AN ETHNOBOTANICAL, PHYTOCHEMICAL, TOXICITY AND EFFICACY STUDY OF SELECTED ANTIBLUE-TICK (*Boophilus decoloratus*) HERBAL REMEDIES FOR CATTLE OF SUBA SUB-COUNTY, KENYA.

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DECLARATION

This thesis is my original work and has not been submitted for a degree in any other university or institution and that all sources of materials used for the thesis have been duly acknowledged.

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To my father Michael Onyango, mother, Linet Onyango, children Trevor, Kelly and Henry and my friend Victor Okelo for their invaluable support throughout the study.

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LIST OF ABBREVIATIONS AND ACRONYMS

BST : Brine Shrimp Test

DCM : Dichloromethane

DMSO : Dimethylsulfoxide

GOK : Government of Kenya

HIV : Human Immunodeficiency Virus

ILRI : International Livestock Research Institute

IR : Independent Reports

LC_{50} : Median Lethal Concentration (concentration required to kill 50% of a population)

NAI : Nairobi University Herbarium

SPSS : Statistical Package for Social Sciences

TM : Traditional Medicine

USD : United States Dollar

WHO : World Health Organization

ABSTRACT

The search for safe pest control options has led to the exploration of potential plants with pesticidal activity. Plants have been reported to exhibit a wide range of biological activities against insect pests, some of which have been validated. Recently, attention has focused on the identification of compounds present in plants with acaricidal properties since blue-tick has remained an economic threat to most smallholder livestock farmers in rural areas in Kenya. Numerous ethnoveterinary practices are harmful, remedies not standardized and dosages are uncertain. Therefore this research investigated the ethnobotany, phytochemistry, toxicity and acaricidal activity crude extracts of three selected plants against blue-tick in Suba Sub-County. A total of 32 herbalists aged between 28 to 87 from four villages were interviewed by use of a questionnaire about their knowledge of acaricidal plants in Suba Sub-County. The local name, part used, traditional mode of preparation and method of administration were documented. There was a high correlation between the age of informants and the number of medicinal plant citations. The study identified 16 plants distributed among 13 families based on independent reports (IR). *Phytolacca dodecandra, Cissus quadrangularis* and *Ipomoea kituiensis* were collected, extracted in methanol in dichloromethane (1:1 v/v) and separately in distilled water for phytochemical studies. Phytochemistry focused on terpenoids, tannins, saponins, flavonoids and alkaloids where all were present in the three plants except flavonoids which were absent in *P.dodecandra.* The *in vitro* acaricidal activity study of crude extracts of these selected plants was done using larvae of *Boophilus* spp. tick to assess their potency. Using 2.5, 5 and 10 mg/mL concentrations of water and methanol in DCM (1:1 v/v) crude extracts of *Phytolacca dodecandra* (leaves), *Cissus quadrangularis* (whole) and *Ipomoea kituiensis* (leaves), their effects were compared with that produced by the standard reference acaricide, almatix® (12.5 %

amitraz) as positive control. The activity of the extracts were tested against the larvae and at 10 mg/mL concentration, the extracts were most active. Mortality was determined within duration of 24 hours. The most potent extract as compared to almatix were *Cissus quadrangularis* (100 kills at 10 mg/mL) and *Phytolacca dodecandra* (100 kills at concentrations, 5 and 10 mg/mL) while *Ipomoea kituiensis* methanol/DCM (1:1v/v) extract was least potent. Analysis of variance revealed that there were significant differences in acaricidal activity of plants' extracts of all the concentrations used (2.5, 5 and 10 mg/mL) ($P \leq 0.05$). *B.decoloratus* larvae's LC_{50} was determined where methanol: DCM (1:1 v/v) extract of *I. kituiensis* displayed mild toxicity while those of *P. dodecandra* and *C. quadrangularis* recorded high toxicity. The conclusion from this study is that *C.quadrangularis* (whole), *I.kituiensis* (leaves) and *P.dodecandra* (leaves) extracts are acaricidal and are as potent at high concentrations as compared to almatix. The mortalities of the larvae increase with increase in concentration of extract and thus the leading three study plants' extracts are effective in blue-tick control. To achieve highly efficacious traditional acaricides, it is recommended that isolation and purification of the crude compounds and bioassay of these isolated compounds be done on the same blue-tick larvae.

Keywords: Amitraz, *Boophilus decoloratus*, acaricidal activity, medicinal plants

CHAPTER ONE

1.0 INTRODUCTION

One of the major constraints to production and improvement in the livestock industry in the tropics and sub-tropics is the transmission of diseases by external and internal parasites (Belay *et al*., 2013). The external parasites transmit pathogens that include bacteria, spirochetes, protozoa, viruses and toxins (Rajput *et al*., 2006). Ticks are the most important external parasites in livestock and the impact of their feeding and disease transmission is responsible for severe economic losses to livestock keepers (Jongejan and Uilenberg, 2004). In eastern Africa, Kenya included, tick-borne diseases such as East Coast Fever (theileriosis), anaplasmosis, ehrlichiosis and babesiosis are a concern to livestock farming leading to massive losses of livestock due to deaths (Gakuya *et al*., 2005).

Several methods to control ticks and tick-borne diseases have been identified and to-date the most effective tick control method is site-specific and repeated acaricide applications (Di Giulio *et al*., 2009). However, a number of problems, including multi-chemical resistance by pest organisms, cost of application, poisoning of treated animals and humans, residues in meat and milk and, environmental contamination especially in water bodies, are associated with their usage (Rajput *et al*., 2006). Secondary metabolites are plants' phytochemicals that are not directly utilized by plants, but are useful as defense mechanisms against many herbivorous organisms including insects. These phytochemicals usually have a broad spectrum of action, hence, pests do not develop resistance easily as commonly found in the application of synthetic pesticides. Ethnoveterinary acaricides offer resource-poor farmers an alternative to synthetic acaricides because they are cheap, familiar to the locals, locally available and easily accessible

(Salwa, 2010). These bio-pesticides are used either in traditional crude forms or as pure active compounds. However, scientific research is needed to provide additional evidence on their safety and efficacy (Green *et al*., 1996).

Bioactive plant extracts are gaining popularity worldwide because they are environment friendly (Sanjay and Tiku, 2009). Saponins, taninns, flavonoids, terpenoids and alkaloids are the most common insecticidal compounds found in plants' extracts and are useful as defense mechanisms against many herbivores, including insects, by acting as anti-feedants, sterilants, repellents and as poisons causing paralysis (Isman, 2006).

Tick prevalence in Suba Sub-County is high due to its proximity to Ruma National Park which contains tick hosts such as buffalos and the region's remoteness in terms of veterinary infrastructures, for example cattle dips. Therefore this study was carried out on ethnoveterinary medicinal plants used by herbalists in Suba Sub-County, Kenya to evaluate the ethnobotanical, phytochemical, toxicity and acaricidal activity of anti-tick and, in particular anti-blue tick, herbal remedies for cattle**.**

1.1 LITERATURE REVIEW

1.1.1 Positive aspects of traditional medicine

Use of herbal medicine has always been part of human culture, since some plants possess important therapeutic properties which can be used to cure human and animal diseases (Rios and Recio, 2005). It is estimated that up to 80 % of the population in some developing countries use traditional medicine (WHO, 2004). In Kenya, rich pharmacopoeia systems have been documented for communities such as the Maasai, Gusii, Luo, Abaluyia and the Kikuyu people (Kiringe, 2006; Njoroge and Bussman, 2007; Kokwaro, 2009).

Application of traditional medicine (TM) is different from those of conventional medicine and varies greatly from country to country, and from region to region, since they are influenced by factors such as culture, history, personal attitudes and philosophy. TM utilizes plants/herbs, animal and mineral substances (Kokwaro 2009). The use of plants, plant extracts or chemicals derived from plants to treat diseases is a therapeutic modality which has stood the test of time (Anwannil and Atta, 2005).

In recent years, the importance of herbal drugs in medicine has tremendously increased because of their fewer side effects **(**Yaday and Rupali, 2011**)**. Consequently, the demand for herbal formulation is increasing. The phytochemical constituent analysis and standardization has been accelerated by the development of instrumental analysis. This field has become important and attractive for further investigations.

1.1.2 Negative aspects of traditional medicine

The global resurgence of interest in herbal medicines has led to an increase in their demand leading to a decline in their quality, primarily due to lack of adequate regulations pertaining to drugs (Rajani and Kanaki, 2008). Ethnoveterinary medicine has also other limitations; some practices are harmful, dosages are uncertain and remedies are not standard. Moreover, ethnoveterinary medicines are often not as fast-working and potent as allopathic medicines (McCorkle, 1986).

Traditional knowledge has been developed through trial and error as well as deliberate experimentation. Therefore, it is less systematic, less formalized and not universally recognized as a valid method of disease control in animals. Further, traditional healers have less to offer in the treatment and control of epidemic and endemic infectious diseases like rinderpest, anthrax

and acute life-threatening bacterial diseases though they can cope with a reasonable spectrum of problems (Sunder *et al*., 2014).

1.1.3 Ethnobotany

Ethnobotany refers to the study of, classification, use and management of plants by people or the study of how people in the traditional society used plants (Kokwaro, 2009; Colombo *et al*., 2012). It has a potential of providing new insights into medically useful plant products. Many plant extracts in modern medicine today were discovered by traditional societies and, ethnobotany is continually being modified and knowledge safeguarded (Gurib-Fakim, 2006). It entails ethnosystematics, which is the ability to know by names and identify the plants in their natural habitats, hence, also referred to as folk knowledge of botanical classification. Ethnosystematics is also concerned with the best practices of conserving medicinal plants to avoid their depletion and extinction. This branch of ethnobotany is particularly well developed in Africa due to its rich and diverse flora (Kokwaro, 2009).

1.1.4 Ethnoveterinary medicine

Ethnoveterinary medicine is defined as traditional animal health care, encompassing the knowledge, skills, methods, practices and beliefs about animal health care (McCorkle, 1986). Traditional knowledge of Ethnoveterinary medicine has been transferred from generations to generations by word of mouth only (Sunder *et al*., 2014).

Livestock owners have an excellent knowledge of ethnobotany and they can cope with a reasonable spectrum of common diseases such as diarrhoea, wounds, colds, worms, coccidiosis and reproductive disorders (Matekaire and Bwakura, 2004). Although an extensive network of veterinary hospitals exist, poor communication infrastructure and shortage of manpower drives

livestock owners to treat animals themselves, consult a local healer, or slaughter the animal if the cost of treatment becomes a significant proportion of the value of the animal. In view of these constraints, the search for alternative drugs become important. Ethnoveterinary medicine offers great potential for development and provides a low-cost alternative to allopathic medications (Manoj *et al*., 2012). WHO, (2004), stated that the use of natural products in the control of animal and human diseases are considerably effective.

1.1.5 Phytochemical perspective

The plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants (Sanjay and Tiku, 2009). They are the source from which discovery of new products with medicinal importance in pharmaceutical industries is based (Kokwaro, 2009). Presently, the structures of close to 50,000 compounds have already been elucidated in plants and it is predicted that the final number will exceed 200,000 of such compounds (Hounsome *et al*., 2008). Medicinal plants contain some secondary metabolites which provide definite physiological action on mammals and these bioactive substances include tannins, alkaloids, terpenoids, steroids and flavonoids (Uma and Sekar, 2014).

Plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs, antimicrobial drugs and antihepatotoxic compounds (Arunkumar and Muthuselvam, 2009). Alkaloids play an important role in the defense system of plants against pathogens and animals. The applications of alkaloids are not limited to biological control of herbivores but they also have pharmacological, veterinary as well as medical importance and among some of their common biological properties are their cytotoxicity, anti-HIV and

antiparasitic activities **(**Bouayad *et al*, 2013). Saponins are widely distributed in the plant kingdom and approximately 70% of all plants produce them. They have a diverse range of properties which includes precipitating and coagulating blood, antimicrobial, insecticidal as well as molluscicidal activities and, in addition, they also have beneficial health effects (Rahman *et al*., 2013).

Terpenoids have been found to be useful in the prevention and therapy of several diseases including cancer and they also have antiparasitic, antimicrobial, antifungal, antiviral and antispasmodic properties (Wagner and Elmadfa, 2003). Current reports show that tannins may have potential value as cytotoxic and antineoplastic agents (Aguinaldo *et al*., 2005). Plant steroids are known to be important for their cardiotonic activities, insecticidal and antimicrobial properties (Sermakkani and Thangapandian, 2010).

Preliminary phytochemical information has been recorded for some Kenyan medicinal plants. Alkaloids were identified in bulb extracts of *Gladiolus dalenii* (Odhiambo *et al*., 2009). The dry leaf powder of *Piliostigma thonningii* has been reported to contain alkaloids, saponins, flavonoids and tannins (Nguta *et al*., 2013). Kaigongi, (2014) carried out a phytochemical analysis of antimicrobial plants and reported presence of tannins, sesquiterpene lactones and saponins. Presence of tannins, saponins and cardiac glycosides have been reported in antihelmintic plants (Sirama *et al*., 2015).

1.1.6 Acaricidal and insecticidal activities of plants

Different pesticidal plant products in form of powders, extracts or distillates could be harnessed as potential toxicants, deterrents, anti-feedants and repellents to prevent insect feeding and oviposition (Isman, 2008). Plants have traditionally been used in the destruction of external micro and macro-organism either by stunning or killing (Kokwaro, 2009). This activity of crude

extracts (*M. azadirach*, *Azadiracta indica* and *C.quadrangularis*) could be partly due to local irritation of the respiratory system and, generally, their toxicity is attributable to ascaridole monoterpenoid. The symptoms of ascaridole poisoning include nausea, depression and extreme fatigue in man. Based on these symptoms, it is likely that this substance has effects on the nervous system of man. This characteristic may be exploited in the control of *B. decoloratus* infestation (Quarles, 1992; Isman, 2006).

Many plant families are known to contain a variety of compounds, which show insecticidal, anti-feedant, growth regulating and development modifying properties as in *M.azedarach* extracts (Carpinella *et al*., 2003) which reportedly has potential for controlling *Spodoptera littoralis* (Abou-Fakhr *et al*., 2000). The presence of several triterpenoids in plants of the Meliaceae family has been described as indicative of insecticidal activity (Mishra *et al.,* 2013).

1.1.7 Toxicity studies and its relevance

Herbal preparations are mostly considered "natural" and are therefore intrinsically harmless. However, some medicinal plants are inherently toxic (WHO, 2004). Overdose of patients due to the imprecise nature of diagnosis and dosage is widespread (Kokwaro, 2009). The effects can be very powerful and potentially lethal if used incorrectly and their use as a substitute for conventional medicines may be ineffective. Toxic effects have been attributed to certain active principles found in plants. These chemical substances interact with living systems and affect normal processes (Kaigongi, 2014).

Brine Shrimp Test (BST) has been used to determine *in vitro* acute toxicity of crude extracts (Odhiambo *et al*., 2014). It is a simple method for natural product research and the procedure determines median lethal concentration values of active compounds and extracts in the

brine medium. This method is rapid, reliable, inexpensive and convenient as an in-house bioassay tool (Musila *et al.,* 2013; Odhiambo *et al.,* 2014).

1.1.8 Ticks

Ticks are considered as the most damaging livestock pests on a global scale and are responsible for a great diversity of livestock health problems (Gakuya *et al.,* 2005). Approximately 80 % of the world's cattle population is at risk of tick infestation and tick-borne diseases (De Castro *et al.,* 1997). Their infestation alone can give rise to severe anaemia, inflammation and swelling at the bite site, irritation and trauma which results in substantial economic losses from reduced milk yield, skin and hide damage as well as reduced weight gain. As vectors of pathogens, ticks are second only to mosquitoes and are responsible for the transmission of protozoal, rikettsial, bacterial and viral diseases among domestic animals (Rajput *et al*., 2006). It is estimated that the annual cost of East Coast Fever in the smallholder dairy system in Kenya and Tanzania is at US$ 54.4 and 4.41 million respectively. In the traditional cattle rearing system in Kenya and Tanzania the annual cost is estimated at US$ 34.1 and 129.5 million respectively (Minjauw and McLeod, 2003).

This study focused on the blue-tick (*B. decoloratus*) (Fig. 1) which belongs to the genus *Boophilu*s as a model parasite. Blue tick transmits the protozoan blood parasites *Anaplasma* and *Babesia* spp. which are responsible for anaplasmosis and babesiosis respectively. High tick infestation may be fatal to cattle especially the young ones. The mature female can lay between 1,000 to 4,500 eggs in a mass. The emptied female then dies after a single oviposition (Walker *et al*., 2002; Junquera, 2014).

Newly hatched larvae (Fig. 2) are six-legged, swollen and take several days to harden (lose a certain quantity of water and eliminate metabolic waste products accumulated during

embryogenesis). Thereafter, it begins to seek the first host and when found, blood meal lasts 3 to 12 days to be fully engorged. Hungry larvae can survive up to 4 months without feeding in dry and cool weather and once appropriate host is found, they attach and start feeding on blood. Development to adult ticks through nymphal stage can be completed in 2 weeks. The nymph is 8 legged and similar to the larva (Junquera, 2014).

Fig. 1: Adult *B. decoloratus* engorged with blood (www.parasitipedia.net, 15th, June, 2014).

Fig. 2: *B. decoloratus* larvae showing three pairs of legs (www. parasitipedia.net, 15th, June, 2014**).**

1.2 Taxonomic description, ethnoveterinary medicine and phytochemistry of selected study plants

1.2.1 *Phytolacca dodecandra* (Phytolaccaceae)

This is a clambering plant, up to 9 m high with long, hanging branches and a tuberous rhizome. Leaves are oval, thick and shiny, up to 15 cm long, the two sides sometimes unequal (Fig. 3). The flowers are greenish-white to yellow, scented, in a spike up to 40 cm long, unisexual with each less than 5 mm long. Male flowers have fifteen stamens and a five-celled infertile ovary while female flowers have a five-celled ovary and eight to fifteen short infertile stamens. The fruit is orange-red, five-lobed and fleshy. They are found in forest edges, scrub patches and termite mounds (Lind and Tallantire, 1975; Kokwaro and Johns, 1998; 2013). The leaf extract of *P.dodecandra* contains alkaloids, saponnins, terpenoids and phenolics (Ogutu *et al*., 2012). *P.dodecandra* is reported to be very poisonous to both people and other animals (Katende *et al.*, 1995).

Fig. 3: *P.dodecandra* shrub showing its flowers and leaves

1.2.2 *Cissus quadrangularis* L. (Vitaceae)

This is a succulent, perennial, climbing shrub, with quadrangular winged stems and leafless when old. The flowers are greenish-yellow and fruits are globose berries (Fig. 4). The genus name is derived from Greek meaning “ivy” while the Latin term *quadrangularis* refers to the rectangular shape of the stems (Shah, 2011). The insecticidal and acaricidal properties of *C.quadrangularis* have previously been reported in Kenya. The stems are crushed in water and the liquid used as a wash to remove fleas in calves while root decoction is given to cattle with East Coast Fever. Its leaf and stem infusion is given to cattle with lung problems (Kokwaro, 2009). The stem of *C. quadrangularis* is considered a termite-repellent by the Turkana people of northern Kenya. The Gerri people of southern Ethiopia use a paste issued from the stems of *C.quadrangularis* to topically treat livestock against tick (Zorloni, 2007). Phytochemical screening has shown presence of tannins and triterpenoids (Shah, 2011).

Fig. 4: *Cissus quadrangularis* plant showing its stems and leaves.

1.2.3 *Ipomoea kituiensis* Vatke (Convolvuliaceae)

This plant is a sub-erect, twining shrub or small bushy tree which grows up to 4 to 6 m tall. The stem has milky latex, pubescent or hirsute with leaf scars as prominent in Zambian specimens (Fig. 5). Leaves are ovate to reniform. The plant's apex is obtuse, apiculate, acuminate or even bilobed while its base is cordate, sparsely pubescent, hirsute or glabrous above and finely pubescent or glabrescent below; margins sometimes pilose; petiole 1 to 10 cm long. Flowers occur in 2.5 to 6 flowered branched inflorescence; peduncle 1.5 to 16 cm long; pedicels 3 to 10 mm long; bracts linear-lanceolate (10 by 2) mm. The sepals are linear-lanceolate to ovate, (7 by 2 to 6 mm), hirsute or appressed velutinous to pilose. Corolla are white, cream, pink to purple or rarely pale blue, centre deeper purple; infundibular, 5 to 8 cm long; midpetaline areas hirsute, pubescent or pilose; tube narrowed below. The fruits are capsule ellipsoid, 15 to 20 mm long, but seeds are ovoid, 7 to 9 by 5 to 6 mm, covered with golden hairs but 6 to 10 mm long along the margins, shorter in other parts (Demissew, 2006).

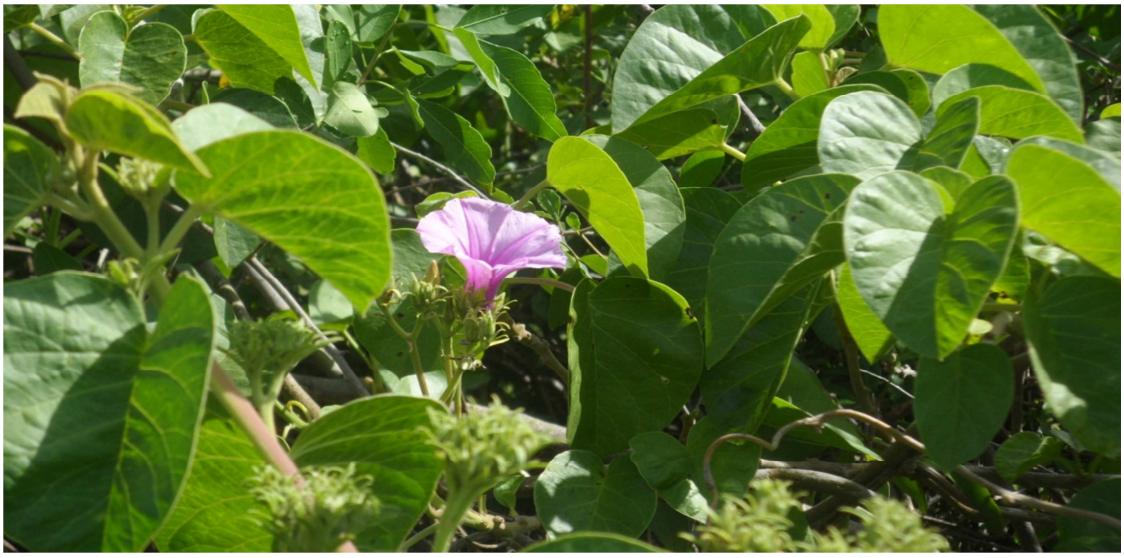

Fig. 5: *Ipomoea kituiensis* showing its stems, leaves and a flower.

1.3 Problem statement

Socio-economic risks from livestock diseases are in the form of losses in production, profitability, cost of treatment, disruptions to markets and zoonosis (Rajput *et al.,* 2006). In the tropical and sub-tropical areas, external parasites transmit diseases. Ticks transmit tick- borne diseases such as anaplasmosis, babesiosis and theileriosis thereby decreasing production and increasing morbidity and mortality of the animals (De Waal, 2002). Heavy parasite infestations lead to anaemia, reduced food intake and emaciation among domestic animals and, in addition, cause inflammation and development of wounds at the bite site which may serve as entry points for secondary infection (Hema, 2006).

In recent years, as a consequence of the financial constraints to chemical acaricide use and the encouraging results obtained from plant extracts, interest in plant with acaricidal properties has increased. Thousands of plants have been screened for acaricidal activities using standard WHO procedure. However, there are still disadvantages in the use of the ethnoveterinary acaricides; several practices are dangerous during administration to the recipient animals and dosage and remedies are not yet standardized in most drugs. Furthermore there is still lack of universal agreement about the most effective plant species in the tick control and also research on acaricidal plant species worldwide is still limited.

1.4 Justification

In Kenya, agriculture is a major occupation for over 70% of the people living in rural areas with smallholder farmers constituting 81% of the farmers in the country. Many large-scale farmers depend on synthetic pesticides despite their inherent socio-economic and environmental concerns challenging their sustainability as reasonable approaches to pest management. In this

connection, it has been pointed out that a sustainable future in agriculture is most likely a shift by farmers to biologically based and diversified approaches that are more stable and with minimal damage to human health and environment (Brookfield, 1991; Pretty, 2008; Kokwaro, 2009).

There is also limited affordability and availability of the conventional veterinary medicine. Over 75 % of 5 billion people cannot afford conventional pharmaceutical products. Most of the world's population cannot afford once-weekly dipping or spraying of cattle against ticks, hence, most cattle go without regular tick control therefore frequently relying upon the use of traditional medicine derived from plants (Isman, 2008; Kokwaro, 2009). In the face of this factor, there is increasing interest in the field of ethnoveterinary research and development as ethnoveterinary medicine often provides cheaper options than comparable conventional drugs since the products are locally available and more easily accessible. This offers the resource-poor farmers an alternative to the conventional pesticides.

1.5. Hypothesis

Phytochemical extracts of the three plants namely *P.dodecandra*, *C.quadrangularis* and *I.kituiensis* are efficacious in controlling blue-tick infestation in domestic animals.

1.6 Research objectives

1.6.1 General objective

To study the ethnobotany, phytochemistry, toxicity and acaricidal activity of anti-blue tick plants of Suba Sub-County.

1.6.2 Specific objectives

1. To identify and record (by interviewing traditional herbalists) the first three major plant species frequently used in the traditional tick control in Suba Sub-County.

2. To extract the three plant materials *Cissus quadrangularis* (whole), *Phytolacca dodecandra* (leaves) and *Ipomoea kituiensis* (leaves) using water and methanol in dichloromethane (1:1 v/v).

3. To determine the efficacy of the selected anti-tick plants on *B. decoloratus* larvae.

4. To determine the toxicity of the crude plant extracts using brine shrimp (*Artemia salina*) lethality assay.

5. To determine the phytochemicals present in the selected anti-tick plant species.

CHAPTER TWO

2.0 METHODOLOGY

2.1. The study area

B. decoloratus adult ticks were collected from Suba Sub-County of Homa Bay County, Kenya which lies between latitudes 0°30'S and 0°50'S and longitudes 34°0'E and 34°20'E (Fig. 6). The mean altitude of the region is 1000m above the sea level with the mean annual rainfall ranging between 250mm and 700mm per annum. Annual temperature ranges from a mean annual minimum of 17.1° C to a mean maximum of 34.8° C.

Suba Sub-County is mapped into nine locations according to the National Boundary Commission namely; Gwassi West, Gwassi East, Gwassi South, Gwassi Central, Kaksingri West, Gwassi North, Kaksingri East, Kaksingri Central and Ruma (Fig. 7). It borders Lake Victoria to the north.

According to 2009 census, Suba Sub-County has a population of approximately 103,789 people out of which 34% live in the urban areas (GoK, 2009; 2012). The Luo ethnic group is demographically dominant but other ethnic groups such as the Luhyia and Kisii also inhabit the area.

Subsistence agriculture together with livestock breeding and trade are the main economic activities of the region. The vegetation is of savannah type with shrub and sparse forests in some areas. The specific study area, Suba Sub-County, was selected because it represents a predominantly free-range smallholder cattle production system and lies in close proximity to Ruma National Park which increased the risk of tick occurrence due to the presence of wild hosts such as buffaloes and rhinos.

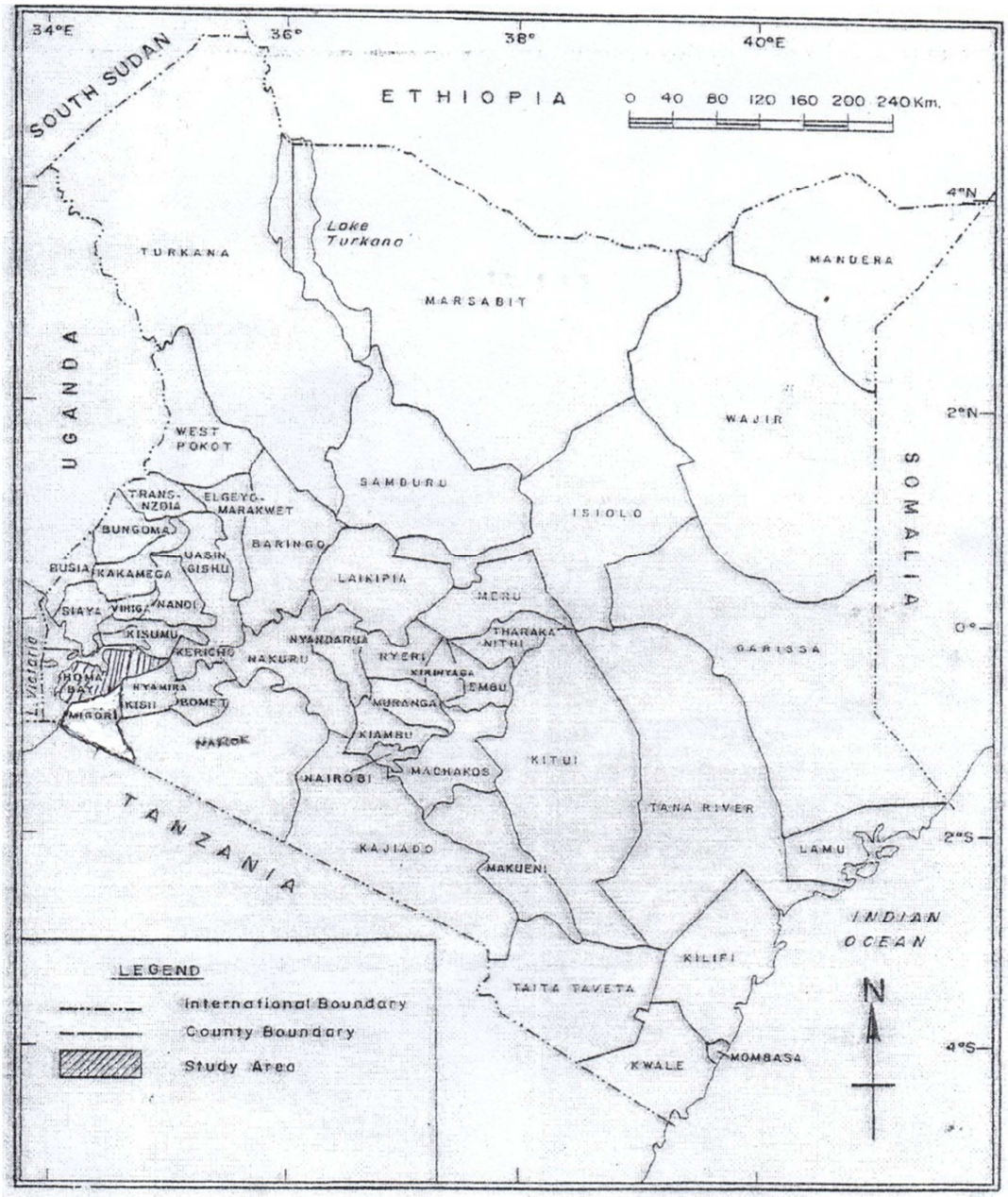

Fig. 6: The map of Kenya showing Homa Bay County (shaded) (Courtesy Department of Geography, University of Nairobi)

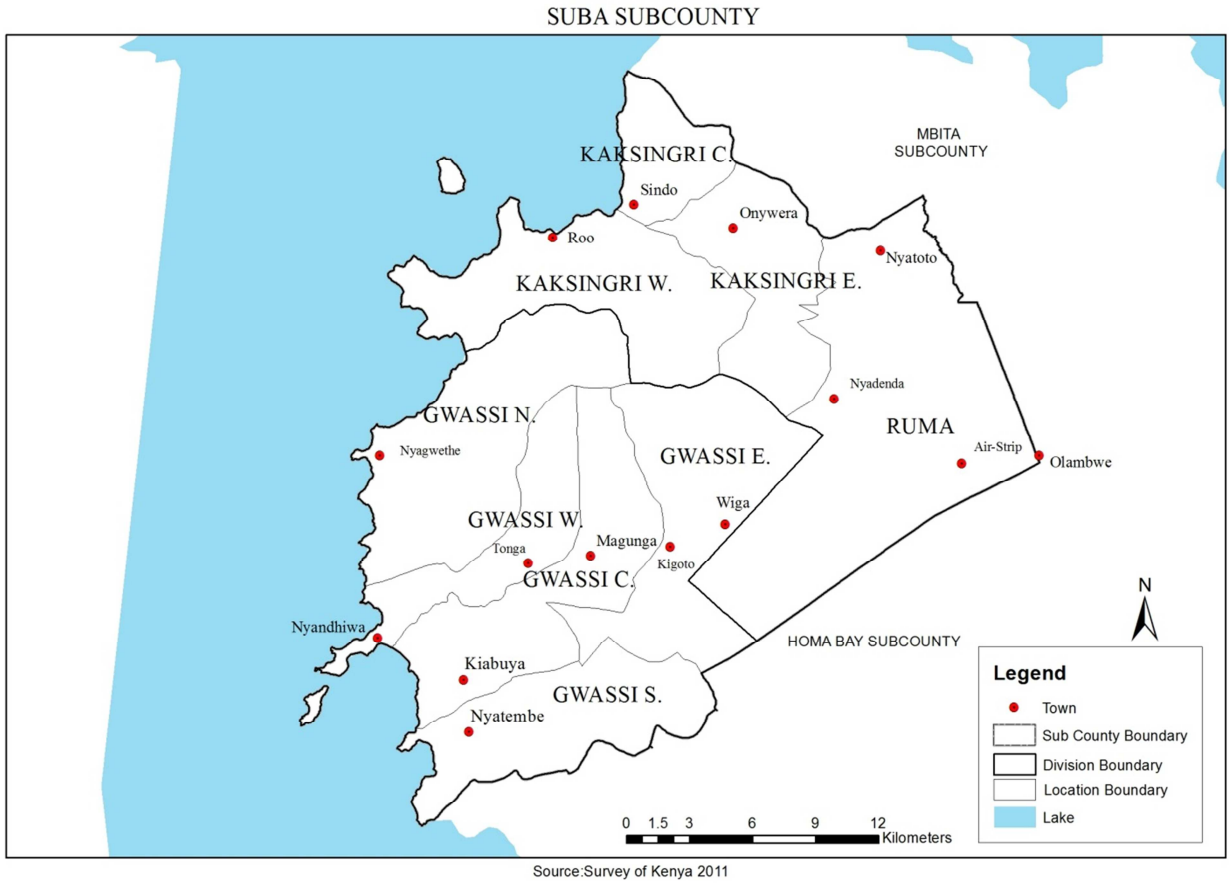

Fig. 7: Map of Suba Sub-County (Courtesy Department of Geography, University of Nairobi)

2.2 Identification of Ethnoveterinary practitioners and collection of ethnobotanical data

The study site was divided into twenty strata based on their recent administrative sub-locations. With the help of local administration (chiefs, assistant chiefs, village elders and the general public), a field survey was conducted to identify traditional medical practitioners and medicinal plant vendors with background knowledge on plants that were useful in this study. Out of these twenty four sub-locations, four sub-locations which also represented four villages were purposively selected since these communities highly depended on traditional healings and possess many skills acquired from fore-parents. The purposive sampling was used to collect ethnoveterinary data from informants according to Orozcoz and Lent, (2005). This is a sampling method which is a non-probability sampling technique. These data were collected from thirty two resource persons in four villages in the study area namely Kiembe village in Gwassi West Location, Kisaku village in Gwassi North Location, Nyadenda village in Kaksingri Central Location and Roo village in Kaksingri West Location. A questionnaire which had 23 questions was made and used to collect information from resource persons (Appendix E). The Chief of Gwassi West Location Mr. Opella directed me to Kiembe village elder that helped me to identify five herbalists whom I used for pre-testing my questionnaire during the pre-survey. The other 27 informants were also identified in the remaining three villages through their respective elders. The questionnaire sort for local names of acaricidal plants and methods of preparation of the acaricides, habits of the plants and part of the plant used in the acaricide preparation. Herbalists provided specific and high quality information on anti-tick plants.

Plant specimens were collected in duplicate; one specimen was used for preliminary identification in the field as previously described (Beentje ,1994 ; Agnew, 2013) while the other

was pressed and carried to the University of Nairobi herbarium (NAI) for authentication and further compared with the available permanent prepared herbarium collections.

2.3 Selection of priority plants

Priority plants were selected based on a survey carried out between December 2014 and January 2015 in Suba Sub-County. The frequency report on plants that are acaricidal agents by the respondents was prepared. The report was arranged in the order of ranks-from highest (rank 1) to the lowest rank (rank 16) according to frequencies in the independent report for each plant. Three plants *Phytolacca dodecandra* (leaves), (*Cissus quadrangularis* (whole) and *Ipomoea kituiensis* (leaves) which had the highest frequency were selected and the parts used as medicine collected and subsequently subjected to chemical as well as bioassay tests. The information on the useful parts of the plants was identified by the herbalists.

2.4 Collection and drying of plant parts

2.4.1 *P.dodecandra* and *I.kituiensis*

The leaves of each of the two plants were plucked and stuffed in a polythene bag which was placed in a cooler box and transported to Nairobi. The materials were then dried in the shade for 14 days and then ground into a fine powder using an electric mill. *P.dodecandra* and *I.kituiensis* dry powder were 354.7 g and 392.8 g respectively.

2.4.2 *C.quadrangularis*

The whole plant was uprooted, roots washed first then the whole plant chopped into pieces with an electric blender and stuffed in a polythene bag which was placed in a cooler box

and transported Nairobi. The material was then shade-dried for 14 days and ground into a fine powder using an electric mill. The dry powder was 401.9 g.

2.5 Extraction of plant crude extracts

Air dried ground material (160 grams) was extracted separately in 2 litres of distilled water, 2 litres of dichloromethane (DCM) and methanol in the ratio of 1:1 (v/v) in 5-litre covered buckets for 72 hours then filtered using Whatman's filter paper No. 1 to obtain solvent extracts. The organic extract was evaporated using a rotary evaporator in a vacuum at 60°C to obtain crude extract which was transferred into separate marked vials and dried at room temperature (26-28°C). The aqueous extract was deep-frozen then freeze-dried to obtain powdered crude extract which was then placed in separate vials and stored at 4°C (Sirama *et al*., 2015). The percentage yield was calculated as a ratio of the extract weight to the plant material weight (160 g). The organic and aqueous extracts of the plant weights were 14.19g and 16.38g respectively.

2.6 Dosage preparation of 2.5, 5 and 10 mg/mL of the crude extracts and 0.0045 % almatix

2.6.1. Preparation of 10 mg/mL

Weigh 10 g of ground crude extract and place in a 1- litre volumetric flask. Make to volume with 990 mL deionized water.

2.6.2 Preparation of 5 mg/mL

Take 50 mL of 10 mg/mL in a 100- mL volumetric flask. Make to volume with 50 mL deionized water.

2.6.3 Preparation of 2.5 mg/mL

Take 50 mL of 5 mg/mL in a 100- mL volumetric flask. Make to volume with 50 mL deionized water.

2.6.4 Preparation of 0.0045 % almatix

Take 0.36 mL of almatix® (12.5 % amitraz, almatix density 1 g/mL) and place in a 1-Litre volumetric flask. Add 999.64 mL of deionized water and stir.

2.7 Tick collection, identification, egg incubation and determination of the LC_{50}

Ten adult engorged female ticks were collected from selected cattle in Suba Sub-County, Kenya. The tick and sex authentication was done at the tick veterinary laboratory of The International Livestock Research Institute (ILRI) and confirmed as adult *B.decoloratus.* Using Ducornez *et al*., (2005) technique with some slight modifications, eggs of blue tick reared at the institution were weighed into 1g samples, placed into test tubes and incubated for 28 days at a temperature range of 28 ±2°C and relative humidity of 80-90 % for egg hatching. One hundred (100) larvae which wer acclimatized for 8 days were subjected to the concentration of 2.5, 5 and 10 mg/mL of crude extracts; where they were exposed to the plant extracts for three minutes by use of larval packet immersion method. Control ticks were prepared in a similar manner as the test specimens and 0.0045 % of 12.5 % amitraz (active ingredient) used as a positive control while 1 % of DMSO and water were used as negative controls (Walker *e t al*., 2002; Ducornez *et al*., 2005). The experiments were done in six replicates. The percentage mortality of *B.decoloratus* larvae was calculated as a function of the concentrations (2.5, 5, and 10 mg/mL) of each crude plant extract within a duration of treatment of 24 hours and LC_{50} determined

(concentration killing 50 % of the ticks). The average mortalities of *B.decoloratus* tick larvae were assessed as a function of the concentrations of crude plant extracts and corresponding LC_{50} calculated for each plant species after a period of 24 hours. Bioassay data subjected to probit analysis program (Finney, 1971) proved that the LC_{50} of the crude plant extracts were dependent on the method of extraction of the crude plant extracts (Table 7 and Appendix D).

2.8 Determination of acute toxicity of crude extracts on brine shrimp

The acute toxicity assay was performed using the phototropic brine shrimp *nauplii* (brine shrimp larvae) based on Meyer's method (Musila *et al*., 2013). Artificial sea water was prepared by dissolving 38 grams of sea salt in 1 litre of distilled water. A tank measuring 14 cm by 9 cm by 5 cm having two unequal chambers with several holes on the divider was used for hatching. The chambers were filled with artificial sea water. Brine shrimp eggs were placed in the larger chamber and yeast added to act as food for the *nauplii*. The larger chamber was then covered with dark background paper while the smaller chamber was illuminated. The incubation was done at a temperature range of 23-29^{o}C for 48h to allow hatching and *nauplii* were collected in the illuminated section. Toxicological studies of brine shrimp can be extrapolated for other animals.

Various concentrations of the crude extract in sea water were used: 10, 100 and 1000 µg/mL (see table 1) in testing toxicity. A stock solution of 10,000 µg/mL for each crude extract was prepared. For the aqueous extract, the stock solution of 10,000 µg/mL was prepared by dissolving 0.1g of the crude extract in distilled water then made to volume using the same distilled water in a 10-mL volumetric flask. For organic extract, 0.1 g of sample was first dissolved in 1% DMSO then further diluted using distilled water to 10 mL in volumetric flask to make stock solution.

Ten brine shrimp larvae were drawn from the hatching tank using Pasteur pipettes and placed in 4.5 mL of sea water then 0.5 mL of stock solution (10,000 μg/mL) added giving a concentration of 1000 μg/mL. To make a concentration of 100 μg/mL, 4.95 mL of sea water and 0.05 mL of stock solution were used. Concentration of 10 μg/mL was made by using 4.995 mL sea water and 0.005 mL of stock solution (Table 1). Control experiments were done using artificial sea water and DMSO for organic extract and artificial sea water only in the case of aqueous extract (Odhiambo *et al.*, 2014). Three replicates for the three serial dilutions of different crude extracts and the control were performed. Surviving *nauplii* were counted after 24 hours using a magnifying glass and the average mortality at each concentration was determined since it essential for estimation of $LC_{50.}$

Table 1: Brine shrimp bioassay set up for each plant extract

Vial	Volume of Artificial sea water (ml)	No of Brine shrimp larvae	Volume of stock solution (ml)	Concentration (µg/ml)	Nature of experiment	Final volume in the vial (ml)
1	4.5	10	0.5	1,000	Trial	5
2	4.5	10	0.5	1,000	Repeat	5
3	4.5	10	0.5	1,000	Repeat	5
4	4.95	10	0.05	100	Trial	5
5	4.95	10	0.05	100	Repeat	5
6	4.95	10	0.05	100	Repeat	5
7	4.995	10	0.005	10	Trial	5
8	4.995	10	0.005	10	Repeat	5
9	4.995	10	0.005	10	Repeat	5
10	5	10	0	0	Control	5
11	5	10	0	0	Control	5
12	5	10	0	0	Control	5

2.9 Phytochemical screening

Phytochemical analysis of the organic and water extracts of the three selected botanicals was done by standard methods as described in Jigna and Sumitra, (2007) and Mariita *et al.*, (2011). The extracts were screened for phytochemicals (saponins, alkaloids, flavonoids, tannins and terpenoids) as described below:

2.9.1 Saponins (Foam test)

Powdered sample of plant extract (1 mg) was added to 10 mL of distilled water in a hot water bath for 10 minutes. The mixture was filtered while hot and allowed to cool. 2.5 mL of the filtrate was diluted to 10 mL with distilled water and shaken vigorously for 2 minutes. A stable 15 minute frothing indicated the presence of saponins in the filtrate (Jigna and Sumitra, 2007; Mariita *et al.*, 2011). The procedure was repeated for all the crude extracts.

2.9.2 Alkaloids (Dragendorff's test)

The dry crude plant extract (0.2 g) of was boiled for 2 minutes with 5 ml of 2 % hydrochloric acid then cooled. It was then filtered and 3 drops of Dragendorff's reagent added to 1 mL of the filtrate. A red precipitate indicated the presence of alkaloids (Jigna and Sumitra, 2007; Mariita *et al.*, 2011). The procedure was repeated for all the crude extracts.

2.9.3 Tannins (Ferric chloride test)

Powdered sample of plant extract (0.5 mg) was boiled for 5 minutes in 10 mL of distilled water in a test tube and then cooled and filtered. 5 drops of 0.1 % ferric chloride was added to the filtrate and observed for brownish green or blue-black coloration that detected the presence of tannins (Jigna and Sumitra, 2007; Mariita *et al.*, 2011). The procedure was repeated for all the crude extracts.

2.9.4 Flavonoids (Alkaline reagent test)

Powdered plant extract (1g) was boiled with 10 mL of distilled water for 5 minutes and filtered while hot. 5 drops of 20 % sodium hydroxide solution were added to 1 ml of the cooled filtrate resulting into a yellow colour. A change to yellow colour which on addition of 5 drops of

dilute hydrochloric acid to colourless is a positive test for the presence of flavonoids (Jigna and Sumitra, 2007; Mariita *et al.*, 2011). The procedure was repeated for all the crude extracts.

2.9.5 Terpenoids (Salkowski test)

Powdered plant extract (1 g) was mixed with 2 mL chloroform. 3 mL concentrated sulphuric acid was then added to form a layer. A redish-brown precipitate colouration formed at the interface indicated the presence of terpenoids (Jigna and Sumitra, 2007; Mariita *et al.*, 2011). The procedure was repeated for all the crude extracts.

2.10 Data analysis

Descriptive statistics was used to analyze the data where MS Excel® 2010 spread sheet application was utilized to make simple calculations, determine proportions of plant families, habit, and preparation methods and to draw graphs. Data were analyzed by ANOVA using SPSS computer programme; mean, standard errors and standard deviations of various mortalities observed after treating *Boophilus* larvae with the various extracts from the three plants at different concentrations were computed. Data from each plant extract were then subjected to one way ANOVA to determine whether there were significant differences between the various concentrations used. Once differences were identified Dunnett t-test was done to compare the treatments with the positive controls.

The lethal concentration (LC_{50}) at 95 % confidence interval of the selected plants was determined using the Finney computer program for brine shrimp and acaricidal activities (Finney, 1971).

CHAPTER THREE

3.0. RESULTS

3.1. Ethnobotany of the identified acaricidal plants

The local administrations in the study area helped identified 32 herbalists who also identified 16 acaricidal plants. From the 16 plants, three with the highest frequencies (ranks 1, 2 and 3) were computed using independent reports to be used for bioassay test. This study also categorized these 16 species of ethnoveterinary medicinal plants into 13 families with their description, traditional mode of preparations, botanical and local names (Table 2).

Table 2: Acaricidal plants identified during the study

Botanical name and voucher number	Vernacular name	Family	Habit	Parts used	Mode of preparation	Number of independent report (IR)	Ranking
Cucumis aculeatus AO 2015/ 001	Otangle	Cucurbitaceae	Herb	Fruit	Decoction	5	15
Senna didymobotrya AO 2015/ 002	Owino	Leguminoseae C.	Shrub	Leaves	Infusion	6	14
Phytolacca dodecandra AO 2015/ 003	Mahoho	Phytolaccaceae	Shrub	Leaves	Infusion	16	1
Tagetes minuta AO 2015/ 004	Nyanjagra	Compositeae	Herb	Whole	Infusion	9	6
Ocimum kilimandscharicum AO 2015/ 005	Mweny madongo	Lamiaceae	Shrub	Whole	Decoction	9	6
Ipomoea kituiensis AO2015/006	Obinju mar nam	Convolvuliaceae	Shrub	Leaves	Decoction	13	3

Azadirachta indica AO 2015/007	Mwarubaine	Meliaceae	Tree	Leaves	Decoction	7	12
Lantana camara AO 2015/008	Onyalo biro	Verbenaceae	Shrub	Leaves	Decoction	7	12
Ricinus communis AO 2015/ 009	Obalandagwa	Euphorbiaceae	Shrub	Leaves, Fruits	Infusion	8	11
Cissus quadrangularis AO 2015/010	Minya	Vitaceae	Herb	Whole	Decoction	15	2
Solanum incanum AO 2015/011	Ochok	Solanaceae	Shrub	Whole	Decoction	12	4
Melia azedirach AO 2015/012	Dwele	Meliaceaea	Tree	Leaves, fruits	Decoction	9	6
Maerua edulis AO 2015/013	Amoyo	Capparaceae	Herb	Leaves	Decoction	4	16
Aloe dawei AO 2015/014	Ogaka	Aloeceae	Shrub	Leaves	Infusion	10	4
Datura stramonium AO 2015/015	Koth-kiyombi	Solanaceae	Herb	Leaves	Infusion	9	6
Euphorbia tirucalli AO 2015/016	Ojuok	Euphorbiaceae	Tree	Leaves, bark	Sap	5	14

NB: The coding is my personal way of plant identification. AO stands for Alfred Onyango

Solanaceae was the most highly used (14.58 %) of the total number of independent reports and the least used was Capparaceae (2.78 %) (Appendix B)

3.1.1 *Cucumis aculeatus* Cogn (Cucurbitaceae) – Otangle –AO 2015/001

This is a perennial herb with spiny yellow- hooked hairs on the stem ridges and major veins underneath the leaves. The leaves are ovate, deeply or shallowly 3- lobed. The male flowers are solitary, yellow- green and about 11 mm long. Its fruit is green to yellow, 7 cm long with scattered bristle-tipped projections .The fruit is cut then decocted and applied to the animal's coat focusing mainly on the infested areas.

3.1.2 *Senna didymobotrya* (Leguminoseae C.) - Owino- AO 2015/002

This is a shrub that grows upto 4.5 m high. Leaves are upto 45 cm long with ten to twenty pairs of oval leaflets, each tipped with a fine point. Flowers are bright yellow which are upto 1.8 cm long, crowded together in a raceme upto 45 cm long. Its unopened buds are almost black and has ten stamens, seven are fully developed (of which two or three are longer) and three are poorly developed. Pods are flattened upto 12.5cm long. It is found in grasslands and scrubs. The leaves, pods and roots are poisonous. Leaves are either dried or used wet. This is pounded and the infusion is used for washing the infested cattle to control lice, ticks and fleas.

3.1.3 *Phytolacca dodecandra* L. Herit (Phytolaccaceae) - Mahoho –AO 2015/003

This is a clambering plant upto 9 m high with long, hanging branches and a tuberous rhizome. The leaves are oval, thick and shiny, upto 15 cm long and the two sides sometimes unequal. The flowers are greenish-white to yellow, scented and unisexual. Male flowers have fifteen stamens and the ovary is five-celled and infertile. Female flowers have a five-celled ovary and eight to fifteen short infertile stamens. The fruit is orange-red, five-lobed and fleshy. They are found on the forest edges, scrub patches and termite mounds. The plant is very poisonous and infusion from leaves is used to control external parasites in livestock in general by washing their whole bodies.

3.1.4 *Tagetes minuta* L. (Compositae) - Nyanjagra –AO 2015/004

This is a stiff herb up to 1.8 m high. The leaves are opposite and compound while the leaflets are narrowly lanceolate, edged toothed and very strong-smelling. The flower-heads are yellow and elongated in a stiff crowded inflorescence. Florets are tubular and ligulate with about two of each

in each flower-head. Pappi have few scales. The whole plant is pounded, the infested animal is then washed severally with the infusion till ticks and lice are completely eradicated.

3.1.5 *Ocimum kilimandscharicum* Guerk (Lamiaceae) - Mweny madongo- AO 2015/005

This is an erect or ascending hairy branching shrub and occasionally a herb. The leaves are ovate to elliptic with spreading hairs often with rounded apex. Its racemes are simple and terminal of rather distant whorls of small white to pinkish flowers. The petals have spreading tubes about 7 mm long and sepals about 6 mm long.

3.1.6 *Ipomoea kituiensis* Vartke (Convolvuliaceae) - Obinju mar nam- AO 2015/006

This is a sub-erect or twining shrub which grows up to 6 m tall. Its leaf-blade is ovate to reniform ranging from 14 cm long and 13 cm wide. The flowers are few to many in cymes with peduncles of about 3.5 to 20 cm long. This plant has linear sepals which are lanceolate to ovate. Its corolla is white, cream or yellow with a purple centre, and funnel-shaped of approximately 5 to 8 cm long. It has ellipsoidal capsule fruit which is 15 mm long 1.3 cm wide and its seed is ovoid (7 mm long) and is covered with hairs. The leaves are pounded and the decoction is used for washing livestock against ticks, lice and fleas.

3.1.7 *Azadirachta indica* (Meliaceae) - Mwarubaine- AO 2015/007

This is a hardy, fast-growing, medium-sized tree growing from 15 to 20 m in height with a dense leafy oval-shaped canopy. It is evergreen flourishing in arid and semi-arid regions. The bark is pale to grey-brown and rough while the leaves are shiny, green, crowded towards the end of branches. It has creamy white flowers hanging down in long sprays. The fruits are oval yellow

berries when ripe, up to 2 cm across, long. A solution from pounded leaves serves as an anti-tick spray on livestock.

3.1.8 *Lantana camara* L. (Verbinaceae) - Onyalo biro- AO 2015/008

This is a scrambling herbaceous shrub upto 2.4 m high. The stem is square and prickly especially near the base. The leaves are opposite and ovate upto 8.7 cm long, rough with toothed edge. The flowers are pink towards the outside of the flattened head and yellowish towards the centre. The fruits are shiny black when ripe. The plant is found in open bushland, wooded-grassland and dry forest margins. Whole leaves are crushed, mixed with hot water and the extract is sprayed to the animal coat as a remedy for lice, mites and ticks.

3.1.9 *Ricinus communis* L. (Eurphorbiaceae) - Obala-ndagwa- AO 2015/009

This is an evergreen shrub measuring upto 5 m high. The leaves are green or redish, glabrous and glaucous with alternating long-petiolate, deeply palmately lobed, 15-60 cm across. The flowers are in large pyramidal pseudo-terminal erect panicles with male flowers below and female ones above. Male flowers have creamy yellow stamens while female flowers have a showy red stigma borne on the upper part of the spike. The fruit is round deep red in colour of capsule type which is ellipsoid or oblong in shape. The seed and seed coat are reported poisonous to cattle (Kokwaro and Johns, 1998; 2013). Mature fruit is pulverized and soaked in cold water and the solution is applied on the animal's coat to control ticks and lice.

3.1.10 *Cissus quadrangularis* L. (Vitaceae) - Minya- AO 2015/010

This is a climbing shrub or herb with a succulent four-sided stem and tendrils. The leaves are simple and variously toothed and are only found on the younger parts. The flowers are in

umbels. The decoction from the pounded whole plant is used for washing livestock against ticks, lice and mange. It has high smarting effects on the skin.

3.1.11 *Solanum incanum* L. (Solanaceae) - Ochok- AO 2015/011

This is an erect shrub upto 2 m high with spiny stems, branches and leaves. The leaves are slightly lobed with hairs on both surfaces, prickly on the midrib of the older leaves. Its flowers are mauve or purple upto 3.8 cm across. The anthers are yellow, joined in the centre of the flower and its inflorescence lateral with three to ten or more flowers. The fruit is a large yellow berry, upto 3.8 cm across. The plant is found on waste ground and along roadside where soil has been eroded or scraped away. Cattle are washed with decoction from pounded whole plant to control ticks and other external parasites. The fruit pulp is applied directly on tick infested areas to kill ticks.

3.1.12 *Melia azedirach* L. (Meliaceae) - Dwele- AO 2015/012

This is a deciduous tree upto 15 m high with a smooth bark and grey-brown in colour. The leaves are usually 2-pinnate with petioles and rachis upto 40 cm long. Its leaflets are upto 5.5 cm long 2.5 cm broad that are opposite or sub-opposite. The inflorescence has small flowers in large axillary cymose panicle with calyx 2.5 mm long and petals upto 8 mm long. The fruit consists of fleshy berries up to 2 cm long and 1.5 cm broad. Berries are extremely poisonous to humans, livestock and poultry. Leaves and fruits are used to control mange, ticks and lice infestations.

3.1.13 *Maerua edulis* (Capparaceae) - Amoyo- AO 2015/013

This is a spreading shrub or woody herb upto 3 m high and glabrous. The leaves are simple petiolate. Its flowers are many with pedicels that are 10 to 25 mm long. The sepals are 4 to 9 mm long with no petals. Its androphore is about 1 mm longer than the receptacle while the stamens are 15 to 30 mm long. Its ovary is spindle-shaped, 4 to 6 mm long and the fruits are globose and yellow or orange in colour. Leaves are pounded and animals are washed with the infusion as remedy to tick control.

3.1.14 *Aloe dawei* Berger (Aloeaceae) - Ogaka- AO 2015/014

This is a much branched leafy shrub 1 to 2 m tall. The leaves are dark green with an inflorescence 60 to 90 cm high. Its stalks are dark red-brown while the racemes are quite densely flowered and the flowers are red. The plant is found most often in rocky bushland in western Kenya. Leaf decoction is used for washing animals to treat skin diseases and control external parasites such as ticks.

3.1.15 *Datura stramonium* L. (Solanaceae) - Koth- kiyombi- AO 2015/015

This is a herb upto 1.5 m high. The stem is smooth and always branching into two. The leaves are ovate, pointed and deeply toothed or lobed. Its flowers are white arising singly where the stem branches with calyx up to 3.8 cm long while corolla is funnel-shaped and folded when young stamens are attached at the base of the corolla-tube. Ovary is four-celled. The fruit is up to 5 cm long and very prickly. The leaves are poisonous to human and animals. They are pounded and the solution used for treatment of ticks and ringworm in livestock.

3.1.16 *Euphorbia tirucalli* (Euphorbiaceae) - Ojuok- AO 2015/016

This is a succulent shrub or tree growing upto 6 m or more, commonly occurring in bushland , thickets and coastal bushland. The bark is dense, straight-stemmed. The branches are smooth, green and cylindrical in dense masses. Its leaves are small upto 6 mm long. The flowers are cream or yellow-green, occur in short, terminal clusters. Its fruit is a 3-lobed capsule, 6 mm across, hard, purple-green. The latex of this plant is highly poisonous and especially harmful to the eyes and is used as fish poison as well as an insecticide.

3.2 Distribution in plant habit and usage as anti-tick

Differences in plants' usage depending on their habit were also recognized (Fig. 8) where the shrubs recorded the highest representation (56.25 %) followed by herbs (29.17 %) and lastly trees (14.58 %).

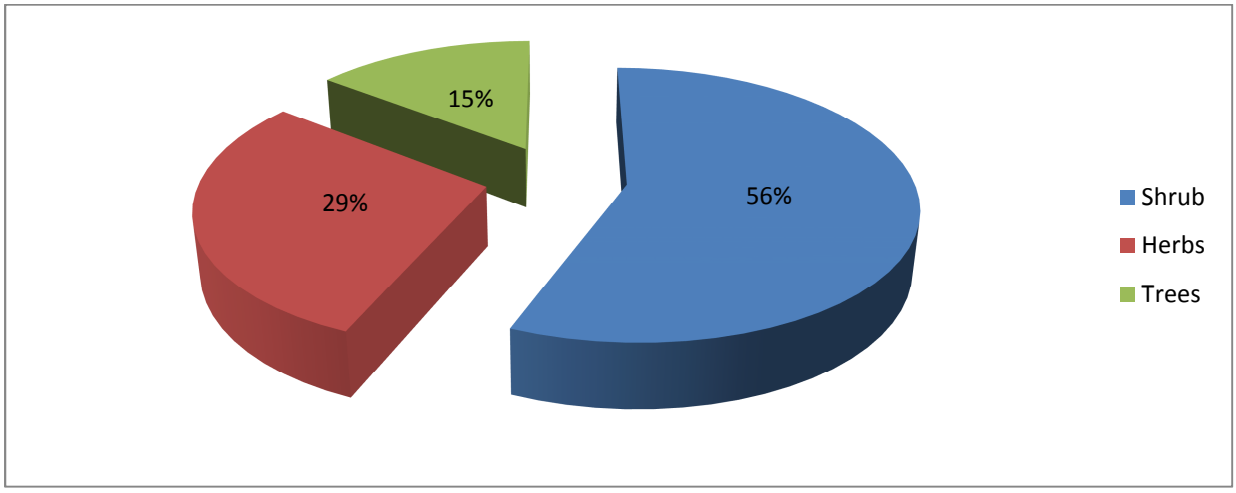

Fig. 8: Plants usage according to their habit

Different plants' parts were used with the leaf being the most frequently used (56.63 %) followed by the whole plant (27.11 %), fruit (13.25 %) and bark (3.01 %). The habit percentage

was calculated as a percentage of the ratio of total number of habits mentioned by all informants in the independent report:

Habit percentage= (Habit independent report /144) X 100;

Where; Habit= total number of times a habit is mentioned in the independent; 144= cumulative number of habits mentioned in the independent report.

3.3 Knowledge on acaricidal plants

From the 32 interviews conducted by use of a questionnaire (see Appendix E) on herbalists between the ages 28 to 87 years, the age groups 68 to 77 and 78 to 87 years had the highest number of plants cited (14 each) while age group 28 to 37 years had the least (4). There was a strong correlation between age and the number of plants cited (r= 0.81). The older the informants the more the plants cited (Fig. 9).

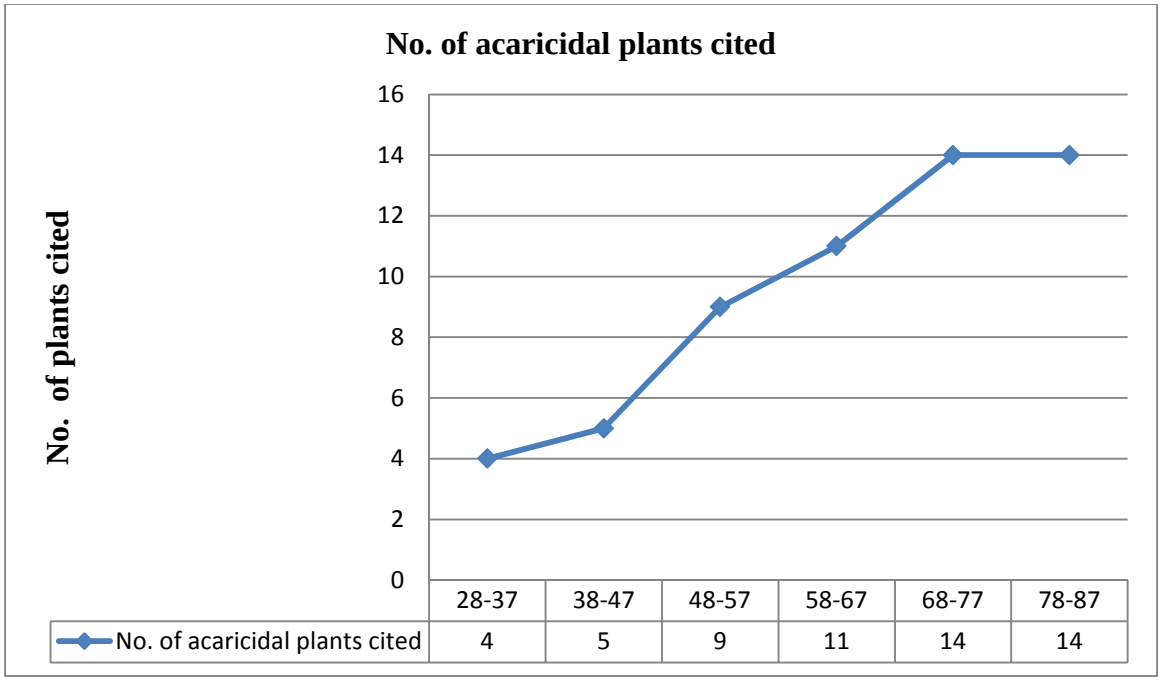

Fig. 9: Age groups of informants and the number of medicinal plants cited

3.4 Traditional methods of preparation of drugs

Various methods of preparation of herbal medicine were applied with the decoction being the most preferred method (56.25 %) followed by infusion (40.28 %) and sap (3.47 %). Pounding was universal as it appeared to be the preliminary method for every medicinal preparation.

Three plants *Phytolacca dodecandra* (leaves), *Cissus quadrangularis* (leaves and stems) and *Ipomoea kituiensis* (leaves) were selected for chemical and bioactivity tests based on their ranking as the top three plants most frequently used (Table 3).

Table 3: Selected priority plants

Plant species	Part collected	Rank
Phytolacca dodecandra	Leaves	1
Cissus quadrangularis	Whole	2
Ipomoea kituiensis	Leaves	3

3.5 Yield of crude plant extracts

P.dodecandra extract had the highest percentage yield of 8.87% among the tested Methanol/ DCM (1:1 v/v) extracts whereas *I.kituiensis* yielded 7.17% and *C.quadrangularis* had the least yield (6.33 %). Among the tested water extracts, *P.dodecandra* recorded the highest percentage yield (10.24 %) as *C.quadrangularis* and *I.kituiensis* yielded only 8.56% and 8.22 % respectively. Between the two solvents, water gave a higher mean percentage yield (9.01 %) compared to methanol in DCM (1:1 v/v) solvent which had 7.46 % yield (Table 4). Original sample taken was 160 g.

Table 4: Yield of organic and aqueous plant extracts measured in grams after extraction

Plant species	**Methanol in DCM extract (1:1 v/v)**		**Water extract**	
	Yield (grams)	**Percentage yield (%)**	**Yield (grams)**	**Percentage yield (%)**
Cissus quadrangularis (whole)	10.13	6.33	13.70	8.56
Ipomoea kituiensis (leaves)	11.47	7.17	13.15	8.22
Phytolacca dodecandra (leaves)	14.19	8.87	16.38	10.24
Average	11.93	7.46	14.41	9.01

3.6 Phytochemical analysis of crude plant extracts for secondary metabolites

Both organic and aqueous extracts of all plants, except *P. dodecandra* showed a positive test for the presence of flavonoids, alkaloids, tannins, terpenoids and saponins. The organic and aqueous extracts of *P.dodecandra* , however, showed negative results for flavonoids (Table 5).

Table 5: Phytochemical analysis of each crude extract for secondary metabolites

Plant	Crude extract	Alkaloids	Flavonoids	Saponins	Tannins	Terpenoids
Cissus quadrngularis	Methanol in DCM (1:1 v/v)	+	+	+	+	+
	Water	+	+	+	+	+
Ipomoea kituiensis	Methanol in DCM (1:1 v/v)	+	+	+	+	+
	Water	+	+	+	+	+
Phytolacca dodecandra	Methanol in DCM (1:1 v/v)	+	-	+	+	+
	Water	+	-	+	+	+

Key: + =Present

- = Abesnt

3.7 Lethal concentration (LC_{50}) of brine shrimp larvae

At 10 µg/ml, all the aqueous extracts as well as organic extracts were highly toxic except organic extracts of *I.kituiensis* which showed comparatively low toxicity (Table 6). Similarly, at 100 µg/mL, all the aqueous and organic extracts showed high toxicity while the organic extracts of *I.kituiensis* were only marginally toxic. At 1000 µg/mL both aqueous and organic extracts of all plants were highly toxic. Overall, all the tested crude extracts (both aqueous and organic) were highly toxic except the organic extracts of *C.quandrangularis* (113.10 µg/mL) and aqueous extracts of *I.kituiensis* (136.96 µg/mL) which were moderately toxic (Table 6 and Appendices C1, C2). However, at high concentration (1000 µg/mL) both aqueous and organic extracts were highly toxic.

Table 6: Lethal concentration (LC_{50}) of brine shrimp larvae

Plant name	Average mortality at various concentrations of extracts (µg/mL)								**LC_{50} (µg/mL)**	
	0 µg/mL		10 µg/mL		100 µg/mL		1000 µg/mL			
	Water	Org	Water	Org	Water	Org	Water	Org	Water	Org
P.dodecandra	0.0	0.0	9.66	8.33	10.0	10.0	10.0	10.0	35.98	**39.97**
C.quadrangularis	0.0	0.0	9.33	8.0	10.0	9.33	10.0	10.0	38.31	**113.1**
I.kituensis	0.0	0.0	10.0	4.0	10.0	6.33	10.0	10.0	136.96	**4.17**
Water	0.0		0.0		0.0		0.0		1708.23	
DMSO		0.00		0.00		0.00		0.00		**1708.23**

Key: LC_{50}< 100 = Strongly/highly toxic

LC_{50} > 100 < 500 = moderately toxic

LC_{50} > 500 < 1000 = weakly toxic

LC_{50} > 1000 = Non toxic

Org= Methanol: DCM (1:1 v/v)

3.8 *In vitro* acaricidal activity of crude plant extracts

An assessment of the acaricidal activity of each crude plant extract in an *in vitro* system showed that *P.dodecandra* leaves ranked number one in terms of efficacy followed by *C.quadrangularis* (whole plant) and *I.kituiensis* leaves in that order (see Table 3).

3.8.1 Larvicidal activitity of *P.dodecandra* extracts

When the larvicidal activity of 5 mg/mL and 10 mg/mL of the organic extracts of *P.dodecandra* were compared with that of the almatix, both concentrations were not significantly ($p > 0.05$) different in activity therefore implying that 5 mg/mL and 10 mg/mL organic extracts of *P.dodecandra* had similar larvicidal activity to that of almatix (Fig.10). However, the aqueous extracts showed a significantly lower activity ($p < 0.05$) compared to almatix. At 2.5 mg/mL, both extracts of *P.dodecandra* showed significantly lower larvicidal activity ($p < 0.05$) compared to almatix.

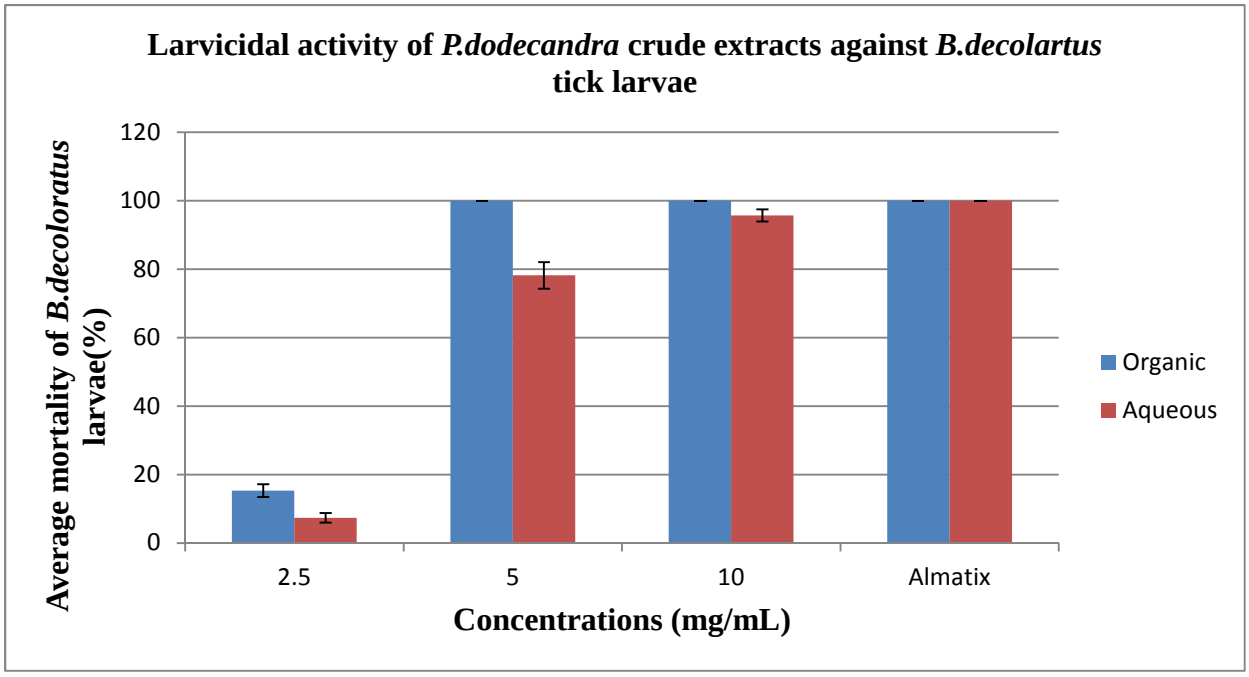

Fig. 10 : Average (±Standard deviation) mortality of *B.decoloratus* tick larvae due to *P.dodecandra* extracts at various concentrations.

3.8.2 Larvicidal activitity of *I.kituiensis* extracts

Both aqueous and organic extracts of *I.kituiensis* showed significantly lower ($p < 0.05$) larvicidal activity at all concentrations compared to almatix (Fig. 11). The difference in larvicidal activity was markedly higher at the lowest concentration of *I.kituiensis* (2.5 mg/mL).

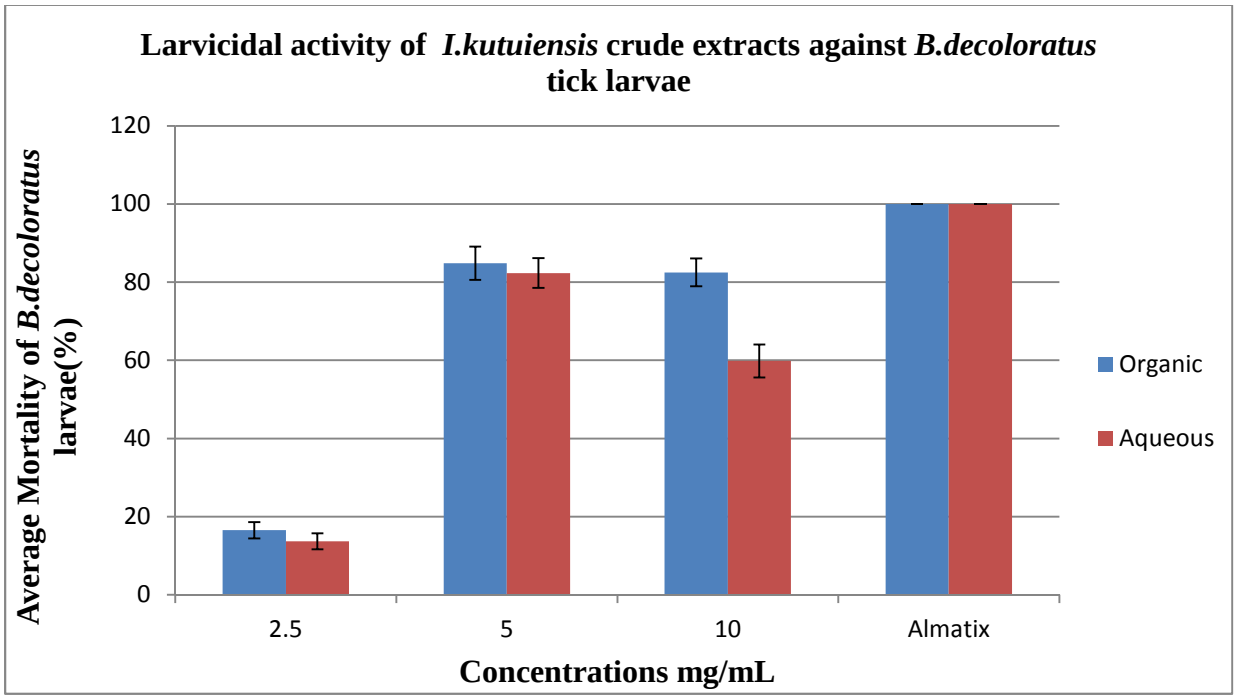

Fig. 11: Average (±Standard deviation) mortality of *B.decoloratus* tick larvae due to *I.kituiensis* extracts at various concentrations.

3.8.3 Larvicidal activitity of *Cissus quadrangularis* extracts

The aqueous and organic extracts of *C.quadrangularis* showed significantly lower larvicidal activity ($p<0.05$) at all concentrations, except for organic extract at 10 mg/mL. At 10 mg/mL, organic extract of *C.quadrangularis* showed 100 % mortality which was not significantly different from almatix activity ($p>0.05$) (Fig. 12). Interestingly, *C.quadrangularis* crude extracts appear to have the lowest larvicidal activity compared to those of *P.dodecandra* and *I.kituensis.*

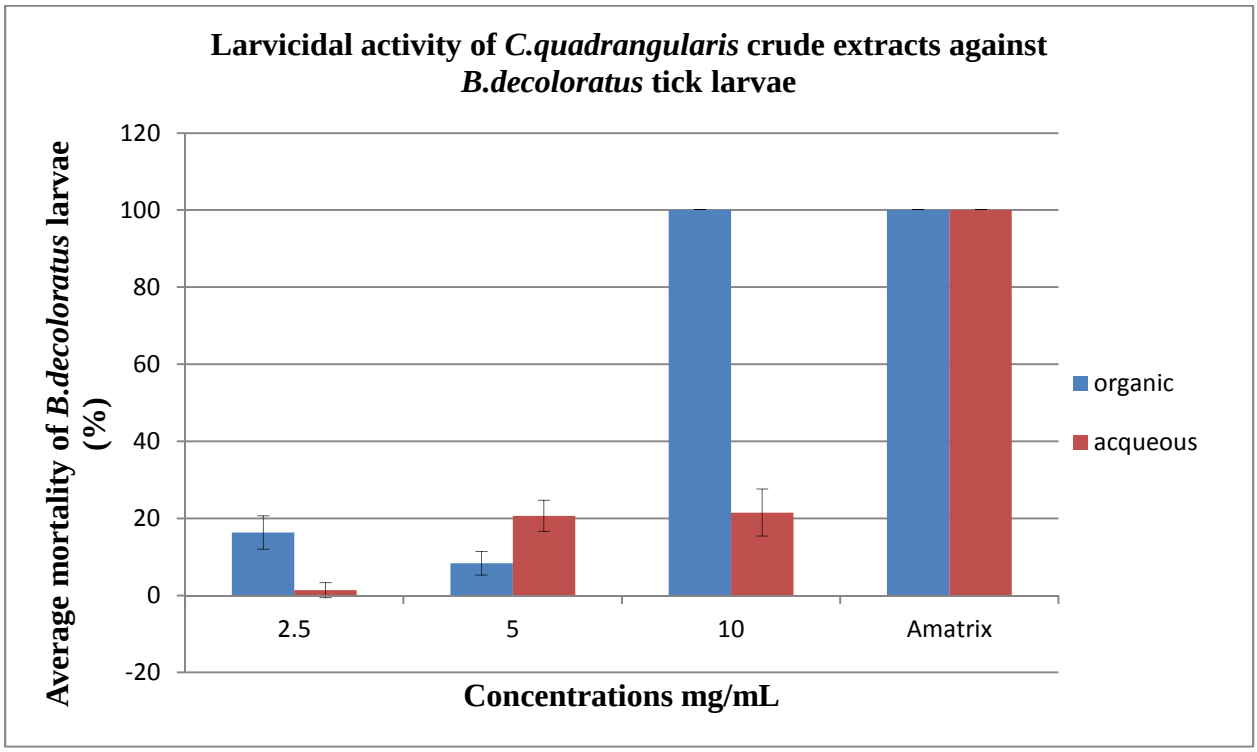

Fig. 12: Average (± Standard deviation) mortality of *B.decoloratus* tick larvae due to *Cissus quadrangularis* extracts at various concentrations**.**

3.9. Acute toxicity of the crude plant extracts to tick larvae and estimation of lethal concentration (LC_{50})

All organic extracts recorded higher mortality compared to aqueous extracts in each plant species except at 5 mg/mL for *C.quadrangularis* where aqueous was more active than organic. Nevertheless *P.dodecandra* organic extracts showed the highest LC_{50} at concentration of 3.85 mg/mL. *C.quadrangularis* aqueous extracts exhibited the lowest LC_{50} at 13.90 mg/mL (Table 7).

Table 7: Acute toxicity and LC_{50} of crude extracts to the *B.decoloratus* tick larvae

Plant name	Average mortality at various concentrations (mg/mL)								**LC_{50} (mg/mL)**	
	0 mg/mL		2.5 mg/mL		5 mg/mL		10 mg/mL			
	Aq	Org	Aq	Org	Aq	Org	Aq	Org	Aq	Org
P.dodecandra	0.0	0.0	7.33	15.33	78.17	100	95.66	100	**4.83**	**3.85**
C.quadrangularis	0.0	0.0	1.33	16.33	20.67	8.83	21.5	100	**13.9**	**5.56**
I.kituensis	0.0	0.0	13.67	16.5	82.33	84.83	59.83	82.5	**6.65**	**5.23**
Almatix	100	100	100	100	100	100	100	100	**2.36**	

Key: Aq= Water

Org= Methanol in dichloromethane (1:1 v/v)

CHAPTER FOUR

4.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

4.1 Discussion

In the past decade, acaricidal and insecticidal properties of plant extracts have found wide application the world over against phytophagous pests (Isman, 2006). Traditional healers in Suba Sub-County have invaluable knowledge on the control of ticks. These local experts on medicinal practices tended to be individuals with specialized plant knowledge. As previously reported (Wanzala *et al*., 2012), they keep the knowledge secret and to themselves and only share with other parties by exchanging with valuables. There is a common belief among herbalists that if herbal knowledge is not jealously guarded, the medicines won't be effective in treatment of various diseases. Nonetheless, during this study most ethno-practitioners were willing to discuss and share their knowledge freely following the award of an inducement fee (ranging from US$ 5 to 10). Most of those interviewed in this study were men aged between 28 and 87 years and out of the 32 people interviewed 5 were women. This is attributable to the fact that, in most cases, in traditional livestock rearing communities, it is men that take care of their sick livestock principally due to their masculine nature (hence can hold animals during drug administration) and better awareness on utilization of medicinal plants in tick control (Magwede *et al.,* 2015). Interestingly, people with high literacy level were found to have same knowledge of medicinal plants as the illiterate ones, presumably because of high interest by both groups. This finding agrees favourably with that of Mesfin *et al.,* (2014) and Beltran *et al*., (2014) who found that the level of education of the informants was not in any way associated to their ethnobotanical knowledge.

The questionnaires had an introductory preamble to explain the intent of the survey to all respondents due to the sensitive nature of interviews related to traditional medicine. Only one respondent was interviewed per household to avoid influence in the responses. There was a significant correlation (r= 0.81) between respondent's age and number of acaricidal plant citation by individuals. The older the individual, the greater the knowledge of plants and their uses. In many cases and in different societies, age is a factor highly correlated with the number and uses of plants known by individuals and this was in agreement with the findings of Magwede *et al*., (2014) and Beltran *et al*., (2014) which showed that the older the individual, the greater the knowledge of plants and their uses as anti-tick preparations because ethnobotanical knowledge tends to accumulate through the life cycle.

The study identified 16 acaricidal plants and gives a concise botanical description of each one of the plants in Suba Sub-County identified as having acaricidal properties. The description of these plants further supplement what has previously been documented (Lind and Tallantine, 1975; Agnew and Agnew, 1994; Kokwaro and Johns, 1998; 2013; Demissew, 2006; Agnew, 2013). Among these plants, the most commonly used plant families in tick control included Solanaceae (14.58 %), Phytolaccaceae (11.11 %) and Meliaceae (11.11 %). Plants from Solanaceae family were frequently employed in tick control more than any other plant family in the sub-county and this can be explained by the fact that the family contains a wide range of secondary metabolites including alkaloids, saponins, terpenoids, tannins and these compounds may independently or jointly contribute to the observed acaricidal activity against ticks. The various plants mentioned fall under three plant habits; shrubs had the highest percentage of the total independent reports (56.63 %) followed by herbs (29.17 %) and trees with the least (14.58 %). Different plant parts used to prepare drugs include leaves, fruits, bark or whole plant. The

leaf was the most frequently used (56.63 %) followed by whole plant (27.11 %) and the fruit (13.25 %). The bark was the least used (3.01 %). This is not consistent with the Magwede *et al*. (2014) which showed that the bark was the most preferred form of plant material followed by leaves, fruits and the root was the least preferred part in tick control. In some plants, for example *C.quadrangularis,* more than one part were (leaves, stems and fruits) administered to cattle. Some plants such as *I.kituiensis, Azaderachta indica and Lantana camara* leaves were used for the preparation of the drugs. It is suggested that different plant parts had various medicinal properties and leaves had high usage because of its ease of preparation.

Fundamentally, drug preparation in this study involved extraction of the active principles from the parent plants that have medicinal value. Pounding was the most common and universally accepted preliminary method for every medicinal preparation. Other methods included the most frequently applied ones such as decoction, infusion and sap. Among these methods, preparation of a decoction was the most preferred method while the use of sap was rather infrequent. Decoction method is widely used because it is simple, convenient and has low cost implications (Mohammed *et al*., 2013). Whichever the method, all the drug preparations were applied by washing the infested cattle on the coats.

The three most commonly used plant species in this research were *P.dodecandra*, *I.kituiensis* as well as *C.quadrangularis* and, frequently, they are used as acaricide. This can be explained by the fact that these species contain a wide range of biologically active principles. Some of these phytochemicals of pharmacological importance are flavonoids, tannins, saponins, terpenoids and alkaloids which have been studied and have shown positive effects as anti-malarial, mulluscicidal, pesticidal and acaricidal compounds (Parveen *et al*., 2014). In this study, preliminary phytochemical screening of *P.dodecandra* crude extracts showed positive results for

tannins, flavonoids, alkaloids, saponins and terpenoids except flavonoids. This is in agreement with what has previously been reported (Mekonnen *et al.,* 2012). *C.quadrangularis* showed positive results for tannins, flavonoids, alkaloids, saponins and terpenoids which were in line with a similar study by Ruskin *et al.* (2014) which also detected the presence of similar compounds. Results of *I.kituiensis* were positive for all the tested secondary metabolites and this is the first time this is being reported. The results of this study favourably agrees with what was reported by Essiett and Obioboho (2014) where in a study of a member of the same genus, *Ipomoea batatas,* it was concluded that the main metabolites of this plant are tannins, flavonoids, alkaloids, saponins and terpenoids.

Phytochemicals have received increasing attention because of interesting new discoveries associated with their biological activities. The three mostly used acaricidal plants were considered for phytochemical screening to assess the presence of tannins, saponins, flavonoids, terpenoids and alkaloids. Tannins are largely distributed in nature, usually being the active principles of plants used in traditional medicine. Condensed tannins have a great ability to interact with metallic ions and macromolecules and to form soluble complexes with electron-donor groups such as those found in alkaloids (Rey *et al*., 1999). This may be one of the reasons explaining their toxicity against different organisms, including insects, fungi and bacteria. Morphological alterations caused by this active fraction on the epithelium of the midgut of larvae of *A. aegypti* resembled those recorded for tannic acid (Rey *et al*., 1999). In a side note, it is worth noting that this *Melia* species has also demonstrated potential on its ethanolic extract of stem barks, as acaricide against the larvae of the common cattle tick, *Rhipicephalus sanguineus and Boophilus* spp. (Kamani *et al*. 2008). The bioactive plant compounds such as phenolics, terpenoids and alkaloids are known to possess insecticidal, growth inhibiting, anti-molting and

repellent activities (Ghosh *et al*., 2007). The potential role of flavonoids in modulating the reproductive functions of ticks was already reported by Juliet *et al*., (2012). Alkaloid in the plant extracts causes mortality and inhibition of fecundity due to its neurotoxic properties while terpenic compounds, for example precocenes, are highly specific chemical substances which attack certain areas of the insect endocrine system causing toxic effects and also disturb its development process and reproduction (Parveen *et al*., 2014).

Over 200,000 people die worldwide each year due to the direct result of pesticide poisoning as estimated by the World Health Organization (Christine *et al*., 2015). To predict toxicity, pharmacological actions and pesticidal effects of crude plants, a brine shrimp test was recommended (Nguta *et al*., 2013). A crude plant extract is considered active upto a concentration of 240 µg/ml. Brine shrimp bioassay is a simple method recommended because it is rapid, reliable, inexpensive and, in most cases, correlates reasonably well with cytotoxic properties of drugs in mammals (Odhiambo *et al.,* 2014). The procedure determines median lethal concentration values of active compounds and extracts in the brine medium (Nguta *et al.,* 2013). The toxicity tests on the plants under study using brine shrimp larvae revealed high toxicity levels. Nevertheless, organic extracts of *C.quandrangularis* with LC_{50} values of 113.10 µg/mL and aqueous extracts of *I.kituiensis* with LC_{50} values of 136.96 µg/mL were moderately toxic. Toxicity can be attributed to presence of secondary metabolites especially alkaloids, saponins and tannins. Alkaloids interfere with membranes of the cells and disrupt the integrity of cells upto apoptosis which is the degradation of the cells because they cannot withstand the osmotic forces as their membranes are destroyed (Rosenkranz and Wink, 2007). In this study, *P.dodecandra* water and organic extracts were highly toxic with LC_{50} values of 35.98 µg/mL and 39.97 µg/mL respectively. However, this was in contrast with report of Namulindwa *et al*.,

(2015) which showed aqueous leaf extract of *P. dodecandra* as moderately toxic. *C.quadrangularis* aqueous extracts were highly toxic with LC_{50} values of 38.31 µg/mL whereas its organic extracts were moderately toxic with LC_{50} values of 113.1 µg/mL. This was not consitent with the results of Enechi *et al.,* (2013) which showed that ethanol extract of *C.quadrangularis* is non-toxic. *I.kituiensis* aqueous extracts were moderately toxic with LC_{50} value of 136.96 µg/mL while its organic extracts were highly toxic with LC_{50} value of 4.17 µg/mL to the brine shrimp. This is the first reported ever toxicity study on this plant species although previously, toxicity reports have been documented on some *Ipomoea* species. For example, *I.carnea* is considered a toxic plant growing in tropical areas (Guilherme *et al*., 2012).

In vitro acaricidal activity of the selected plant species from the area based on their wide usage was tested using *Boophilus decoloratus* tick larvae. The criteria for *in vitro* acaricidal activity was based on Ducornez *et al*., (2005) with some modifications and larvae mortality was established by lack of movement after observation for 30 minutes after exposure to crude extracts (*I.Kituiensis*, *P.dodecandra* and *C.quadrangularis*) and the standard drug, almatix. The data illustrated that the larvae mortality significantly correlated to the concentrations of the treatments ($p<0.05$). As the concentration of the plant extracts increased, the mortality of the larvae also increased. This result concurs with Belmain *et al*., (2001) whose results showed low tick deaths with the lower concentrations of 5 to 10 % dose in trials with *Lippia javanica* as compared to the higher doses. In this current study, the lowest dose (2.5 mg/mL) caused low mortalities (below 20 %) while higher doses recorded higher mortalities.

All the aqueous extracts tested for larvicidal activity were significantly different from almatix. However, among the tested organic extracts for larvicidal activity, only organic

C.quadrangularis extracts at (10 mg/mL) and *P.dodecandra* organic extracts at 5 mg/mL and 10mg/mL were not significantly different ($p > 0.05$) from the positive control.

There were significant differences among various concentrations of each plant in terms of larvicidal activity in both aqueous and organic extracts except two concentrations (5 and 10 mg/mL) of *P.dodecandra* which were not significantly different since they evoked 100 % kill ($p>0.05$). Results from one-way ANOVA followed by Dunnet t-test revealed that there was significant difference in bioactivity between the three groups of plants. The types of secondary metabolites in the plant extracts determine the effectiveness of drugs. The larvicidal activity of these plant extracts on the larvae may be partly due to the direct repressive action on its cardiovascular and respiratory systems activity and due to possible nervous systems attacks by ascaridole monoterpenes. This substance has shown effects on the nervous system of *Rhipicephalus lunulatus* (Miegoue *et al*., 2013).

Results of contact toxicity bioassays revealed that *P.dodecandra, C.quadrangularis* and *I.kituiensis* organic extracts at the end of 24 hours were significantly ($P<0.05$) influenced by plant species and concentration of the crude plant extracts. *P.dodecandra, C.quadrangularis* and *I.kituiensis* at a dose of 5 and 10 mg/mL each evoked a maximal (100 %) kill of tick larvae for organic extract. This could be explained by contact toxicity properties of the chemical constituents of the other related member of the same genus, *Ipomoea alba*. This plant was found to be rich in indolizidine alkaloids assessed to cause acute toxicity to central nervous system as characterized by convulsions and tremor among others in mice and rats (Guilherme *et al.,* 2012). Based on bioassays and concentration-mortality regression, the results estimated the actual susceptibility of the tick larvae populations to the effects of the botanicals. All the plant extracts exhibited lower LC_{50} between 3.85 and 6.65 mg/mL, indicating that *Boophilus* spp. was very

susceptible to the effects of the crude plant products. However, water extract of *C.quadrangularis* (whole) revealed the highest LC_{50} and this can be explained by the fact that the extraction method used did not concentrate active ingredients well before use since the same plant in organic solvent showed a lower acute lethal concentration. The LC_{50} was 3 fold greater in *C.quadrangularis* organic extracts compared to aqueous extracts of *P.dodecandra*. Acaricidal effect was higher in all organic extracts than aqueous extracts. The toxicity of the crude extracts against the study tick was indicated by the LC_{50} estimates. The larvae tick stage populations were more susceptible to botanical extracts.

4.2. Conclusions

The results provide a scientific rationale for the use of botanicals in the mitigation of the blue tick burden in cattle. It is concluded that both LC_{50} of organic and aqueous crude extracts of *P.dodecandra* (LC_{50}: aqueous, 4.83 and organic, 3.85 mg/mL), *C.quadrangularis* (LC_{50}: aqueous, 13.90 mg/mL and organic, 5.56 mg/mL) and *I.kituiensis*' LC_{50}: aqueous, 6.65 mg/mL and organic, 5.23 mg/mL) demonstrate larvicidal effects in ticks and hence could be used *in vitro* in the mitigation of blue tick problem and justifies the reason why these medicinal plants have been used to control this species of tick affecting cattle in Suba Sub-County. The fact that, the crude extracts at higher concentrations had significant mean percentage larvicidal activity against the study tick is interesting and lends support to the traditional usage of these plant materials as cattle protectants against destructive tick pests. All evaluated extracts represent attractive candidates for evaluation as protectants of livestock in general against *B.decolocrotus* tick as the results confirm the acaricidal potency of the three plant species in blue tick control.

Water as a solvent gave higher yield than methanol in DCM (1:1 v/v) solvent, therefore, it should be preferred to the latter. Phytochemical analysis confirmed that all the tested plants had all the compounds (except *P.dodecandra* which lacked flavonoids) associated with acaricidal activity and this confirmed their relevance as anti-blue tick strategy in Suba. *P.dodecandra*, *C.quadrangularis* and *I.kikuiensis* were all toxic to brine shrimp.

It is expected that the crude plant extracts could offer suitable alternatives to conventional acaricides. This proves the herbalists' competence as a repository of knowledge in acaricidal plants because the plants they cited displayed the ability to control blue ticks. This is likely to assist in improving livestock health and, by extension, increased livelihood. This means less hunger and improved nutrition for humans.

4.3 Recommendations

From the research undertaken the following recommendations were made:

1. Use of other solvents for extraction which may lead to improvement in yield and biological activity.
2. Identify other phytochemicals giving rise to activity.
3. Using other concentrations in brine shrimp test, for example, 20, 30, 40, 50, 60, 70, 90 µg/mL among others.
4. Using other concentrations in acaricidal test such as 7, 8, 9 mg/mL is required.
5. Conduct toxicity tests in livestock and humans.
6. Isolation and purification of the crude compounds.
7. Structure elucidation and bioassay of isolated compounds.
8. Same plants to be tested against other tick species.

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APPENDICES

Appendix A: Independent report on parts of plants used in the acaricide preparation

Part used	**IR**
Leaves	94
Fruits	22
Bark	5
whole	45

Appendix B: The distribution of plant families as enumerated by the herbalists

Plant families	Total no of IR	% of IR of family mentioned as anti-ticks
Cucurbitacea	5	3.47
Leguminoseae	6	4.17
Phytolaccaceae	6	11.11
Compositae	9	6.25
Lamiaceae	9	6.25
Convolvulaceae	13	9.03
Meliaceae	16	11.11
Verbanaceae	7	4.86
Euphorbiaceae	13	9.03
Vitaceae	15	10.42
Solanaceae	21	14.58
Capparaceae	4	2.78
Aloeceae	10	6.94
Total	144	100

Appendix C: Toxicity of the test extracts on Brine shrimp

Appendix C1: Aqueous extracts

	Phytolacca dodecandra							
Conc.	10 µg/mL		100 µg/mL		1000 µg/mL		Water	
Trial	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
1st	0	10	0	10	0	10	10	0
2nd	0	10	0	10	0	10	10	0
3rd	1	9	0	10	0	10	10	0
Average Mortality		9.7		10		10		0

	Cissus quadrangularis							
Conc.	10 µg/mL		100 µg/mL		1000 µg/mL		Water	
Trial	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
1st	0	10	0	10	0	10	10	0
2nd	2	8	0	10	0	10	10	0
3rd	0	10	0	10	0	10	10	0
Average Mortality		9.3		10		10		0

	Ipomoea kituiensis							
Conc.	10 µg/mL		100 µg/mL		1000 µg/mL		Water	
Trial	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
1st	0	10	0	10	0	10	10	0
2nd	0	10	0	10	0	10	10	0
3rd	0	10	0	10	0	10	10	0
Average Mortality		10		10		10	10	0

Appendix C2: Methanol in DCM (1:1 v/v) extracts

	Phytolacca dodecandra							
Conc.	10 µg/mL		100 µg/mL		1000 µg/mL		DMSO	
Trial	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
1st	4	6	0	10		10	10	0
2nd	1	9	0	10		10	10	0
3rd	0	10	0	10		10	10	0
Average Mortality		8.3		10		10	10	0

	Cissus quadrangularis							
Conc.	10 µg/mL		100 µg/mL		1000 µg/mL		DMSO	
Trial	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
1st	3	7	2	8	0	10	10	0
2nd	2	8	0	10	0	10	10	0
3rd	1	9	0	10	0	10	10	0
Average Mortality		8		9.33		10	10	0

	Ipomoea kituiensis							
Conc.	10 µg/mL		100 µg/mL		1000 µg/mL		DMSO	
Trial	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
1st	6	4	2	8	0	10	10	0
2nd	7	3	4	6	0	10	10	0
3rd	5	5	5	5	0	10	10	0
Average Mortality		4		6.33		10	10	0

Appendix D: *Boophilus decoloratus* larvicidal activity of plant extracts

Appendix D1: Methanol in DCM (1:1 v/v) extracts

	Phytolacca dodecandra									
Conc.	2.5 mg/mL		5 mg/mL		10 mg/mL		DMSO		Almatix	
Trial	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
1st	85	15	0	100	0	100	100	0	0	100
2nd	79	21	0	100	0	100	100	0	0	100
3rd	83	17	0	100	0	100	100	0	0	100
4th	90	10	0	100	0	100	100	0	0	100
5th	90	10	0	100	0	100	100	0	0	100
6th	81	19	0	100	0	100	100	0	0	100
Average Mortality		15.33		100		100		0		100

	Cissus quadrangularis									
Conc.	2.5 mg/mL		5mg/mL		10 mg/mL		DMSO		Almatix	
Trial	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
1st	90	10	87	13	0	100	100	0	0	100
2nd	83	17	90	10	0	100	100	0	0	100
3rd	80	20	93	7	0	100	100	0	0	100
4th	88	12	91	9	0	100	100	0	0	100
5th	81	19	90	10	0	100	100	0	0	100
6th	80	20	96	4	0	100	100	0	0	100
Average Mortality		16.33		8.83		100		0		100

	Ipomoea kituiensis									
Conc.	2.5 mg/mL		5 mg/mL		10 mg/mL		DMSO		Almatix	
Trial	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
1st	76	24	35	65	7	93	100	0	0	100
2nd	84	16	16	84	12	88	100	0	0	100
3rd	90	10	15	85	24	76	100	0	0	100
4th	87	13	10	90	12	88	100	0	0	100
5th	85	15	9	91	20	80	100	0	0	100
6th	79	21	6	94	30	70	100	0	0	100
Average Mortality		16.5		84.83		82.5		0		100

Appendix D2: Water extracts

	Phytolacca dodecandra									
Conc.	2.5 mg/mL		5 mg/mL		10 mg/mL		Water		Almatix	
Trial	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
1st	94	6	5	95	8	92	100	0	0	100
2nd	90	10	30	70	4	96	100	0	0	100
3rd	96	4	28	74	2	98	100	0	0	100
4th	89	11	18	82	11	89	100	0	0	100
5th	90	10	30	70	0	100	100	0	0	100
6th	97	3	22	78	1	99	100	0	0	100
Average Mortality		7.33		78.17		95.67		0		100

	Cissus quadrangularis									
Conc.	2.5 mg/mL		5mg/mL		10 mg/mL		Water		Almatix	
Trial	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
1st	98	2	77	23	81	19	100	0	0	100
2nd	100	0	81	19	88	12	100	0	0	100
3rd	95	5	85	15	73	27	100	0	0	100
4th	100	0	80	20	78	22	100	0	0	100
5th	99	1	80	20	71	29	100	0	0	100
6th	100	0	73	27	80	20	100	0	0	100
Average Mortality		1.33		20.67		21.5		0		100

	Ipomoea kituiensis									
Conc.	2.5 mg/mL		5 mg/mL		10 mg/mL		Water		Almatix	
Trial	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
1st	94	6	11	89	28	72	100	0	0	100
2nd	83	17	15	85	35	65	100	0	0	100
3rd	90	10	35	65	50	50	100	0	0	100
4th	85	15	21	79	48	52	100	0	0	100
5th	80	20	14	86	30	70	100	0	0	100
6th	86	14	10	90	50	50	100	0	0	100
Average Mortality		13.67		82.33		59.83		0		100

Appendix E: Interview guide

A: Background information

1. Gender

   Male [ ] Female [ ]

2. Level of education

   Below primary [ ] Primary [ ] Secondary [ ] College [ ] University [ ] Post graduate [ ]

3. Age

   18-23 years [ ] 24-28 years [ ] 29-33 years [ ] 34-40 years [ ] 41-45 years [ ] 46-55 years [ ]

   Over 55 years [ ]

4. Experience in herbal medicine

   1-4 years [ ] 5-9 years [ ] 10-14 years [ ] 15-20 years [ ] Over 20 years [ ]

B: Health practice information

5. How do you rate your current performance in your herbal medicine?

   High [ ] Moderate [ ] Low [ ]

6. What are the factors affecting your performance in herbal medicine?

   Resources [ ] Working environment [ ]

7. How do the current conventional health practices affect your occupation as a herbalist?

   Lead to decreased performance [ ] Lead to increased performance [ ] No effect on performance [ ]

   Briefly explain your response for question (7) above

   ……

8. Briefly explain your response for question (7) above

   ……

9. Do your health practices have a health training to guide its operation in the livestock husbandry?

   Yes [ ] No [ ] Don't know [ ]

10. Do you know different types of ticks with their specific local names?

    Yes [ ] No [ ]

11. If yes, which one is most prevalent in your region?

    ……..

12. Do you have knowledge of the plants that are used as traditional acaricides?

    Yes [ ] No [ ]

13. If yes, which plants provide remedies for tick infestation in livestock in your area?

    (i)……. (ii)…… (iii)……..……

14. What is the level of efficacy of these drugs from botanicals you have mentioned?

    High [ ] Moderate [ ] Low [ ]

15. At what stage of tick infestation do you apply the traditional acaricides?

    Larval stage [ ] Nymphal stage [ ] Adult stage [ ]

16. When do you start noticing disappearance of ticks from the infested cattle after application of the traditional acaricides s?

    Immediately [ ] Later [ ], Specify……..……

17. Is the effectiveness of the traditional acaricides maintained after repeated application on same cattle?

    Yes [ ] No [ ]

18. If No, what are the other strategies used to control ticks?

    ……

19. What part of the plant(s) do you use in preparing the crude extracts of the traditional acaricides?

    ……

20. Briefly explain how you prepare the medicine?

    ……

21. What other ingredients apart from water do use for the preparation of the plant crude extracts?

    ……..

22. What is the habit of the plant (in 12 above)?

Herb [ ] Shrub [ ] Lianas [ ] Tree [ ]

23. Is the plant easily available?

    Yes [ ] No [ ] specify…….