DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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DEDICATION

To my Dad Bonventure, Mum Betty for their commited parenthood, Brothers Archduke and Alex, Sister Linet for support and encouragement in my academic endeavours. To my late twin brother Odongo "Norola soluya".

"Blessed is the man who perseveres under trial".....James 1: 17

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LIST OF ABBREVIATIONS

AIT	Adult Immersion test
BOD	Biological oxygen demand
CDC	Center for Disease Control
CSIRO	Commonwealth Scientific and Industrial Research Organization
ERD	Ethnoveterinary Research and Development
EVM	Ethnoveterinary Medicine
FAO	Food and Agricultural Organization
GIS	Geographical Information Systems
GPS	Global Positioning System
ICTTD	Integrated Consortium on Ticks and Tick-borne Diseases
IDRC	International Development Research Centre
ILRI	International Livestock Research Institute
LC_{50}	Lethal concentration that kills 50% of the population
LPT	Larval Packet Test
MDG	Millenium Development Goals
NMR	Nuclear Magnetic Resonance
TAM	Traditional African Medicine
TCE	Trichloroethylene
WARRC	World Acaricide Resistance Reference Centre
WHO	World Health Organization

ABSTRACT

Ticks resistance to acaricides is an increasing problem in Kenya and other parts of the world. It poses an economic burden due to livestock losses and enormous costs of developing novel acaricides. Traditional healers in Samburu district have utilised herbs for management of tick infestation despite the lack of scientific evidence of efficacy. The objectives of this study were to develop an inventory guide for Samburu plant use and scientifically validate the pharmacological and toxicological activity of two preferentially used herbal acaricides.

A sensitization workshop was held in February 2007 involving the University research team, local leaders and 15 herbalists at Nomotio Livestock and Improvement Center in Maralal. Indigenous knowledge, intellectual property rights on bioprospection, plant conservation and integration of ethnopractises in mainstream disease mitigation were deliberated on. Data on plant use and voucher specimens were collected using Participatory Rural Approaches from 15th to 19th of May 2007 in which 70 informants including pastoralists, healers, housewives and opinion leaders participated. Oral interviews and pretested semi-structured questionnaires were admitted for qualitative data gathering.

Using standard botanical procedures *Acokanthera schimperi* (*Lmorijoi*) and *Psiadia punctulata (Labaai)* were selected out of the sixty two (62) plants documented since they were ranked highly by the locals in management of ticks.Voucher specimens of all the plants were deposited at East African Herbarium and Botany department, University of Nairobi for confirmatory identification.

In-vitro bioassays were conducted at the International Livestock Research Institute Laboratories to determine the plants potential acaricidal activity. The plant specimens were dried, pulverised, methanol extracted then lyophilized and homogenized. Twelve (12) grams of fine powder of each plant was used to prepare a stock solution of 5000 ppm of both extracts was formulated and lower concentrations of 4000 to 1000 ppm prepared by serial dilution in deionised water 24 hours prior to the bioactivity tests.

Thirty (30) unfed 14 day old larvae of *Rhipicephalus appendiculatus* and *Boophilus decolaratus* were enclosed in filter paper packets impregnated with 2ml of each extract at stated concentrations and controls in triplicate. They were incubated at 27-28^{0}C and 90-95% RH for 24 hours at 12:12 hour photoperiod (Light: Day). *A. schimperi* at 5000 ppm produced 63% and 53% mortality to *B. decoloratus* and *R. appendiculatus* respectively. *Psiadia punctulata* at 5000 ppm produced 90% and 60% mortality to larvae of *B. decoloratus* and to *R. appendiculatus* respectively. Probit analysis and linear regression modelling at ($p<0.05$) was calculated by interpolation of mortality counts to establish lethal concentrations (LC). *A. schimperi* produced larvicidal activity with LC_{50}/LC_{99} values of 4509/10611 ppm and 2787/8945 ppm, while *P. punctulata* had LC_{50}/LC_{99} values of 3918/10020 ppm and 1857/8347 ppm on *R. appendiculatus* and *B. decoloratus*, respectively. Data on plant use from the informants yielded 990 citations on 62 medicinal plants used to treat animal and human diseases

The documented use of these plants is promising for search of alternative acaricides. Further pharmacological, toxicological and phytochemical cross-screening to develop effective and cheaper pharmaceuticals of medical and veterinary importance is

CHAPTER 1

1.0 INTRODUCTION

Traditional plant use in disease management is of extreme importance and it is practiced by various East African communities. Ethnoveterinary medicine (EVM) focuses on livestock keepers' and traditional healers' knowledge and approaches to animal health care. It covers information on diseases and their control; remedies and clinical practices for treatment and prevention strategies. Ethnoveterinary information is a key aspect of participatory epidemiology that promises to improve epidemiological surveillance in remote areas and encourage community participation in disease control. EVM practices offer an alternative to conventional veterinary medicine; it encompasses a large body of information and experience on animal management and disease prevention (Martin, 2001).

Herbal remedies provide readily available low cost alternatives to the poor society of developing nations. Eighty percent (80%) of Sub Saharan Africa population relies on traditional remedies, it is therefore imperative to focus on scientific validation of traditional medicines (Wanyama, 1997a; WHO, 2002). Despite the fact that EVM has been very crucial for the animal healthcare of most developing countries, it has not yet been well documented and much effort is needed in research and integration activities of EVM. Most of the *materia medica* used in EVM is derived from plants (McCorkle *et al.*, 1996).

It is estimated that eighty percent (80%) of the world's 1.3 Billion cattle suffer from deleterious effects caused by ticks. They transmit diseases such as heart water *(cowdria/Ehrlichia ruminatum)*, bovine babesiosis (*Babesia bovis* and *B. bigemina*), anaplasmosis (*Anaplasma marginale*), theileriosis (*Theileria parva parva*), corridor disease (*T. parva lawrenci)* and January disease (*T. parva bovis)* (Mekonnen, 2002).

Worldwide, the economic losses caused by ticks and TBDs in cattle are estimated at US$13.9–18.7 billion annually. The annual cost for importing acaricides have been estimated at US$ 16, 26 and 26 million in Kenya, Tanzania and Uganda respectively Tick borne diseases (TBD) constitute a major constraint to livestock production in developing countries within tropical areas especially in the smallholder sector of East, Central and Southern Africa. Tick resistance to acaricides poses a real economic threat to the livestock and veterinary pharmaceutical industries as the cost of developing a new acaricide is estimated at US$230 million per compound (FAO, 2004; Rajput *et al.*, 2006; Jongejan *et al* ., 2007).

Tick infestations are controlled with chemical acaricides administered by dipping or spraying. This is increasingly unsustainable due to the high incidence of acaricide resistance within tick populations, toxic residues in foods of animal origin and escalating costs of maintaining dips,spray races and labour .Traditionally ticks are also controlled using a variety of methods such as ethnobotanicals, handpicking, burning pastures, quarantine, cleaning cattle sheds, burning or burying of residues, bird predation, feeding animals on naturally salty soils and applying kerosene, *magadi* (soda ash) or grease on cattle (Tamboura *et al*., 2000; George *et al*., 2004; Graf *et al*., 2004).

Ethnopharmacopiae can be put to commercial use but it should be scientifically validated to verify its' safety and efficacy. However Herbal medicines are difficult to standardize, prepare, and require large doses which may vary greatly. The required plants may be unavailable through the year and the pharmacological active ingredients may vary according to season, site, harvest time, maturity and other factors (Cunningham, 2001; Martin, 2001). Indigenous knowledge particularly on medicinal plants is speeding up as firms and researchers and bioprospectors bypass the communities to commercialize and patent these remedies without indicating their commercial intent. The Samburu flora and traditional knowledge has hitherto been a victim of this overexploitation due to poverty and illiteracy (WHO, 2002; Per.Commu).

Currently there is concern over the human health and environmental contamination associated with use of some pesticides, such as the organochlorines and organophosphates and pyrethroids leading to their gradual withdrawal and restriction in some areas of the world (Eisler *et al.*, 2003; Rajput *et al.*, 2006). This has been counterbalanced by the development of new compounds, such as the phenylpyrrazoles, chloro-nicotinyl insecticides, semicarbazones and the use of compounds such as the insect growth inhibitors. The concern of drug development is heightened by the rapid development and spread of insecticide resistance in major groups of ectoparasites (Geary *et al.*, 2004).

The laboratory acaricide resistance testing methods usually involve either larval or adult ticks and include Shaw Larval Immersion Test (SLIT), Larval Packet test (LPT) and Adult Immersion Test (AIT) respectively. The SLIT and FAO-adapted LPT are the most important larval acaricide resistance testing methods currently being used worldwide (Mekonnen, 2002).

Traditional livestock healers in Samburu District of Kenya have utilised herbal remedies for management and treatment of both external and internal parasites including ticks based on their indigenous knowledge there is still need for documenting and testing potential plants. A major advantage of herbal preparations is that they are cheap, readily available in pastoral areas and solve the problem of resistance to commercial parasiticides. Their major drawback is lack of sufficient scientific data on efficacy, therapeutic index, toxic effects and other pharmacological and toxicological properties to support their use (Wanyama, 1997b, Gathuma *et al.*, 2004).

In order to reduce the high costs of importing acaricides in Kenya and to replace those rendered unusable by tick resistance, it is important to consider the possibilities for using indigenous botanicals as sources of acaricides. Some plants and plant extracts have been reported to be capable of causing tick mortality, tick repellence and immobilization (Wanyama, 1997b; Carroll, 2007).

1.1 JUSTIFICATION:

Ticks are important ectoparasites of domestic animals and man; they affect cattle, sheep, dogs and other animals. They affect their hosts in many ways like damaging hides, reducing their growth rates and milk production, transmitting disease organisms, causing paralysis and zoonoses Worldwide, the economic losses caused by ticks and TBD in cattle are estimated at US$13.9–18.7 billion annually (George *et al.*, 2004; Graf *et al.*, 2004). This can be reduced substantially by adopting effective integrated tick management strategies (Wanyama, 2000; George *et al.*, 2004).

Plant extracts have been reported to be capable of causing tick mortality, repellence and immobilization (Fernandez *et al.*, 2005, 2007b). Integration of these biopesticides in sustainable pests' management strategy will alleviate poverty in accordance with United Nations MDGs (UN, 2006).

Ethnopractitioners in Samburu District have successfully utilized various plants for ectoparasite control despite lack of pharmacological and toxicological proof. Therefore the feasibility of in-cooperating ethnobotanicals in an integrated tick control strategy necessitates the elucidation and validation of their scientific rationale.

1.2 OBJECTIVES:

The general objective of the current study was to document and assess the acaricidal potential of extracts of *Psidia punctulata* (*Labaai*) and *Acokanthera schimperi* (*Lmorijoi*) against prevalent tick species in Samburu district using the recommended *in vitro* bioassay techniques.

Specific objectives:

1. To document the plants claimed to possess acaricidal effects based on Samburu traditional healers' indigenous knowledge.

2. To determine the *in vitro* susceptibility profiles of ticks *B. decolaratus* and *R. appendiculatus* populations to the extracts of *Psiadia punctulata* (*Labaai*) and *Acokanthera schimperi* (*Lmorijoi*).

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Ethno veterinary medicine

Ethnoveterinary medicine (EVM) is the holistic, interdisciplinary study of local knowledge and its associated skills, practices, beliefs, practitioners and social structures pertaining to the healthcare and healthful husbandry of food, work, and other income-producing animals. It focuses on practical development applications within livestock production and livelihood systems, with the ultimate goal of increasing human well-being via increased benefits from stock raising (McCorkle *et al.*, 1996).

The paramount goal of ethnoveterinary research and development (ERD) is to further the search for alternative medicines at the intersection of medicine and agriculture by building upon local veterinary and husbandry knowledge, *materia medica* and their associated human and socio-structural resources so as to increase the number of reliable animal healthcare options that are readily, cost effectively and sustainably available (McCorkle *et al.*, 1996).

Livestock farming remains the major source of food in Africa. Pastoralists have compiled substantial knowledge about animal healthcare and production and their economic significance. Inadequate provision of modern medicines and lack of access to the available medicines to the rural poor in Africa has also accelerated the use of alternative medicines (Tafesse and Mekonnen, 2001).

The economic constraints have forced the majority of livestock owners in Kenya to rely chiefly on traditional animal health practices to control common livestock diseases. This is because modern veterinary inputs and services are inaccessible or unaffordable by the poor pastoralists (Wanyama, 1997 a).

Plants by means of various secondary metabolites are certainly the main source of medicinal treatment. Some of the drugs include; Warfarin a dicoumarol from sweet clover, anticancers like Paclitaxel an from yew tree, Vinca alkaloids(vincristine and vinblastine) from periwinkle, Antimalalarials like quinine from the cinchona tree and artemisinin derivatives from sweet wormword and several antibiotics from fungal metabolites. (TDR, 2007) Their biological activities, alongside the different practices in terms of preparing and applying herbal remedies have been developed by certain ethnic groups throughout the world and form a reliable database for the evaluation of new pharmaceuticals (Sneˇzana *et al.*, 2007).

Plant species and associated traditional knowledge are threatened by habitat modification and unsustainable rates of exploitation. The transmission of this knowledge is jeopardised by loss of plant diversity and modernization (Tabuti, 2007). The global increase in the use of herbal remedies is set to continue at a tremendous pace well into future despite the fact that most of the plant remedies have not been scientifically validated. This raises important public health concerns especially relating to safety issues including adverse effects and herb-drug interactions (WHO, 2002).

The Lusaka Declaration of the Decade for TAM (2001-2010) has resulted in creation of the Network on Medicinal Plants and Traditional Medicine. Its main objectives are to strengthen collaborative activities and to share information on medicinal plants. The WHO Alma Ata Declaration of 1978 recommended full utilization of all resources including traditional medicine and its practioners to achieve the goal of "health for all". International agreements and legal frameworks should consider the needs of herders to enable them maintain their livelihoods and not exclude them from international markets (WHO, 2002; IDRC, 2003).

Several scientific studies are currently in progress across the African continent to examine ways in which EVM may be used to reinforce classical veterinary services (Tamboura *et al.*, 2000). Pastoralists in Samburu district will continue to utilize Ethnoveterinary medicines (EVM) until better alternative boiacaricides in terms of efficacy; low cost, availability and ease of administration are found (Wanyama, 1997a). Samburu represents a very important dry season grazing area for ethnopharmacological studies (Bussmann *et al.*, 2006; Njoroge and Bussmann, 2006).

2.2 Taxonomy of ticks

Ticks are obligate, non permanent ectoparasites of terrestrial vertebrates and hematophagus in all-feeding stages of their life cycle. Ticks belong to the phylum *Arthropoda,* class *Arachnida*, order *Acari,* suborder *Ixodida* and include three families namely *Argasidae* (soft ticks), the *Nuttaliellidae* and the *Ixodidae* (hard ticks). There are nearly 800 tick species of which 150 belong to the family *Argasidae* and 650 to the family *Ixodidae* and one to the family *Nuttaliellidae* (Mekonnen, 2002).

2.3 Economic significance of ticks

Ticks comprise a veterinary problem because they transmit diseases, produce paralysis or toxicosis and cause physical damage to livestock and zoonoses to man. Losses attributable to ticks are caused either directly, through tick worry, blood loss, damage to hides and udders and the injection of toxins or indirectly through mortality or debility caused by Tick borne diseases (TBD) (Graf *et al.*, 2004; Rajput *et al.*, 2006).

Economic impact of ticks/TBD have been expressed in terms of grams of live weight gain or milk production lost per tick engorging (for example 0.7g/tick) or the total average financial loss per animal per year (e.g. US$ 7.3/head/year) (Rajput *et al.*, 2006). The economic losses caused by ticks and TBD worldwide in cattle are estimated at US$13.9–18.7 billion annually. The annual cost for importing acaricides has been estimated at US$ 16, 26 and 26 million in Kenya, Tanzania and Uganda respectively (FAO, 2004; George *et al.*, 2004).

2.3.1 Direct effects

Feeding by large numbers of ticks causes reduction in live weight and anemia among domestic animals, while tick bites also reduce the quality of hides. Heavy infestations can cause severe dermatitis (L'Hostis and Seegers, 2002; FAO, 2004; Peter *et al.*, 2005).

2.3.1.1 Tick-bite paralysis

Tick-bite paralysis is an acute ascending flaccid motor paralysis caused by the injection of a toxin by ticks. Paralysis includes those caused by *Dermacentor andersoni*, sweating sickness (*Hyalomma truncatum*), Australian tick paralysis (*Ixodes holocylus*) and tick toxicosis (*Rhipicephalus* spp.) (Jevsk *et al.*, 2005).

2.3.1.2 Physical damage

Ticks are attached to the body of the host for a blood meal and thereby cause irritation and serious physical damages to livestock health. These damages include: "tick worry", irritation, unrest, and weight loss due to massive infestation of ticks; the direct injury to hides due to tick bites, loss of blood due to the feeding of ticks (Rajput *et al* ., 2006).

2.3.1.3 Vector of pathogens

Ticks transmit various pathogens from host to host during feeding. In the host, TBD generally affects the blood and lymphatic systems. The major TBD include babesiosis, anaplasmosis, theileriosis and cowdriosis. These organisms may be transmitted transtadially (stage to stage) in the tick, a typical example being *Theileria parva* transmitted by *R. appendiculatus* or Transovarially (from the female tick through the egg to the larvae) an example is *Babesia equi* transmitted by *Anocentor nitens* (FAO, 2004).

2.4 Acaricide resistance.

The World Health Organisation Committee on Insecticide Resistance defines resistance as the "the development of an ability in a strain of insects or other arthropods to tolerate doses of toxicants, which would prove lethal to the majority of individuals in a normal population of the same species". Resistance to a given acaricide or insecticide can also be described as a reduction in susceptibility of a parasite to the acaricide or insecticide when it is used at the recommended concentration (Mekonnen, 2002; FAO, 2004).

Tick resistance can exist in the absence of chemical pressure and this suggests that resistant genes pre-exist in a population and can be selected for by exposure to insecticides. Ticks resistance to acaricide is a common phenomenon globally with resistance to arsenicals, organochlorines, organophosphates, carbamates, amidines and pyrethroids having been reported (FAO, 2004; Foil *et al.*, 2004). Acaricide resistance occurs due to the phenotypic expression of an evolutionary process accelerated by chemical selection and often involves inherited characters. The process occurs primarily through the selective effect of chemicals favouring pre-existing low frequency resistant mutants in field populations (Mekonnen, 2002; Odongo *et al.*, 2007).

The rate at which resistance develops is influenced by the strength of acaricide used, the life cycle of the ticks, residual activity of pesticides, the degree of dominance of resistant alleles and the dose levels applied . High gene frequency for resistance in a population of ticks will result in an increased selection pressure (Odongo *et al.*, 2007; Spickett *et al.*, 2007).

2.4.1 Mechanisms of acaricide resistance

Ticks resistance to acaricides is due to a range of different parameters including increased detoxification by metabolic breakdown of the acaricide and reduced sensitivity to the acaricide by the target system. Interference with nerve conduction is the main mode of action of synthetic pyrethroids and biochemical findings indicate that decreased target sensitivity is the predominant pyrethroid resistance mechanism in ticks (Mekonnen, 2002).

Tick resistance has a genetic basis and mutation or sometimes amplification of structural genes occur. Resistance is quicker if the genes are dominant or slower if they are recessive. Resistance can also arise from different types of mutation affecting the same gene (FAO, 2004). The resistance to organophosphates is suggested to be as a result of cytochrome P450 detoxification (*cytP450*) of coroxon and diazoxon and an insensitive acetylcholine esterase is involved (Foil *et al.,* 2004; Li *et al.*, 2004).

Resistance to Pyrethroids is conferred by target sight mediated mutation on the Na^+ channel and metabolic mechanism due CzEst9 esterase activity confers resistance by over expression of the esterase (Guerrero *et al.*, 2002). Formamidine resistance is the least understood, Triphenylphosphate (TPP) has been shown to synergize amitraz toxicity 6-fold, yet in the susceptible population of ticks TPP only synergized amitraz toxicity 2-fold. This suggested that esterase plays some role in amitraz resistance (Jamroz *et al.*, 2000).

2.5 Management of acaricide resistance

Strategies for resistance management are designed to delay or restrict the development of resistance to a new acaricide. The strategies involve reducing the dipping frequency and avoiding high dosing rates in order to minimize the period of selection by retaining acaricide susceptible ticks within a tick population. If tick resistance can be identified with reliable resistance test methods, the preferred alternative acaricide should be used. Lack of suitable alternatives may allow use of higher concentrations of the active ingredient or addition of synergists (Mekonnen, 2002).

Complacency and imprudent use of acaricides has led to resistance and concerns over environmental contamination and effects on human health. It is important to develop parasite management programmes, drawing on a multiplicity of techniques into which insecticides use can be integrated rationally (Wall, 2007).

The impact of prudent strategies that delay resistance is enhanced if tick control strategies are integrated chemical and with non-chemical control measures these include the use of tick resistant Zebu cattle, herbal plants. A lack of standardized techniques for diagnosing acaricide resistance appears to be the main difficulty in creating and maintaining a tick resistance monitoring system (FAO, 2004).

2.5.1 Diagnosis of resistance in ticks

A suitable laboratory test for acaricide resistance should be simple, inexpensive and sensitive enough to identify resistance early in its emergence cover the full range of chemical groups that are in use especially novel active ingredients. It should provide a rapid and reliable result and be suitable for intra-laboratory standardization. It should also be adaptable for several tick species of concerns and the results for each species must be reproducible. The most widely used *in vitro* tests do not meet all of these requirements and improvement of protocols for diagnosis of acaricide resistance should be a continuing goal (FAO, 2004). Resistant strains of ticks can be diagnosed without having internationally recognized standardized test protocols. To facilitate global monitoring and provide a basis for comparison of test results, standardized diagnostic methods should be adopted. FAO has promoted the use of the LPT for field investigations of acaricide resistance (Baxter *et al.*, 1999).

2.5.2 *In vitro* diagnostic methods:

2.5.2.1 The Larval Packet Test (LPT)

The Larval Packet Test (LPT) is considered to be the most repeatable; however it is limited by the length of time that it takes to implement. It is the test of choice for surveys and for definitive confirmation of a diagnosis of resistance. Results for the larval bioassay for the diagnosis of resistance in *Boophilus spp.* takes about 6 weeks. Following the adoption of this test by the FAO as the preferred means of diagnosis of resistance in ticks, it was promoted in the form of the FAO Acaricide Resistance Testing Kit. In this test, tick larvae are exposed to chemically impregnated filter papers and their subsequent mortality is quantified 24 hours post exposure (FAO, 2004).

2.5.2.2 The Larval Immersion Test (LIT)

This involves submerging the larvae into acaricides. The LPT can be used for Macrocyclic Lactones but preliminary results at CSIRO, Australia, have shown that the LIT is much more sensitive. Comparative studies have indicated a good agreement between LIT and LPT. The inability of the LPT to diagnose potential resistance to fluazuron also applies to LIT. LIT provides a result in 6 weeks (FAO, 2004).

2.5.2.3 The Adult Immersion Test (AIT)

The AIT is a bioassay applied to engorged, female ticks. It has been recommended as a preliminary screening test for resistance because it is relatively rapid. It was used to determine the relative effectiveness of new acaricides against a number of tick species. In the near future, the Resistance ratios of the FAO will use the classical AIT (with different concentrations of product) and a standardized AIT that uses discriminating doses (DD) (FAO, 2004; Jonsson and Hope, 2007).

2.5.2.4 Modified Larval Packet Test

The test follows the LPT protocol but the Packets are enclosed in plastic Petri dishes (with each replicate of packets for one concentration in a separate dish). The exposure time is extended to 48 hours. In the past, the paper packets were enclosed in polythene bags but some polythene has been found to be toxic to larvae (Miller *et al.*, 2002; FAO, 2004).

2.6 Epidemiology of Tick Control.

The successful implementation of rational and sustainable tick control programmes in grazing animals is dependent upon a sound knowledge of the ecology or epidemiology of the parasite and appropriate bio-control methods (Quijada *et al.*, 1997; Lodos *et al.*, 2000; Jonsson and Hope, 2007).

Substantial ecological and epidemiological knowledge bases helps in control, the understanding of the host parasite interaction in specific climatic, management and production systems (Graf *et al.*, 2004; Sonenshine *et al.*, 2007).

Acaricide treatments are commonly used in a *suppressive approach*, applying multiple treatments at regular intervals during the height of infestation and a *threshold approach* is when the application of acaricide is initiated after tick infestations exceed an acceptable level. A *strategic approach* is used where there is sufficient ecological information and annual population trends. It involves the application of acaricides on F1 generation of ticks (Quijada *et al.*, 1997).

2.7 Chemical control of ticks

Most neurotoxins are acaricides that act either systemically or by direct contact with ticks following external application since. Acaricides that act systematically may be given parenterally or topically. Due to differences in pharmacokinetics and pharmacodynamics different acaricides formulations may be indicated for different target parasites (Taylor, 2001).

2.7.1 Acaricides

2.7.1.1 Arsenic

Use of arsenic was the first effective method for controlling ticks and TBD. It was used in the form of water soluble compounds like sodium arsenite. Arsenic dips were used successfully to eradicate *Boophilus* ticks from the southern United States. Unfortunately, arsenic has a very short residual activity and therefore develops resistance rapidly (Mekonnen, 2002).

2.7.1.2 Amidines (Formamidines)

The main member of this group is amitraz, which acts at octopamine receptor sites in ectoparasites resulting in neuronal hyperexcitability and death. Amitraz has been widely used in dips, sprays or pour-on formulations for the control of single and multi-host tick species. In dipping baths, it can be stabilized by the addition of calcium hydroxide and maintained by standard replenishment methods for routine tick control (Taylor, 2001).

2.7.1.3 Chlorinated hydrocarbons

They fall into three main groups: (a) chlorinated ethane derivatives such as dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD) (b) the cyclodienes that include chlordane, aldrin, dieldrin, hepatochlor, endrin, toxaphene; (c) the hexachlorocyclohexanes (HCH) such as benzene hexachloride (BHC) which includes the g-isomer, lindane. Chlorinated ethanes cause inhibition of Sodium (Na^+) conductance along sensory and motor nerve fibres by holding Na^+ channels open, resulting in delayed repolarization of the axonal membrane. The cyclodienes act by inhibition of gamma (λ)-amino butyric acid (GABA) stimulated Cl2 flux and interference with Calcium (Ca^{2+}) flux. Lindane binds to the picrotoxin side of the GABA receptor, resulting in an inhibition of GABA-dependent Chloride (Cl) flux into the neuron. Because of their high toxicity and long residual effect, these compounds have mostly been withdrawn from the market (Mekonnen, 2002).

2.7.1.3 Organophosphorous compounds

Organophosphates (OP) are neutral esters of phosphoric acid or its thio analogue and act by inhibiting the action of acetyl cholinesterase (AchE) at cholinergic synapses and at muscle end plates causing transphosphorylation of the enzyme. The transphosphorylated AchE is unable to break down accumulating Acetylcholine at the post-synaptic membrane leading to neuromuscular paralysis. This is not an irreversible process as eventually the AchE is metabolized by oxidative and hydrolytic enzyme systems. OP compounds can be extremely toxic in animals and humans (FAO, 2004).

2.7.1.4 Carbamates

Carbamate insecticides are closely related to the OP as anticholinesterases, which cause a spontaneously reversible block on AchE. Commonly used carbamates include carbaryl and propoxur; carbaryl has low mammalian toxicity but may be carcinogenic (Taylor, 2001).

2.7.1.5 Pyrethrins and Pyrethroids

Synthetic pyrethroids are derivatives of naturally occurring pyrethrins from pyrethrum (chrysanthemum flowers); they include Bifenthrin, Cypermethrin, Deltamethrin, and Permethrin. The insecticidal properties of pyrethrins are derived from ketoalcoholic esters of chrysanthemic and pyrethroic acids (Mekonnen, 2002). Pyrethrins act on the insect's Central Nervous System (CNS) by exciting the cell membranes resulting in extended depolarization. Some act as synergists by helping to prevent the pyrethrin break-down by microsomal mixed function oxidase (MFO) system in insects (Taylor, 2001).

2.7.1.6 Nitroguanidines

Imidacloprid is a chloronicotinyl insecticide; it specifically binds to nicotinic acetylcholine (Ach) receptors in the insect's Central Nervous System (CNS) leading to inhibition of cholinergic transmission resulting in paralysis and death. It is selectively toxic by binding to the Ach receptors of insects and no effect on mammalian receptors (Taylor, 2001).

2.7.1.7 Phenylpyrazoles

The phenylpyrazole, fipronil, is the first of a new class of broad-spectrum insect control agents that act to block the GABA gated chloride channel in the insect nervous system (Taylor, 2001).

2.7.2 Alternative methods of tick control

2.7.2.1 Genomics and selective breeding

Genomics has an impact on tick research through the generation of new resources, including genome information and experimental techniques. The application of genomics has led to acquisition of new insights and the development of practical new interventions in the tick–host–pathogen triangle (Jongejan *et al.*, 2007).

The greatest single impact on tick genomics research is RNA interference (RNAi). RNAi has been used successfully to transiently suppress the expression of specific tick genes. RNAi has been used to study gene function and the role of tick genes in pathogen transmission and as a screening tool for the identification of potential tick antigens for vaccine development (Aljamali *et al.*, 2002; De la Fuente and Kocan, 2006; Nijhof *et al.*, 2007).

On tick borne pathogen spectrum it is recognised that the post-genomics era of *Theileria* spp. offers an opportunity to develop new vaccines by following 'reverse vaccinology' strategies. Using the genome sequence data on *T. parva,* several promising candidate vaccine antigens that are recognized by bovine CD8+ cytotoxic T-lymphocytes have been identified (Graham *et al.*, 2006). Genomics will lead to control technologies with characteristics that differ from those of current acaricides and drugs. Vaccines, RNAi and transgenics will typically have greater specificity for a limited range of tick species (Willadsen, 2006; Jongejan *et al.*, 2007).Considerable progress has been made in evolving resistant *Bos indicus* × *Bos taurus* beef and dairy cattle that limit the effects of ticks while retaining high productivity (Bock *et al.*, 2004).

2.7.2.2 Immunological control

The two commercially available vaccines (TickGARD Plus®, Hoechst, Australia) and (GavacTM, Heber Biotec, Cuba) are now on the market in a limited number of countries. It is possible that they can reduce tick burdens however they lack the knockdown effect of traditional chemical acaricides (FAO, 2004). On TBD control using ECF as an example, the Muguga cocktail is a three strain *T. parva* stabilate vaccine that has been used in combination with an antibiotic treatment, known as the infection-and-treatment method. The mild Boleni strain ECF vaccine in Zimbabwe is used without the treatment component (Odongo *et al.*, 2007; FAO, 2004).

The Bm86 antigen based vaccine against *Boophilus microplus* has proved its efficacy in a number of experiments when combined with acaricides (García-García *et al.*, 2000). The isolation of the Bm95 gene from the *B. microplus* strain A and Bm95 antigen from strain A was able to protect against infestations with Bm86-sensitive and Bm86-resistant tick strains. This suggested that Bm95 could be a more universal antigen to protect cattle against infestations by *B. microplus* strains from different geographical areas (García-García *et al.*, 2000).

The identification of protective antigens for the control of *Ixodes scapularis* infestation using cDNA expression library immunization and insect control based on sterile males or genetic manipulations offer little promise, while pheromone attractants could be useful for domestic pets, or for ticks attached on specialized sites (Aljamali *et al.*, 2002).

2.7.2.3 Herbal acaricides

2.7.2.3.1 *Azadirachta indica* (Neem)

Derivatives of neem tree *Azadirachta indica* (A. Juss) comprise a complex array of novel compounds with diverse behavioural and physiological effects on insects, including repellence, feeding and oviposition deterrence and inhibition of growth and reproduction (Abdel-Shafy and Zayed, 2002).

The i*n vitro* acaricidal effect of plant extract of neem seed oil on egg, immature and adult stages has been examined and shown to exhibit antitick activities against *R. appendiculatus, R. pulchellus*, *Amblyomma variegatum* and *Boophilus decoloratus* (Handule *et al*., 2002; Abdel-Shafy and Zayed, 2002).

2.7.2.3.2 *Margaritaria discoidea (Eurphorbiacea)*

Studies assessing the water soluble and hexane extracts of *Margaritaria discoidea* induced high mortalities in nymphs of *R. appendiculatus* and *A. variegatum* and adults of *R. pulchellus* (Kaaya and Hassan, 2000).

2.7.2.3.3 *Nicotiana tabacum*

A ground mixture of dried tobacco leaves and a mineral called *Magadi* (soda ash), proved to be effective as an acaricide against all stages of *R. appendiculatus*. It prevented the completion of all feeding phases of the tick, suppressed the oviposition capacity of the engorged ticks and reduced the hatchability of eggs. Larvae and nymphs were killed within 24 h of the application of the substance on calves' ears (Kaaya and Hassan, 2000).

2.7.2.3.4 *Commiphora species*

Hexane oil extracts from the gum of the tree *Commiphora myrrh* Neels was repellent to adult *R. appendiculatus. C. erythraeae* Egler was found to possess larvicidal and repellent activities against *A. americanum, Dermacemtor variabilis,* and repellence activities against adults of *Ixodes dammini, D. variabilis* and *A. americanum* (Kaaya and Hassan, 2000).

2.7.2.3.5 *Callicarpa americana (Lamiaceae)*

Two terpenoid compounds Callicarpenal and intermedeol isolated from *Callicarpa americana* (Lamiaceae) were evaluated in laboratory bioassays for repellent activity against host-seeking nymphs of the blacklegged tick, *Ixodes scapularis*, and *Amblyomma americanum*. Callicarpenal and intermedeol, at 155 nmole/cm2 cloth repelled 98% and 96% of *I. scapularis* nymphs, respectively (CDC, 2002; Carroll *et a*l., 2007).

2.7.2.3.6 *Aloe marlothii*

The effect of a decoction of pulverized aloe leaves (*Aloe marlothii*) mixed with tap water and administered orally through a gastric tube was determined against *Boophilus decoloratus*. Fertility estimates were determined for 30 engorged females. The treatments had no significant effect on total or daily numbers of engorged females collected per group. Fertility estimates showed the treatment group yielded a marginally higher, yet insignificant, egg laying response (ELR) and reproductive estimate (RE) (Spickett *et al* ., 2007).

2.7.2.3.7 *Copaifera reticulata*

Following the FAO LPT the acaricidal activity of oleoresinous extract from the copaiba tree, *Copaifera reticulata* on *Rhipicephalus (Boophilus) microplus* larvae exposed to filter paper envelopes impregnated with different concentrations. Mortality was observed 24 hours after treatment. Oleoresin LC_{50} /LC_{99} values were 1579/3491 ppm, respectively (Fernandes *et al.*, 2007b).

2.7.2.3.8 *Sapindus saponaria*

Evaluations of the larvicidal potential of a crude ethanol extract (CEE) of soapberry *Sapindus saponaria* stem peel on the cattle tick *Boophilus microplus* and *Rhipicephalus sanguineus* 14-21 days old fasted tick larvae following FAO LPT. Mortality was observed after 48 hours. LC_{50}/LC99 values of 1,258 ppm/6,360 ppm were obtained on *B. microplus*, 1994/3922ppm on *R. Sanguineus* respectively (Fernandes *et al.*, 2005; 2007a).

2.7.2.4 Biological control

Bioassays using fungal isolates of *Metarhizium anisopliae* killed various tick stages of *Boophilus annulatus* and *Rhipicephalus sanguineus* (Gindin *et al.*, 2002). Isolates of *M. anisopliae* were also tested against *R. sanguineus* and found to cause 82.6% and 60% mortalities in engorged larvae and nymphs, respectively and 92–100% mortality in adults and unfed larvae and nymphs (Kaaya and Hassan, 2000). *M. anisopliae* against *B. microplus* larvae resistant to pyrethroid have been studied and produced mortality rates ranges from 10 to 96.9%. *M. anisopliae* was used at concentrations of 105, 106, 107 and 108 conidia/ml (Babiense *et al.*, 2006).

2.8 Tick species used in susceptibility testing

2.8.1 *Boophilus decolaratus* (blue tick)

B. decoloratus is the predominant one-host tick species present in East, Central and Southern Africa and the major vector of the protozoan *Babesia bigemina* (Texas or red water fever), the rickettsia *Anaplasma marginale* (gall sickness) and *B. theileri* (Spirochaetosis) in horses, sheep and dogs (Bock *et al.*, 2004; Kockan *et al.*, 2004; FAO, 2004). It shows predilection for the dewlap and neck, or else at the tip and outer edge of the pinnae of the ears. Since it is a one- host tick it can be effectively controlled by three-weekly treatment of cattle (Mekonnen, 2002).

2.8.2 *Rhipicephalus appendiculatus* (brown ear tick)

Rhipicephalus appendiculatus is a three host tick with cattle as the preferred domestic hosts of all stages. Shoats and equines are also parasitized; the African buffalo eland and waterbuck are the preferred wild hosts. It is the main vector of *Theileria parva parva* (East Coast Fever) and *T. parva lawrenci* (Corridor disease) and *T. parva bovis* (January disease) of cattle. Adults attach to the ears of their hosts while the nymphae and larvae are commonly found on the ears, head and extremities; it is confined to the Eastern, Central and Southern regions of Africa (Mekonnen, 2002).

2.9 Test plants description

2.9.1 *Acokanthera schimperi* (D.C) Oliv.

Family: *Apocynaceae,* **Samburu name:** *Lmorijoi*

A shrub or small deciduous tree up to 3.5 m height, it can reach occasionally 6 m, it occurs all over eastern and central Africa. It has green simple, ovate succulent leaves (Plate 1). It produces round fruits up to 2 cm in diameter and they ripen to dark purple but this plant is extremely poisonous (Prelude, 2007; Per.Commu.).

Uses: Ethnopractioners apply it as an ectoparisticide in control of ticks, fleas and lice. It is also used for arrow poisons (Per.Commu.; Wanyama, 1997a; Prelude, 2007; IPNI, 2007)

Phytochemistry: *A. schimperi* contains cardiac digitalis glycosides of which Acovenoside A is the major compound and *ouabaïn* ($C_{30}H_{46}O_{12}$) an amorphous crystalline glycoside as a minor constituent. Two classes have been observed in nature - the cardenolides and the bufadienolides. *A. schimperi* contains cardenolides which an unsaturated butyrolactone ring (Melero *et al.*, 2000).

Mechanism of action: Digitalis glycosides are inhibitors of cellular Na^+/K^+-ATPase, which causes increased intracellular Na^+ and decreased intracellular K^+. In myocytes, elevated intracellular Na^+ concentrations produce increased intracellular Ca^{2+} concentrations via a Na^+- Ca^{2+}-exchanger. Inhibition of Na^+-K^+-ATPase in skeletal muscle results in increased extra cellular potassium and contributes to hyperkalemia. Cardiotoxicity involves prolonging refractory period in atrioventricular node (AVN), shortening refractory periods in atria and ventricles, and decreasing resting membrane potential (increased excitability) (Melero *et al.*, 2000)

Figure 1: Structures of diterpenes and flavones in *Psiadia punctulata* leaf surface exudate (Midiwo *et al.*, 2002).

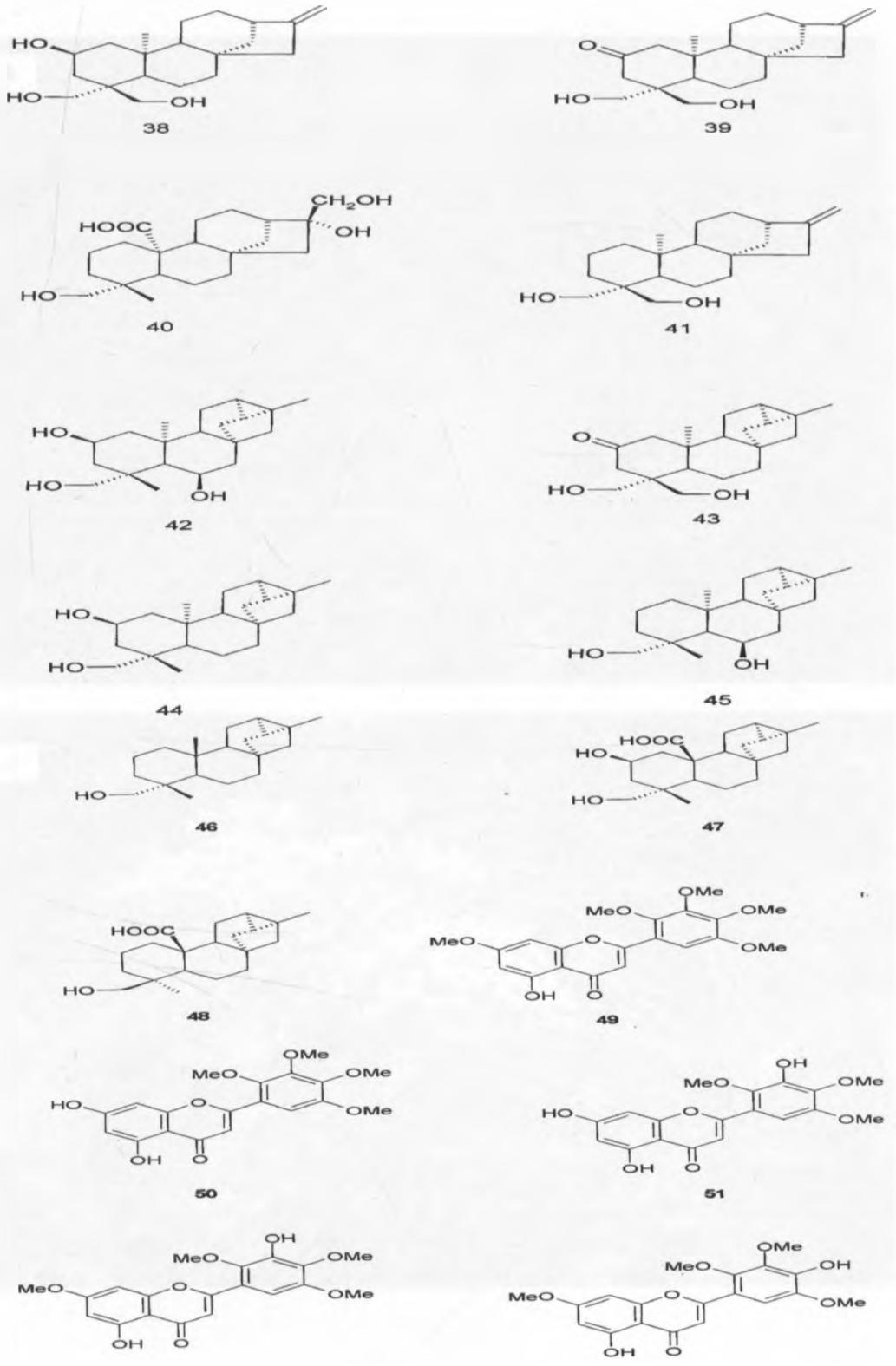


Plate 1 : Voucher specimens of *Acokanthera schimperi* (D.C) Oliv. (A) and *Psiadia punctulata* (DC) Vatke. (P).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Ethnobotanical Survey

3.1.1 Study area geographical location:

Samburu District is situated in the northern half of the Rift Valley Province. It is bordered by 5 districts; to the northwest is Turkana District while to the southwest is Baringo District. Marsabit District is to the northeast, Isiolo District to the east and Laikipia District to the south. The area of study lies between Latitudes 0°40" north and 2°50" north of the equator and Longitudes 36°'20" east and 38° 10" east of the Prime Meridian. It covers approximately 21,126.5 square kilometres. It is divided into six divisions, 39 locations and 108 sub-locations. Samburu district has a population of approximately 156,125 people (CBS, 2001) mainly as pastoralists (Appendix V (Figure 1) and Plate 2).

3.1.2 Physiographic and Natural Conditions:

Samburu district is characterised by high-level plateaus, hills and the Rift valley and Kirisia Hill, rising up to 2000m above sea level. January to March are dry followed by Long rains from April to May, Short rains occur during July, August and September. This short rain succeeds a fairly dry spell during the month of June. The south-western plains and the Lerroghi plateau receive between 500 and 700mm of rain annually. District annual rainfall is between 250 and 500 mm. Temperatures in the district vary with altitude and are between 24°C and 33°C, with a mean of 29°C (www.aridlands.go.ke).

3.2 Qualitative data collection

This was based on Participatory Rural Appraisal (PRA) techniques as described below:

3.2.1 Sensitisation workshop

This was held on 15th to 17th February 2007 at Nomotio Livestock improvement center, after prior consenting with the respective authorities. It was attended by Local leaders, 15 Traditional healers (10 men: 5 women), contact organizations' staff and the University of Nairobi research team. The workshop focused on the understanding of the indigenous knowledge of the healers, intellectual property rights on bioprospection and biopiracy, integration of the ethno practises in the government's mainstream policy for control of livestock and human diseases and sustainable environmental conservation of the existing flora.

3.2.2 Geographical Mapping

A map of Samburu district was constructed to define the target area of research using Geographical Information System (GIS) (Arc View version 9.2, ESRI, Inc., New York, NY), (GCS -ARC 1960 /Projection- UTM 37 South) to illustrate the geographical boundaries of the divisions in the district with emphasis on forested areas, the principal site of sample collection and interviews. This was used as a guide in identification of the areas by the help of the locals (Appendix V (Figure 1).

3.2.3 Semi structured interview and questionnaires

Standardized semi-structured and pretested questionnaires in Appendix (I) were used to conduct interviews with assistance of Samburu translators. Focus group discussions were used to verify the survey's concepts in the local context. Homogeneity in selection of informants ensured equal representation (Ssegawa and Kasenene, 2007).

Plate 2: Satellite images of Maralal town (L) and Lerroghi forest (R); sites of plant samples collection (White arrows)

Source Google earth©2007

A total of 70 informants (Male: Female, 1:1) in Appendix (II) were involved in the study by systematic and random sampling techniques (Eregae, 2003). They ranked five medicinal plants based on the efficacy of the plants against the ticks.

The interviews were conducted according to Yinenger *et al.*, (2007) to collect relevant data on: age, sex and occupation of informants as well as indications treated, local name of plants, growth form, plant part used, methods of preparation, form used (fresh/dried), route of administration (Appendix I and Table 1 in Appendix V), threats to medicinal plants, conservation efforts, beliefs and indigenous knowledge transfer.

These interviews and transect walks with the locals were employed to corroborate the field survey data to avoid the probable confusions with regard to the identity of the medicinal plants. The morphological characteristics and habitats of medicinal plants were observed, recorded and photographed during and after the fieldwork.

3.3 Collection and Preparation of plant specimens

Succulent leaves of *A. schimperi* and *P. punctulata* were sustainably harvested from 19th to 20th May 2007 in four different sites on Lerroghi plateau and Kirisia forest by cutting and handpicking just before the rainy season to obtain optimum constituents. Sites GPS coordinates : **A**-1˚05.17.94°N 36°42˚20.14 E elevation 6296 ft, **B**- 1˚05.17.13° N 36°42˚26.13 E elevation 6293 ft, **C**- 1˚05.10.63° N 36°42˚42.49 E elevation 6246 ft, **D**-1˚06.26.24° N 36°41˚36.48 E elevation 6374 ft (GARMIN Inc,Olathe, Kansas,USA).

Botanical identification was done with the help of the experienced traditional herbalists, followed by digital photography and labelling, pressing and allotment of voucher numbers (MN018, MN019) for *A. schimperi* and *P. punctulata* respectively. Duplicates of the specimens were submitted to the East African Herbarium and Botany department, University of Nairobi for confirmatory taxonomical identification. Others were kept in the Department of Public Health Pharmacology and Toxicology for future reference.

Drying was done *ex situ* under shade for 2 months in a well aerated dimly lit basement room which was restricted due to the potential toxicity of *Acokanthera schimperi*.

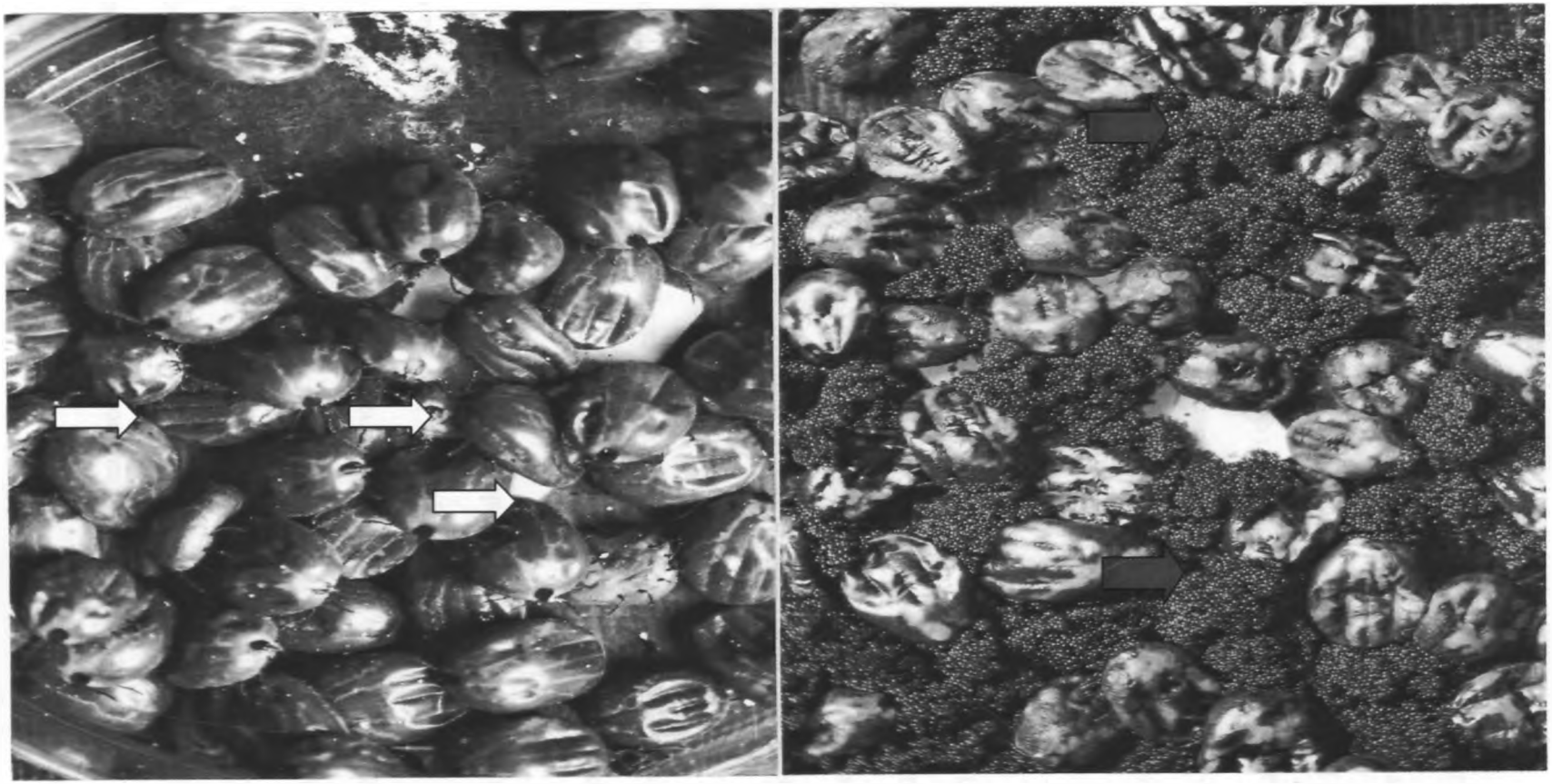
3.4 Collection of field strain ticks from Study areas.

Engorged female ticks of *B. decoloratus* and *R. appendiculatus* were removed manually from their respective predilection sites from freely grazed animals in Maralal according to ICTTD-3 (2007). GPS coordinates of the sites of collection were determined as 1°06.15.04° N 36°41′20.95 E, elevation 6384 ft (GARMIN Inc, Olathe, Kansas, USA).

The ticks were stored in small plastic containers and data including the tick species, date/site of collection and code number were all recorded before transfer to the Kabete veterinary Laboratories, Acarology unit and ILRI Tick vector unit for confirmatory identification.

The ticks were washed in distilled water to remove any eggs laid during transportation. Identification was done using a dissecting microscope (Wild Heartburn, 355110, Switzerland). Ticks were then air-dried in absorbent paper, placed in a petri dish and incubated at 27–28^{0}C and 85–90% Relative humidity in a BOD incubator and maintained until egg-laying and hatching were completed (Plate 3).

Plate 3 : *Rhipicephalus appendiculatus* Engorge adult females (White arrows) and Egg masses (Red arrows) in petridishes.



Source : ILRI Tick vector Laboratory © 2007

3.4.1 Propagation of ticks

Boophilus decolaratus

Five, 6-9 months old cattle that had been kept off acaricides for at least two weeks were selected and held in ranch cubes with solid partitions to prevent cross infection of ticks, at ILRI tick unit. The dorsum of the cattle was shaved using clippers. Cloth ear bags and cloth back patches were stuck in place using zinc oxide plasters and rubber glue respectively. Up to 40,000 unfed larvae were applied to each of the five back patches and 20,000 larvae in ear bags. This was allowed for three weeks for a complete life cycle and then engorged female ticks were collected (Plate 4).

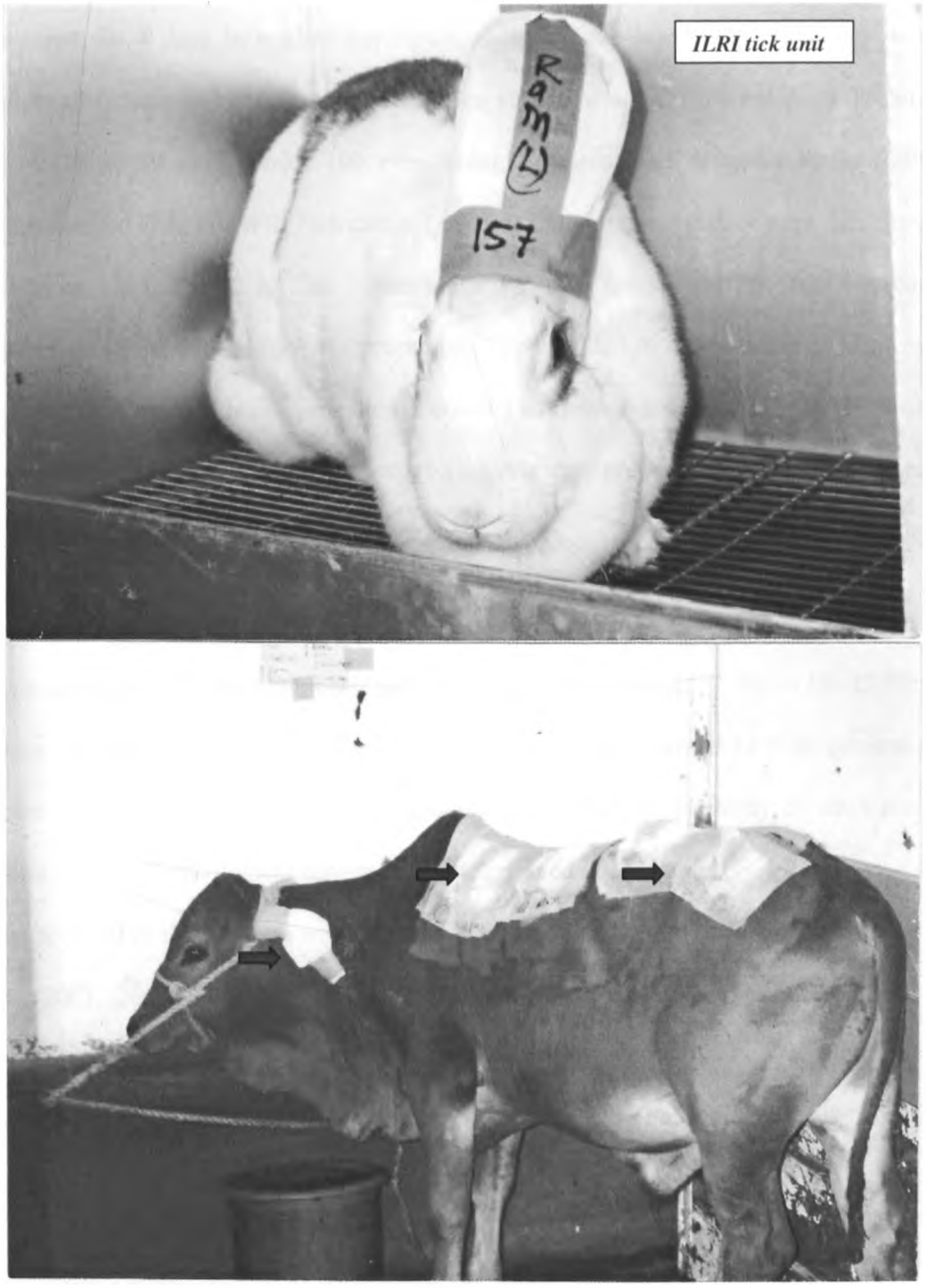
Rhipicephalus appendiculatus

New Zealand white rabbits (*Oryctolagus cuniculis*) were placed in clean cages in the tick unit rabbit rooms. The base of the rabbit ears was shaved with small animal electric clippers and cloth cotton ear bags attached using zinc oxide plaster. 10,000 unfed larvae were separately applied on ears. The back legs of rabbits were taped together loosely (2-3 cm) to prevent animals from scratching the bags. It took 3 weeks to collect engorged adults (Plate 4).

3.4.2 Rearing of tick larvae.

Engorged female ticks of *B. decoloratus* and *R. appendiculatus* were collected from infested animals, washed with water and dried in absorbent paper towels. They were stuck to the lid of a Petri dish with double sided sticky tape and the dish was closed. The lid had holes to allow air exchange and kept at 27–28^{0}C and 85–90% R.H. The eggs were laid over 2 weeks, collected and incubated in a 150mm glass tube with a nylon gauze lid to allow air and moisture exchange. Larvae were ready after 4 weeks.

Plate 4: Propagation of *Rhipicephalus appendiculatus* on New Zealand white rabbit ear bags (**Ram (L) 157**) and *Boophilus decoloratus* on a steers' backpatches and earbags (Arrows).



3.5 Extraction procedure.

The dried leaves were garbled, hand crushed and pulverized using an electric grinder (Arthur H Thomas Company, 750404, Philadelphia PA. USA.) to obtain 200 g of *A. schimperi* and *P. punctulata.* The plant powders were extracted with 3× 500 ml of 70% methanol for 4 days in a glass percolator under a fume hood. The percolates were collected, filtered and concentrated using rota vapour at 50 °C (Laboratorium Technik Ag, 211864, Sweden); under 100 mbar using Edwards High Vacuum Pump (GEC Machines Ltd D.53114 WK, Newcastle, UK).The aqueous concentrates were then deep-frozen at −20 °C for 12 h. The frozen mass was then transferred into freeze drying equipment (Beta, Medizinischer Apparatebau Type 1102, 336 osterode/Harr, Germany) for lyophilisation and homogenization according to Gebre-Mariam *et al*., (2006). Stock solutions of 5000 ppm of plant extracts (10 mg/ml) were prepared in distilled and stored at 4 °C until use (Fernandes *et al* ., 2007b).

3.6 *In vitro* bioassay tests

A preliminary adult immersion test was conducted prior to the LPT. Up to 10- 15 Field strains of both tick species were immersed in crude water extracts of both extracts to assess the potential acute toxicity of crude extracts and susceptibility of adult ticks, mortality and morphological changes were determined after 6 hours.

The protocol of FAO (2004) was followed with modifications according to Fernandes *et al*., (2005; 2007b). Twelve (12) grams of fine powder of each extract was used to prepare a stock solution of 5000 ppm and diluted serially from 4000 to 1000 ppm in two parts trichloroethylene (TCE) (Sigma, St. Louis, MO, USA) and one part olive oil, 24 hours before each bioassay.

Two (2) ml of 1000,2000,3000,4000 and 5000 ppm was pipetted evenly on the surface of standard sized filter papers 150mm in diameter (Whattman, Maidstone, Kent, UK) in an ascending order. The control papers were impregnated with solvents (TCE, olive oil and distilled water) only. Three envelopes were impregnated with each test solution according to the tick species. The filter papers were dried for 2 hours under a fume hood to allow evaporation of TCE, they were double-folded to form packets and with identification mark (test solution, concentration, number of larvae, tick species and date) on the outside.

A colony of each species of tick larvae was picked from the tube using a pair of forceps and floated in water in 90 mm diameter Petri dish immersed in ice cubes to immobilize the larvae. The larvae examined for viability and under a dissecting microscope (Wild Heartburn, 355110, Switzerland) shown in Plate 5.

They were transferred on to a white enamel tray where a manual counter and an entomological needle were used in counting approximately 30 unfed 14-21 day old larvae which were transferred into the formed packets and the top was sealed with a metallic clip.

Three replicates were conducted on three different days; new stock solutions were prepared for each replicate. This was placed in a BOD incubator (Memmert BK 50, GmbH and Company, Germany) at 27-28^{0}C and 90-95% RH for 24 h at 12:12 hour photoperiod (Light : Day) (Fernandes *et al.*, 2005, 2007b).

3.6.1 Mortality determination

Mortality was determined by counting dead and alive larvae. According to FAO (2004), if counting reveals control larval mortality to be "very low" (<5%) then the direct mortality figures will be utilized. If the control mortality is "low" (5–10%), then the percentage mortality in all of the experimental groups of larvae will be corrected by applying Abbott's formula:

$$\textit{Corrected \% mortality} = \% \frac{\textit{test mortality} - \textit{\% control mortality}}{100 - \textit{\% control mortality}} \times 100$$

Counting was done under a dissecting microscope with aid of an entomological needle (Plate 5). In the response criteria all immobile larvae or those moving limbs but could move on stroking were scored as dead (Fernandes *et al*., 2007b)

If the larval mortality was found to be "high" (>10%) in the control, the bioassay would be annulled and repeated (Fernandes *et al*., 2007b).

The mean mortalities for each of the replicate counts for control and each test concentration were recorded in the standard FAO kit forms in Table 1.

Plate 5: Counting of the larval mortality using dissecting microscopes in the tick unit laboratory.

3.7 Data and Statistical analysis

Data collected in the field was captured on to specifically prepared forms and stored in Microsoft Excel© 2007 for convenient statistical analysis. The ethnobotanical data was qualitatively analyzed using Ethnograph version 5® (Qualis Research). Descriptive statistics like percentage and frequency distribution were used to analyze the data collected through semi-structured interviews. The Spearman rank correlation test was used to determine whether there was a significant correlation between the age of informants and the number of ethnoveterinary medicinal plant species used on ticks according to Tabuti, (2007).

The probability of susceptibility of *B. decolaratus* and *R. appendiculatus* larvae to *A. schimperi* and *P.punctulata* were modelled using the probit analysis based on individual and aggregated cases according to tick species. The goodness of fit for the model was assessed using the Pearson chi-square test ($\chi 2$). Regression analysis generated Probit transformed mortalities which were plotted against log dose of the extracts to determine LC_{50}, $LC_{99.9}$ values and their 95% confident limits ($P< 0.05$), this also assessed the relative mean potency (RMP) and parallelism of both plant extracts.

PROBIT model: PROBIT (p) = Intercept + BX

This was analyzed using statistical software STATA 9.2 IE (Stata Corporation, College Station, Texas, USA).

CHAPTER 4.0

4.0 RESULTS

4.1 Ethnobotanical Survey

The use of medicinal plants in management of veterinary and medical conditions is highly preferred by residents of Samburu district. It was evident that 65 % of the local population had profound knowledge on traditional plant use. Lack of standardized dosages and protocols for preparation and administration has led to significant adverse reactions that are preferentially underreported.

Most plants are used in combination prepared by pounding, powdering and hot decoction. Leaves are used frequently (50%) followed by stembarks (35%), roots (10%) and others (5%). The use of herbal concoctions of *A. schimperi* (*Lmorijoi*), *P. punctulata* (*Labaai*), *Nicotanium tabacum* (*Lkumpao), Adenum obesum* (*Lperintai*) and *Solanum incanum* (*Ltulelei*) as acaricides was preferred by 80% of the informants, combination with commercial acaricides is also practised for management of ectoparasites (Appendix V[Table 1]).

There was no significant correlation (Spearman correlation test $r = -0.030$, $p = 0.840$) between the age of informants and the total number of medicinal plants reported to be used by each informant for tick management. The average number of bioacaricides known and used by female and male informants was similar ($\chi 2 = 9.262$, d.f. = 69, $p = 0.932$).

4.2 Tick borne diseases (TBD)

Pastoralists ranked *lipis* (theileriosisis) *mporoto ndiss* (anaplasmosis), *ngula* (babesiosis) and *lmilo* (cowdriosis) respectively as the most frequent cattle diseases caused by ticks. *R. appendiculatus* (brown ear tick) and *B. decolaratus* (Blue tick) were collected and identified from freely grazing livestock found in Maralal, with concurrent infestation being most frequent.

4.3 *In vitro* bioacaricidal tests.

4.3.1 Adult Immersion Test (AIT)

Preliminary adult immersion test (AIT) experiments using crude water extracts of both plants on adult field strains. *P. punctulata* produced 93% and 80% mortality on adult *B. decolaratus* and *R. appendiculatus*, while *A. schimperi* produced 78% and 77% on *B. decolaratus* and *R. appendiculatus* respectively. After 24 hours morphological malformations were observed in some of the adults due to the chemical damage of the cuticle and appendages, there was also observable detachment of appendages.

4.3.2 Larval Packet Test (LPT)

Mortality counts revealed that *A. schimperi* at 5000 ppm produced 63% and 53% mortality to *B. decoloratus* and *R. appendiculatus* respectively. *Psiadia punctulata* at 5000 ppm produced 90% and 60% mortality to larvae of *B. decoloratus* and to *R. appendiculatus* respectively. No significant mortality was observed in the control groups (0 – 6%) at ($p < 0.05$) (Table 1 and Figures 2 &3).

Table 1: *In vitro* larval bioassays mean mortalities of *B. decolaratus* and *R. appendiculatus* larvae to extracts of *A. schimperi* and *P. punctulata*

Extracts: *Acokanthera schimperi* , *Psiadia punctulata*							
A. schimperi	***B. decoloratus***			***R. appendiculatus***			**Time**
Concentration (ppm)	**Number dead**	**Total larvae**	**% mortality**	**Number dead**	**Total larvae**	**% mortality**	**Hours**
5000	19	30	63	16	30	53	24
4000	17	30	57	13	30	43	24
3000	16	30	53	12	30	40	24
2000	14	30	47	5	30	17	24
1000	10	30	33	2	30	7	24
0	2	30	6	0	30	0	24
P. punctulata							
5000	27	30	90	18	30	60	24
4000	25	30	83	15	30	50	24
3000	18	30	67	12	30	40	24
2000	14	30	60	8	30	27	24
1000	12	30	40	4	30	13	24
0	1	30	3	2	30	6	24

4.3.2.1 *Acokanthera schimperi*

Methanolic extracts of *A. schimperi* produced acaricidal activity to larvae of *B. decoloratus* with LC_{50} and LC_{99} values of 2.80 mg/ml (2,787 ppm, -5287- 4857= CI 95%) and 8.95 mg/ml (8,945 ppm, 6828 –17552 = CI 95%), while against *R. appendiculatus* it had LC_{50} and LC_{99} values of 4.50 mg/ml (4,509 ppm, 3645 -5320 = CI 95%) and 10.6 mg/ml (10,611 ppm, 8871 –14253 = CI 95%) (Table 2).

4.3.2.2 *Psiadia punctulata*

Methanolic extracts of *P. punctulata* produced LC_{50} and LC_{99} values of 2.0 mg/ml (2,055 ppm, -4320-3830= 95% CI) and 8.35 mg/ml (8,347 ppm, 6354-17966 = 95% CI) respectively on *B. decoloratus* and 3.90 mg/ml (3,918 ppm, 2883 – 4720 = 95% CI) and 10.0 mg/ml (10,020 ppm, 8410-13350 = 95% CI) on *R. appendiculatus* respectively (Table 2)

Table 2:

Lethal concentrations of extracts from *Acokanthera schimperi* and *Psiadia Punctulata* to larvae of *R.appendiculatus* and *B.decolaratus* and respective chi-squares and Relative median potency values, calculated starting from the interpolation of the mortality results by Probit analysis

Lethal concentration (LC)	***Acokanthera schimperi(Aa)***		***Psiadia Punctulata(Pp)***	
	LC Values on		LC Values on	
	R.appendiculatus	*B.decolaratus*	*R.appendiculatus*	*B.decolaratus*
LC_{01}	382.0	47.0	312.0	31.0
LC_{05}	797.0	145.0	650.0	94.0
LC_{10}	1178	262.0	962.0	171.0
LC_{15}	1534	392.0	1252	255.0
LC_{20}	1893	539.0	1545	351.0
LC_{25}	2267	709.0	1850	461.0
LC_{30}	2665	906.0	2175	404.0
LC_{35}	3095	1138	2526	801.0
LC_{40}	3569	1412	2912	1170
LC_{45}	4095	1740	3342	1519
LC_{50}	4509	2787	3918	1857
LC_{55}	4839	3120	4248	2055
LC_{60}	5174	3458	4583	2522
LC_{65}	5520	3807	4929	2860
LC_{70}	5885	4175	5294	3209
LC_{75}	6279	4572	5688	3577
LC_{80}	6717	5015	6126	3975
LC_{85}	7228	5530	6637	4417
LC_{90}	7871	6179	7280	5582
LC_{95}	8824	7141	8233	6543
LC_{99}	10611	8945	10020	8347

R.appendiculatus

Pearson Goodness-of Fit Chi Square ($\chi 2$) = 1.237; d.f = 7; P = 0.990
Parallelism Test Chi Square ($\chi 2$) = 0 .295; d.f = 1; P = 0 .587
RMP of *Aa* Vs *Pp* =1.2254 (0.90 - 1.80)

B.decolaratus

Pearson Goodness-of Fit Chi Square ($\chi 2$) = 9.040; d.f = 7; P = 0.250
Parallelism Test Chi Square ($\chi 2$) = 3.976; d.f = 1; P = 0 .046
RMP of *Aa* Vs *Pp* =1.5363 (0.95 - 3.0)

Statistics based on aggregated cases of larvae responses to grouping plant variables. ($p < 0.05$)

4.4 Comparative acaricidal potency

4.4.1 Boophilus decolaratus

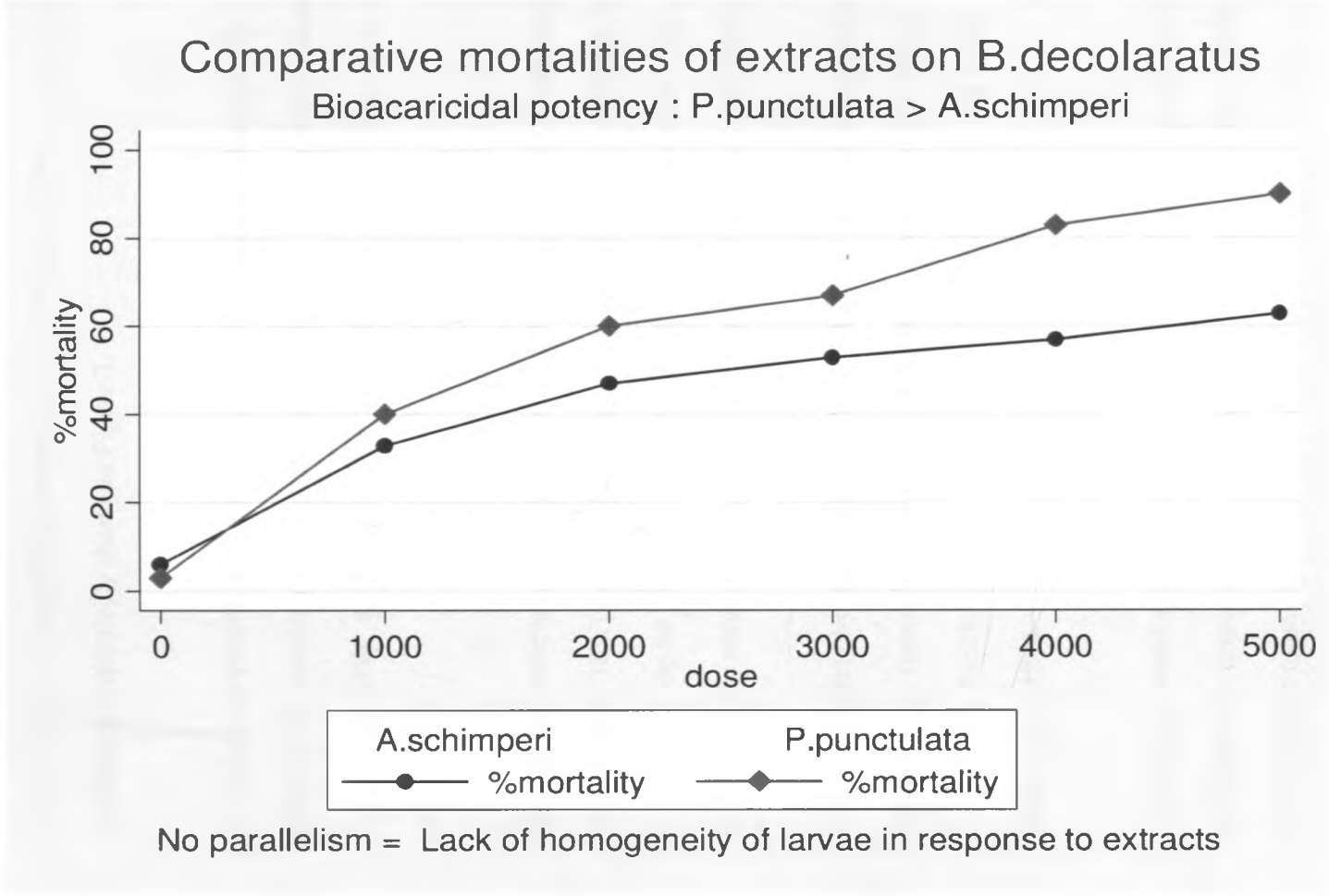
Direct percentage mortalities plotted against the dose revealed a non sigmoid curve for *Boophilus decolaratus* in response to the plants. There were similar responses in the control groups with no significant mortalities, an observable positive correlation proportionate (dose-percent mortality) relationship shown in Figure 2.

P. punctulata produced higher overall mortality than *A. schimperi*. It required 1800 ppm of *P. punctulata* to kill 50 % of the *Boophilus decolaratus* tick larvae population while up to 2200 ppm of *A. schimperi* was required to kill the same population.

The larvae mortality of *Boophilus decolaratus* demonstrated incremental susceptibility to both extracts suggesting a non linear response.

B. decoloratus response to both extracts produced Pearson Goodness-of-Fit Test $\chi 2$ = 9.040, d.f. = 7, p = 0.250, Parallelism Test $\chi 2$ = 3.976, d.f. = 1, p = 0.046 and RMP = 1.54 mg/ml (0.95-3.0 = 95% CI) (Table 2)

Figure 2: Comparative larval mortality of *B. decolaratus* to *A. schimperi* and *P. punctulata* extracts after 24 hours exposure.



4.4.2 Rhipicephalus appendiculatus

Comparative percentage mortality responses of *R. appendiculatus* larvae plotted against the dose revealed a positive correlation. There were similar responses in the control groups with no significant mortalities of 2% and 4.5% for *A. schimperi* and *P. punctulata* respectively shown in Figure 3.

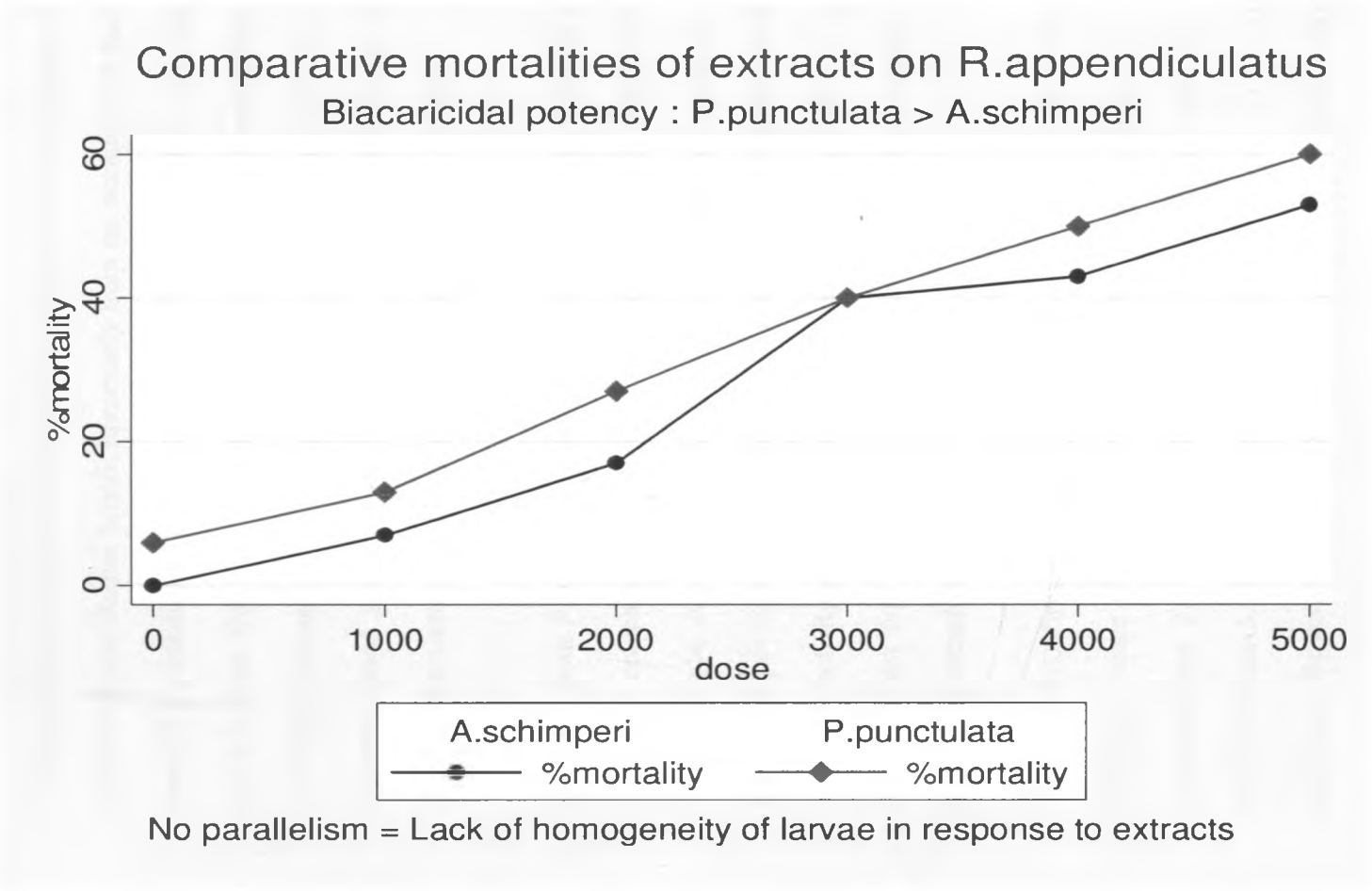
P. punctulata produced higher overall mortality than *A. schimperi.* Approximately 4000 ppm of *P. punctulata* was required to kill 50% of *R. appendiculatus* tick larvae population while up to 4800 ppm of *A. schimperi* was required to kill the same population.

Both plants extracts produced equal mortalities of 40% of the *R. appendiculatus* larvae at 3000 ppm. This is suggestive of equilibrium in their activity that may be attributed to a similar mode of action or possibly target receptors saturation. The differences in mortality at the remainder of the concentrations was significant at (p<0.05).

Probit modelling by aggregation of data on basis of tick species revealed a non linear regression. *R. appendiculatus* produced Pearson Goodness-of-Fit Test $\chi 2 = 1.23$, d.f. = 7, $p = 0.99$, Parallelism Test $\chi 2 = 0.295$, d.f. = 1, $p = 0.587$ and RMP = 1.22 mg/ml (0.88 - 1.8 = 95%).

Regressions transformations of the log-dose and Probit plots clearly indicated lack of parallelism in response using aggregated larval mortality counts to both plant species with reference to tick species (Appendix III).

Figure 3: Comparative larval mortality of *R. appendiculatus* to *A. schimperi* and *P. punctulata* extracts after 24 hours exposure.



CHAPTER 5

5.0 GENERAL DISCUSSION

The documented use of plants as veterinary and medical remedies in Samburu District, Kenya provides a rich flora for future bioprospection and drug discovery research. Ethnobotanical studies benefit immensely from the accumulated indigenous knowledge base of the inhabitants on plant use (Bussmann *et al*., 2006). The ethnomedicinal use of plant species was documented in the study area for treatment of both human and veterinary diseases. The local population has high ethnobotanical knowledge and has adopted sound management conservation practices. The major threatening factors reported were anthropogenic and natural (Nanyingi *et al* ., 2008)

Although both *P. punctulata* and *A. schimperi* have been reported to be useful in the control of ectoparasites in domestic animals (Wanyama, 1997a; 2000; Midiwo et al., 2000; Prelude, 2007) this is the first record assessing the acaricidal properties of both plants using *in vitro* bioassays (LPT) against *R. appendiculatus* and *B. decolaratus*. The acaricidal activity of both plant extracts varied directly with the concentration when tested against tick larvae. Higher concentration produced higher mortality than the lower concentration.

The results confirm the greater efficacy of *P. punctulata* as a botanical acaricide over *A. schimperi*, since it produced 50% and 99% larval mortality for *B. decoloratus* at concentrations 2.0 mg/ml and 8.35 mg/ml respectively and *R. appendiculatus* at concentrations 3.90 mg/ml and 10.0 mg/ml respectively. There have been previous studies on the activities of Brazilian medicinal plants against tick larvae; Chagas *et al*., (2002) obtained promising results using commercial formulations of essential oils from

three *Eucalyptus* spp. (*Myrtaceae*) as emulsified concentrates against *R. (B.) microplus*. They reported 100% larval mortality when exposed to 10% (≈100,000 ppm) concentration of the oils from *E. staigeriana* and *E. citriodora* and 20% from *E. globules*. In a related study the crude ethanol extract of soapberry, *Sapindus saponaria* (Sapindaceae), also had *R. (B.) microplus* larvicidal activity with LC_{50} and LC_{99} values of 1258 and 6360 ppm, respectively (Fernandes et al., 2005). This plant also demonstrated larvicidal activity for *Rhipicephalus sanguineus* (Acari: Ixodidae), with LC_{50} and LC_{99} values of 1994 and 3922 ppm, respectively (Fernandes *et al.*, 2007).

Other plants have also demonstrated tick repellency and toxicity under laboratory conditions *Callicarpa americana* on nymphs of *Ixodes scapularis* and *Amblyomma americanum*, *Commiphora myrrh* on *R. appendiculatus* (Kaaya and Hassan, 2000; Carroll *et al.*, 2007). The i*n vitro* acaricidal effect of plant extract of neem (*Azadirachta indica*) seed oil on egg, immature and adult stages has been examined and shown to exhibit anti-tick activities against *R. appendiculatus, R. pulchellus*, *Amblyomma variegatum* and *Boophilus decoloratus* (Handule *et al.*, 2002; Abdel-Shafy and Zayed, 2002).

In vitro efficacy tests against *Boophilus decoloratus* using latex of *Euphorbia obovalifolia* and *Ficus brachypoda*, juice of crushed leaves of *Phytolaca dodecandra* and *Vernonia amygdalina*, fruit juice of *Solanum incanum*, crushed seeds of *Lepidium sativum* mixed with fresh cattle faeces, juice of crushed leaves and bark of *Calpurnea aure have* produced significant acaricidal effects in Ethiopia (Regassa, 2000).

The effects of both *Psiadia punctulata* and *Acokanthera schimperi* extracts are considered promising when compared with the effects of commercial pesticides and other botanical materials that have been tried against *Boophilus, Rhipicephalus* and *Hyalomma spp.* (Abdel-Shafy and Zayed, 2002; Chagas, 2002; Fernandes *et al.*, 2005 2007a, 2007b). The observed mortality denotes the different mechanism of action of the chemical constituents of the two plants, *P. punctulata* being more potent as contact and inhalant poison suggesting a neurotoxic potential compared to the cardiotoxic *A. schimperi* which had relatively less potency by the same routes. The bioacaricidal properties of *Psiadia* spp. may be attributed to a group of phytochemicals known as flavones, flavonoids, diterpenes and phenylpropenoids (Midiwo *et al.*, 1997, 2002; Juma *et al.*, 2001, 2006). They may be involved in a synergistic action against *B. decolaratus* and *R.appendiculatus* larvae (Scheidt *et al.*, 2004).

The larvicidal action of *A. schimperi* could be related to the presence active principle ouabaïn ($C_{30}H_{46}O_{12}$) a very poisonous, amorphous cardiac glycoside which at toxic amounts decreases the electric conductivity through the heart causing irregular heart contractility and arrest (Melero *et al.*, 2000). The cardenolides of *A. schimperi* act by inhibition of Na^+- K^+ ATPase, may be exploited in detecting other structurally simpler compounds with the same mode of action. It is expected that such compounds may have broad-spectrum pesticidal activity against ticks, mites and insects (Melero *et al.,* 2000). These findings of the promising acaricidal properties of *A. schimperi* attract attention to cardiac glycosides. These compounds may provide an impetus for the synthesis of novel effective acaricides by modifying glycoside group, analogues of cardiac glycosides (Abdel-Shafy and Zayed, 2002).

5.1 CONCLUSIONS

5.1.1 Ethnobotanical documentation

The results of the current study support the documented use of acaricidal ethnobotanicals for management of diseases of veterinary importance in Samburu district. Pertinent conclusions drawn include:

- Samburu district represents a very important dry season grazing area for ethnobotanical studies (Appendix V)
- The Samburu pastoralists are still among the most traditional communities of Kenya that have retained their EVM practices though it lacks standardized dosages and adverse effects are frequently ignored or underreported.
- The Samburu ethnobotanical knowledge is threatened due to change from a nomadic to more sedentary lifestyle, overgrazing and over-exploitation and passing on of knowledge by word of mouth to successive generations.

5.1. 2 Ectoparasites management

Plant concoctions in the treatment of ectoparasites are confidently used by Samburu pastoralists despite lack of scientific validation. The deduction is that:

- The bioacaricidal tests of *Labaai* and *Lmorijoi* as ectoparasiticides demonstrated that they had significantly killed the tick larvae; they could potentially be an auto-sustainable resource of novel acaricidal compounds further supporting preservation of the Samburu flora and phytochemical isolation of active ingredients.

5.2 RECOMMENDATIONS

- There is need to undertake vast ethnopharmacological surveys in Samburu District and other geographical areas to identify other potential anti-tick botanicals, this will prompt scientific exploitation and validation of other plants that have largely been ignored or underutilised. Development of a Samburu inventory plant index translated in other languages can be a useful source of information for academic and public utility.
- Tick control strategies must be designed to both prolong the effectiveness of current commercial and herbal acaricides. Epidemiological data on tick borne diseases should be related to ecological data as a basis for a recommended tick control program. Subsequent more satisfactory results may be obtained with other parts of these plants for example seeds, fruits, roots and bark or with fractions and sub fractions of the crude extracts tested.
- Phytochemical isolation of *A. schimperi*, *P. punctulata* and other potential acaricidal plants and the evaluation of their bioactive fractions against prevalent tick species should be explored as a steadfast search for alternatives in the control of ticks and other arthropods of medical and veterinary importance.
- Safety and toxicity studies should be conducted in the target animals to determine any adverse effects that may occur under the proposed use of bioacaricides studied and establish a margin of safety by dosages standardization and specific selective toxicity models established.
- Environmental impact assessment (EIA) should be conducted to provide details on the risk of environmental exposure during manufacture, use and disposal of the acaricides.

- The elucidation of the structures of biologically active substances in these plants can lead to development of combination drugs after standardisation of pharmacodynamic and toxicodynamic profiles. Experiments on the synergism or potentiation effects of these plants would be vital in pharmacophores modelling and economical drug discovery.
- There is need to generate sufficient scientific data on efficacy, mode of action, acute and chronic toxic effects and other pharmacological and toxicological properties to support widespread use of these and other plant preparations.
- Pharmaceutical development of these herbal acaricides should be certified by a proper official agency after extensive standard scientific testing before product registration.
- To ensure the quality and consistency of studies undertaken to support registration of herbal acaricides, studies should be performed in compliance with the Good Clinical Practice Guidelines (GCP) for efficacy studies and the Good Laboratory Practice Guidelines (GLP) for safety studies. Standards for manufacturing processes and the handling of ingredients under the Good Manufacturing Practice Guidelines (GMP).
- Any attempts of commercialization of the products developed from these ethnobotanicals must be in tandem with WHO international agreement promoting the harmonization of national intellectual property rights (IPR) regimes concerning patents, geographical indications, undisclosed information (trade secrets) and trademarks.

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LIST OF APPENDICES

Appendix I: Data acquisition and Survey Questionnaire

PROJECT TITLE: RESEARCH AND VALIDATION OF ETHNOVETERINARY REMEDIES FOR ECTOPARASITES AND ENDOPARASITES OF LIVESTOCK IN KENYA.

PART 1:

1. **RESPONDENTS DETAILS:**
   Name..... Sex...M/F Age.....Years.
   Occupation..... Location.....
2. **EFFICACY/TOXICITY DATA**
   Type of Plant (Local name):
   .....
   Preparation method :
   .....
   Administration form :
   .....
   Part of plant used :
   .....
   Used on : Humans ☐ Animals ☐
   Species.....
   Route of application :
   .....
   Approximate dosage :
   .....
   Response of Patient : Good ☐ Fair ☐ Poor ☐
   Duration of response : ..... Seconds
   .....Minutes.....Hours
   Complications :
   .....

PART 2: **RESPONDENTS CONSENT AGREEMENT:**

I ..... Hereby agree to participate in this study with my full consent and conscious and declare that to the best of my Knowledge the information that I have provided is true, accurate and complete.

Signature / Thumb print ..... Date ...../May/2007

RESEARCHER'S DECLARATION

1. The following research will be undertaken with respect to the indigenous knowledge and intellectual proprietary of the Samburu Community
2. We will at no given time initiate or conduct practices that are deemed to obtain information from the respondents by intimidation, coercion or false pretence.
3. The respondents will be informed of the intended project elaborately prior to questionnaire administration and in confidence to eliminate any degree of conspiracy
4. We will be no under any obligation to edit or tamper the information provided by the respondents.
5. Translation and transcription will be necessary for clarification due to the language barrier.
6. The information collected will be used for the described research purpose and not any undisclosed intentions

Researchers:

1. Dr.Nanyingi M.O (UON) 2. Dr. Mbaria J.M (UON) 3. Dr.Ogara O.W (UON)

Appendix II: List of informants

ID	Name	S	A	O
1	Isaac Juma	M	52	TH
2	Aplea Lewaso	M	65	HV
3	Lesirima Lewaso	M	42	HV
4	Daniel Leshampuri	M	60	TH
5	Priscilla Letarkush	F	20	HV
6	Prisca Aplea	F	35	HV
7	Priscilla Lemar	F	32	HV
8	Amina Abdi	F	30	HV
9	Emily Lwatai	F	35	HV
10	Ltampis Nesirikwat	F	42	HW
11	Magret Echom	F	60	HV
12	Katrina Kamar	F	60	HV
13	Magret Kamar	F	45	HV
14	Saire Lenguru	F	54	FM
15	Ekwam Ibuya	M	36	TH
16	Loiture Lanyasunya	M	82	TH
17	Daniel Sedimo	M	18	BS
18	James Lengarusi	M	40	HD
19	Kastella Lenamalda	F	35	HW
20	Naisunguru Lugurusi	F	20	HW
21	Selina Lessamana	F	28	HW
22	Stella Lessamana	F	29	HW
23	Paul Lessamana	M	35	MO
24	Mary Elakata	F	45	HW
25	Lekur Elakata	F	40	HW
26	Monica Lelamunya	F	33	HW
27	Lucy Lenamalda	F	29	TC
28	Miriam Letongot	F	28	HW
29	Veronica Letunyo	F	35	HW
30	Ntiglai Lamanyo	F	80	TH
31	Loipan Lenamalda	F	40	HW
32	Josephine Lenamalda	F	32	HW
33	Joseph Letongot	M	65	VE
34	Lenamalda Leipan	M	70	VE
35	Veronica Letunyo	F	35	HW

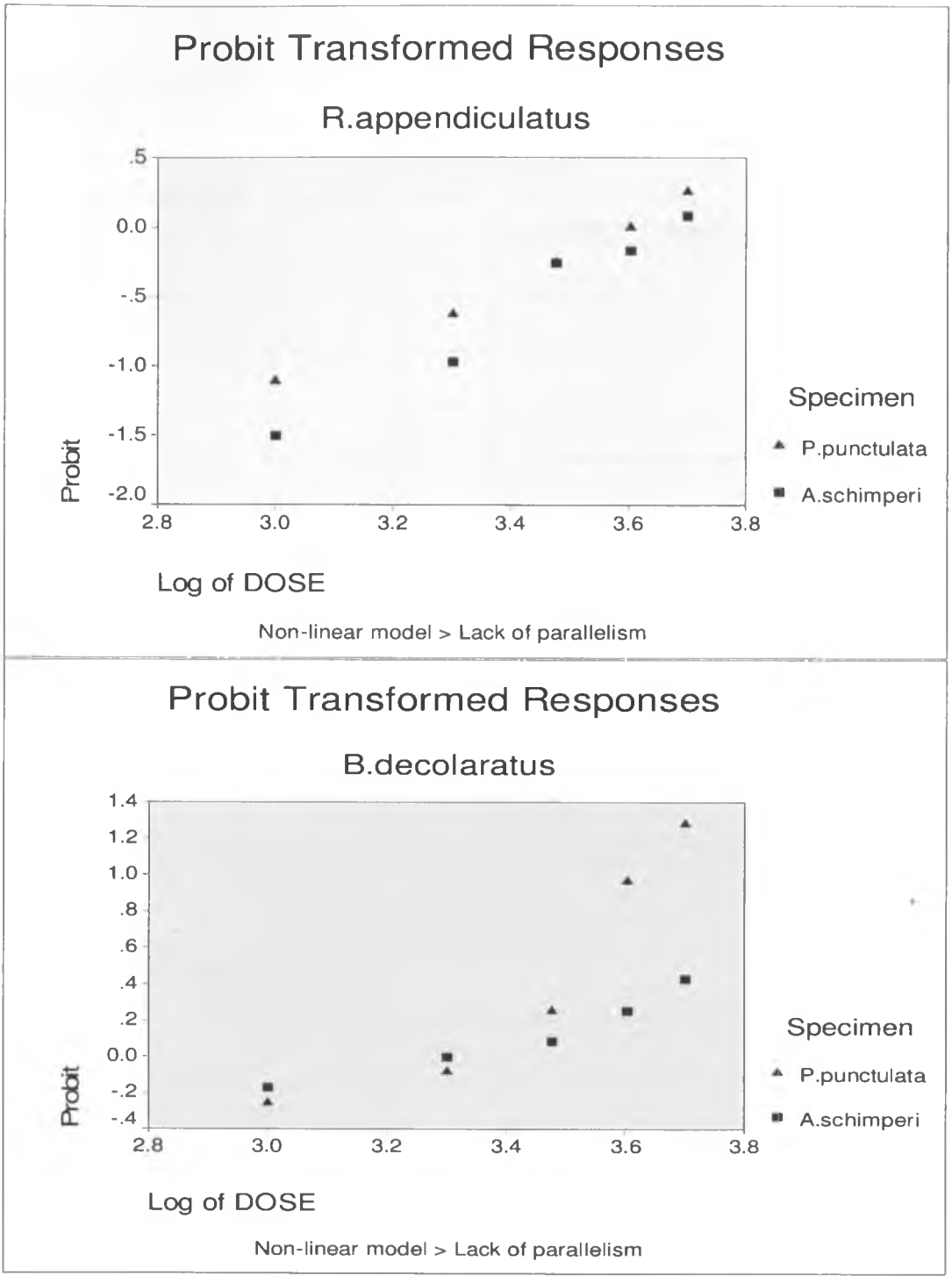
ID	Name	S	A	O
36	Selina Lessamana	F	28	HW
37	Elizabeth Lekiman	F	26	HW
38	Alex Lakaram	M	31	MO
39	Sarah Sein	F	28	SC
40	Peter Lelanguya	M	16	BS
41	Sarun Lelanguya	M	18	BS
42	Yusuf Lelanguya	M	24	BS
43	John Lelanguya	M	19	BS
44	Thomas Lelanguya	M	28	BS
45	Simon Lelanguya	M	45	BS
46	Nanlei Lelanguya	M	65	BS
47	James Lelanguya	M	22	BS
48	Mary Lelanguya	F	62	TH
49	Daniel Sitati	M	35	SK
50	Lemakuya Lesrima	F	32	HV
51	Lenyasunya Adams	M	36	OL
52	Andrew Lekapano	M	29	OL
53	David Lengala	M	43	PS
54	Joseph Lenaseitan	M	33	AS
55	Kristina Letiwa	F	66	TH
56	Boniface Lochok	M	28	CL
57	Simon Lesorokit	M	45	CL
58	Lewaso Lekupano	M	68	TH
59	Joseph Oltajiri	M	65	TH
60	Marko Ibuya	M	45	TH
61	Ekiru Samar	M	24	CL
62	Lenaingo'ingo'i	M	50	FT
63	Lekaint Lekangurusi	M	29	MO
64	Loildemet Yappas	F	34	HV
65	Lketilo Ltampasi	M	50	TH
66	Latangasi Lamiuga	M	33	MO
67	Antonella Lekupe	F	31	HV
68	Masianai Lepaya	F	30	HV
69	Nasikwa Leiron	F	35	TC
70	Elizabeth Sinantei	F	26	SC

Key :

ID= Respondents Identity, **S** = Sex, **A**= Age, **O** = Occupation**TH** = Traditional Healer, **HV** = HJerbal Vendor, **HW** = House Wife, **FM** = Farmer, **MO** = Moran

TC = Teacher, **VE** = Village elder, **OL** = Opinion leader, **FT** = Forester, **SK** = ShopkeeperPS = Pastor, **SC** = Secretary, **AS**= Animal health assistant, **BS** = Blacksmith, CL= Casual Labourer.

Appendix III: Observed Log-Probit transformed larval mortality of *R.appendiculatus* (Top) and *B.decolaratus* (Bottom) over 24 hours by *A.schimperi* and *P.punctulata*



Appendix IV: FAO Larval Packet test form for detection of ticks' resistance to Amitraz (CSIRO, Australia).

CHEMICAL: Amitraz **Laboratory No:** **Date tested :**

% CONCENTRATION	TICK SPECIES (A)			TICK SPECIES (B)		
	No. dead	Total	% mortality	No. dead	Total	% mortality
0.4						
0.2						
0.1						
0.05						
0.025						
CONTROL						

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Ethnopharmacological survey of Samburu district, Kenya

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Abstract

Background: Ethnobotanical pharmacopoeia is confidently used in disease intervention and there is need for documentation and preservation of traditional medical knowledge to bolster the discovery of novel drugs. The objective of the present study was to document the indigenous medicinal plant utilization, management and their extinction threats in Samburu District, Kenya

Methods: Field research was conducted in six divisions of Samburu District in Kenya. We randomly sampled 100 consented interviewees stratified by age, gender, occupation and level of education. We collected plant use data through semi-structured questionnaires; transect walks, oral interviews and focus groups discussions. Voucher specimens of all cited botanic species were collected and deposited at University of Nairobi's botany herbarium.

Results: Data on plant use from the informants yielded 990 citations on 56 medicinal plant species, which are used to treat 54 different animal and human diseases including; malaria, digestive disorders, respiratory syndromes and ectoparasites.

Conclusion: The ethnomedicinal use of plant species was documented in the study area for treatment of both human and veterinary diseases. The local population has high ethnobotanical knowledge and has adopted sound management conservation practices. The major threatening factors reported were anthropogenic and natural. Ethnomedical documentation and sustainable plant utilization can support drug discovery efforts in developing countries.

Background

The Samburu pastoralists of Kenya are still among the traditional communities of the country that have retained most of their knowledge about the use of a large part of the plants in their environment for a wide variety of purposes. This knowledge is however dwindling rapidly due to changes towards a more western lifestyle, overgrazing, and overexploitation of plant resources have already led to a decline of the plant material available [1].

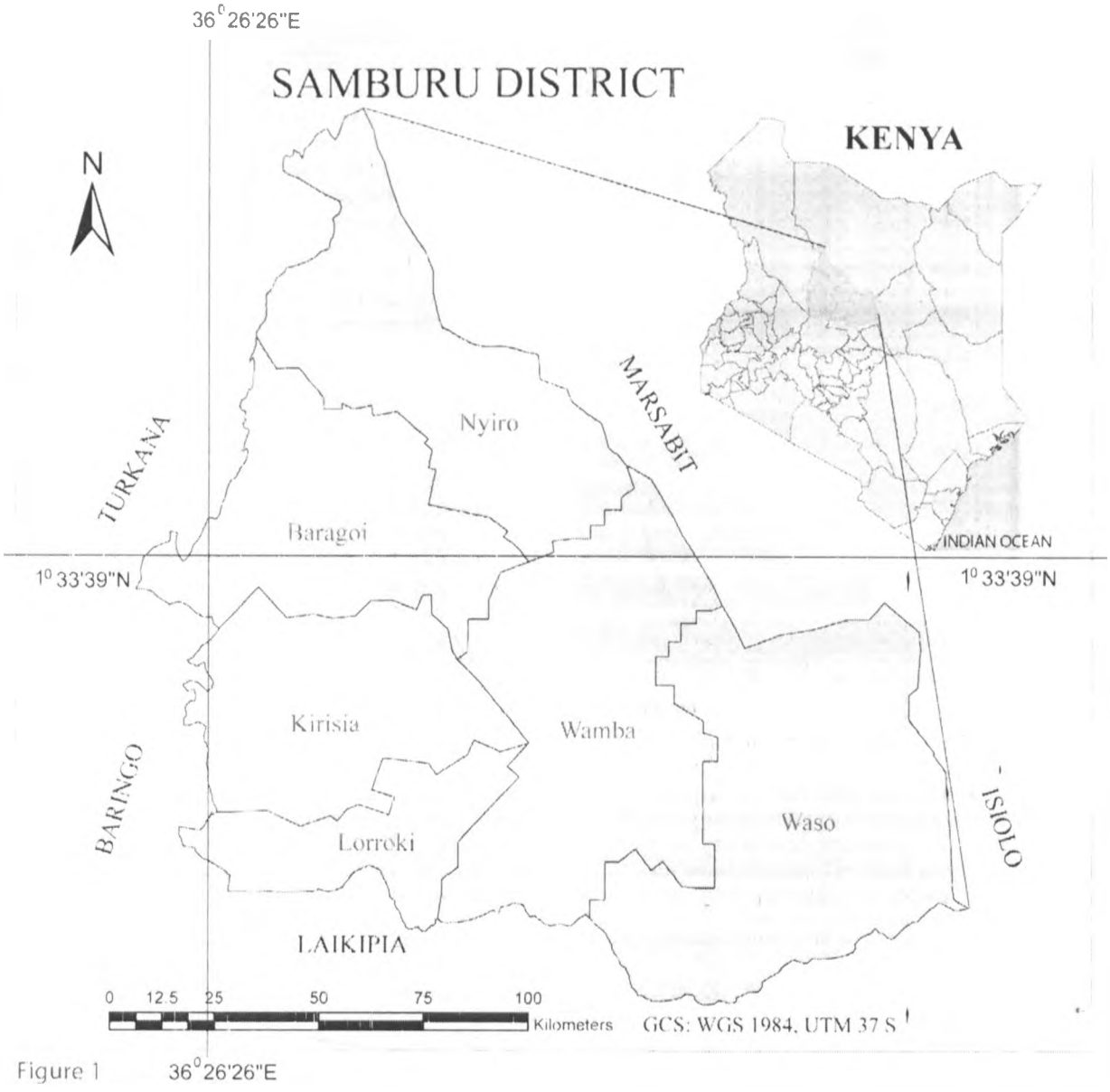

Figure 1

Map of research area. Right: Map of Kenya illustrating the geographical position of Samburu District. Left: Samburu District indicating the divisional administrative boundaries.

characteristics of the study area. The fieldwork was done in January, February, May and August 2007.

A total of 100 informants in figure 2 were selected purposively [18] based on knowledge, attitudes and practices (KAP) survey with the help of local administrators. They included 14 traditional medicine practitioners (4 females and 10 males), 86 locals (Male: Female = 2:1). Information on knowledge depth of respondents was collected from local elderly people, opinion leaders and the local administrators. Similar responses obtained from the three groups were used to identify knowledgeable traditional healers. The respondents and traditional healers identi-

Wilcoxon's test was used to determine if there was a difference age of respondents and knowledge of medicinal plants used. Chi-square test was used to evaluate the average number of medicinal plant species reported and used by each informant, to determine if there is any significant difference between female and male practitioners with respect to the knowledge and use of medicinal plants. The Spearman rank correlation test was used to determine whether there was a significant correlation between the disease reported and the number of ethnoveterinary medicinal plant species used by each informant for management of the disease. STATA 9.2 IE (Stata Corporation, College Station, Texas, USA) software was used.

Results and discussion

Medicinal plants diversity and Ethnobotanical knowledge

There was a highly significant difference between age of respondents and knowledge of medicinal plants (Wilcoxon's test, $p < 0.001$). The average number of medicinal plants known and used by female and male practitioners was similar ($\chi^2 = 8.262$, d.f. = 13, $p = 0.932$). It was observed that informants between 58 and 77 years old mentioned more species than younger informants: 58–67 years old: about 10 per informant; 48–57: about 6; 38–47: about 5; and 28–37: 2 quoted plants, due to larger experience of older individuals. These results also agree with other previous studies[25]. It was observed that some plants had more than one vernacular name due to use of the Maasai and Turkana dialects in the area.

Diseases treated in the study area

A total of 28 animal and 26 human ailments were reported by the informants respectively. The frequency of the most cited ailments and the number of medicinal plant species used are also given in figure 3. The most frequently cited animal health problems were; Retained afterbirth (9), Ectoparasites (8), gastrointestinal disorders (5), Theileriosis (4) and helminthosis (3). Human ailments treated cited frequently included; Malaria (18), gastrointestinal disorders (10), helminthosis (5) and rheumatism (4). Respondents had good knowledge and remote diagnosis of the disease and could readily distinguish them on the basis of accepted signs and symptoms. Ailments such as convulsions, hypertension, asthma, yellow fever and infertility were beyond the scope of the present study, it was considered important to record plants that were frequently mentioned for the treatment of such health conditions (see table 1).

Ticks (*Intunturi*) were the main cattle ectoparasites that the local people controlled using traditional plant extracts. The most frequently used plants for tick control were found to be: *Acokanthera schimperi* (*Lmoriyoi*), *Adenium obesum* (Lperantai), *Aloe secundiflora* (Sukuroi),*Psiadia*

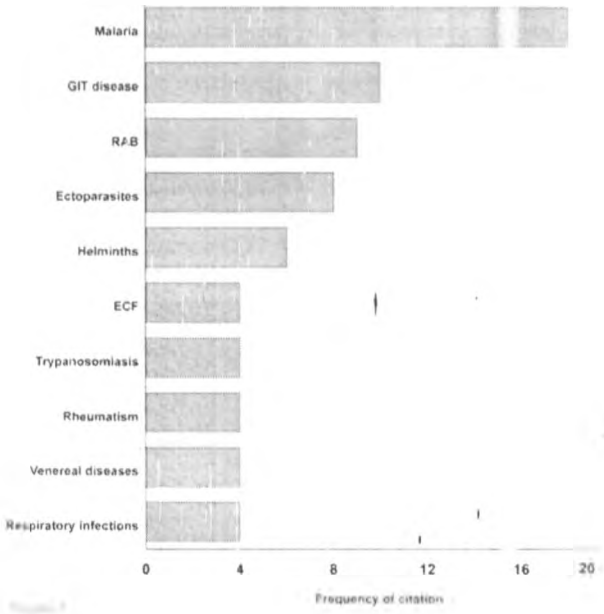

Figure 3
Frequency citation (n = 990) of therapeutic indications of plant remedies based on informants knowledge (n = 100) and traditional healers (n = 14).

punctulata (Labaai),*Nicotiana tabacam* (Lkumbao), *Euphorbia herechroma* (Lpara) represented in table 1.

The respondents classified all intestinal worms under one local name, *ntubui* and therefore use the same plant extracts for all helminths. The main species used for this were: seeds of *Myrsine africana* L (*Seketet*) (45%), *Albizia anthelmintica* (*Lmungutan*) (30%) and *Warburgia ugandensis* (*Sokorioi*) (18%).

Medicinal plants used by the locals

Fifty four (54) plant species of ethnopharmacological importance were gathered and documented throughout the study period (table 1). These medicinal plants were distributed among 50 genera and 33 families. Analysis of the growth forms of these medicinal plants revealed that, shrubs constituted the largest number or proportion with 31 species (56%), followed by trees 15 (28 %), herbs 5 (9%) and lianas 4 (7%) respectively shown in figure 4. Ethnobotanical knowledge was passed on by word of mouth. Knowledge of ailments such epilepsy, hypertension, venereal diseases, impotence, was generally restricted to the elders and traditional medicine practitioners represented in figure 5.

Leaves were the most frequently used plant parts constituting 4 %, followed by roots (3 0%), stems (10%), fruit/

Table 1: Plants of veterinary and medical utility in Samburu District. *(Continued)*

Podocarpaceae	*Podocarpus falcatus* (Thunb.)	Masanduku	MN 38	SH	Leaves	Hot decoction, Fumigation	Measles	pc
Rhamnaceae	*Cissus quadrangularis* L	Sukurtuti	MN 50	SH	Leaves, Fruits	Crushing, homogenizing for hot/cold decoction	Wounds, gastric ulcers, schistosomiasis, neurosis, ECF, rheumatism, epilepsy, TB, Asthma, collibacillosis	po
	Helinus integrifolius (Lam.) Kuntze	Lmekori	MN 12	SH	Root bark	Grinding, hot decoction, mix with milk	Arthritis, paralysis	po
	Rhamnus staddo L.	Lkukulai	MN 10	SH	leaves, fruits	hot decoction	Malaria, fevers!	po
	Scutia myrtina (Burm. F.) Kuntz	Laturdiai	MN 20	SH	Leaves	Hot decoction	Retained afterbirth	po
Rubiaceae	*Rubia cordifolia* L.	Loitunenei	MN 9	L	Leaves, Roots	Hot decoction	URTI	po
Rutaceae	*Teclea simplicifolia* (Engl.)	Lgelai	MN 42	TR	Rc, Flowers	Hot decoction	Cerebral malaria, Fevers	po
	Zanthoxylum usambarrense (Engl.)	Loisuk	MN 31	SH	Seeds	Grinding, Hot decoction	URTI, Malaria, Malignant catarrhal fever	po
Salvadoraceae	*Salvadora persica* L.	Sekotei	MN 41	SH	Roots	Grinding, hot decoction	RAB, ulcers, seizures, toothbrush, mange, Trypanosomosis, Brucellosis, and Anthrax	po
Simaroubaceae	*Harrisonia abyssinica* Oliv.	Lasaramai	MN 24	SH	Roots, Leaves	Grinding, hot decoction	Abscess, ECF, Malaria, Lumbago, Rheumatism, RAB	po
Solanaceae	*Nicotiana tabacum* L.	Lkumbao	MN 34	SH	Leaves	Crushing, smoke bath, chewing	Snuff, Ectoparasites, wounds, Babesiosis, gastro-enteritis, chronic cough, gingivitis, candidosis, glossitis	Pc,
	Solanum incanum L.	Ltulelei	MN 22	H	Fruit	Burn and drip sap on skin	Ectoparasites	pc
Verbanaceae	*Lippia javanica* (Burr.)	Sunoni	MN 4	SH	Leaves	Fumigation, decoction	Migraines, Measles	in, pc
	Clerodendrum myricoides (Hochst.)	Lmakutikuti	MN 25	TR	Root	Powdering, hot decoction, chewing	GIT, Lumbago, Venereal diseases.	po
Viscaceae	*Viscum tuberculatam*	Larrudenyai	MN54	SH	Root bark	Hot decoction	RAB	po
Vitaceae	*Rhoicissus tridentata* (L.F.)	Nkilenyai	MN 1	L	Leaves	Crushing, cold homogenization	URTI, Malaria	
	Hilderbrantia sepalosa	Nyirrnan	MN 30	SH	Roots	Crushing, hot decoction	URTI and GIT complications	Po

Habit (H – *Herb*, L – *Liana*, SH – *Shrub*, TR – *Tree*); **Therapeutic indications** (ECF – *East Cost Fever*, GIT – *Gastrointestinal*, RAB – *Retained Afterbirth*, TB – *Tuberculosis*, URTI – *Upper Respiratory Tract Infections*, VD – *Venereal Diseases*); **Routes** (in -*Intranasal*, pc – *Per cutaneous*, po – *Per os*).

seeds (8%) and whole plant (4%) in figure 6. The majority of informants (42%) mentioned *Myrsine africana* L. (*Seketet*) as medicinal for the treatment of various animal and human ailments.*Seketet* was thus the most popular remedy in the study area, followed by *Carissa edulis* F. (*Lamuriai*) (5%), *Salvadora persica* L. (*Sekotei*) (30%), *Albizia anthelmintica* Brongn. (*Lmungutan*) (27%) and *Clerodendrum myricoides* Hochst. (*Lmakutikuti*) (22%).

The number of species frequently used in each family was cited as; Apocynaceae (6), Mimosaceae (5), Euphorbiaceae and Rhamnaceae (4) and Asteraceae (2) other families were represented by at most one species shown in figure 7.

The preparation of the medicines employed several methods; hot decoction (48%) followed by cold decoction (19.4%) and homogenization by pounding or powdering (6.5%) respectively in (table 1). The majority of these preparations were drawn from mixtures of different plant species for the treatment of a single ailment. Oral administration (8%) was the predominant route of administration followed by dermal and nasal administrations (20%).

Medicinal plants extinction threats

Many medicinal plants in the study area were mainly threatened by anthropogenic and natural factors. The majority of medicinal plants declined due to deforestation for construction, tools, firewood, fodder, agricultural expansion and ceremonial purposes. Drought, overgrazing, bush fires had reportedly affected a significant number of medicinal plant species.

Conservation efforts and indigenous knowledge transfer

About 47% of the informants had sufficient awareness in conserving some medicinal plant species that were relatively scarce in their surroundings. *In situ* protection of plants (fencing plants in their natural habitat, refraining from excessive cutting, debarking and uprooting and protection from fire) and *ex situ* conservation by cultivation of some plants as live fence and in nurseries were undertaken by the locals. Moreover, some of them were keen to inform responsible bodies or authorities of any illegal logging, deforestation and bush fires.

Majority of local healers preferred to collect medicinal plants solely to preserve their secrecy sometimes accompanied by the chosen family member(s). The ethnobotanical knowledge is transferred to that trustworthy family

Figure 4
Percentage distribution of the habit growth forms of medicinal plants.

from a single species [29]. This could also be ascribed to the differences in the socio cultural landscapes, indigenous knowledge on synergetic effect of different medicinal plants and vegetation types in the current study area [29].

The most frequently used methods of preparation were hot decoctions, cold decoctions, powdering and grinding respectively. The prepared medicines were mainly administered through oral (98%), dermal (1.5%), and nasal (0.5%), routes concurring with the previous findings in Ethiopia [30].

The measurements used to determine the dosages were not standardized and depended on the age and physical appearance of the patient, sociocultural explanation of the illness, diagnosis and experience of individual herbalist [27].

The naming of diseases by local people when compared to conventional systems, at times did not distinguish between diseases and symptoms of diseases. This is because local disease nomenclature is based on symptoms

Figure 5
Lewaso Aplea (66 years), The most revered and knowledgeable of the remaining traditional healers in Samburu District displaying, *Ximenia caffra* Sond.(*Ledat*) and *Myrsine africana* L.(*Seketet*) during the field collection.

of diseases and not according to aetiological information [31,32].

While conducting this study, some informants raised some concern on false promises about getting the feedback. They agreed that scientific methods are better in

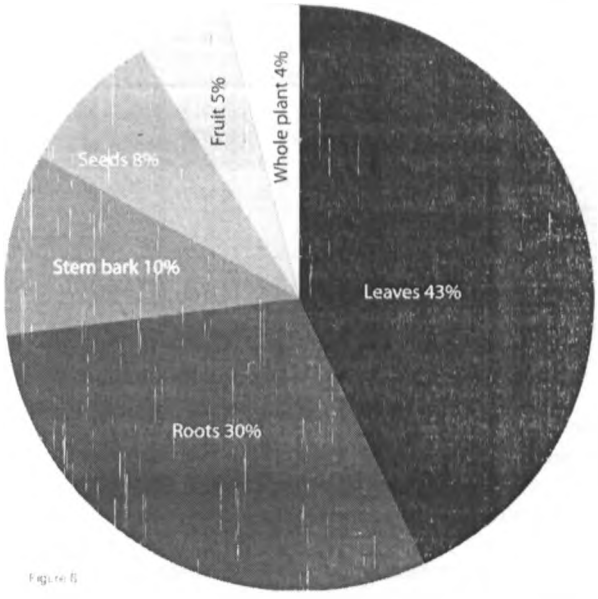

Figure 6
Percentage distribution of Plant parts used in Samburu District.

knowledge so as to minimize the eminent fragmentation and biodiversity loss.

The lack of standardized posology of the traditional medicines should encourage pharmacological and toxicological tests to develop formulations that can be administered in measurable dosages whose clinical efficacy can be monitored and pharmacovigilance mechanisms instituted to eliminate development of resistance to these novel compounds. Scientific feedback studies should be encouraged to instil confidence in the increasingly suspicious local populations to eliminate the apparent hostility observed among some the informants during the field research.

The data presented in this paper form a basis for further ethnopharmacological research in this region especially in studies dealing with efficacy, dosage, quality and toxicology. Those plants found empirically to be particularly effective can be used in preparation of commercial indigenous-based pharmaceuticals. We recommend that ethnopharmacologists project pharmacologic data against a backdrop of medical ethnography and anthropology. Relevant evidence generated from literature review and these biological tests will be passed back in order to improve the proper use of medicinal plants and create a good relationship for future ethnobotanical studies. The local community of Samburu District, Kenya is the owner of the traditional knowledge presented in this paper, consequently any benefits that may arise from the use this knowledge must be shared with them.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MON carried out the field research, analyzed the data and wrote the manuscript, JMM and WOO designed the study, conducted fieldwork, supervised the research and revised the manuscript, CGW reviewed the manuscript and conducted the field research, KBK reviewed the manuscript and assisted in data analysis, HFK, RWM and AAL assisted in the fieldwork and taxonomic identification of the botanic specimens. All authors read and approved the final manuscript.

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