the hrs shown below			
			6 hrs	12 hrs	24 hrs	48 hrs
5	1	20	0	0	0	0
	2	20	0	0	0	0
	3	20	0	0	0	0
10	1	20	0	0	0	2
	2	20	0	0	0	0
	3	20	0	0	0	2
15	1	20	0	0	0	4
	2	20	0	0	0	0
	3	20	0	0	0	4
20	1	20	0	0	0	4
	2	20	0	0	0	6
	3	20	0	0	0	6
25	1	20	0	0	0	6
	2	20	0	0	0	4
	3	20	0	0	0	8
30	1	20	0	0	0	10
	2	20	0	0	0	8
	3	20	0	0	0	10
35	1	20	0	0	0	10
	2	20	0	0	0	10
	3	20	0	0	0	14
40	1	20	0	0	0	12
	2	20	0	0	0	10
	3	20	0	0	0	16
45	1	20	0	0	0	16
	2	20	0	0	0	10
	3	20	0	0	0	14
50	1	20	0	0	0	12
	2	20	0	0	0	16
	3	20	0	0	0	16
55	1	20	0	0	0	16
	2	20	0	0	0	16
	3	20	0	0	0	18
60	1	20	0	0	0	20
	2	20	0	0	0	16
	3	20	0	0	0	18
65	1	20	0	0	0	20
	2	20	0	0	0	20
	3	20	0	0	0	18
70	1	20	0	0	2	20
	2	20	0	0	0	20
	3	20	0	0	0	20
75	1	20	0	0	6	20
	2	20	0	0	0	20
	3	20	0	0	0	20
Amitraz (0.2 % v/v) [P]	1	20	0	10	20	20
	2	20	0	14	20	20
	3	20	0	10	14	20
(2% DMSO)[Q]	1	0	0	0	0	0
	2	0	0	0	0	0
	3	0	0	0	0	0

[P] Positive control ;[Q] Negative control

Appendix 6: Generated LC values of methanol extract of *P. viridiflorum* at 48 hrs

Confidence Limits

	Probability	95% Confidence Limits for con			95% Confidence Limits for log(con)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
	.010	8.147	4.329	11.647	.911	.636	1.066
	.020	9.510	5.372	13.162	.978	.730	1.119
	.030	10.491	6.158	14.228	1.021	.789	1.153
	.040	11.295	6.823	15.089	1.053	.834	1.179
	.050	11.994	7.415	15.831	1.079	.870	1.199
	.060	12.623	7.959	16.492	1.101	.901	1.217
	.070	13.201	8.468	17.096	1.121	.928	1.233
	.080	13.742	8.950	17.658	1.138	.952	1.247
	.090	14.252	9.411	18.186	1.154	.974	1.260
	.100	14.739	9.856	18.687	1.168	.994	1.272
	.150	16.939	11.924	20.933	1.229	1.076	1.321
	.200	18.918	13.854	22.941	1.277	1.142	1.361
	.250	20.800	15.735	24.849	1.318	1.197	1.395
	.300	22.649	17.616	26.736	1.355	1.246	1.427
	.350	24.509	19.528	28.657	1.389	1.291	1.457
	.400	26.415	21.495	30.663	1.422	1.332	1.487
	.450	28.399	23.537	32.808	1.453	1.372	1.516
PROBIT	**.500**	**30.498**	**25.671**	**35.152**	**1.484**	**1.409**	**1.546**
	.550	32.752	27.918	37.773	1.515	1.446	1.577
	.600	35.213	30.300	40.773	1.547	1.481	1.610
	.650	37.951	32.854	44.290	1.579	1.517	1.646
	.700	41.067	35.635	48.520	1.613	1.552	1.686
	.750	44.718	38.737	53.764	1.650	1.588	1.730
	.800	49.166	42.329	60.531	1.692	1.627	1.782
	.850	54.912	46.733	69.807	1.740	1.670	1.844
	.900	**63.106**	**52.684**	**83.917**	**1.800**	**1.722**	**1.924**
	.910	65.262	54.200	87.783	1.815	1.734	1.943
	.920	67.688	55.885	92.204	1.831	1.747	1.965
	.930	70.459	57.786	97.343	1.848	1.762	1.988
	.940	73.688	59.971	103.447	1.867	1.778	2.015
	.950	77.552	62.547	110.908	1.890	1.796	2.045
	.960	82.352	65.695	120.400	1.916	1.818	2.081
	.970	88.661	69.756	133.243	1.948	1.844	2.125
	.980	97.803	75.504	152.544	1.990	1.878	2.183
	.990	114.163	85.457	188.979	2.058	1.932	2.276

a. Logarithm base = 10.

Appendix 7: Bioassay results of methanol extract of *Phytolacca dodecandra*

Concentration in mg/ml	**No of test**	**No of larvae per petri dish**	**No of larvae dead at the hrs shown below**			
			6 hrs	**12 hrs**	**24 hrs**	**48 hrs**
5	1	20	0	0	0	0
	2	20	0	0	0	0
	3	20	0	0	0	0
10	1	20	0	0	0	2
	2	20	0	0	0	0
	3	20	0	0	0	0
20	1	20	0	0	0	4
	2	20	0	0	0	4
	3	20	0	0	0	8
30	1	20	0	0	0	6
	2	20	0	0	0	4
	3	20	0	0	0	10
40	1	20	0	0	0	8
	2	20	0	0	0	10
	3	20	0	0	0	6
50	1	20	0	0	0	12
	2	20	0	0	0	12
	3	20	0	0	0	10
60	1	20	0	0	0	10
	2	20	0	0	0	14
	3	20	0	0	0	18
70	1	20	0	0	0	14
	2	20	0	0	0	16
	3	20	0	0	0	18
80	1	20	0	0	0	18
	2	20	0	0	0	18
	3	20	0	0	0	20
90	1	20	0	0	0	20
	2	20	0	0	0	20
	3	20	0	0	0	18
100	1	20	0	0	2	20
	2	20	0	0	0	20
	3	20	0	0	2	20
Amitraz (0.2 % v/v) [P]	1	20	0	10	20	20
	2	20	0	14	20	20
	3	20	0	10	14	20
(2% DMSO)[Q]	1	20	0	0	0	0
	2	20	0	0	0	0
	3	20	0	0	0	0

[P] Positive control; [Q] Negative control

Appendix 8: Generated LC values of methanol extract of *P. dodecandra* at 48 hrs

Confidence Limits

	Probability	95% Confidence Limits for con			95% Confidence Limits for log(con)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
	.010	9.664	4.037	14.960	.985	.606	1.175
	.020	11.385	5.175	16.948	1.056	.714	1.229
	.030	12.632	6.055	18.351	1.101	.782	1.264
	.040	13.660	6.813	19.487	1.135	.833	1.290
	.050	14.557	7.498	20.467	1.163	.875	1.311
	.060	15.367	8.133	21.342	1.187	.910	1.329
	.070	16.115	8.734	22.144	1.207	.941	1.345
	.080	16.815	9.308	22.889	1.226	.969	1.360
	.090	17.477	9.862	23.591	1.242	.994	1.373
	.100	18.111	10.399	24.259	1.258	1.017	1.385
	.150	20.986	12.942	27.261	1.322	1.112	1.436
	.200	23.593	15.372	29.963	1.373	1.187	1.477
	.250	26.087	17.787	32.548	1.416	1.250	1.513
	.300	28.550	20.240	35.124	1.456	1.306	1.546
	.350	31.040	22.768	37.769	1.492	1.357	1.577
	.400	33.603	25.399	40.558	1.526	1.405	1.608
	.450	36.284	28.156	43.571	1.560	1.450	1.639
PROBIT	**.500**	**39.131**	**31.060**	**46.907**	**1.593**	**1.492**	**1.671**
	.550	42.202	34.133	50.692	1.625	1.533	1.705
	.600	45.569	37.401	55.094	1.659	1.573	1.741
	.650	49.332	40.904	60.345	1.693	1.612	1.781
	.700	53.634	44.710	66.782	1.729	1.650	1.825
	.750	58.698	48.937	74.923	1.769	1.690	1.875
	.800	64.902	53.804	85.655	1.812	1.731	1.933
	.850	72.966	59.739	100.711	1.863	1.776	2.003
	.900	**84.550**	**67.717**	**124.251**	**1.927**	**1.831**	**2.094**
	.910	87.614	69.744	130.818	1.943	1.844	2.117
	.920	91.068	71.996	138.384	1.959	1.857	2.141
	.930	95.023	74.535	147.251	1.978	1.872	2.168
	.940	99.644	77.452	157.879	1.998	1.889	2.198
	.950	105.188	80.889	171.000	2.022	1.908	2.233
	.960	112.098	85.087	187.893	2.050	1.930	2.274
	.970	121.218	90.501	211.075	2.084	1.957	2.324
	.980	134.500	98.164	246.556	2.129	1.992	2.392
	.990	158.451	111.439	315.372	2.200	2.047	2.499

a. Logarithm base = 10.

Appendix 9: Screening results of hexane, ethyl acetate and aqueous extracts

Extracts at 25 mg/ml	**Test**	**No of larvae per petri dish**	**No of ticks dead at the hrs shown below**			
			6hrs	**12 hrs**	**24 hrs**	**48 hrs**
Aqueous extract of *L. kituiensis*	1 2 3	20 20 20	0 0 0	0 0 0	0 0 0	0 0 0
Hexane extract of *L. kituiensis*	1 2 3	20 20 20	20 20 20	20 20 20	20 20 20	20 20 20
Ethly acetate extract of *L. kituiensis*	1 2 3	20 20 20	0 0 0	0 0 0	0 0 0	4 0 2
Aqueous extract of *P. viridiflorum*	1 2 3	20 20 20	0 0 0	0 0 0	0 0 0	2 0 2
Hexane extract of *P. viridiflorum*	1 2 3	20 20 20	0 0 0	0 0 0	0 0 0	16 12 8
Ethly acetate extract *P. viridiflorum*	1 2 3	20 20 20	0 0 0	0 0 0	0 0 0	4 0 6
Aqueous extract of *P. dodecandra*	1 2 3	20 20 20	0 0 0	0 0 0	0 0 0	16 18 20
Hexane extract of *P. dodecandra*	1 2 3	20 20 20	0 0 0	0 0 0	0 0 0	0 2 0
Ethly acetate extract of *P. dodecandra*	1 2 3	20 20 20	0 0 0	0 0 0	0 0 0	0 2 2
Amitraz (0.2 % v/v) [P]	1 2 3	20 20 20	0 0 0	10 14 10	20 20 14	20 20 20
(2% DMSO)[Q]	1 2 3	10 10 10	0 0 0	0 0 0	0 0 0	0 0 0

[P] positive control ;[Q] negative control

Appendix 10: Larval bioassay results of hexane extract of *L. kituiensis*

Concentration in mg/ml	**No of test**	**No of larvae per petri dish**	**No of ticks dead at the hrs shown below**			
			6 hrs	**12 hrs**	**24 hrs**	**48 hrs**
5	1	20	0	2	2	10
	2	20	0	0	0	14
	3	20	0	2	4	12
7.5	1	20	0	2	8	12
	2	20	4	6	14	20
	3	20	4	4	8	16
10	1	20	6	12	18	20
	2	20	4	8	16	20
	3	20	6	6	14	20
12	1	20	6	12	18	20
	2	20	12	10	20	20
	3	20	6	12	20	20
14	1	20	8	8	20	20
	2	20	10	12	20	20
	3	20	12	16	20	20
16	1	20	16	20	20	20
	2	20	16	16	20	20
	3	20	10	14	20	20
18	1	20	20	20	20	20
	2	20	16	20	20	20
	3	20	16	18	20	20
20	1	20	20	20	20	20
	2	20	18	20	20	20
	3	20	18	20	20	20
23	1	20	20	20	20	20
	2	20	20	20	20	20
	3	20	20	20	20	20
25	1	20	20	20	20	20
	2	20	20	20	20	20
	3	20	20	20	20	20
Amitraz (0.2 % v/v) [P]	1	20	0	10	20	20
	2	20	0	14	20	20
	3	20	0	10	14	20
(2% DMSO)[Q]	1	20	0	0	0	0
	2	20	0	0	0	0
	3	20	0	0	0	0

[P] Postive control ;[Q] Negative control

Appendix 11: Generated LC values of hexane extract of *L. kituiensis* at 6 hrs

Confidence Limits

	Probability	95% Confidence Limits for con			95% Confidence Limits for log(con)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
	.010	5.713	3.534	7.294	.757	.548	.863
	.020	6.268	4.061	7.831	.797	.609	.894
	.030	6.647	4.435	8.194	.823	.647	.913
	.040	6.947	4.737	8.479	.842	.676	.928
	.050	7.202	4.998	8.720	.857	.699	.941
	.060	7.425	5.231	8.931	.871	.719	.951
	.070	7.627	5.444	9.121	.882	.736	.960
	.080	7.813	5.641	9.295	.893	.751	.968
	.090	7.985	5.826	9.458	.902	.765	.976
	.100	8.148	6.001	9.610	.911	.778	.983
	.150	8.855	6.781	10.274	.947	.831	1.012
	.200	9.461	7.463	10.847	.976	.873	1.035
	.250	10.014	8.094	11.377	1.001	.908	1.056
	.300	10.537	8.696	11.889	1.023	.939	1.075
	.350	11.047	9.281	12.399	1.043	.968	1.093
	.400	11.554	9.860	12.922	1.063	.994	1.111
	.450	12.066	10.437	13.470	1.082	1.019	1.129
PROBIT	**.500**	**12.592**	**11.017**	**14.059**	**1.100**	**1.042**	**1.148**
	.550	13.141	11.605	14.703	1.119	1.065	1.167
	.600	13.724	12.206	15.425	1.137	1.087	1.188
	.650	14.353	12.827	16.250	1.157	1.108	1.211
	.700	15.048	13.479	17.214	1.177	1.130	1.236
	.750	15.835	14.180	18.371	1.200	1.152	1.264
	.800	16.760	14.959	19.809	1.224	1.175	1.297
	.850	17.906	15.872	21.694	1.253	1.201	1.336
	.900	**19.461**	**17.045**	**24.403**	**1.289**	**1.232**	**1.387**
	.910	19.857	17.333	25.118	1.298	1.239	1.400
	.920	20.295	17.650	25.921	1.307	1.247	1.414
	.930	20.789	18.001	26.839	1.318	1.255	1.429
	.940	21.354	18.399	27.907	1.329	1.265	1.446
	.950	22.018	18.860	29.183	1.343	1.276	1.465
	.960	22.824	19.411	30.764	1.358	1.288	1.488
	.970	23.855	20.105	32.837	1.378	1.303	1.516
	.980	25.298	21.056	35.824	1.403	1.323	1.554
	.990	27.753	22.632	41.124	1.443	1.355	1.614

a. Logarithm base = 10.

Appendix 12: Generated LC values of hexane extract of *L. kituiensis* at 12 hrs

Confidence Limits

	Probability	95% Confidence Limits for con			95% Confidence Limits for log(con)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
	.010	4.341	2.492	5.770	.638	.397	.761
	.020	4.821	2.914	6.253	.683	.465	.796
	.030	5.152	3.217	6.583	.712	.507	.818
	.040	5.416	3.465	6.843	.734	.540	.835
	.050	5.641	3.681	7.063	.751	.566	.849
	.060	5.840	3.874	7.257	.766	.588	.861
	.070	6.020	4.052	7.432	.780	.608	.871
	.080	6.185	4.218	7.592	.791	.625	.880
	.090	6.340	4.374	7.742	.802	.641	.889
	.100	6.486	4.523	7.883	.812	.655	.897
	.150	7.127	5.190	8.500	.853	.715	.929
	.200	7.681	5.783	9.035	.885	.762	.956
	.250	8.190	6.340	9.531	.913	.802	.979
	.300	8.676	6.877	10.011	.938	.837	1.000
	.350	9.153	7.407	10.489	.962	.870	1.021
	.400	9.629	7.936	10.979	.984	.900	1.041
	.450	10.113	8.472	11.492	1.005	.928	1.060
PROBIT	**.500**	**10.613**	**9.018**	**12.042**	**1.026**	**.955**	**1.081**
	.550	11.138	9.580	12.643	1.047	.981	1.102
	.600	11.698	10.163	13.317	1.068	1.007	1.124
	.650	12.307	10.773	14.090	1.090	1.032	1.149
	.700	12.983	11.421	14.999	1.113	1.058	1.176
	.750	13.753	12.123	16.099	1.138	1.084	1.207
	.800	14.666	12.909	17.482	1.166	1.111	1.243
	.850	15.806	13.835	19.321	1.199	1.141	1.286
	.900	**17.367**	**15.028**	**22.011**	**1.240**	**1.177**	**1.343**
	.910	17.766	15.322	22.728	1.250	1.185	1.357
	.920	18.211	15.645	23.538	1.260	1.194	1.372
	.930	18.712	16.005	24.467	1.272	1.204	1.389
	.940	19.289	16.413	25.555	1.285	1.215	1.407
	.950	19.968	16.886	26.862	1.300	1.228	1.429
	.960	20.797	17.453	28.492	1.318	1.242	1.455
	.970	21.863	18.170	30.645	1.340	1.259	1.486
	.980	23.366	19.157	33.780	1.369	1.282	1.529
	.990	25.946	20.802	39.425	1.414	1.318	1.596

a. Logarithm base = 10.

Appendix 13: Generated LC values of hexane extract of *L. kituiensis* at 24 hrs

Confidence Limits

	Probability	95% Confidence Limits for con			95% Confidence Limits for log(con)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
	.010	2.853	1.258	4.024	.455	.100	.605
	.020	3.154	1.496	4.328	.499	.175	.636
	.030	3.361	1.668	4.534	.526	.222	.656
	.040	3.526	1.811	4.697	.547	.258	.672
	.050	3.666	1.936	4.834	.564	.287	.684
	.060	3.789	2.049	4.954	.579	.311	.695
	.070	3.901	2.153	5.062	.591	.333	.704
	.080	4.004	2.250	5.162	.602	.352	.713
	.090	4.100	2.342	5.254	.613	.370	.721
	.100	4.190	2.431	5.341	.622	.386	.728
	.150	4.585	2.830	5.721	.661	.452	.757
	.200	4.926	3.190	6.049	.693	.504	.782
	.250	5.238	3.532	6.353	.719	.548	.803
	.300	5.536	3.866	6.645	.743	.587	.822
	.350	5.826	4.198	6.936	.765	.623	.841
	.400	6.116	4.534	7.233	.786	.656	.859
	.450	6.410	4.877	7.544	.807	.688	.878
PROBIT	**.500**	**6.714**	**5.231**	**7.878**	**.827**	**.719**	**.896**
	.550	7.031	5.598	8.243	.847	.748	.916
	.600	7.369	5.983	8.654	.867	.777	.937
	.650	7.736	6.388	9.129	.889	.805	.960
	.700	8.142	6.818	9.695	.911	.834	.987
	.750	8.604	7.282	10.393	.935	.862	1.017
	.800	9.150	7.793	11.289	.961	.892	1.053
	.850	9.830	8.382	12.511	.993	.923	1.097
	.900	**10.757**	**9.117**	**14.346**	**1.032**	**.960**	**1.157**
	.910	10.994	9.294	14.843	1.041	.968	1.172
	.920	11.257	9.488	15.408	1.051	.977	1.188
	.930	11.554	9.702	16.060	1.063	.987	1.206
	.940	11.894	9.942	16.829	1.075	.997	1.226
	.950	12.295	10.218	17.758	1.090	1.009	1.249
	.960	12.783	10.547	18.927	1.107	1.023	1.277
	.970	13.410	10.957	20.484	1.127	1.040	1.311
	.980	14.291	11.517	22.777	1.155	1.061	1.357
	.990	15.798	12.436	26.967	1.199	1.095	1.431

a. Logarithm base = 10.

Appendix 14: Generated LC values of hexane extract of *L. kituiensis* at 48 hrs

Confidence Limits

	Probability	95% Confidence Limits for con			95% Confidence Limits for log(con)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
	.010	1.991	.138	3.315	.299	-.859	.520
	.020	2.206	.192	3.530	.344	-.717	.548
	.030	2.353	.236	3.675	.372	-.626	.565
	.040	2.471	.276	3.788	.393	-.558	.578
	.050	2.571	.314	3.884	.410	-.503	.589
	.060	2.660	.350	3.967	.425	-.456	.598
	.070	2.740	.384	4.042	.438	-.415	.607
	.080	2.814	.418	4.110	.449	-.378	.614
	.090	2.882	.452	4.174	.460	-.345	.621
	.100	2.947	.485	4.234	.469	-.314	.627
	.150	3.231	.650	4.492	.509	-.187	.652
	.200	3.476	.819	4.714	.541	-.087	.673
	.250	3.701	.998	4.917	.568	-.001	.692
	.300	3.916	1.191	5.112	.593	.076	.709
	.350	4.126	1.401	5.305	.615	.146	.725
	.400	4.335	1.633	5.502	.637	.213	.741
	.450	4.548	1.890	5.709	.658	.277	.757
PROBIT	**.500**	**4.768**	**2.179**	**5.931**	**.678**	**.338**	**.773**
	.550	4.998	2.506	6.177	.699	.399	.791
	.600	5.243	2.877	6.462	.720	.459	.810
	.650	5.510	3.301	6.807	.741	.519	.833
	.700	5.805	3.784	7.250	.764	.578	.860
	.750	6.141	4.328	7.862	.788	.636	.896
	.800	6.539	4.926	8.781	.815	.692	.944
	.850	7.035	5.562	10.286	.847	.745	1.012
	.900	**7.713**	**6.247**	**13.019**	**.887**	**.796**	**1.115**
	.910	7.886	6.397	13.841	.897	.806	1.141
	.920	8.079	6.555	14.815	.907	.817	1.171
	.930	8.296	6.723	15.988	.919	.828	1.204
	.940	8.546	6.905	17.434	.932	.839	1.241
	.950	8.840	7.108	19.274	.946	.852	1.285
	.960	9.198	7.342	21.722	.964	.866	1.337
	.970	9.658	7.625	25.210	.985	.882	1.402
	.980	10.306	7.998	30.806	1.013	.903	1.489
	.990	11.416	8.590	42.415	1.058	.934	1.628

a. Logarithm base = 10.

Appendix 15: Larval bioassay results of hexane extract of *P. viridiflorum*

Concentration in mg/ml	**No of test**	**No of larvae per petri dish**	**No of ticks dead at the hrs shown below**			
			6 hrs	**12 hrs**	**24 hrs**	**48 hrs**
5	1 2 3	20 20 20	0 0 0	0 0 0	0 0 0	0 0 0
10	1 2 3	20 20 20	0 0 0	0 0 0	0 0 0	2 4 0
15	1 2 3	20 20 20	0 0 0	0 0 0	0 0 0	6 6 4
20	1 2 3	20 20 20	0 0 0	0 0 0	0 0 0	6 12 6
25	1 2 3	20 20 20	0 0 0	0 0 0	0 0 0	10 12 12
30	1 2 3	20 20 20	0 0 0	0 0 0	0 0 0	12 16 10
35	1 2 3	20 20 20	0 0 0	0 0 0	0 0 0	16 10 16
40	1 2 3	20 20 20	0 0 0	0 0 0	0 0 0	16 14 18
45	1 2 3	20 20 20	0 0 0	0 0 0	0 2 0	20 20 18
50	1 2 3	20 20 20	0 0 0	0 0 0	4 0 0	20 20 20
Amitraz (0.2 % v/v) [P]	1 2 3	20 20 20	0 0 0	10 14 10	20 20 14	20 20 20
(2% DMSO)[Q]	1 2 3	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0

[P] Positive control ;[Q] Negative control

Appendix 16: Generated LC values of hexane extract of *P. viridiflorum* at 48 hrs

Confidence Limits

	Probability	95% Confidence Limits for con			95% Confidence Limits for log(con)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
	.010	6.257	2.728	9.357	.796	.436	.971
	.020	7.269	3.441	10.476	.861	.537	1.020
	.030	7.994	3.985	11.259	.903	.600	1.051
	.040	8.587	4.450	11.888	.934	.648	1.075
	.050	9.102	4.867	12.429	.959	.687	1.094
	.060	9.564	5.252	12.910	.981	.720	1.111
	.070	9.989	5.614	13.349	1.000	.749	1.125
	.080	10.385	5.958	13.756	1.016	.775	1.138
	.090	10.759	6.289	14.138	1.032	.799	1.150
	.100	11.115	6.609	14.501	1.046	.820	1.161
	.150	12.720	8.109	16.122	1.104	.909	1.207
	.200	14.158	9.523	17.569	1.151	.979	1.245
	.250	15.521	10.912	18.947	1.191	1.038	1.278
	.300	16.857	12.308	20.315	1.227	1.090	1.308
	.350	18.197	13.731	21.716	1.260	1.138	1.337
	.400	19.567	15.196	23.192	1.292	1.182	1.365
	.450	20.991	16.713	24.789	1.322	1.223	1.394
PROBIT	**.500**	**22.493**	**18.290**	**26.558**	**1.352**	**1.262**	**1.424**
	.550	24.102	19.936	28.570	1.382	1.300	1.456
	.600	25.856	21.659	30.912	1.413	1.336	1.490
	.650	27.803	23.479	33.704	1.444	1.371	1.528
	.700	30.013	25.427	37.115	1.477	1.405	1.570
	.750	32.596	27.565	41.404	1.513	1.440	1.617
	.800	35.734	30.002	47.009	1.553	1.477	1.672
	.850	39.776	32.945	54.789	1.600	1.518	1.739
	.900	**45.517**	**36.863**	**66.789**	**1.658**	**1.567**	**1.825**
	.910	47.023	37.853	70.107	1.672	1.578	1.846
	.920	48.716	38.949	73.916	1.688	1.591	1.869
	.930	50.649	40.182	78.361	1.705	1.604	1.894
	.940	52.897	41.594	83.665	1.723	1.619	1.923
	.950	55.584	43.252	90.181	1.745	1.636	1.955
	.960	58.916	45.269	98.521	1.770	1.656	1.994
	.970	63.286	47.858	109.886	1.801	1.680	2.041
	.980	69.602	51.498	127.124	1.843	1.712	2.104
	.990	80.861	57.746	160.116	1.908	1.762	2.204

a. Logarithm base = 10.

Appendix 17: Larval bioassay results of aqueous extract of *P. dodecandra*

Concentration of extracts in mg/ml	**No of test**	**No of larvae per petri dish**	**No of ticks dead at the hrs shown below**			
			6	**12**	**24**	**48**
7	1 2 3	20 20 20	0 0 0	0 0 0	0 0 0	0 0 0
10	1 2 3	20 20 20	0 0 0	0 0 0	0 0 0	2 4 2
13	1 2 3	20 20 20	0 0 0	0 0 0	0 0 0	0 6 6
16	1 2 3	20 20 20	0 0 0	0 0 0	0 0 0	6 8 4
19	1 2 3	20 20 20	0 0 0	0 0 0	0 0 0	12 12 10
22	1 2 3	20 20 20	0 0 0	0 0 0	0 0 0	18 12 12
25	1 2 3	20 20 20	0 0 0	0 0 0	0 0 0	16 20 16
27	1 2 3	20 20 20	0 0 0	0 0 0	0 0 0	20 20 16
30	1 2 3	20 20 20	0 0 0	0 0 0	0 0 2	20 20 20
Amitraz (0.2 % v/v) [P]	1 2 3	20 20 20	0 0 0	10 14 10	20 20 14	20 20 20
(2% DMSO)[Q]	1 2 3	20 20 20	0 0 0	0 0 0	0 0 0	0 0 0

[P] Positoive control; [Q] Negative control

Appendix 18: Generated LC values of aqueous extract of *P. dodecandra* at 48 hrs

Confidence Limits

	Probability	95% Confidence Limits for con			95% Confidence Limits for log(con)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
	.010	7.795	4.773	9.957	.892	.679	.998
	.020	8.558	5.503	10.694	.932	.741	1.029
	.030	9.081	6.021	11.192	.958	.780	1.049
	.040	9.495	6.441	11.584	.977	.809	1.064
	.050	9.846	6.803	11.915	.993	.833	1.076
	.060	10.154	7.127	12.206	1.007	.853	1.087
	.070	10.433	7.423	12.467	1.018	.871	1.096
	.080	10.689	7.698	12.707	1.029	.886	1.104
	.090	10.927	7.956	12.931	1.039	.901	1.112
	.100	11.151	8.200	13.141	1.047	.914	1.119
	.150	12.128	9.287	14.060	1.084	.968	1.148
	.200	12.966	10.239	14.855	1.113	1.010	1.172
	.250	13.730	11.118	15.594	1.138	1.046	1.193
	.300	14.455	11.956	16.311	1.160	1.078	1.212
	.350	15.160	12.769	17.031	1.181	1.106	1.231
	.400	15.862	13.569	17.772	1.200	1.133	1.250
	.450	16.571	14.363	18.556	1.219	1.157	1.268
PROBIT	**.500**	**17.301**	**15.158**	**19.402**	**1.238**	**1.181**	**1.288**
	.550	18.062	15.960	20.334	1.257	1.203	1.308
	.600	18.870	16.775	21.381	1.276	1.225	1.330
	.650	19.743	17.614	22.580	1.295	1.246	1.354
	.700	20.707	18.493	23.982	1.316	1.267	1.380
	.750	21.800	19.437	25.665	1.338	1.289	1.409
	.800	23.085	20.489	27.753	1.363	1.312	1.443
	.850	24.679	21.726	30.488	1.392	1.337	1.484
	.900	**26.842**	**23.318**	**34.421**	**1.429**	**1.368**	**1.537**
	.910	27.392	23.711	35.458	1.438	1.375	1.550
	.920	28.002	24.141	36.625	1.447	1.383	1.564
	.930	28.689	24.620	37.959	1.458	1.391	1.579
	.940	29.476	25.162	39.512	1.469	1.401	1.597
	.950	30.400	25.790	41.368	1.483	1.411	1.617
	.960	31.523	26.542	43.671	1.499	1.424	1.640
	.970	32.961	27.489	46.691	1.518	1.439	1.669
	.980	34.973	28.790	51.051	1.544	1.459	1.708
	.990	38.398	30.944	58.806	1.584	1.491	1.769

a. Logarithm base = 10.

Appendix 19: Larval bioassay results of *L. kituiensis* essential oil

Concentration used in mg/ml	**test**	**No of larvae per petri dish**	**No of ticks dead at the hrs shown below**			
			6 hrs	**12 hrs**	**24 hrs**	**48 hrs**
1.5	1	20	0	0	0	0
	2	20	0	0	0	0
	3	20	0	0	0	0
2.0	1	20	0	0	0	0
	2	20	0	0	0	0
	3	20	1	1	1	1
2.2	1	20	2	2	2	2
	2	20	0	0	0	0
	3	20	0	0	0	0
2.5	1	20	2	2	2	3
	2	20	3	3	3	3
	3	20	2	2	2	2
2.8	1	20	5	5	6	6
	2	20	3	3	4	6
	3	20	4	4	4	5
3.0	1	20	8	9	10	10
	2	20	7	7	8	9
	3	20	6	7	9	9
3.3	1	20	7	9	10	13
	2	20	10	11	12	11
	3	20	9	10	9	10
3.5	1	20	10	11	12	12
	2	20	9	10	12	13
	3	20	12	13	13	14
3.7	1	20	13	14	15	16
	2	20	12	13	13	14
	3	20	13	13	14	15
3.9	1	20	17	17	17	17
	2	20	15	16	17	18
	3	20	17	18	19	20
4.0	1	20	19	20	20	20
	2	20	18	19	20	20
	3	20	17	19	20	20
4.2	1	20	19	20	20	20
	2	20	20	20	20	20
	3	20	20	20	20	20
4.5	1	20	20	20	20	20
	2	20	20	20	20	20
	3	20	20	20	20	20
Amitraz	1	20	0	10	20	20
(0.2 % v/v) [P]	2	20	0	14	20	20
	3	20	0	10	14	20
(2% DMSO)[Q]	1	20	0	0	0	0
	2	20	0	0	0	0
	3	20	0	0	0	0

[P] positive control ;[Q] Negative control

Appendix 20: Generated LC values of *L. kituiensis* essential oil at 6 hrs

Confidence Limits

	Probability	95% Confidence Limits for con			95% Confidence Limits for log(con)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
	.010	2.106	1.840	2.303	.323	.265	.362
	.020	2.217	1.963	2.404	.346	.293	.381
	.030	2.290	2.045	2.470	.360	.311	.393
	.040	2.346	2.108	2.521	.370	.324	.402
	.050	2.394	2.162	2.564	.379	.335	.409
	.060	2.434	2.208	2.601	.386	.344	.415
	.070	2.471	2.249	2.634	.393	.352	.421
	.080	2.504	2.286	2.664	.399	.359	.426
	.090	2.534	2.321	2.691	.404	.366	.430
	.100	2.562	2.353	2.717	.409	.372	.434
	.150	2.683	2.491	2.827	.429	.396	.451
	.200	2.783	2.605	2.918	.445	.416	.465
	.250	2.872	2.707	3.000	.458	.432	.477
	.300	2.954	2.800	3.077	.470	.447	.488
	.350	3.032	2.887	3.151	.482	.460	.498
	.400	3.108	2.971	3.225	.492	.473	.509
	.450	3.183	3.053	3.300	.503	.485	.519
PROBIT	**.500**	**3.259**	**3.134**	**3.378**	**.513**	**.496**	**.529**
	.550	3.337	3.215	3.460	.523	.507	.539
	.600	3.418	3.297	3.548	.534	.518	.550
	.650	3.504	3.381	3.645	.545	.529	.562
	.700	3.596	3.468	3.752	.556	.540	.574
	.750	3.699	3.562	3.876	.568	.552	.588
	.800	3.817	3.667	4.021	.582	.564	.604
	.850	3.959	3.790	4.201	.598	.579	.623
	.900	**4.145**	**3.946**	**4.442**	**.618**	**.596**	**.648**
	.910	4.192	3.985	4.503	.622	.600	.654
	.920	4.243	4.027	4.571	.628	.605	.660
	.930	4.299	4.073	4.646	.633	.610	.667
	.940	4.364	4.125	4.732	.640	.615	.675
	.950	4.438	4.185	4.832	.647	.622	.684
	.960	4.527	4.257	4.953	.656	.629	.695
	.970	4.639	4.346	5.106	.666	.638	.708
	.980	4.792	4.467	5.318	.681	.650	.726
	.990	5.043	4.664	5.671	.703	.669	.754

a. Logarithm base = 10.

Appendix 21: Generated LC values of *L. kituiensis* essential oil at 12 hrs

Confidence Limits

	Probability	95% Confidence Limits for con			95% Confidence Limits for log(con)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
	.010	2.114	1.858	2.303	.325	.269	.362
	.020	2.219	1.975	2.400	.346	.296	.380
	.030	2.289	2.053	2.463	.360	.312	.391
	.040	2.343	2.114	2.512	.370	.325	.400
	.050	2.388	2.164	2.552	.378	.335	.407
	.060	2.426	2.208	2.588	.385	.344	.413
	.070	2.461	2.247	2.619	.391	.352	.418
	.080	2.492	2.283	2.647	.397	.359	.423
	.090	2.521	2.316	2.673	.402	.365	.427
	.100	2.548	2.346	2.698	.406	.370	.431
	.150	2.662	2.477	2.802	.425	.394	.447
	.200	2.757	2.584	2.888	.440	.412	.461
	.250	2.840	2.680	2.965	.453	.428	.472
	.300	2.918	2.767	3.037	.465	.442	.483
	.350	2.991	2.850	3.107	.476	.455	.492
	.400	3.063	2.929	3.176	.486	.467	.502
	.450	3.133	3.006	3.246	.496	.478	.511
PROBIT	**.500**	**3.205**	**3.083**	**3.319**	**.506**	**.489**	**.521**
	.550	3.277	3.159	3.395	.516	.500	.531
	.600	3.353	3.236	3.476	.525	.510	.541
	.650	3.433	3.315	3.565	.536	.521	.552
	.700	3.520	3.398	3.664	.547	.531	.564
	.750	3.616	3.487	3.777	.558	.542	.577
	.800	3.725	3.586	3.910	.571	.555	.592
	.850	3.857	3.702	4.074	.586	.568	.610
	.900	**4.030**	**3.849**	**4.295**	**.605**	**.585**	**.633**
	.910	4.073	3.885	4.351	.610	.589	.639
	.920	4.120	3.924	4.413	.615	.594	.645
	.930	4.173	3.967	4.482	.620	.598	.651
	.940	4.232	4.016	4.560	.627	.604	.659
	.950	4.301	4.072	4.652	.634	.610	.668
	.960	4.383	4.139	4.762	.642	.617	.678
	.970	4.486	4.222	4.901	.652	.626	.690
	.980	4.627	4.335	5.093	.665	.637	.707
	.990	4.859	4.517	5.413	.687	.655	.733

a. Logarithm base = 10.

Appendix 22: Generated LC values of *L. kituiensis* essential oil at 24 hrs

Confidence Limits

	Probability	95% Confidence Limits for con			95% Confidence Limits for log(con)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
	.010	2.094	1.845	2.280	.321	.266	.358
	.020	2.197	1.958	2.374	.342	.292	.375
	.030	2.264	2.034	2.435	.355	.308	.387
	.040	2.317	2.092	2.482	.365	.321	.395
	.050	2.360	2.141	2.522	.373	.331	.402
	.060	2.397	2.184	2.556	.380	.339	.408
	.070	2.431	2.222	2.586	.386	.347	.413
	.080	2.461	2.256	2.614	.391	.353	.417
	.090	2.489	2.288	2.639	.396	.359	.421
	.100	2.515	2.317	2.663	.401	.365	.425
	.150	2.626	2.443	2.763	.419	.388	.441
	.200	2.717	2.547	2.847	.434	.406	.454
	.250	2.798	2.639	2.921	.447	.421	.466
	.300	2.872	2.723	2.991	.458	.435	.476
	.350	2.943	2.802	3.058	.469	.448	.485
	.400	3.012	2.879	3.124	.479	.459	.495
	.450	3.080	2.954	3.191	.489	.470	.504
PROBIT	**.500**	**3.149**	**3.027**	**3.261**	**.498**	**.481**	**.513**
	.550	3.219	3.101	3.333	.508	.492	.523
	.600	3.292	3.176	3.411	.517	.502	.533
	.650	3.369	3.253	3.495	.527	.512	.543
	.700	3.452	3.333	3.589	.538	.523	.555
	.750	3.544	3.420	3.696	.549	.534	.568
	.800	3.649	3.516	3.823	.562	.546	.582
	.850	3.776	3.628	3.979	.577	.560	.600
	.900	**3.942**	**3.770**	**4.189**	**.596**	**.576**	**.622**
	.910	3.983	3.804	4.242	.600	.580	.628
	.920	4.028	3.842	4.301	.605	.585	.634
	.930	4.078	3.884	4.366	.610	.589	.640
	.940	4.135	3.931	4.441	.616	.595	.647
	.950	4.201	3.985	4.527	.623	.600	.656
	.960	4.280	4.050	4.632	.631	.607	.666
	.970	4.378	4.130	4.764	.641	.616	.678
	.980	4.513	4.238	4.946	.654	.627	.694
	.990	4.734	4.413	5.249	.675	.645	.720

a. Logarithm base = 10.

Appendix 23: Generated LC values of *L. kituiensis* oil at 48 hrs

Confidence Limits

	Probability	95% Confidence Limits for con			95% Confidence Limits for log(con)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
	.010	2.066	1.822	2.248	.315	.260	.352
	.020	2.166	1.932	2.340	.336	.286	.369
	.030	2.232	2.006	2.400	.349	.302	.380
	.040	2.282	2.063	2.446	.358	.314	.388
	.050	2.325	2.110	2.484	.366	.324	.395
	.060	2.361	2.151	2.517	.373	.333	.401
	.070	2.394	2.188	2.547	.379	.340	.406
	.080	2.423	2.221	2.574	.384	.347	.411
	.090	2.450	2.252	2.598	.389	.353	.415
	.100	2.476	2.281	2.621	.394	.358	.419
	.150	2.583	2.403	2.719	.412	.381	.434
	.200	2.672	2.504	2.801	.427	.399	.447
	.250	2.750	2.593	2.873	.439	.414	.458
	.300	2.823	2.674	2.941	.451	.427	.468
	.350	2.892	2.752	3.006	.461	.440	.478
	.400	2.958	2.826	3.070	.471	.451	.487
	.450	3.025	2.898	3.135	.481	.462	.496
PROBIT	**.500**	**3.091**	**2.970**	**3.202**	**.490**	**.473**	**.505**
	.550	3.159	3.042	3.272	.500	.483	.515
	.600	3.230	3.115	3.346	.509	.493	.525
	.650	3.304	3.190	3.428	.519	.504	.535
	.700	3.385	3.269	3.518	.530	.514	.546
	.750	3.474	3.353	3.621	.541	.525	.559
	.800	3.576	3.447	3.742	.553	.537	.573
	.850	3.699	3.556	3.892	.568	.551	.590
	.900	**3.859**	**3.695**	**4.094**	**.587**	**.568**	**.612**
	.910	3.899	3.728	4.145	.591	.572	.617
	.920	3.943	3.765	4.201	.596	.576	.623
	.930	3.992	3.806	4.264	.601	.580	.630
	.940	4.047	3.852	4.335	.607	.586	.637
	.950	4.110	3.904	4.418	.614	.592	.645
	.960	4.186	3.967	4.519	.622	.598	.655
	.970	4.282	4.045	4.646	.632	.607	.667
	.980	4.412	4.150	4.821	.645	.618	.683
	.990	4.625	4.320	5.111	.665	.635	.709

a. Logarithm base = 10.

Appendix 24: larval bioassasy results of *L. javanica* essential oil

Concentration used in mg/ml	**test**	**No of larvae per petri dish**	**No of ticks dead at the hrs shown below**			
			6 hrs	**12 hrs**	**24 hrs**	**48 hrs**
1.5	1	20	0	0	0	0
	2	20	0	0	0	0
	3	20	0	0	0	0
2.0	1	20	0	0	0	0
	2	20	0	0	0	1
	3	20	0	0	1	2
2.2	1	20	3	3	3	3
	2	20	0	0	0	1
	3	20	2	2	2	2
2.5	1	20	4	4	4	4
	2	20	2	2	3	3
	3	20	2	3	2	3
2.8	1	20	5	6	8	8
	2	20	7	7	7	8
	3	20	5	7	7	7
3.0	1	20	8	8	9	10
	2	20	8	8	9	10
	3	20	7	7	8	9
3.2	1	20	11	11	13	13
	2	20	11	11	11	11
	3	20	8	9	10	12
3.4	1	20	14	14	15	16
	2	20	11	11	12	14
	3	20	12	13	13	14
3.6	1	20	15	16	15	16
	2	20	13	13	16	17
	3	20	14	14	15	15
3.7	1	20	18	18	18	19
	2	20	17	17	17	20
	3	20	16	17	19	20
3.9	1	20	20	20	20	20
	2	20	19	20	20	20
	3	20	19	20	20	20
4.0	1	20	20	20	20	20
	2	20	20	20	20	20
	3	20	20	20	20	20
Amitraz (0.2 % v/v) [P]	1	20	0	10	20	20
	2	20	0	14	20	20
	3	20	0	10	14	20
2% DMSO)[Q]	1	20	0	0	0	0
	2	20	0	0	0	0
	3	20	0	0	0	0

[P] Positive control ;[Q] Negative

Appendix 25: Generated LC values of oil extract of *L. javanica* at 6 hrs

Confidence Limits

	Probability	95% Confidence Limits for con			95% Confidence Limits for log(con)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
	.010	2.025	1.756	2.220	.307	.245	.346
	.020	2.130	1.873	2.314	.328	.273	.364
	.030	2.199	1.951	2.376	.342	.290	.376
	.040	2.252	2.012	2.424	.353	.304	.385
	.050	2.296	2.063	2.464	.361	.314	.392
	.060	2.335	2.107	2.499	.368	.324	.398
	.070	2.369	2.146	2.529	.375	.332	.403
	.080	2.400	2.182	2.557	.380	.339	.408
	.090	2.429	2.215	2.583	.385	.345	.412
	.100	2.456	2.246	2.607	.390	.351	.416
	.150	2.569	2.377	2.710	.410	.376	.433
	.200	2.663	2.486	2.795	.425	.396	.446
	.250	2.746	2.582	2.872	.439	.412	.458
	.300	2.823	2.671	2.944	.451	.427	.469
	.350	2.897	2.754	3.014	.462	.440	.479
	.400	2.968	2.833	3.084	.472	.452	.489
	.450	3.039	2.910	3.155	.483	.464	.499
PROBIT	**.500**	**3.110**	**2.986**	**3.229**	**.493**	**.475**	**.509**
	.550	3.183	3.062	3.308	.503	.486	.520
	.600	3.259	3.138	3.392	.513	.497	.530
	.650	3.339	3.216	3.484	.524	.507	.542
	.700	3.426	3.297	3.588	.535	.518	.555
	.750	3.522	3.384	3.706	.547	.529	.569
	.800	3.632	3.481	3.845	.560	.542	.585
	.850	3.765	3.594	4.017	.576	.556	.604
	.900	**3.939**	**3.738**	**4.249**	**.595**	**.573**	**.628**
	.910	3.982	3.773	4.307	.600	.577	.634
	.920	4.029	3.812	4.372	.605	.581	.641
	.930	4.082	3.855	4.444	.611	.586	.648
	.940	4.142	3.903	4.527	.617	.591	.656
	.950	4.212	3.958	4.623	.624	.597	.665
	.960	4.294	4.023	4.738	.633	.605	.676
	.970	4.399	4.105	4.885	.643	.613	.689
	.980	4.541	4.216	5.088	.657	.625	.707
	.990	4.775	4.396	5.426	.679	.643	.734

a. Logarithm base = 10.

Appendix 26: Generated LC values of oil extract of *L. javanica* at 12 hrs

Confidence Limits

	Probability	95% Confidence Limits for con			95% Confidence Limits for log(con)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
	.010	2.009	1.744	2.201	.303	.242	.343
	.020	2.112	1.859	2.294	.325	.269	.361
	.030	2.179	1.936	2.355	.338	.287	.372
	.040	2.232	1.996	2.402	.349	.300	.381
	.050	2.276	2.045	2.441	.357	.311	.388
	.060	2.313	2.089	2.475	.364	.320	.394
	.070	2.347	2.127	2.506	.371	.328	.399
	.080	2.378	2.162	2.533	.376	.335	.404
	.090	2.406	2.195	2.559	.381	.341	.408
	.100	2.432	2.225	2.582	.386	.347	.412
	.150	2.544	2.354	2.683	.405	.372	.429
	.200	2.636	2.460	2.767	.421	.391	.442
	.250	2.718	2.555	2.843	.434	.407	.454
	.300	2.793	2.641	2.913	.446	.422	.464
	.350	2.865	2.723	2.982	.457	.435	.475
	.400	2.935	2.801	3.051	.468	.447	.484
	.450	3.005	2.877	3.120	.478	.459	.494
PROBIT	**.500**	**3.075**	**2.951**	**3.192**	**.488**	**.470**	**.504**
	.550	3.146	3.026	3.268	.498	.481	.514
	.600	3.220	3.101	3.350	.508	.491	.525
	.650	3.299	3.178	3.440	.518	.502	.537
	.700	3.384	3.258	3.541	.529	.513	.549
	.750	3.478	3.344	3.655	.541	.524	.563
	.800	3.586	3.439	3.791	.555	.536	.579
	.850	3.717	3.551	3.958	.570	.550	.598
	.900	**3.887**	**3.693**	**4.184**	**.590**	**.567**	**.622**
	.910	3.929	3.728	4.240	.594	.571	.627
	.920	3.976	3.766	4.303	.599	.576	.634
	.930	4.028	3.808	4.374	.605	.581	.641
	.940	4.086	3.855	4.454	.611	.586	.649
	.950	4.154	3.909	4.547	.618	.592	.658
	.960	4.235	3.974	4.660	.627	.599	.668
	.970	4.337	4.054	4.803	.637	.608	.681
	.980	4.477	4.163	5.000	.651	.619	.699
	.990	4.706	4.340	5.328	.673	.638	.727

a. Logarithm base = 10.

Appendix 27: Generated LC values of oil extract of *L. javanica* at 24 hrs

Confidence Limits

	Probability	95% Confidence Limits for con			95% Confidence Limits for log(con)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
	.010	1.970	1.713	2.159	.295	.234	.334
	.020	2.071	1.825	2.250	.316	.261	.352
	.030	2.138	1.900	2.311	.330	.279	.364
	.040	2.189	1.958	2.357	.340	.292	.372
	.050	2.232	2.007	2.396	.349	.303	.379
	.060	2.269	2.049	2.430	.356	.312	.386
	.070	2.303	2.087	2.459	.362	.320	.391
	.080	2.333	2.121	2.487	.368	.327	.396
	.090	2.360	2.153	2.511	.373	.333	.400
	.100	2.386	2.183	2.535	.378	.339	.404
	.150	2.495	2.308	2.634	.397	.363	.421
	.200	2.586	2.413	2.717	.413	.382	.434
	.250	2.667	2.505	2.791	.426	.399	.446
	.300	2.741	2.589	2.861	.438	.413	.457
	.350	2.812	2.669	2.928	.449	.426	.467
	.400	2.880	2.746	2.995	.459	.439	.476
	.450	2.948	2.820	3.063	.470	.450	.486
PROBIT	**.500**	**3.017**	**2.894**	**3.133**	**.480**	**.461**	**.496**
	.550	3.087	2.967	3.208	.490	.472	.506
	.600	3.160	3.041	3.287	.500	.483	.517
	.650	3.238	3.118	3.375	.510	.494	.528
	.700	3.321	3.197	3.472	.521	.505	.541
	.750	3.414	3.282	3.583	.533	.516	.554
	.800	3.520	3.377	3.714	.547	.529	.570
	.850	3.648	3.488	3.877	.562	.543	.588
	.900	**3.815**	**3.629**	**4.096**	**.582**	**.560**	**.612**
	.910	3.857	3.663	4.151	.586	.564	.618
	.920	3.903	3.701	4.212	.591	.568	.625
	.930	3.954	3.742	4.281	.597	.573	.631
	.940	4.011	3.789	4.358	.603	.579	.639
	.950	4.078	3.843	4.449	.610	.585	.648
	.960	4.158	3.907	4.559	.619	.592	.659
	.970	4.258	3.986	4.697	.629	.601	.672
	.980	4.395	4.094	4.889	.643	.612	.689
	.990	4.620	4.269	5.208	.665	.630	.717

a. Logarithm base = 10.

Appendix 28: Generated LC values of oil extract of *L. javanica* at 48 hrs

Confidence Limits

	Probability	95% Confidence Limits for con			95% Confidence Limits for log(con)[a]		
		Estimate	Lower Bound	Upper Bound	Estimate	Lower Bound	Upper Bound
	.010	1.902	1.653	2.087	.279	.218	.320
	.020	2.002	1.763	2.178	.301	.246	.338
	.030	2.068	1.837	2.238	.315	.264	.350
	.040	2.119	1.894	2.284	.326	.277	.359
	.050	2.161	1.941	2.322	.335	.288	.366
	.060	2.198	1.983	2.356	.342	.297	.372
	.070	2.230	2.020	2.385	.348	.305	.378
	.080	2.260	2.053	2.412	.354	.312	.382
	.090	2.287	2.084	2.437	.359	.319	.387
	.100	2.313	2.113	2.460	.364	.325	.391
	.150	2.421	2.237	2.559	.384	.350	.408
	.200	2.511	2.339	2.642	.400	.369	.422
	.250	2.591	2.430	2.716	.413	.386	.434
	.300	2.664	2.513	2.785	.426	.400	.445
	.350	2.735	2.592	2.852	.437	.414	.455
	.400	2.803	2.668	2.918	.448	.426	.465
	.450	2.870	2.741	2.986	.458	.438	.475
PROBIT	**.500**	**2.939**	**2.814**	**3.055**	**.468**	**.449**	**.485**
	.550	3.009	2.887	3.129	.478	.461	.495
	.600	3.081	2.961	3.207	.489	.472	.506
	.650	3.158	3.038	3.293	.499	.483	.518
	.700	3.241	3.117	3.389	.511	.494	.530
	.750	3.334	3.203	3.498	.523	.506	.544
	.800	3.439	3.298	3.628	.536	.518	.560
	.850	3.567	3.409	3.788	.552	.533	.578
	.900	**3.734**	**3.551**	**4.004**	**.572**	**.550**	**.603**
	.910	3.776	3.586	4.059	.577	.555	.608
	.920	3.821	3.624	4.119	.582	.559	.615
	.930	3.872	3.665	4.187	.588	.564	.622
	.940	3.930	3.713	4.264	.594	.570	.630
	.950	3.997	3.767	4.354	.602	.576	.639
	.960	4.076	3.831	4.462	.610	.583	.650
	.970	4.177	3.911	4.600	.621	.592	.663
	.980	4.314	4.020	4.789	.635	.604	.680
	.990	4.540	4.197	5.106	.657	.623	.708

a. Logarithm base = 10.

Appendix 29: Essential oil components

NAME OF COMPOUND	RETENTION TIME	AREA% CONCENTRATION
MONOTERPENES		
delta3 carene	6.44	0.08
2-methyl-5-(1-methylethyl)-Bicyclo(3.1.0)hex-2-ene	6.60	0.24
alpha-pinene, (-)-	**6.77**	**2.04**
Camphene	**7.23**	**7.26**
Sabinene	**7.91**	**2.15**
beta-myrcene	**8.36**	**1.49**
l-Phellandrene	**8.72**	**1.11**
alpha-terpinene	9.08	0.55
dl-limonene	**9.52**	**6.52**
cis-ocimene	9.69	0.07
beta-trans-ocimene	10.01	0.24
gamma-terpinene	**10.31**	**1.22**
trans-sabinene hydrate	**10.71**	**4.45**
cis-sabinene hydrate	10.77	0.88
alpha.-terpinolene	**11.20**	**1.44**
linalool	12.11	0.20
neo-allo-ocimene	**12.45**	**2.39**
camphor, (1S,4S)-(-)-	**13.26**	**18.29**
camphore	**13.35**	**3.49**
Pinocarvone	13.57	0.45
14.29 borneol (=endo-borneol)	**13.87**	**1.77**
4-methyl-1-(1-methylethyl)- 3-Cyclohexen-1-ol	**13.97**	**1.43**
4-terpineol	**14.10**	**3.03**
p-cymen-8-ol	14.18	0.39
l-verbenone	14.82	0.36
trans-(+)-carveol	15.01	0.17
dill ether	15.27	0.09
7-(1-methylethylidene)- Bicyclo[4.1.0]heptane	15.63	0.23
TOTAL	**28**	**36.36%**
SESQUITERPENES		
alpha-Cubebene	18.43	0.03
Ylangene	19.01	0.06
Copaene	19.16	0.48
beta-bourbonene	**19.43**	**1.36**
beta-Cubebene	19.50	0.26
Italicene	19.85	0.05
alpha-cedrene	20.08	0.06
Germacrene D	**20.35**	**3.20**
alpha-Guaiene	20.75	0.02
alpha-Elemene	21.01	0.06
gamma-Cadinene	**21.17**	**1.00**
allo-aromadendrene	21.35	0.88
gamma muurolene	21.70	0.54
ar-Curcumene	21.83	0.64
1H-Cyclopropa[a]naphthalene, decahydro-1,1,3a-trimethyl-7-methylene-, [1as-(1a.alpha.,3a.alpha.,7a.beta.,7b.alpha.)]-	21.98	0.04
2-isopropyl-5-methyl-9-methylene- Bicyclo[4.4.0]dec-1-ene	**22.26**	**1.05**
(E,Z)-alpha-farnesene	22.41	0.09
Zingiberene	22.51	0.10

Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)-	22.59	0.06
30.19 cubebol<10-epi->	22.66	0.42
delta-Cadinene	22.81	0.22
alpha-selinene	22.86	0.03
cadina-1,4-diene	23.00	0.04
Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,[1R-(1.alpha.,4a.alpha.,8a.alpha.)]-	23.14	0.07
Cadala-1(10),3,8-triene	23.28	0.03
2,4a,8,8-tetramethyl-1,1a,4,4a,5,6,7,8-octahydro-cyclopropa[d]naphthalene	23.47	0.09
1,5-epoxysalvial-4(14)-ene	23.85	0.07
beta-Gurjunene	23.98	0.12

Appendix 30: Absorbance values at 562 and 690 nm

a) 562 nm

Wells	MeoH extract of *P. viridiflorum*		-Ve control	MeoH extract of *L. kituiensis*		-Ve control	MeoH extract of *P. dodecandra*		-Ve control
A	0.807	0.793	0.514	0.866	0.974	0.572	0.897	0.851	0.433
B	0.971	0.966	0.488	1.181	1.232	0.492	0.868	0.884	0.443
C	1.196	1.3	0.502	1.554	1.51	0.486	1	0.987	0.452
D	1.694	1.909	0.472	1.933	1.944	0.495	1.171	1.169	0.501
E	2.586	2.436	0.457	2.15	2.124	0.508	1.471	1.707	0.491
F	2.671	2.671	0.481	2.193	2.231	0.508	1.793	1.56	0.48
G	2.041	2.041	0.442	2.036	1.969	0.579	1.39	1.548	0.592
H	1.557	2.302	0.54	1.319	1.477	0.611	2.946	3.047	0.487

b) 690 nm

Wells	H_20 extract of *P. dodecandra*		-Ve control	Hexane extract of *P. viridiflorum*		-Ve control	Hexane extract of *L. kituiensis*		-Ve control
A	0.583	0.528	0.543	0.875	0.773	0.479	0.94	0.71	0.506
B	0.514	0.547	0.57	0.872	1.081	0.516	0.731	1.098	0.543
C	0.598	0.638	0.589	1.05	0.898	0.48	1.094	1.127	0.487
D	0.966	0.979	0.621	0.827	1.004	0.54	1.168	1.101	0.502
E	1.236	1.265	0.557	2.278	1.306	0.505	0.898	1.05	0.538
F	1.545	1.441	0.509	2.114	2.043	0.521	1.788	2.025	0.533
G	1.848	1.782	0.527	0.99	1.03	0.556	2.22	1.512	0.506
H	2.612	2.686	0.499	2.333	2.333	0.604	1.67	1.802	0.62