Acaricidal characteristics of ethnoveterinary plants used for tick control in southern Africa

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Declaration

I declare that the experimental work described in this thesis is my original work (except where the input of others is acknowledged), conducted partly in the Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science , University of Pretoria, South Africa and partly in the University of Zimbabwe in Harare. This work has not been submitted in any other form to any University or academic institution.

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List of abbreviations

AGRITEX	Agricultural, Technical and Extension Services
BHC	Benzene hexachlorine
CBD	Central Business District
CVL	Central Veterinary Laboratory
DDT	Dichlorodiphenyltrichloroethane
DMSO	Dimethyl sulphoxide
FCS	Foetal calf serum
FGD	Focus group discussions
GC-MS	Gas chromatography-mass spectroscopy
IBM	International business machines
LC_{50}	Lethal concentration for 50% of organisms
LSM	Least square means
MEM	Minimal essential medium
MTT	3-(4, 5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NGO	Non-governmental organisations
NMR	Nuclear Magnetic Resonance spectroscopy
PBS	Phosphate-buffered saline
POPs	Persistent organic pollutants
RH	Relative Humidity
SAS	Statistical Analysis system
SD	Standard deviation
SLIT	Shaw Larval Immersion Test
SPSS	Statistical Package for the Social Sciences
TTBD	Ticks and tick-borne diseases
TBD	Tick-borne diseases
UNDP	United Nations Development Programme
WHO	World Health Organisation

Abstract

Ticks and tick borne diseases remain a huge threat to livestock productivity the world over. While several efforts have been made to control ticks, current control measures are still not adequate. Conventionally, tick control programmes are heavily reliant on the use of synthetic chemical acaricides while the impact of other less frequently used control methods has not been fully established. Unfortunately, heavy chemical use has led to a number challenges that include: unsustainable high costs of acaricides, development of tick resistance, environmental pollution, contamination of animal products with chemical residues and many other topical issues. Ethnoveterinary plants are however an alternative but possibly effective, environmentally benign and safe option that can complement and in some cases substitute synthetic chemical acaricides. In this study, plant species identified in Zimbabwe and found elsewhere in southern Africa were characterised for anti-tick properties with the aim of developing an ethnobotanical product for use.

The initial step involved the identification of plants through an ethnobotanical survey carried out in 4 arid and semi-arid districts of Zimbabwe, namely Muzarabani, Chiredzi, Matobo and Kadoma. These areas were purposively selected on the basis of high cattle production and high likelihood of use of traditional practices in primary animal health care. More than 51 plant species were recorded and a ranking according to frequency of mention showed that *Cissus quadrangularis*, *Aloe* sp., *Lippia javanica* and *Psydrax livida* were the most popular plants mentioned by farmers. The most common method for preparation was crushing and soaking in water before spraying the animals. Despite the farmers acknowledging that they had access challenges to the normal government-provided dipping services and having knowledge on traditional practices of tick control, the actual use of these practices was low. It was concluded that farmers and other knowledgeable people do have plants they know that have anti-tick properties, thus providing a good basis for the development of ethno-based tick control products.

In order to confirm farmer claims of efficacy of the plant extracts and to find ways of increasing that efficacy, three *in vitro* screening experiments were done using the modified Shaw Larval Immersion Test on *Rhipicephalus (Boophilus) decoloratus* tick larvae. Different extraction methods were used in the screening: crude water extracts, acetone extracts and solvent – solvent fractions of acetone extracts of *Maerua edulis*. Results showed that contrary to the high activity reported by farmers in the surveys, water extracts were not toxic to the tick larvae. Perhaps the high activity reported by farmers, if confirmed may be associated with the repellence of volatile emissions from the plants. The addition of a liquid soap as a surfactant however increased the efficacy of the *M. edulis* tuber aqueous extract to activity levels comparable with those of an amitraz-based commercial acaricide, which was the positive

control. The use of the organic solvent acetone as an extractant markedly increased the efficacy of 13 of the plant species under study, particularly *M. edulis*, *Monadenium lugardae* and *Kleinia* species. The chloroform and hexane fractions from *M. edulis* exhibited very high activity, possibly indicating that less-polar compounds are responsible for the observed activity. Thus, the use of water as a sole extractant is limited in terms of extracting compounds active against ticks, but organic solvents and acetone in particular increase the efficacy of the extracts. In the case of *M. edulis* less polar extracts and fractions were most active against the ticks.

Because *Maerua edulis* consistently showed good activity in all prior testing, it was further tested using low-cost optimisation strategies like the use of hot water, a surfactant and a different organic solvent (methanol). Hot water extraction and use of a surfactant increased efficacy of the crude extracts of the *M. edulis* leaves against ticks to satisfactory levels compared to cold water extracts. There was no significant difference between the positive control and methanol-extracted *M. edulis.* It is, however, the use of ordinary soap that may bring relief to rural farmers who are generally unable to have access to organic solvents.

From the observation that the hexane and chloroform extracts of the *M. edulis* leaf and roots were very active against the ticks, cytotoxicity of the extracts on African Green monkey kidney (Vero) cells and bovine dermal cells was determined to shed some preliminary insights on safety aspects of the plant. Neither extract had high toxicity against these cell lines. The LC_{50} was greater than 20 µg/ml which is considered as a maximum threshold for indicating toxicity of plant extracts.

After confirmation that non-polar fractions of *M. edulis* were active against ticks and that cytotoxicity results showed that the extracts are relatively non-toxic to animal cell lines, attempts to isolate and identify the active compounds in the chloroform fractions of *M. edulis* were made without much success. Using column chromatography, an impure compound was isolated in the chloroform fraction but the amount was too low for characterisation by NMR. When the compound was analysed using Gas Chromatography-Mass Spectrometry, a number of chemicals in the isolate were avident but which did not have the pre requisite high similarities with the compound library to be considered. Because of the low quantities no further work was done to further purify and test the compounds against the ticks.

For purposes of confirming laboratory activity under field conditions, *M. edulis*, *C. quadrangularis* and *Aloe vera* crude water extracts combined with a surfactant (liquid soap), were tested on Mashona cattle at Henderson Research Station (Zimbabwe) over 7 weeks during the period of peak tick infestation. Only *M. edulis* tuber extracts with a surfactant were as effective as the amitraz-based positive control. There was no significant difference in activity between the other plant extracts and the negative control.

It can be concluded that there is scope to use *M. edulis* tubers extracted with locally available surfactant as a tick control product. This whole study therefore shows that ticks can be controlled using locally available plant materials provided they are prepared and applied properly. While the overall aim of the study of producing a working plant based tick control product was not met, there is sufficient data from the study to justify developing crude formulations from *M. edulis* that can be used to control ticks.

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Output from research for this thesis

A. Conference presentations

1. Nyahangare E T and Mvumi BM, 2015. Ethnoveterinary tick control options in Zimbabwe – Research, Issues and challenges: Presented at the "Human-Livestock - Wildlife interfaces: an Ethno-science perspective" Seminar/Round Table, 5th May 2015. University of Zimbabwe, Harare, Zimbabwe

2. Nyahangare E T., Mvumi B M., Magona C., Nota B., Eloff J N. 2017. Indigenous acaricidal plants and cattle tick control**.** 2nd International Conference on Pesticidal Plants. 6-9 February 2017. Victoria Falls, Zimbabwe.

3. Eloff, J N., Nyahangare, ET., Mvumi BM., Adenubi, O., Naidoo,V., McGaw, L., 2017. Plant extracts and isolated compounds that are as active as commercial acaricides in controlling ticks in vitro and in vivo. 65th Annual Meeting of the society for Medicinal Plant and Natural Product Research (GA). 3 - 7 September 2019. Basel, Switzerland

4. Eloff JN, Nyahangare ET, Mvumi BM, McGaw LJ, 2019 The extractant is the main limiting factor in using plants by rural people for human and animal health. International Society for Ethnopharmacology Congress, June 2019. Dresden, Germany

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C. Publications

1. Nyahangare, E.T., Mvumi, B.M., McGaw, L.J. and Eloff, J.N., 2019. Addition of a surfactant to water increases the acaricidal activity of extracts of some plant species used to control ticks by Zimbabwean smallholder farmers. *BMC Veterinary Research*, *15*(1), pp.1-7.

2. Nyahangare, E. T., B. M. Mvumi, C. Magona, and J. N. Eloff. 2017. An aqueous extract of *Maerua edulis* (Gilg and Ben) DeWolf tuber is as effective as a commercial synthetic acaricide in controlling ticks on cattle in vivo. Industrial Crops and Products 110:88–93.

3. Nyahangare, E. T., B. M. Mvumi, and T. Maramba. 2016. Acute oral mammalian toxicity and effect of solvents on efficacy of *Maerua edulis* (Gilg. and Ben.) de Wolf against *Rhipicephalus (Boophilus) decoloratus* Koch, 1844 (Acarina: Ixodidae), Tick Larvae. BioMed Research International: 1–8. Available from: http://dx.doi.org/10.1155/2016/7078029

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CHAPTER 1

1 Introduction and Problem statement

1.1 Background

Livestock plays a crucial role for 70% of the world's resource-poor population mainly living in rural areas (Randolph et al., 2007; Herrero and Thornton, 2013). The role of livestock, particularly cattle, is magnified even more in the drier ecological zones where crop production is restricted by low and irregular rainfall coupled with recurring droughts. Productivity of animals in these regions is furthermore often hampered by many factors that include poor health service delivery and animal disease (Adenubi et al., 2016). One of the major health concerns affecting cattle is ticks and tick-borne diseases (TTBD). Ticks act as vectors of fatal diseases, for example, babesiosis and theileriosis, which have serious economic impacts to farmers (Jabbar et al., 2015; Luseba et al., 2016). According to a report by Minjauw and McLeod (2003), the challenges posed by ticks have a huge impact on the livelihoods of disadvantaged smallholder farmers in developing countries across the world. In Africa, TTBD were identified ahead of tsetse fly and trypanosomiasis as the biggest animal health challenge (Young et al., 1988). Efforts to control ticks have been underway for a long time and it is widely accepted that complete eradication of ticks is impossible under the current efforts (Mapholi et al., 2014). Conventionally, intensive use of commercial chemical acaricides has been the main control method of ticks but several negative associated effects are emerging (Adenubi et al., 2016). Some of the issues include widespread development of acaricidal resistance, ever-increasing cost of acaricides, high toxicity of chemicals, chemical residues in meat and milk, and harm and threat to non-target organisms (Willadsen, 2006; Fuente et al., 2007; Adehan et al., 2016). It has also been realized that the current tick control methods are not well-suited for marginalised smallholder farmers in developing countries, thereby creating many challenges in the control of ticks. This is because most of the foci in the management of ticks and acaricide development have been largely focused on vulnerable exotic and commercial cattle breeds and not so much on the smallholder sector and indigenous cattle breeds where the challenges are somewhat different (Perry et al., 2005). All these factors have made intensive dipping and spraying using commercial acaricides unsuitable for smallholder farmers. Several options have been postulated, including the use of vaccines, genomics and integration of several systems with various degrees of adoption and success (Godfray et al., 2010; Merino et al., 2013; Mapholi et al., 2014).

One innovative approach that has received wide approval from many stakeholders is consideration of integrating existing conventional methods of animal health management with ethnoveterinary practices and products (Isman, 2008; Moyo and Masika, 2009; Madzimure et al., 2011; 2013). From time immemorial, plants have been used to control agricultural pests but this has been overtaken by use of synthetic acaricides and pesticides (Kiss et al., 2012). There is renewed interest in them due to the afore-mentioned challenges with commercial products. Available evidence suggests that pesticidal plants have considerable potential in ecto-parasite control programs, particularly for resource-poor smallholder farmers (Isman, 2008; Kiss et al., 2012). An important challenge now is to find out which plants are effective against what pest/parasite from the wide selection of plant species found across the world.

Application of ethnoveterinary technologies, like the use of acaricidal and tick repellent plants, has not been tested under controlled conditions and is not considered in the current conventional acaricide industry. As a result, these technologies are often regarded as backward, ineffective and generally not applicable in serious farming operations (Wanzala et al., 2005). Unless issues like efficacy (in laboratory bioassays and on-farm trials), phytochemistry, safety and toxicity of these ethnobotanical plants are scientifically established, it remains difficult to have them contribute to the wide-spread primary health care of livestock.

The comprehensive study of characteristics of ethnoveterinary plants used for controlling ticks may offer a good opportunity for the development of alternative tick control remedies that can improve smallholder livestock productivity and reduce over-reliance on the use of chemical acaricides in conventional tick control programmes.

1.2 Hypothesis

Safe and effective tick control products can potentially be developed from locally available traditionally used acaricidal and tick-repellent plants.

1.3 Aim and Objectives

1.3.1 Aim

The aim of this study was to confirm acaricidal activity of plants used traditionally against ticks and to establish the groundwork for developing a product that would be effective against ticks under field conditions.

1.3.2 Specific objectives

The specific objectives were to:

1. Identify ethnoveterinary plants used for tick control in Muzarabani, Chiredzi, Sanyati and Matobo districts of Zimbabwe through an ethnobotanical survey.

2. Determine the acaricidal activity of ethnoveterinary plant extracts ranked highly by rural farmers against ticks *in vitro.*

3. Evaluate the effect of different extractants on acaricidal efficacy of *Maerua edulis* (Gilg and Ben) De Wolf against *Rhipicephalus (Boophilus) decoloratus* tick larvae and acute oral mammalian toxicity.

4. Evaluate *in vitro* cytotoxicity of active fractions of *M. edulis* extracts against Vero monkey kidney and bovine dermal cells

5. Determine efficacy of extracts prepared using different extractants of plants considered to be most effective by rural farmers against cattle ticks in on-station trials

CHAPTER 2

2 Literature review and current state of knowledge

2.1 Introduction

The threat of ticks and tick borne diseases (TTBD) to the productivity of a large number of cattle globally is not a new phenomenon but well-documented (Pegram et al., 1993; Ghosh et al., 2007; Simuunza et al., 2011; Atif et al., 2012; Jabbar et al., 2015). It is also very clear that effective management and control of ticks continues to be a very costly exercise, requiring significant amounts of money. Aware of the costly nature of controlling ticks and the need to protect cattle especially among smallholder farmers, governments of most poor and developing countries like Zimbabwe and Zambia have assumed the responsibility of controlling ticks by heavily subsidizing acaricides required for communal cattle-dipping (Pegram et al., 1993; Moyo and Masika, 2009). This has somewhat prevented and cushioned smallholder farmers from loss of their livestock and, in the process, enhanced food security. At a global level, many countries are spending large amounts of money in tick control programmes estimated to be in the range of approximately US$13.9 – US$18.7 billion annually (Jongejan and Uilenberg, 2004; Ghosh et al., 2007). Africa is estimated to use approximately US$720 million annually (Adenubi et al., 2016). For most poor countries, the costs of treating and managing TTBD are often prohibitive and largely unsustainable. In Zimbabwe, for example, there was a national annual loss of US$5.6 million due to cowdriosis (Mukhebi et al., 1999), a tick-borne disease, during the 1988 – 1989 farming season.. The total Government of Zimbabwe expenditure for communal cattle-dipping accounted for 57% (US$9.2 million) of the total budget allocated to the Department of Veterinary Services (Perry et al., 1990, Norval et al., 1992). These financial statistics and the threat to livestock productivity are the reason for research efforts into alternative holistic methods of tick control. However, accurate and current data on economic losses associated with TTBD are scarce. In this review, the general control of TTBD and the developments surrounding use of ethnoveterinary plants for the control of ticks is examined with particular emphasis on southern Africa.

2.2 Synthetic chemical control of ticks

2.2.1 Overview

A good understanding of the circumstances surrounding acaricidal plant usage can be informed by what is known about the current conventional tick control methods dominated by synthetic acaricides. This has been the preferred method of tick control in many places over the world since the early 1890s where they were first used in Australia and the United States of America (George et al., 2004; Bissinger and Roe, 2010). Dipping

vats for immersing cattle were used with a wide range of chemicals in a system of trial and error. Some of the earlier unsophisticated chemicals used included cotton seed oil, fish oil, crude petroleum, kerosene, creosote, tobacco extracts and many similar products (Mohler, 1906; Angus 1996). These products became precursors to the production of the first arsenic acaricide in South Africa in 1896, which was a very popular product for close to 50 years until problems related to resistance surfaced. This led to the development of the organochloride insecticides, dichloro-diphenyltrichloroethane (DDT) and benzene hexachlorine (BHC) which were superseded later by organophosphates e.g. chlorpyrifos and chlorfernvinphos introduced almost at the same time with carbamate acaricides. Other synthetic acaricides which have been used extensively are the synthetic pyrethroids (based on pyrethrin isolated from *Chrysanthemum* (previously *Pyrethrum*) e.g. permethrin and amitraz (Adehan et al., 2016). With amitraz, some populations of *Boophilus micropus* have already been reported to be resistant (Li et al., 2007; Adehan et al., 2016).

This synopsis on the different types of acaricides that have been developed shows that the development of acaricides has been an on-going response to the different challenges that are faced with developed products on the market; chief amongst them being development of tick resistance and banning of certain chemicals deemed environmentally or host-animal unfriendly. There are other negative issues associated with the use of these products that include high costs of the acaricides, environmental pollution, residual contamination of animal products and the unavailability of the chemicals especially for resource challenged farmers (Graf et al., 2004; Abbas et al., 2014; Grzywacz et al., 2014). Most of these concerns are increasingly becoming pertinent and requiring considerable research into alternative products.

2.2.2 Development of tick resistance

The problem of tick resistance development is a critical issue for the livestock industry because the prospects of having ticks totally resistant to all forms of acaricides will cause an unprecedented threat to livestock productivity. Abbas et al. (2014) reviewed how ticks develop resistance; corroborating earlier work reported by George (1990). It is a well known fact that ticks, like many other organisms, respond to selection pressure by developing resistance after continuous exposure challenges. Therefore, instead of researching methods to kill them, scientists are investing time and reosurces into methods of controlling the ticks (Ntondini et al., 2008; Madzimure et al., 2013; Abbas et al., 2014; McNair, 2015). There is not much that can be done concerning development of natural resistance but more can be done to address resistance that is caused by incorrect and inconsistent use of acaricides. There are several factors that can contribute to this, including adulteration and inability of users to adhere to acaricide manufacturer's instructions because of failure to read, interpret and

implement label instructions (Ssekitto and Mwayi, 2004; Addah et al., 2009; Grzywacz et al., 2014; Sola et al., 2014). This is mainly perhaps because most acaricides come with instructions given in foreign languages and not in the local vernacular African languages.

The language barrier is a big disadvantage because the bulk of cattle and generally livestock producers are smallholder communal farmers whose literacy levels are usually low (Isman, 2008). Geographical location also plays an important role because some farmers cannot access veterinary extension services because of where they live (Perry et al., 2005). It is not uncommon for some farmers in these circumstances to think that they can save their acaricides by using less than recommended concentrations thereby unwittingly contributing to development of acaricide resistance (Ssekitto and Mwayi, 2004; Grzywacz et al., 2014). Unfortunately, the performance of acaricides is generally dependent on quality and quantity administered to the animal and inadequate doses can cause development of resistance. There should be more programmes that educate farmers on how to use acaricides correctly and the looming dangers of having ticks that are resistant to available chemicals.

2.2.3 Disposal of obsolete acaricides and environmental pollution

Despite acaricides playing a very critical role in animal health programmes, they pose dangers to the environment and people alike through water, soil, air pollution and reducing beneficial populations of insect species (Karstensen et al., 2006; Shah and Devkota, 2009; Damalas and Eleftherohorinos, 2011). Regarding human health, it is estimated that at least 1 million people are victims of pesticide poisoning globally, with the bulk of these people in Africa (Isman, 2008; Damalas and Eleftherohorinos, 2011). It is known that many active ingredients are used to make acaricides and over time these formulations begin to degrade and more often than not, they form by-products which can be more toxic than the original pesticide (Shah and Devkota, 2009). Many of the obsolete pesticides are persistent organic pollutants (POPs) which have toxic properties and can be transferred across international boundaries, officially becoming "*everybody's problem"* (Karstensen et al., 2006). In many countries there are stockpiles of these obsolete pesticides which need to be safely disposed of. Another reason why countries end up with chemicals that cannot be used is because some chemicals are prematurely banned because of the negative effects they have on animal and human health while countries still have huge stock piles. A good example was the global ban on the use of DDT in agriculture through the Stockholm Convention on Persistent Organic Pollutants in 2001. Prior to the ban, DDT was a multipurpose pesticide used globally and boosted agricultural productivity but studies linking the chemical to health problems, particularly cancer, prompted its ban (Richardson, 1998). Despite this

development, some developing countries still have stock piles of DDT today because they lack capacity and expertise to dispose of them, thereby predisposing the environment to pollution. Disposal in developing countries often involves repackaging and transportation to Europe for incineration in appropriate facilities at very high costs, estimated at US$3 000 – US$5 000 per ton of waste (Richardson, 1998). It is estimated that the continent requires at least US$100 million to expedite the discarding of these obsolete pesticides. As it is, Africa is a net importer of agricultural pesticides but is the least prepared continent to deal with the growing stockpiles of unwanted pesticides.

2.2.4 High cost and accessibility of acaricides

The pesticide industry is a multi-billion dollar business based on sound scientific research and development programmes that require scientific expertise and adequate finances. These investments should be realized with a profit from sales of the chemical products. This is the reason why these products come at high costs which may be beyond the reach of resource poor farmers. As a mitigation measure, some governments highly subsidize acaricides for communal dipping of animals but, as anticipated, this has not been a sustainable approach (Ntondini et al., 2008; Moyo and Masika, 2009; Madzimure et al., 2011). In most southern African countries, for example South Africa, Namibia, Zambia and Zimbabwe, authorities have already started revising this approach, forcing farmers to bear the full costs of tick control on their animals (Chamboko et al., 1999; Kumba, 2003; Makala et al., 2003; Moyo and Masika, 2009; Gunjal et al., 2009). Apart from the high costs, the products may sometimes not be available in the veterinary shops. This was experienced in Zimbabwe during the serious economic slump experienced between 2004 and 2010 (Gunjal et al., 2009). Even if someone had money to buy products, they were not available on the market and the cattle industry relied on acaricides imported from South Africa, Namibia, Botswana and cheap versions from as far as China.

2.2.5 Inadequate water supply

In the colonial past, African governments made significant investment in the construction of community dipping and spraying infrastructure for the purposes of tick and other ectoparasite control. However, a new challenge of insufficient water has arisen with climate change in some areas. Most farmers are concerned that it is becoming increasingly drier and incidences of drought are on the increase in the tropics and subtropics (Collier et al., 2008; Dantas-Torres, 2015). Communities that have been using communal dip tanks are now faced with another challenge since dip tanks require large volumes of water to dilute the chemical acaricides (Muhammad et al., 2008; Sungirai et al., 2016). Adequate water is important for effective full body immersion and spraying of the animals and also prevention of fracture injuries as the animals plunge into the dip tank.

This is perhaps the reason why most dip tanks were conveniently sited close to reliable perennial water sources. Recent trends however show that even those reliable water sources are now compromised as the water table continues to go down. In a survey reported by Nyahangare et al. (2015), farmers in Zimbabwe stated that water was a limiting factor in normal dipping programs because of its unavailability due to recurrent droughts and prolonged dry spells. As a result, cattle may go for months on end without dipping, predisposing the animals to TTBD (Maroyi, 2012; Nyahangare et al., 2015; Sungirai et al., 2016). Disruption of dipping services always have disastrous consequences as experienced during the liberation war of independence in Zimbabwe, where because of the fighting and sabotage of key infrastructure, cattle were not dipped regularly causing deaths of close to a million head of cattle due to TBD (Pegram et al., 1993). Most farmers now have abandoned community dipping to using smaller knapsack sprayers which use less water and are better suited for fewer animals.

2.3 Alternatives to synthetic chemical tick control method

It is clear that no single method is adequate to deal with the complexities of tick control (Estrada-Peña and Salman, 2013). Despite the use of chemical acaricides being the dominant method available today, there are several other methods that have been hypothesized and used in different parts of the world with different levels of success. A review by several authors show that cattle have been immunized with vaccines to prevent TTBD (Rajput et al., 2006; Nene et al., 2016); pheromones combined with toxicants have also been used (Sonenshine, 2004; Benelli et al., 2016) while other methods based on the disruption of the tick's life cycle have been tried. There is another report on pheromones being used to attract ticks combined with entomopathogenic nematodes which have been used with some success (Nchu et al., 2009). Correct management of pastures and the purposive breeding for animals which are tick and tick borne disease resistant are also very important measures that are being used in non-chemical control of ticks (George et al., 2004; Ghosh et al., 2007; Shyma et al., 2013; Van Zyl et al 2017). It is widely accepted that indigenous Zebu breeds are more resistant to ticks than exotic breeds and therefore crossing extremes of breeds may produce animals fairly resistant to TTBD without overly compromising productivity of the animals. Over the years, plunge dipping programmes have also been replaced by newer methods for application of acaricides that involve the use of ear tags, neck bands and pour-ons (Makala et al., 2003; Willadsen, 2006; Moyo and Masika, 2009). The different methods used therefore support the conclusion that it is not one but an integration of a number of methods that seems to be the future for effective tick control programmes (Magadum et al., 2009).

2.4 Relevance of potential new technologies to smallholder communal farmers

The use of newer technologies is not without its challenges to particular production systems. Synthetic acaricides and most of the technologies used in the control of ticks are biased towards tick control programmes for exotic breeds of cattle in commercial cattle farming enterprises (Chamboko et al., 1999; Perry et al., 2005). Under these systems in Africa and other developing countries, farmers raise exotic specialized breeds (*Bos taurus*) which have low resistance to TTBD. These farms are normally well serviced with electricity, good roads and have access to modern veterinary services. Under these circumstances these modern technologies give optimum productivity results, but the same cannot be said for poorly resourced smallholder farmers who produce the bulk of cattle on the market in developing countries and whose farms are located in marginal areas which may be geographically and logistically less accessible (Mapiye et al., 2009). The road networks are often bad and the areas may not have access to amenities like electricity. These places may also have very limited access to conventional veterinary services (Hlatshwayo and Mbati, 2005; Moyo and Masika, 2009; Gumbochuma et al., 2013). This shows that while the acaricidal products on the market are good, they may not be the best for the main beneficiaries of improved cattle rearing technologies in some developing countries. There is therefore a real need to investigate alternative cost effective measures that can be used to help the smallholder farmers control external parasites of cattle. This explains the high activity towards research and development of ethnoveterinary practices and the use of plants for the control of external parasites (Adenubi et al., 2016; Adenubi et al., 2018).

2.5 Global use of acaricidal / pesticidal plants

From time immemorial, plants have been used to control agricultural pests and parasites but this practice had been overtaken by use of well formulated synthetic acaricides and pesticides (Isman, 2006; Dubey et al., 2010). Terrestrial plants are a great reservoir of secondary chemicals developed over time as a response by plants to prevent herbivory and pathogenic attack (McGaw and Eloff, 2008; Sultana et al., 2009; Dubey et al., 2010; Baetz and Martinoia, 2014; Grzywacz et al., 2014). It is estimated that there are over 100 000 secondary plant metabolites of which hundreds have tested positively to bioactivity against internal and external parasites (Isman, 2006; Koul, 2008; Adenubi et al., 2018). There are renewed efforts to develop the botanical pesticide industry as a primary health care alternative for animals because of the challenges associated with using commercial synthetic acaricides (Bissinger and Roe, 2010; Abbas et al., 2014; Ghosh et al, 2015). Already, the demonstration of efficacy and other bioactivity characteristics of plant chemicals is a growing industry that has attracted many professional scientists including botanists, biochemists, toxicologists and social scientists (Isman and Grieneisen, 2014). In the period between 1980 and 2011, a total of 4 997 and

2 180 journal paper articles on neem and essential oils of different plants have been published the world over showing the growing interest in botanical pesticides (Isman, 2006; Miresmailli and Isman, 2014).

Most of the issues around the research and development of acaricidal plant products have already been extensively reviewed by many authors (Miresmailli and Isman, 2014; Mkindi et al., 2015; Benelli et al., 2016). Recently, a global review of published articles on plants used for tick control showed more than 200 species have acaricidal properties (Adenubi et al., 2016). Other reviews have also showed that researchers in different laboratories use different *in vitro* bioassay techniques, extractants and tick species in efficacy testing (Adenubi, et al., 2018a; Adenubi, et al., 2018b). The absence of standardised procedures however does not overshadow the important critical data that is being generated on the plants that have acaricidal activity. However, using standard procedures is essential to be able to compare activity of different extracts.

2.6 Botanical pesticides developed for the agricultural industry

Despite the high number of plant secondary metabolites identified, there has not been a corresponding high number of developed commercial botanical acaricides on the official market (Koul et al., 2008; Pavela, 2016). The earliest known botanical pesticide products include pyrethrum, rotenone and nicotiana while neem and essential oils from different plant species form the bulk of botanical insecticides widely used today (Isman, 2006). Pyrethrum (from *Tanacetum cinerariaefolium* reclassified from *Chrysanthenum)* in Kenya, Tanzania and Australia (Tasmania) is a contact acaricide whose effect is through pyrethrum esters found in them. Rotenone is also a natural chemical mainly found from the subtropical and tropical pea plants e.g. *Derris elliptica* and *Lanchocarpus* sp. (Ott, 2008). It has also been isolated from *Tephrosia vogelii* in Malawi (Belmain et al., 2012). Nicotiana mainly comes from the tobacco crop *Nicotiana tabacum* and other *Anabis* species. The use of nicotiana and rotenone has come under strong negative criticism as they are highly toxic and therefore no longer used in industrialised countries (Ott, 2006; Isman, 2006). Later work has shown that in fact rotenone is not as highly toxic to humans and birds as earlier suspected, but fish are highly susceptible to it. It is the possible link to Parkinson's disease in humans popularized by the press that led to its acceptability challenges on the market and the subsequent ban (Belmain et al., 2012).

Products from *Azadirachta indica* (neem) are probably the most well-known biological insecticides today. Neem is found mainly in India, Australia, Central America and East and West Africa. It has anti-feedant properties and also acts as a growth regulator, mainly due to the presence of limonoids and azadirachtin (Webb and David, 2002; Benavides et al., 2001). Essential oils are also significantly contributing to botanical pesticides and are found worldwide in the flavouring and fragrance industries (Isman, 2008). Most essential

oils have contact toxicity, fumigant, deterrent and repellent activities and are most effective against sucking insects (Bissinger and Roe, 2010).

Besides neem and essential oils from plant species, there are also other plant species which have been tested and shown to have acaricidal activity, e.g. *Stylosanthes scarbra* (Khudrathulla and Jagannath, 2000); *Solanum dasypyllum* (Kaposhi, 1992), *Gynandropsis gynandra* (Malonza et al., 1992) and *Melinis minutiflora* (de Barros and Evans 1989). *Melinis minutiflora, Stylosanthese* spp. and *Cassia absus* have also been used as repellants in the control of ticks (Ghosh et al., 2007). The challenge however, is still that there have not been many products commercially produced for the market and yet the potential is there. There are also many more plant species traditionally used for tick control which have not yet been identified and tested. This justifies the need to continue engaging knowledgeable people in communities so that this important information is not lost.

2.7 Research on anti-tick plants in southern Africa

It is evident that research on botanicals differs across the world. If publications are used as a measure of progress in acaricidal research, then India, China and Brazil are the biggest players accounting for 40.9% of the 1207 botanical insecticide articles published in 2012. In Africa, Egypt and Nigeria seem to be the leading countries (Isman and Grieneisen, 2014; Miresmailli and Isman, 2014). However, it cannot be said with certainty that areas with few publications don't use acaricidal plants and traditional methods for pests and parasite control, but it could be an issue of poor documentation and availability of resources for formal scientific research studies. It is estimated by the World Health Organization (WHO) that in the order of 80% of the people in developing countries rely on traditional medicines, mainly plants, for their health care (Bannerman, 1983; Bodeker et al., 2005).

Generally, scientific research and development of anti-tick and pesticidal plant products in sub-Saharan Africa is still limited in scope and scale (Sola et al., 2014). In the southern African region, South Africa has done much more compared to other countries with regard to the botanical industry development (Gurib-Fakim et al., 2010). Formal commercialisation of botanical products in South Africa started in the 1990s and to date several products ranging from teas, medicines and food products have been commercialised through companies like PhytoNova, Thebe Medicare and Afriplex (Van Wyk, 2011). This is perhaps because South Africa has one of the biggest economies across the African continent with a stable economy and excellent research infrastructure and expertise which are prerequisites for this type of work (Van Wyk, 2011; Sarkar and Kshirsagar, 2014). Moreover, many technical people who should otherwise be leading researchers in their own countries in the region prefer working in this country because of better remuneration and research

opportunities resulting in brain drain in their native countries. Besides product development, South Africa has several documented ethnobotanical surveys (McGaw and Eloff, 2008; Moyo and Masika, 2009; Van Wyk, 2011; Luseba and Tshisikhawe, 2013; Muyobela et al., 2016), *in vitro* efficacy evaluation reports (Mkolo and Magano 2007; Muyobela et al., 2012; Adamu et al., 2013; Fouche et al., 2017; Wellington et al., 2017; Adenubi et al., 2018) and *in vivo* generated data (Ntondini et al., 2008; Moyo et al., 2009; Luseba et al., 2016). There are also other aspects of acaricidal plant research such as safety and phytochemistry analysis that are in the public domain.

In the other countries in the region, commercialisation and research on botanical pesticides is very marginal and often uncoordinated. There are few isolated efforts to identify potentially useful plants from ethnobotanical surveys and other indigenous knowledge sources to produce marketable commercial products. It must be noted however that there are potentially many more plant uses to be "discovered" and screened for efficacy because of the richness of Africa's biodiversity and the risks of bio-piracy (McGaw and Eloff, 2008; Dubey et al., 2010).

The work on ethnobotanicals used for tick control in Zimbabwe is still limited to a few surveys (Maroyi, 2012; Gumbochuma et al., 2013; Ndhlovu and Masika, 2012; Matekaire and Bwakura, 2014; Nyahangare et al., 2015). Some of the identified plants have been screened for efficacy *in vitro* with good results e.g. *L. javanica* (Madzimure et al., 2011); *S. incanum* and *S. spinosa* (Madzimure et al., 2013) and *M. edulis* (Nyahangare et al., 2016). Efforts to get the activity replicated *in vivo* have not been many but *T. vogelii* and *M. edulis* have been tested and have shown positive activity (Gadzirayi et al., 2009; Mgocheki, 2017; Nyahangare et al., 2017).

The situation in Zambia is also not very different with a few isolated reported cases of acaricidal plant research. The most consistent plant to have received attention in laboratory experimentation is *T. vogelii* (Kaposhi et al., 1992; Siame et al., 2019). The activity of *Bobgunnia madagascariensis* has also been tested against ticks (Muyobela et al., 2016). There are other reports of plants used in the management of other agricultural pests. It is fair then to say that this industry is still very much in its infancy judging by the amount of published material available. Similar trends are observed in other countries like Namibia and Botswana where again there are few surveys and screening for efficacy experiments of reported acaricidal plants. Botswana has recorded efficacy of *Azadirachta indica* (Neem) (Webb and David, 2002). Other works recorded focus on ectoparasites in poultry and goats but not necessasrily ticks (Moreki, 2013; Setlalekgomo and Setlalekgomo, 2013). Documentation of ethnoveterinary practices in Namibia was recorded by Chinsembu et al (2014). Earlier, *Margaritaria discoidea* had been found to have acaricidal effect on ticks *in vitro* (Kaaya et al 1995).

Other plant species that have been tested successfully include *Gynandropis gynandra* and *Ocimum suave* (Kaaya, 2003) and *Azadirachta indica* (Kaaya et al., 2007).

2.8 Bio-prospecting challenges of anti-tick plants in southern Africa

It is not surprising that identifification of anti-tick plants dominates many publications in this part of the world because this is arguably the "*easiest*" component of acaricidal plant products research (Adenubi et al., 2016). Several authors have given accounts from surveys that show different plant materials with potential anti-tick properties (Kaposhi, 1992; Chamboko et al., 1999; Hlatshwayo and Mbati, 2005; McGaw and Eloff, 2008; Moyo and Masika, 2009; Matlebyane et al., 2010; Chinsembu et al., 2014; Nyahangare et al., 2015). This is not to say that there are no challenges but it appears that Africa does have enough human capital to deal with these types of research. Bio-prospecting on the other hand offers bigger challenges in achieving sufficient quantitative and qualitative data required for acaricidal product formation. Issues like toxicity, efficacy, and characterization of active components require adequate financial investment, enabling infrastructure and laboratory equipment supported by scientific expertise capital to be adequately addressed (Sola et al., 2014). Unfortunately, many countries in the region fall short of these requirements. There is therefore still great potential in southern Africa to grow and benefit from the botanical acaricide industry. It is in the best interests of the region that this happens because analysis by Isman (2008) showed that the biggest beneficiaries of pesticidal/acaricidal plant research are the poor who need low cost pest/parasite control products to improve their livelihoods.

2.9 Challenges to development of anti-tick plants

While pesticidal plants are proving to be useful in controlling agricultural pests and there is scope to increase their use, this field is not without its own challenges. There are some grey areas which include limited finances, inadequate human capital, inadequate infrastructure and lack of an enabling policy which limit their full exploitation (Sola et al., 2014).

2.9.1 Research finances

Production of plant based acaricides requires a good financial support base. Bio-prospecting experiments are generally very expensive and it is estimated that producing a novel plant product requires at least US$100 million and in a period no less than 10 years (Graf et al., 2004; Njoroge and Bussmann, 2006). Most of the recorded studies available therefore are not conclusive and limited to efficacy of extracts *in vitro,* but still there is a need to identify the actual active ingredients, understand their modes of action, determine the residual period and the effective application interval of the extracts (Miresmailli and Isman 2014; Stevenson et al.,

2018). At the end of it, the plant extracts must demonstrate efficacy under field conditions too because *in vitro* data by itself is not sufficient. All this requires strong research and development budgets which unfortunately may not be available in most developing countries and research institutions.

2.9.2 Research expertise

Research finances must always be complemented by expertise to design and execute experiments along the product development value chain. In a review by Isman and Grieneisen (2014), it was reported that there were too many inconsistencies in bio-prospecting procedures leading to accumulation of a lot of manuscripts but unfortunately with practically useless data. The same review also noted that most papers published did not have chemical composition data, thus further limiting the usefulness of these manuscripts. Botanical pesticides development, just like synthetic drugs value chains, require biochemists, toxicologists, chemists, botanists, pharmacologists, economists, lawyers and many other relevant experts. Unfortunately, these scarce skills follow where the money is, posing serious challenges in economies with financial problems in some parts of the world. Any person, organisation or institute seriously thinking about producing a botanical product will have to think of how the experts can be recruited for the success of the bio-prospecting and product development. The problem of brain drain, particularly in Africa, does not make the situation any easier for the continent notwithstanding the potential that these technologies have in revolutionising communal and small holder agriculture (Maisonneuve et al., 2001).

2.9.3 Infrastructure

The work of bio-prospecting requires fully furnished laboratories. The reason why some countries in developing nations have lagged behind is because, even if they wanted to test some material, they don't have proper laboratories. As a result, there is always a risk of bio-piracy as researchers expose themselves by seeking collaboration with scientists from other countries. Many laboratories in Africa are in a sorry state of disrepair and some do not have even basic equipment for phytochemical analysis even in institutes of higher learning like universities. It would have been good for the private sector to invest into this but it seems that they are not sure of the return on investment on bio-pesticidal products (Sola et al., 2014).

2.9.4 Policy and Regulatory challenges

Existing policy and resultant regulatory frameworks on the use of bio-pesticides have been formulated to guide synthetic pesticide use the world over for many years (Isman, 1997). As a result, because most bio-pesticides do not comply with these regulations, they are therefore illegally produced, informally traded and used and as

such do not influence policy much (Sola et al., 2014). It is perhaps time policy makers deliberately create conducive policies and legislature for the growth of the botanical acaricide industry. There is no doubt that the botanical pesticide industry has a very important role to play in agricultural production because there is overwhelming scientific evidence that some are efficacious, safe, environmentally benign and relatively cheap compared to synthetic products on the market. There are a few countries in the world that exempt botanical products from being regulated like the United States of America but the majority of countries use the same legislation for synthetics to apply to botanical pesticides (Koul et al., 2008). This gives an unfair advantage to synthetics and downplays the many advantages that botanical pesticides may have over synthetic products. As a way forward, Sola et al. (2014) put the challenge to private and public research institutions to invest in more comprehensive research that should nudge policy makers to believe that botanicals are potentially safe and effective products that can be used in the market. Funding for research in these areas is limited because investors are sceptical about the returns that can come out of this industry (Isman, 1997; Sola et al., 2014).

2.9.5 Intellectual property rights

Patent rights have always been a thorny issue associated with plant based product development, largely because a lot of information about use of these plants is based on indigenous knowledge platforms with knowledge emanating largely from the farmers and rural communities (Isman, 2005; Wanzala et al., 2005; Nyahangare et al., 2015). However, these communities have no capacity to develop and produce a competitive product for the formal markets which is where scientists and the private sector play an important role. Now in the event of developing a promising product, it becomes very difficult to share potential profits and decide who should get patent rights to the product between the farmers/communities and the scientists and the private sector. The latter are the people with the funds and expertise to produce the final product but the truth of the matter is that the original information belongs to the former. If the knowledge is not applied and developed at a huge cost it does not have much value. There is also a substantial risk in these investments because in many cases application of traditional knowledge cannot be confirmed under controlled conditions. It is therefore a contentious issue with no standard template to follow, and no wonder that the private sector has been hesitant to invest in these ventures because of these uncertainties (Isman and Machial 2006). Patents provide market exclusivity which increases the chances of making more profits (Isman, 1997; Sola et al., 2014). There is also growing mistrust between researchers and farmers, mostly from the farmers' side, because they think when researchers conduct ethno-surveys it is to milk them of information which they use to make themselves rich while the farmers get nothing but more expensive acaricides on the market (Nyahangare et al., 2015). This is despite the fact that the application of very few plant materials actually

passes the rigorous quality assurance aspects that is necessary for the development of a new product and that a lot of money and time is needed to produce a single product from plant materials (Graf et al., 2004).

2.10 Summary

This review has shown a steadily growing botanical acaricide industry globally is backed by a genuine need for alternative and complementary tick controlling remedies necessitated by the numerous cropping challenges emanating from the use of synthetic acaricides. However, the development of these acaricidal products from plants also has challenges which include finances, standardization of protocols, policy and regulatory issues that hamper commercialisation of the products. There are also performance variations in both *in vitro* and *in vivo* studies for the same plant species. Because of these bottlenecks, there is apparently a lot of information on acaricidal plants but very few products actually available on the market. The region is also lagging behind in botanical acaricides research mainly because of issues regarding research finance and expertise. South Africa is probably the leading light in this area of research and one hopes that other countries will benefit eventually from the strides that they have made. In the other southern African countries, the scope for identifying potentially useful plants and screening them through efficacy and toxicity experiments and potentially making commercial products is still promising and should be explored.

CHAPTER 3

3 Ethnoveterinary plants used to control ticks in Zimbabwe

Preface

Many rural people are knowledgeable about traditional plants used to control ticks based on their real life experiences. In this chapter, the first objective of the study is addressed, namely to identify from knowledgeable farmers plants with known application to control ticks. While the objective focuses on southern Africa, the survey was done using Zimbabwe as a case study. Because of similarities in geography and culture most of the plants in Zimbabwe are also found across the other southern African countries. The result of this study has been published: *Nyahangare, E. T., B. M. Mvumi, and T. Mutibvu. 2015. Ethnoveterinary plants and practices used for ecto-parasite control in semi-arid smallholder farming areas of Zimbabwe. Journal of Ethnobiology and Ethnomedicine. 11:30* and is copied below.

Abstract

The inclusion of traditional plant-based ecto-parasite control methods in primary health care of livestock is increasingly becoming an important intervention for improving livestock productivity in resource challenged smallholder farming areas. In this study, commonly used plants used for the control of cattle ticks and other pests were identified through a survey in four semi–arid districts of Zimbabwe. A standard structured questionnaire with details of demographics, socio-economic status of households, livestock parasites, control practices and list of ethnoveterinary plants used was used to interview 233 knowledgeable smallholder farmers in four districts. Focus group discussions with community members further provided insights on how the plants were being used and other issues surrounding ecto-parasite control and indigenous knowledge systems in the study areas. The older generation (>40 years) of the respondents were knowledgeable about ethnoveterinary plants and practices. Overall, 51 plant species were reportedly effective against cattle ticks and other livestock parasites. The most frequently mentioned plants were in descending order, *Cissus quadrangularis* (30.1%), *Lippia javanica* (19.6%), *Psydrax livida* (14.9%) and *Aloe* sp. (14.9%). Most of the plant materials were prepared by crushing and soaking in water and spraying the extract on animals. Despite the knowledge of these useful pesticidal plants, the preferred animal health care for cattle and other highly ranked livestock species is still the use of commercial acaricides. Cattle dipping services were reported sporadic by 48% of the

respondents. Traditional knowledge and plants are considered only as an alternative in the absence of conventional synthetic products. It can be concluded that livestock farming communities know of plant species used for livestock ecto-parasite control. The plant species are mostly used to complement commercial products. More work is required to confirm the acaricidal properties claimed by the farmers in order to optimize and promote sustainable use of these plants.

Keywords: Acaricidal plants, Cattle ticks, Indigenous knowledge, Smallholder farmers

3.1 Introduction

The general appreciation of the potential of ethnoveterinary plants in ecto-parasite control has not been comprehensively supported by formal documentation and scientific research in most developing countries (Wanzala et al., 2005; Sindhu et al., 2010; Marandure, 2016). This is notwithstanding the fact that since time immemorial different communities have used plants in agricultural parasite management. While efforts are underway to collate information from knowledgeable stakeholders, it is generally accepted that a lot more ethno botanical surveys covering a wider range of communities collecting available information on plants that are useful for the control of livestock ecto-parasites still need to be done. Sub-Saharan Africa has a rich plant and cultural biodiversity which are key pillars of ethnoveterinary practices and there are worries that if this knowledge is not captured and documented future generations will be clueless on traditional pest control measures (McGaw and Eloff, 2008; Gradé et al., 2009; Van Wyk, 2011; Robbertse et al., 2016). This is largely because normally, traditional knowledge is passed orally from generation to generation but in the process some vital information can be lost (Matekaire and Bwakura 2004; Masimba et al., 2011; Abbasi et al., 2013). It is also a fact that with globalisation and modernisation, traditional practises and knowledge have been shunned for being backward, baseless and therefore useless (Wanzala et al., 2005; Isman, 2006; Maroyi, 2012). In a nut shell, while the prevailing operating environment does not encourage preservation and documentation of traditional practices, there is recognition that such practices have an important role to play in the future of agricultural pests, parasites and diseases control (Wanzala et al., 2005; Koul et al., 2008; Isman et al., 2014). Ethnobotanical surveys are therefore excellent tools of documenting existing knowledge on useful plants providing a good base for the development of the botanical pesticide industry. The potentially effective plants in different parts of the world need to be identified and documented. In this study, acaricidal plants particularly from areas with significant cattle production in Zimbabwe were sought from knowledgeable people. Information was also gathered on how the plants

were harvested and prepared for administration and whether the plants were easily available. While the survey was conducted in Zimbabwe, most of the plants are found in the whole southern African region owing to the close and shared cultural practices and plant biodiversity in the region. The knowledge generated from this survey will help further research and development on alternative pest and parasite control options most likely to benefit smallholder farmers in developing countries (Isman, 2008).

3.2 Materials and methods

3.2.1 Study sites

The surveys to document ethnoveterinary practices used to control ticks and other parasites in livestock were conducted in four semi-arid to arid districts of Zimbabwe covering natural agro ecological regions 4 and 5. The basis for selection of these two regions was that livestock production is the key to livelihoods of farmers in these zones because of the sporadic and unreliable rainfall which make crop production and other water dependent enterprises a huge challenge. Any intervention that helps improve livestock productivity will also most likely directly improve livelihoods of the communities. In ecological region 4, Matobo and Kadoma districts were randomly selected while Chiredzi and Muzarabani districts were randomly selected from natural ecological region 5 (Fig. 3.1). In each district, four wards where ethnoveterinary practices are prevalent were purposely selected for administration of the questionnaire. District Agricultural, Technical and Extension Services (AGRITEX) officials played a key role in identifying the areas best suited for the surveys. This approach was used to increase the likelihood of getting information since traditional practices are very area specific. With regard to human development, the districts in natural region 4 (Kadoma and Matobo) are regarded number 43 and 36 poorest districts in Zimbabwe respectively while Chiredzi and Muzarabani occupy positions 52 and 61 out of a total of 77 districts (UNDP, 1998). A short description of the study sites is as shown in Table 3.1.

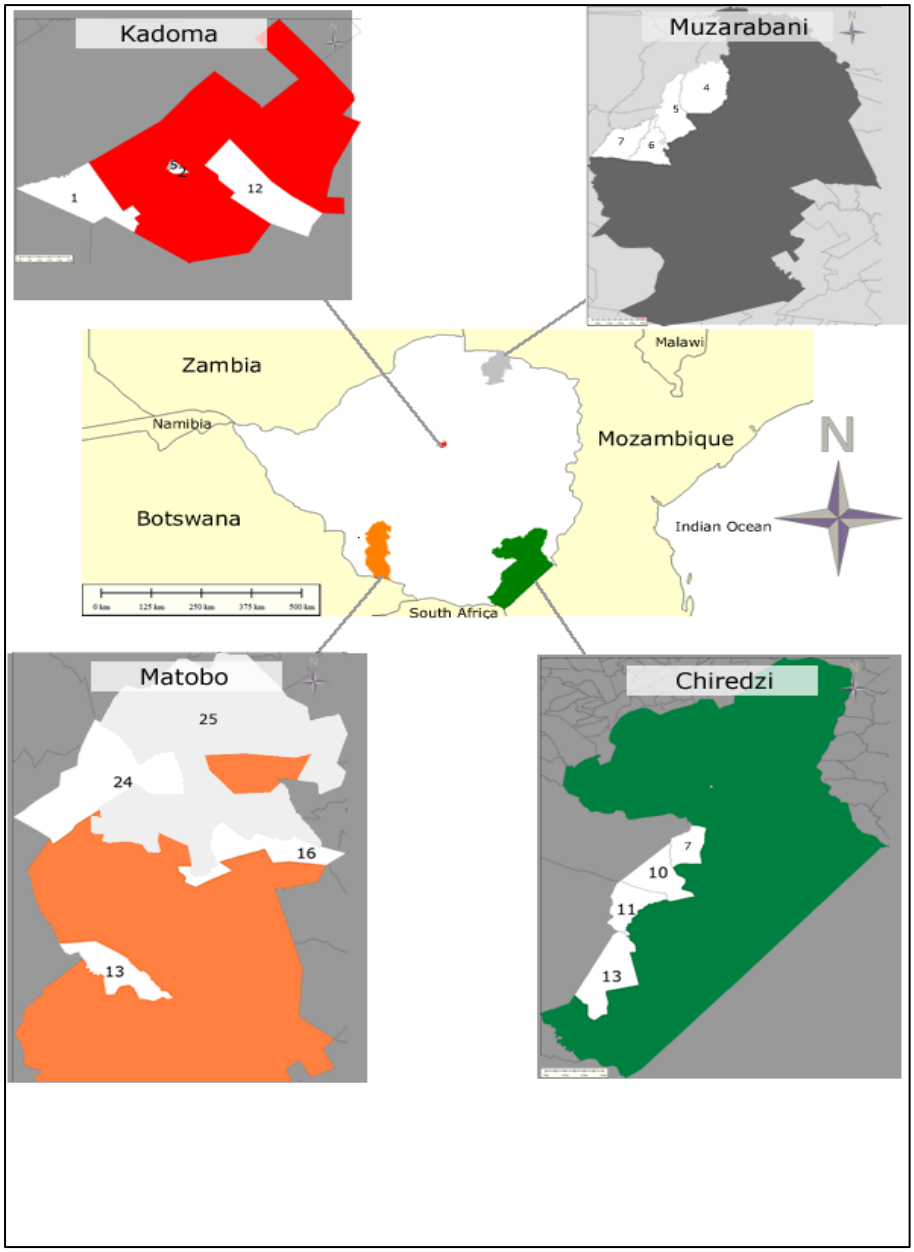

Figure 3.1: Map of Zimbabwe showing the study sites

3.2.2 Ethnobotanical data collection

A total of 233 households (60 from Muzarabani, Sanyati and Matobo districts and 53 from Chiredzi district) were purposively selected depending on whether they kept livestock and if they had knowledge about ethnoveterinary practices. They were interviewed using a pre-tested standard structured questionnaire (Martin, 1995). The purposive sampling technique was chosen in order to increase the likelihood of obtaining detailed and useful information from respondents. The survey sought information on ethno tick and other livestock pest control practices and issues surrounding their use. Local

agricultural and veterinary extension staff assisted in the identification and selection of respondents, and subsequent administration of the questionnaires. Apart from the questionnaires, further information on pesticidal plants was collected through focus group discussions with knowledgeable persons from the respective districts. The knowledgeable persons were pre identified by AGRITEX officials. Information regarding the plant vernacular names, part(s) used, methods of preparation and mode of application was documented during the interviews. Each plant was correctly identified by a qualified botanist and voucher specimens deposited at the National Herbarium and Botanical Gardens in Harare, Zimbabwe.

Table 3.1: Some characteristics and description of the survey study sites

District	Ward	Province	Agro-ecological Zone	Characteristics
Kadoma	1 2 5 12	Mashonaland West	III - IV	Found on approximately 18°19'S longitude and latitude 29°53' E, moderate to low average rainfall 450 - 600mm, semi-extensive farming of livestock and drought resistant fodder crops (Pedersen and Madsen, 1998)
Matobo	13 16 24 25	Matabeleland South	IV	District is found at 20°23`È latitude and 28°30`S longitude. Average rainfall is 450 - 650 mm, area is good for semi-extensive livestock and game ranching, common crops include drought resistant maize, sorghum and millet (Mugandani et al., 2012)
Chiredzi	7 10 11	Masvingo	V	Area is found at about 31°30'S longitude, 21°10'E latitude. It is one of the largest districts in Zimbabwe with an average uncertain annual rainfall < 450 mm; suitable for extensive cattle production and game ranching (Mugandani et al., 2012)

3.2.3 Data analyses

Data collected were analyzed using SPSS version 21 (IBM Statistics, 2012). Frequencies, means and tables were generated for variables such as livestock species kept, major parasites affecting the livestock and the methods used for controlling them. Identified plants were ranked according to frequency of mention in all the districts.

3.3 Results

3.3.1 Household demographics

A total of 233 household heads responded to the questionnaire in the four districts of which 78.5% were males and 21.5% were females. Their average age was about 51 years and the household size averaged 7 people (Table 3.2). The age distribution of respondents showed that they were mostly of the older generation with the bulk of them past 40 years of age (Fig 3.2). About 6% were found in the youngest age group (21 – 30) and only 2% were in the oldest group (81 – 90).

Table 3.2: Summary of household demographics (N = 233)

Household details	Mean	Std. Deviation
Age of household head (years)	51.3	15.23
Household size	7.2	4.46
Number of adult males (> 18years)	2.0	1.52
Number of adult females (> 18years)	2.0	1.57
Number of children (0 - 18 years)	3.4	2.62

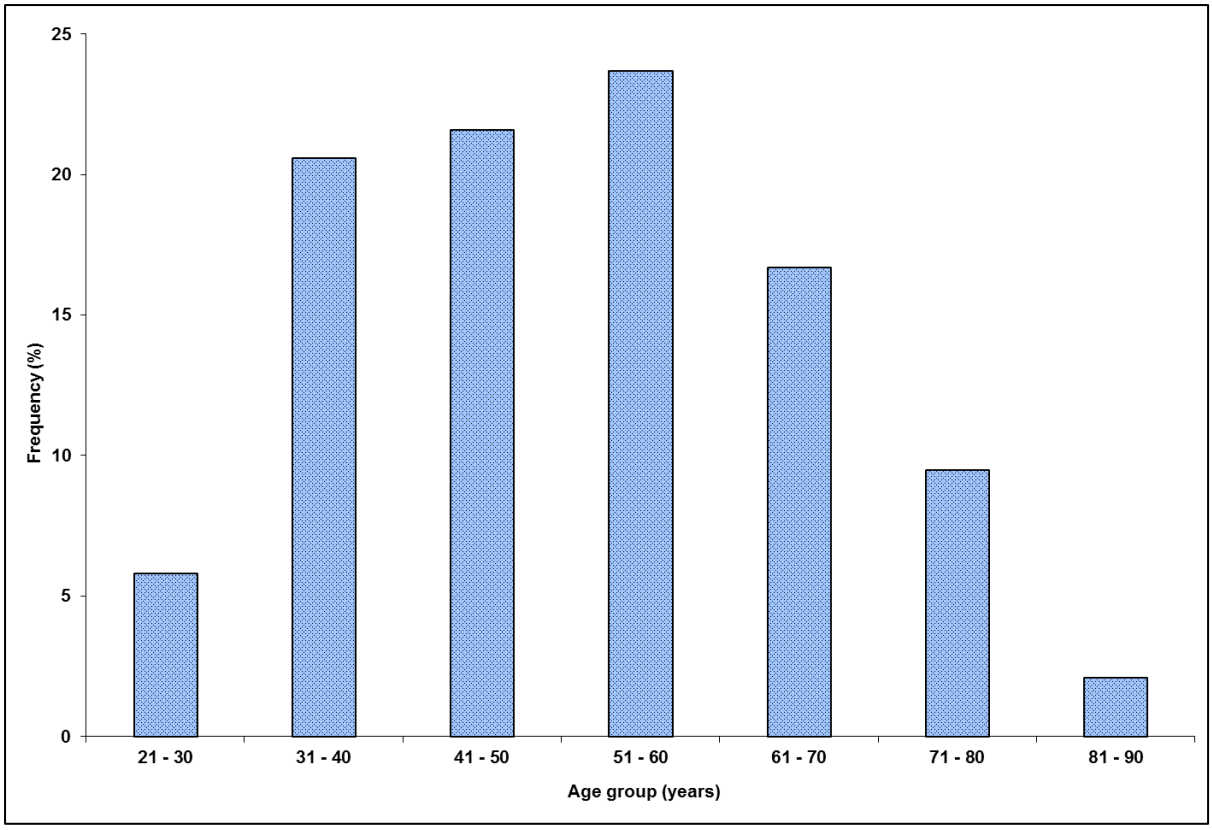

Figure 3.2: Age distribution of respondents across survey area

3.3.2 Livestock species kept by the farmers

Most households owned different types of livestock which consisted mainly of poultry, cattle, goats, sheep, donkeys and pigs. Indigenous chickens were the most populous species kept per household with a mean estimate of 17 (Fig 3.3). The highest population of cattle was found in Matobo district while goats were most populous in Chiredzi. There were more indigenous chickens kept in Kadoma than any other district. Across the districts, the mean number of sheep was very low. A detailed picture of the distribution of the livestock species by district is as shown in Fig 3.4.

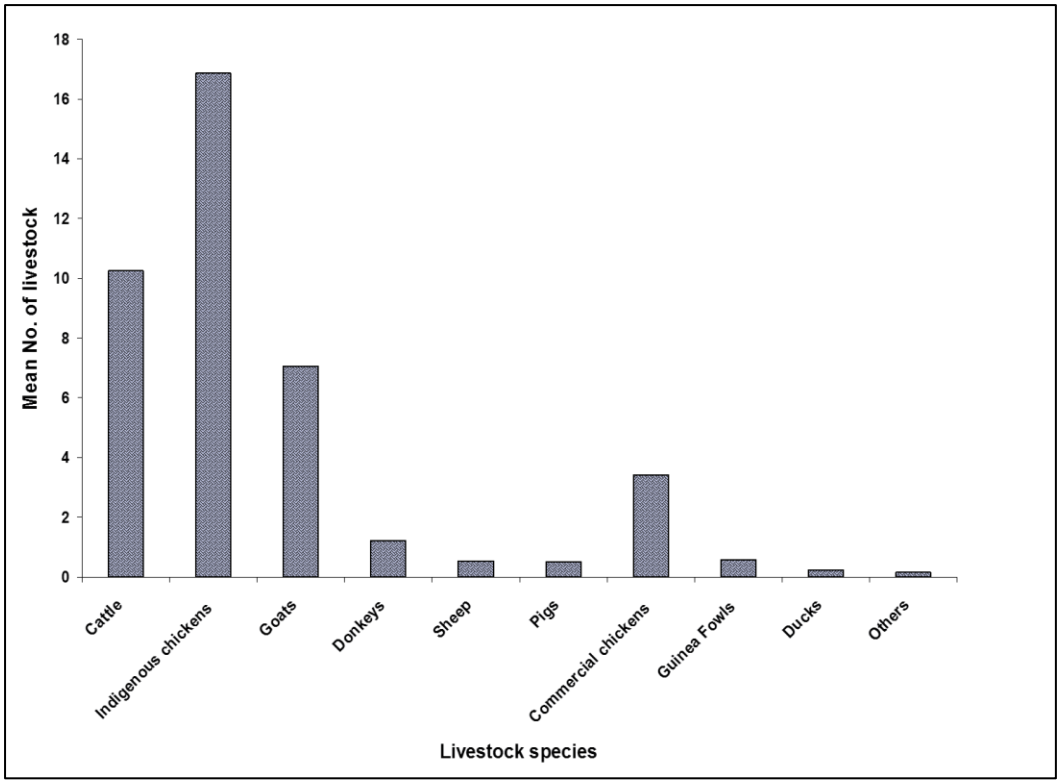

Figure 3.3: Average livestock numbers kept per household across the survey districts (N =233)

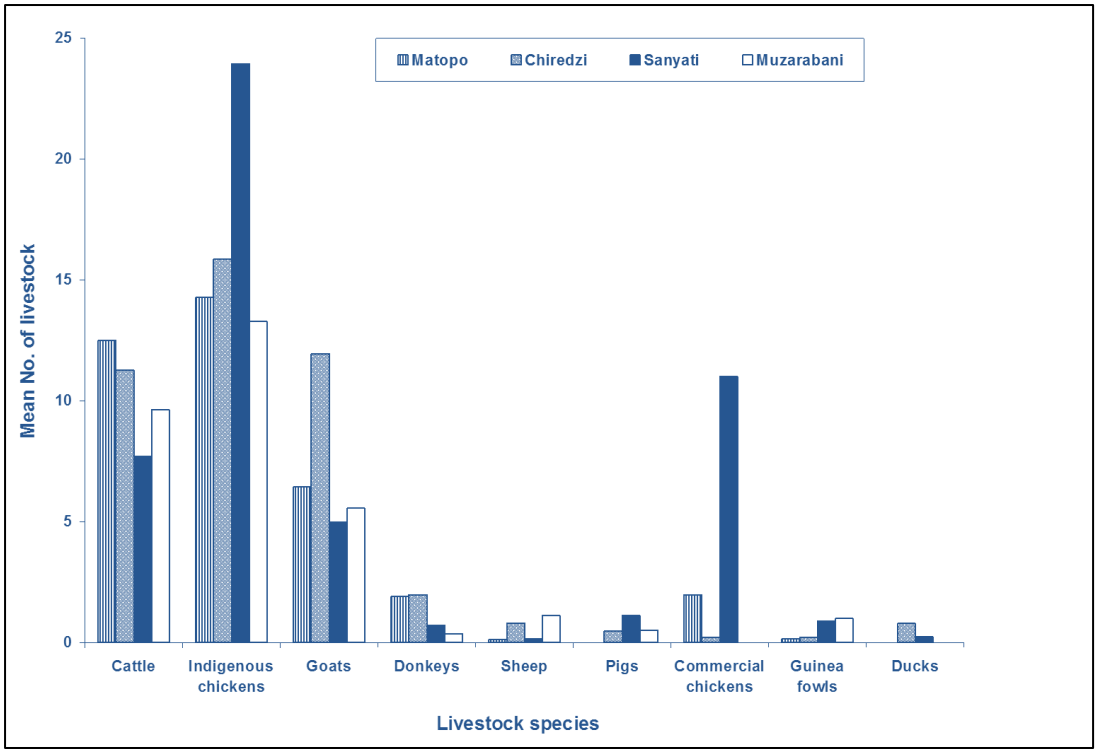

Figure 3.4: Mean number of livestock species by district (N= 233)

3.3.3 Ranking of animal species

Despite chickens being the most populous species kept by respondents, cattle were ranked highest of all the species in terms of importance followed by indigenous chickens and then goats (Table 3.3). Other species kept by respondents in the survey areas and ranked lower include commercial chicken breeds, pigs, donkeys and various other less popular poultry species like guinea fowl and ducks.

Table 3.3 : Ranking of livestock species according to importance by respondents

Species	Rank	Frequency (%)
Cattle	1	77.7
Indigenous chickens	2	76.9
Goats	3	56.6
Donkeys	4	28.4
Sheep	5	13.8
Pigs	6	10.7
*Other species	7	7.2
Commercial chickens	8	5.7

*Refers to livestock species that were available in very small numbers which include; turkeys, ducks, rabbits, pigeons and guinea fowls

3.3.4 Prevalent livestock parasites and their management

Ticks were the most commonly identified parasites affecting mostly cattle, goats, donkeys and sheep (Table 3.4). Other parasites also reported include common flies and tsetse flies. Mites, fleas and lice were the most prevalent parasites to poultry species with the most common parasites for indigenous chickens being fleas and lice at 27.5% and 22.7% respectively (Table 3.4). Generally, parasites were controlled mostly by synthetic acaricides only for the highly ranked animal species (cattle, indigenous chickens and goats) with the lowly ranked animals receiving little or no remedial action at all (Fig 3.5). The use of traditional practices alone was prevalent mainly in poultry production compared to other systems. A mixture of traditional practices and commercial products was prevalent mostly in cattle, indigenous chickens and goats. It was however minimal in pigs, sheep and donkeys (Fig 3.5).

Table 3.4: The most prevalently mentioned livestock parasites by species in the 4 districts (N=233)

Livestock Species	Common parasites as identified by respondents (%)					
	Ticks	Flies	Tsetse flies	Mange mites	Fleas	Lice
Cattle	81.0	0.9	0.4	0.4	-	-
Sheep	5.6	0.4	-	-	-	-
Goats	49.8	-	0.4	3.9	0.4	0.4
Indigenous chickens	-	1.3	-	3.4	27.5	22.7
Commercial chickens	-	-	-	-	0.4	0.9
Pigs	-	-	-	2.2	-	1.3
Donkeys	13.3	0.4	-	-	0.4	0.4
*Other species	-	0.9	-	-	0.9	2.1

*Refers to livestock species that were available in very small numbers. These include; turkeys, rabbits and guinea fowls
"-"means zero respondents mentioned the parasites in the particular animal species

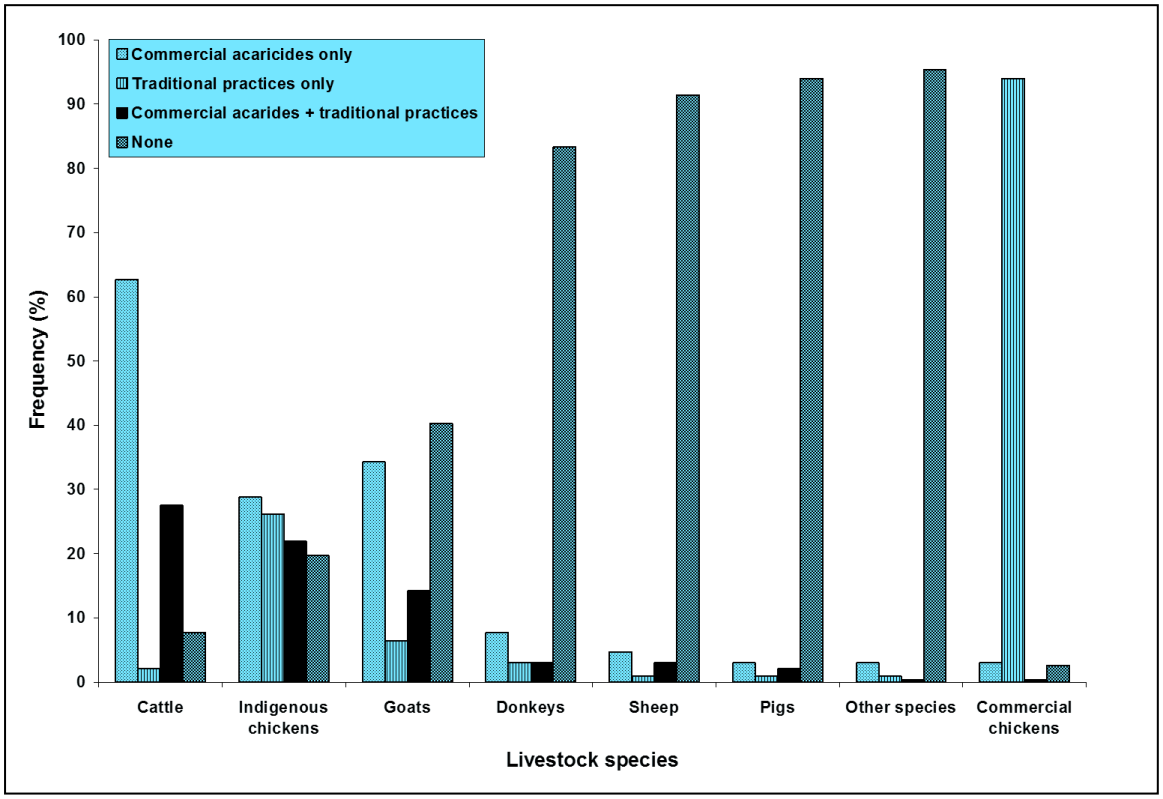

Figure 3.5: Livestock ectoparasite management by livestock species (N=233)

3.3.5 Status of cattle dipping in the survey areas

A significant percentage of the respondents highlighted that dipping services were sporadic (48%) and in some areas not available at all (12%). However, 40% of the respondents had no challenges with availability of dipping services. Chief amongst the reasons cited for inconsistent dipping services was the lack of veterinary services through Government support and high cost of acaricides (Fig 3.6). Other reasons included long distances to dip tanks, political instability, dysfunctional dipping infrastructure (Fig 3.7) and unavailability of water.

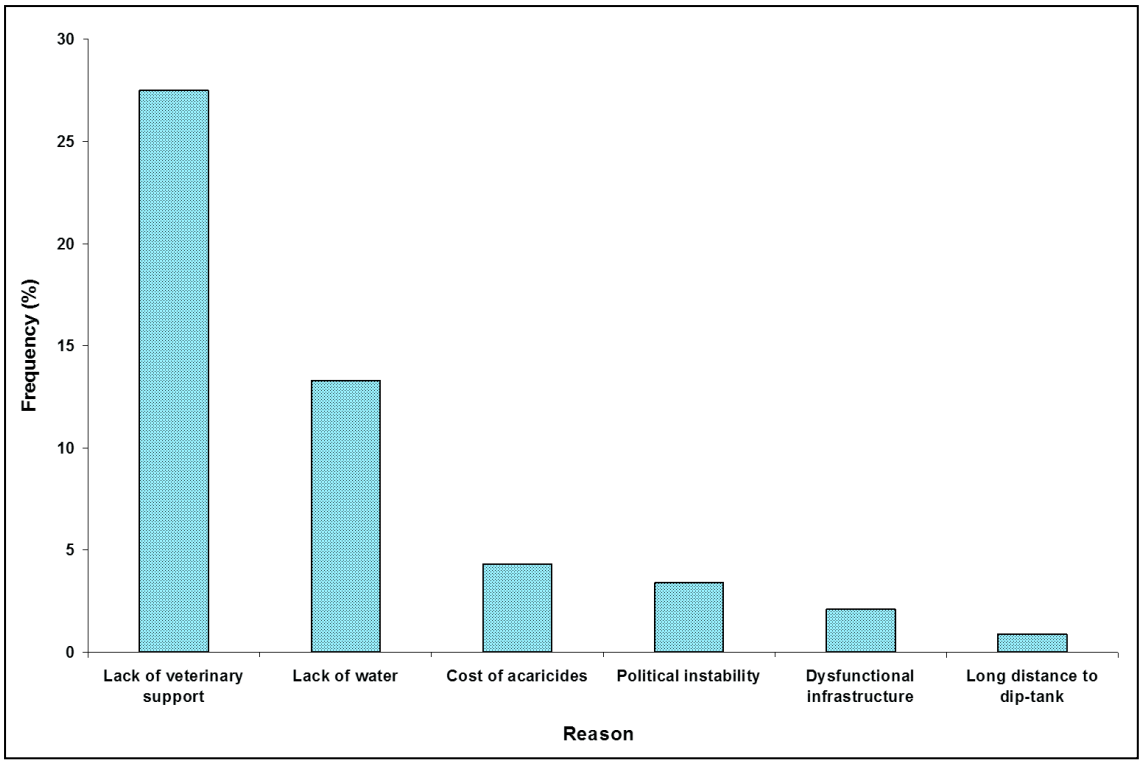

Figure 3.6: Reasons cited for inconsistent dipping services in the survey areas (N=233)

3.3.6 Use of pesticidal plants

Many respondents (72.1%) had some knowledge of plants used in the control of animal parasites where-as 27.9% were totally unaware. The knowledge was attributed to come mainly from the older generation of parents and grandparents (75.3%). Other knowledge sources of pesticidal plants were extension workers, relatives, NGOs, friends and the media in descending order as is in Fig. 3.8

Figure 3.7: Communal dip tank in disrepair in Honde Valley, Zimbabwe (*Photograph by ET Nyahangare*)

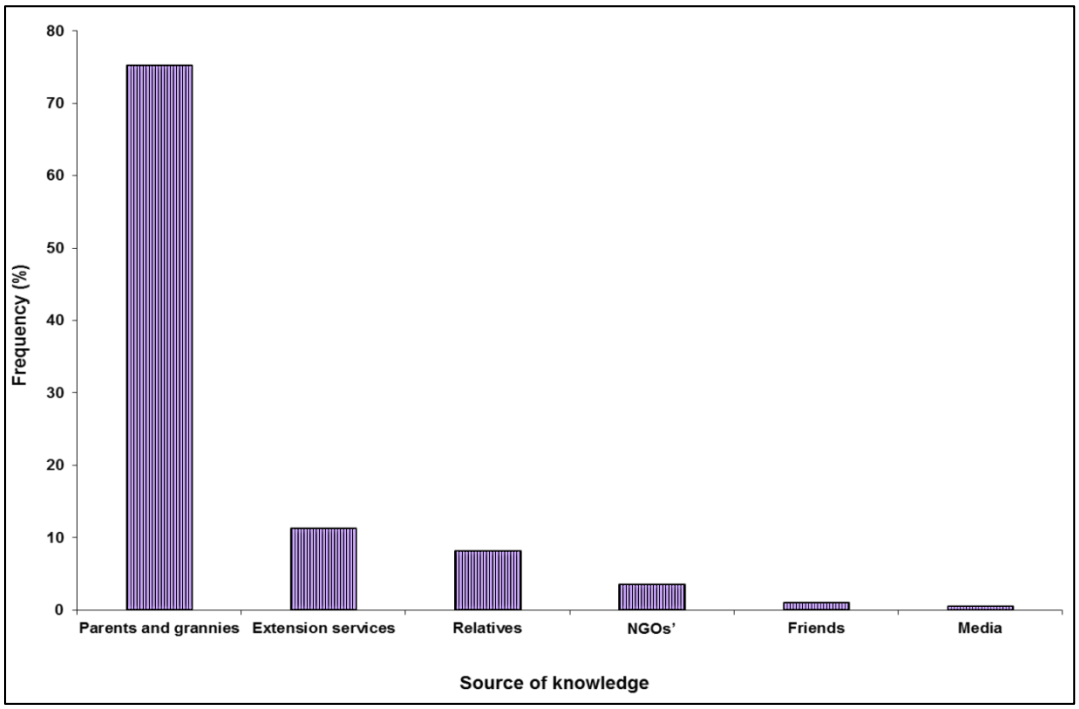

Figure 3.8: Sources of knowledge of acaricidal plants in the survey areas (N=168)

A total of 51 different plant species reportedly effective against livestock parasites were identified from this survey. The most popular plants by frequency of mention were *Cissus quadrangularis* L. (30.1%), *Lippia javanica* (19.6%)*, Psydrax livida* Willd. (14.9 %) and *Aloe* sp (14.9 %) (Fig 3.9). *Cissus quadrangularis* was particularly singled out as a very effective plant acaricide in Muzarabani, Kadoma and Chiredzi districts in FGDs with the farmers. Many other plant materials were not as well-known and were less mentioned by the respondents (Fig 3.9). The information on the specific pesticidal plants (trees, shrubs and grasses), preparation methods, parts of the plant used, targeted parasites, availability and other issues based on farmers experiences are presented in Table 3.5. The most common method of preparation of the plant materials was crushing the leaves/stems, soaking in water for variable times and then spraying the animals. Other methods included dusting ashes of certain trees, shrubs and herbs over the animals and birds. In poultry, twigs and leaves of *L. javanica* were laid as bedding in the fowl run to control fleas and mites.

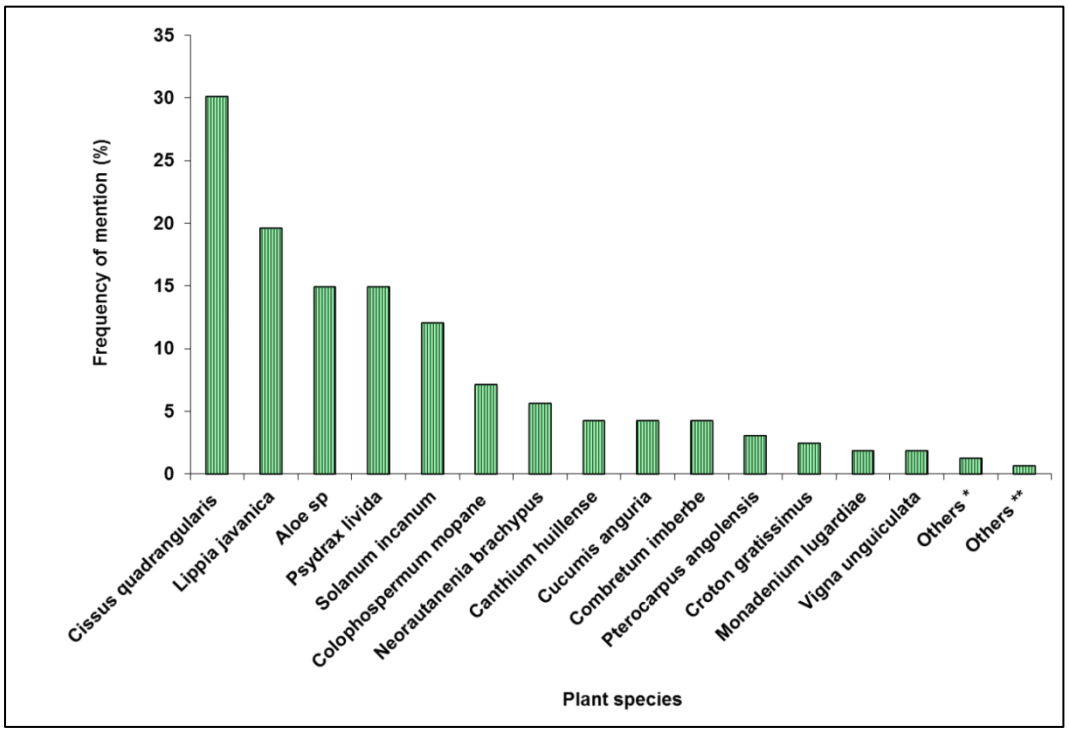

Figure 3.9: The most common acaricidal plants by frequency of mention (N=168)

3.3.7 Focus group discussions

During discussions, farmers acknowledged that they were more knowledgeable about livestock medicinal plants than pesticidal plants in general. The practice of using ashes of different plants as

acaricides, for example *C. mopane* tree, was based on the observation that donkeys regularly roll or bath in ashes in the villages and consequently, are rarely tick-infested despite not being dipped at all. Farmers in Muzarabani district showed awareness of aspects to do with intellectual property rights from previous exposure to studies on medicinal plants by other researchers. They felt that the scientists took advantage of them by extracting information from them and making money out of it with no acknowledgement of their input in monetary terms or otherwise. They demanded assurance that whatever came out from the deliberations would remain community property and that they should benefit as well. Separately, there was concern by farmers in all the districts about political interference and general lack of Government support to arrest deteriorating health care provision to livestock. In newly resettled areas, farmers lamented the long distances they must travel to access communal dip tanks.

Table 3.5 Summary of plants used for ticks and other ecto parasites control, how they are used and status of availability

Scientific name and Voucher Number	Family name	Local names *Shona - S Ndebele -N*	Part used	Preparation methods	Target parasites	Availability status	Comments and precautions
Acacia karroo Hayne. **(Nyahangare E 11)**	Fabaceae	Muzunga (S)	Root	Leave root in fowl run	Fleas and mites	Always	Safe to use
Monadenium lugardiae N.E. B. **(Nyahangare E 15)**	Euphorbiaceae	Chisvosve (S)	Whole plant	Crush and mix with water 24h	Ticks , fleas	Always	Handle with care
*Albizia amara (*Roxb.) Boiv. **(Nyahangare E 38)**	Fabaceae	Umbola (N)	Leaves	Crush leaves + water + spray	Ticks , fleas	Seasonal	Effective
Aloe excelsa A. Berger. **(Nyahangare E 29)**	Aloaceae	Gavakava, Mhangani(S)	Stemmy leaves	Crush leaves, mix with water for 24h and spray	Fleas , ticks	Seasonal	Safe to use
Aloe chabaudii Schoenland. **(Nyahangare E 37)**	Aloaceae	Inhlaba (N)	fresh stems	Grind and soak and smear the bird with sticky water	Lice	Always	Very effective
Bauhinia petersiana Bolle.**(NyahangareE23)**	Fabaceae	Mutyatyambe (S)	Leaves	Crush leaves, mix with water	Ticks, Goats	Always	Safe to use
Capsicum annuum L. **(Nyahangare E 69)**	Solanaceae	Mhiripiri (S)	Fruits	Crush the fruits and mix with soot in water and spray	Ticks	Always	Causes eye irritation
Carissa edulis (Forssk.) Vahl. **(Nyahangare E39)**	Apocynaceae	Umlugulu (N)	Leaves	Grind leaves, mix with water in the ratio 1:4 and spray	Lice and ticks	Seasonal	Wash hands after use
Cucumis anguria L. **(Nyahangare E47)**	Cucurbitaceae	Mujachacha (S) Amagaka (N)	Fruits	Collect ripe fruits (yellow), crush and mix with water and spray	Ticks	Seasonal	Have an itching effect on animals
Ornithogalum sp **(Nyahangare E59)**	Asparagaceae	Chihanyanisi (S)	Roots	Crush and mix with water	Ticks	Always but scarce	Very effective
*		Chimwamaruka (S)	Leaves	Crush the leaves and branches and mix with water	Fleas and ticks	Always but scarce	Very effective

** At the time of the survey, samples of these plants could not be found and require further follow up for positive identification*

(**Table 3. 5 Continued)**

Scientific name and Voucher number	**Family Name**	**Local names *Shona- S Ndebele – N Shangani - C***	**Part used**	**Preparation method**	**Target parasites**	**Availability status**	**Comments and precautions**
Cissus quadrangularis L. **(Nyahangare E6)**	Vitaceae	Chiololo (C), Murunjurunju (S)	Stems	Crush and mix with water to spray	Ticks	Always	Handle with care, has an itchy effect
*Psydrax livida (*Hiern) Bridson **(Nyahangare E20)**	Rubiaceae	Muvengahonye (S) Umhlahlampethu (N)	Leaves	Crush, mix with water and spray or crush leaves and put on wound or tick infestation site	Ecto parasites	Always	
Rotheca eriophylla **Nyahangare E35**	Lamiaceae	Umnukanja (N) Munukanja (S)	Leaves	Macerate, soak with water and spray	Ticks, Lice and tsetse fly	Always	Very effective and safe to use
Colophospermum mopane (J. Kirk ex Benth.) J. Kirk ex J. Leonard. **(Nyahangare E41)**	Fabaceae	Mopani (S)	Branches and twigs	Burn and apply ashes on animal skin	Ticks , fleas, mites	Always	Safe to use
Combretum imberbe Wawra. **(Nyahangare E55)**	Combretaceae	Muchenarota / Mutsviri (S)	Bark	Ash of the bark and twigs dusted on infestation sites	Ticks	Always	Very effective
Croton gratissimus Burch. **(Nyahangare E48)**	Euphorbiaceae	Inkiza emhlope (N)	Leaves and twigs	Use as bedding in the fowl run	Lice	Always	Very effective
*Ptaeroxylon obliquum (*Thunb.) Radlk. **(Nyahangare E27)**	Rutaceae	Umpahla, Umpandula N,	Leaves	Crush leaves, soak in water and spray	Ticks, Fleas, Lice	Always but scarce	Very effective
Euphorbia cooperi L. **(Nyahangare E71)**	Euphorbiaceae	Umhlonhlo (N)	Branches	Smoke the walls of the fowl run with the branches	Fleas	Always	Handle with care
Euphorbia ingens E.Mey.ex Boiss. **(Nyahangare E43)**	Euphorbiacaea	Mukondekonde (S)	Stems	Mix milk sap with water and place on infested sites	Fleas, ticks	Always	Avoid contact with the eyes
Gnidia kraussiana Meisn. **(Nyahangare E70)**	Thymelaeaceae	Chitupatupa (S)	Whole plant	Crush and mix with water to spray	Ticks	Always	Very effective

(Table 3.5 continued)

Scientific name and Voucher number	Family name	Local names *Shona- S, Ndebele – N Shangani - C*	Part used	Preparation method	Target parasites	Availability status	Comments and precautions
Lippia javanica (Burm.f.) Spreng. **Nyahangare E46**	Verbenaceae	Zumbani (S) Umsuzwane (N)	Leaves and branches	Crush leaves mix with water and spray, twigs can also be used as bedding in fowl runs	Ticks , fleas, lice	Always	Safe to use
Maerua edulis GilgandGilg-Ben. DeWolf **Nyahangare E5**	Capparidaceae	Katunguru (S)	Roots	mix *M. edulis* roots and *S. incanum* fruits with water and spray	Ticks	Always	Very effective
Ornithogalum sp **Nyahangare E59**	Alliaceae	Masimbe (S)	Bulb	crush bulb, soak in water and spray	Ticks	Always	Very effective
Datura stramonium L. **Nyahangare E51**	Solanaceae	Iyoyi (N), Chowa (S)	Leaves	Crush leaves mix with water and spray on animal	Ticks and Lice	Always but scarce	Safe to use
Euclea divinorum Hiern. **Nyahangare E15**	Ebenaceae	Muchekesani(S)	Roots	Crush leaves soak in water and spray	Fleas , cattle	Always	
*		Mundorani (S)	Roots	Crush dry roots and mix with water (50g in 300ml for 3 days)	Ticks	Always but scarce	Very effective
Vernonia colorata (Willd.) Drake. **Nyahangare E16**	Compositae/ Asteraceae	Munyatera (S)	Roots	Crush mix with water for an hour and spray	Ticks	Always but scarce	Very effective
*		Muvuwavuwa (S)	Whole plant	Just put the plant in the fowl run	Fleas and lice	Always but scarce	Very effective
Neorautanenia brachypus (Harms) C.A. Sm. **Nyahangare E65**	Fabaceae	Zhombwe (S)	Bulb	Crush the bulb , mix with water and spray	Fleas , ticks	Always	Avoid water contamination
Nicotiana tabacum L. **Nyahangare E68**	Solanaceae	Tobacco (E)	Water	Break and mix with water	Ticks	Always	Safe to use

** At the time of the survey, samples of these plants could not be found and require further follow up for positive identification*

(Table 3.5 Continued)4.5

Scientific name and Voucher Number	Family name	Local names *Shona - S Ndebele – N Shangani - C*	Part used	Preparation method	Target pests	Availability status	Comments and precautions
Spirostachys africana Sond. **(Nyahangare E63)**	Euphorbiaceae	Mutovhoti (S)	Barks	Cut barks break and mix with water	Ticks	Seasonal	Very effective
Strychnos spinosa Lam. **(Nyahangare E10)**	Strychnaceae	Matamba (S)	Unripe fruits	Crush unripe fruits, mix with water and spray	Ticks	Always	Very effective
Tagetes minuta L. **Nyahangare E66)**	Compositae/ Asteraceae	Munyakambanje (S)	Whole plant	Crush and mix with water	Ticks, fleas, mites	Always	Safe to use
Terminalia sericea Burch. ex DC. **(Nyahangare E36)**	Combretaceae	Mususu (S)	Leaves	Crush mix with water for spray	Ticks	Always	Very effective
Strychnos cocculoides Baker. **(Nyahangare E45)**	Strychnaceae	Ubuhlali (N)	Fruit	Squash the fruit and smear contents on animal infested	Lice	Seasonal	Very effective
Vigna unguiculata L. **(Nyahangare E67)**	Leguminosae Papilionoideae	Cowpea (E)	Pods	Empty pods burnt and ash applied on tick sites	Ticks	Always	Very effective
*Xeroderris stuhlmannii (*Taub.) Mendonca and E.P.Sousa **(Nyahangare E7)**	Papilionaceae	Murumanyama (S)	Barks	Crush stems and spread on infestation sites	Fleas, ticks	Always	Very effective
Zantedeschia albomaculata (Hook.) Baill. **(Nyahangare E62)**	Araceae	Mufanawembudzi (S)	Stem	Crush , mix with water and drench	Ticks	Always	Very effective
Zanthoxylum capense (Thunb.) Harv. **(Nyahangare E22)**	Rutaceae	Mukundanyoka (S)	Stems	Crush stems and spread on infestation sites	Fleas, ticks	Always	Safe to use

Scientific name and Voucher Number	**Family name**	**Local names *Shona - S Ndebele – N Shangani - C***	**Part used**	**Preparation method**	**Target pests**	**Availability status**	**Comments and precautions**
Xanthocercis zambesiaca (Baker) Dumaz-le-Grand **(Nyahangare E8)**	Fabaceae	Muturufuwa (S)	Bark	Crush the bark and soak in water to form a soapy solution for spraying	Fleas and ticks	Available	Safe to use
Pterocarpus angolensis DC. **(Nyahangare E32)**	Papilionoideae	Umvagazi (N) Mubvamaropa (S)	Barks, branches	Mix with water	Ticks	Always	Safe to use
Sansevieria hyacinthoides L. Druce. **(Nyahangare E58)**	Asparagaceae	Chikwenga (S) / Bushfibre (E) Mashamhanda (S)	Stemmy rhizomes	Squash the stems, mix with water and spray on ticks	Ticks	Always	Handle with care
*Senna singueana (*Del.) Lock **(Nyahangare E13)**	Fabaceae	Mudyamhungu Mukundanyoka (S)	Bark	Crush bark and mix with water	Ticks	Always	User friendly
Solanum incanum L. **(Nyahangare E61)**	Solanaceae	Nhundurwa (S)	Fruits	Crush fruits and mix with water	Ticks	Seasonal	Handle with care
Solanum panduriforme E.Mey. **(Nyahangare E60)**	Solanaceae	Nhundurwa (S)	Fruits	Crush fruits and mix with water	Ticks	Seasonal	Handle with care
*		Chinyaride (S) (fibre like)	Roots and the bulb	Crush the roots/bulb and mix with water	Ticks	Always but scarce	Handle with care
Kleinia sp **(Nyahangare E50)**	Asteraceae	Iphunja (N)	Leaves	Crush leaves , mix with water and rub on affected areas	Ticks	Scarce	Very effective
Ricinus communis L. **(Nyahangare E42)**	Euphorbiaceae	Umtshafuto (N)	Leaves	Grind leaves and paste on tick infested site	Ticks	Always	Very effective
Osyris lanceolata Hochst. **(Nyahangare E49)**	Santalaceae	Ingobamakhosi (N)	Roots	Pestle roots , soak in water and spray	Ticks	Always but scarce	Very effective

3.4 Discussion

3.4.1 Household demographics

The study showed that most of the respondents were of an older age ranging between 41-70 years of age. Similarly high, was the mean age (51.3 years) of the household head. This is due to the purposive nature of the sampling where the focus was on livestock farmers and younger generations were unlikely to own livestock as they were in the process getting an education and pursuing other developmental avenues of life. The older generation were also more knowledgeable about the use of traditional plants in the provision of primary health care of animals. It is known that traditional knowledge and practices are normally found in the older generations as the younger generations generally disregard traditional practices as a result of direct and indirect effects of modernisation and globalisation (Njoroge and Bussmann, 2006; Quave et al., 2008; Confessor et al., 2009). The fact that more males were the respondents in the survey reflects the patriarchal nature often seen in some communities in Zimbabwe (Chirisa, 2013). In such areas, males are considered the family heads and attend to the weightier matters of the family that include family wealth management. Cattle and large stock in general are considered as a sign for wealth in most rural communities and are hardly surrendered to the care of women (Mutopo, 2011). It is therefore not surprising that they featured more in the survey and would naturally have more knowledge on traditional practices used to manage the health of the cattle. Women usually take management roles in poultry and small stock.

3.4.2 Livestock production in the survey areas

3.4.2.1 Species kept by the farmers

Overall, indigenous chickens were the most populous livestock species averaging 16.9 birds per household. This figure is almost similar to the established national average in rural areas of Zimbabwe of around 17 birds (Muchadeyi, 2007). Mutibvu et al. (2012) found an average of 16.1 birds per household in a survey carried out in a district in Zimbabwe. These numbers are because these types of chickens generally require minimal initial capital injection compared to other species and can also perform relatively well even under poor management. Almost every farmer can afford to raise the birds on the farm regardless of social status. Chickens provide the household with a readily available cheap protein and can also be used as a

form of quick off-take, thus playing a critical role in the livelihoods of farmers (Muchadeyi, 2007). Kadoma district had the highest mean average of chickens kept per household. This can be explained by the fact that indigenous chickens also rely to a lesser extent on maize grain, sunflower and other grains. In the study area, crop production is most favourable in Kadoma district than the other districts.

3.4.2.2 Livestock species ranking in order of importance

Despite chickens being the most populous livestock species, cattle were ranked as the most important on the farms. Cattle are considered very important because of the many socio-cultural and economic roles they play in the context of the African society. They have multiple uses for communal farmers providing draught power, used in traditional rituals, providing milk, meat, manure and acting as social security (Dovie et al., 2006; Mavedzenge et al., 2006; Ndebele et al., 2007; Svotwa et al., 2007). The importance of cattle has also been underscored by other researchers who made similar observations (Ndlovu et al., 2004; Mlambo et al., 2011). The other livestock types were ranked lower and had lower mean herd and flock sizes. Muchadeyi et al. (2004) reported that even though farmers keep other species such as commercial chicken breeds, pigs, donkeys and various other less popular poultry species like guinea fowl and ducks, they are generally ranked lower and their populations are also very low. Despite being lowly ranked, these species play important roles in the livelihoods of rural farmers. Donkeys for example were ranked fourth but are critical in the drier areas of the country where they provide a steady supply of draught power (Mogotsi et al., 2013). They are well known to be drought resistant and easier to manage than cattle. The study areas experience frequent droughts hence donkeys occupy a very important niche. The only disadvantage of donkeys is that while other species can be used for milk and meat, not many people are known to prefer donkey meat or milk in Zimbabwe.

3.4.3 Livestock parasites and control methods

3.4.3.1 Common livestock parasites and pests identified in the survey

Most farmers identified ticks as the most problematic pest in cattle and other animals. Other authors also found ticks to be the major problematic external parasites in cattle rearing in South Africa and Zimbabwe

respectively (Moyo and Masika, 2009; Maroyi, 2012). There is a long history of the negative effects of ticks on productivity of cattle. Young et al. (1988) put the economic effects of ticks in sub-Saharan Africa higher than tsetse flies. The effects of other parasites are not as high and debilitating as those of ticks which can cause several weakening and fatal conditions to the animals (Peter et al., 2005; Muchenje et al., 2008). The high incidence of fleas and lice in poultry is not uncommon as these are the most common parasites affecting chickens. Maroyi (2012) also reported a high presence of these pests in chickens in a survey in Nhema area of the Midlands province of Zimbabwe.

3.4.3.2 Methods of controlling parasites

There seemed to be a deliberate inclination to manage ecto-parasites in the highly ranked livestock species (cattle, indigenous chickens and goats) with commercial acaricides while there were few interventions in the other livestock species with some of them not receiving any attention at all. The preference of commercial acaricides can be explained by the fact that over a long period of time commercial products have been proven to be very effective and are the default pest control remedies. On the contrary, farmers perceive the efficacy of traditional practices to be on the low side even if those thoughts are not backed by hard scientific facts (Gumbochuma et al., 2013). Government has also played a critical role in the promotion of commercial products over traditional practices through extension agencies as government policy. In most developing countries, there is no formal policy on the use of pesticidal plants and traditional practices in general. It must also be noted that through the veterinary services department most acaricides are heavily subsidised for communal farmers (Chamboko et al., 1999). This implies that, for as long as the services are available farmers will take this route for animal health care. It is when commercial acaricides are not available that farmers start using plants and other ethnoveterinary practices. Isman (2008) noted that the use of pesticidal plants is most prominent in the absence of commercial pesticides due to various reasons.

Challenges arise when national governments no longer have capacity to meet this need and instances of this nature are occurring more frequently. Between the years 2000 and 2009 for example, Zimbabwe went through serious economic challenges and cattle herds would go for months without dipping because the

government could not supply the required chemicals (Gunjal et al., 2009). Prior to the economic challenges, community dipping infrastructure was also dilapidated due to neglect of the past three decades (Chatikobo et al., 2004). Availability of acaricides has improved slightly as a result of economic stability that has happened over the last couple of years. Failure of governments to meet the needs of farmers is not a Zimbabwean problem alone but a feature in other countries as was observed in some South African communities (Hlatshwayo and Mbati, 2005).

The pronounced use of acaricidal plants in combination with commercial acaricides observed in the survey shows the complementary nature of the two practices in holistically dealing with ectoparasites. Other researchers have reported this trend in other ethnoveterinary studies (Moyo and Masika, 2009; Masimba et al., 2011; Gumbochuma et al., 2013).

It is not surprising that the major reason for inconsistent dipping was ascribed to lack of government support. As alluded to before, farmers had gotten used to government providing acaricides and making sure that animal health is well ensured through the Department of Veterinary Services but this has since changed as the economic climate changes and government ministries are always underfunded. As farmers try and adapt to the new set up it is inevitable that they feel the acaricides are overpriced and that the government is not fully supporting them. The issue of inadequate water supply as another challenge will likely increase as we anticipate more frequent droughts and erratic rainfall patterns due to the effects of climate change. The design of the plunge dip tanks in the country is such that they must be filled with water (about 20 000 litres) so that many cattle can be immersed when they dip in. In the absence of sufficient quantities of water it will be impossible to dip the cattle. The reasons of high cost of acaricides, dilapidated infrastructure and political instability are likely to improve in the long term as the economy and political atmosphere in the country improves.

3.4.4 Use of acaricidal plants for control of parasites

The high proportion of respondents (72.1%) with knowledge of plants with pesticidal properties is perhaps indicative of a very good link between primary animal health care and traditional practices. A 100% affirmation to knowledge of pesticidal plants was anticipated but it became apparent during the survey that some respondents had mistaken pesticidal and medicinal plants. Nonetheless the knowledge can be explained by that many people (> 80%) in the world rely on traditional practices to cater for health problems (Jabbar et al., 2005). Plants are a critical component of traditional medicines and pesticides (Sindhu et al., 2010). Most traditional knowledge is not as yet comprehensively documented and is passed on orally from generation to generation which could be the reason why parents and grandparents are the biggest source of this knowledge in this study. In a separate study on ethnoveterinary medicines, Masimba et al. (2011) showed that 61.5% of the sampled population reported also getting knowledge from parents and grandparents. The various media platforms were cited as the least source of information but it must be noted that it can be the most powerful tool in the promotion and development of traditionally based remedies especially if the younger generation are to be involved in this information age.

3.4.5 Plants reported to have acaricidal properties

Some of the plants that were reported were well-known while others were known by a few people. In Table 5, there is a possibility of inter-use of the plants identified. Some are stated as useful in controlling fleas, lice, flies etc. with no specific mention of ticks but it is possible that the pesticidal properties can also work against ticks. Future studies should investigate this possibility. The most common plants determined by frequency of mention across the districts were *C. quadrangularis*, *L. javanica*, *P. livida* and *Aloe* sp. It is important to note that it does not necessarily mean that the most mentioned plants are the most effective as only efficacy experiments can determine that. Some plant species have over time been well-known to work in different places of the world thus exposing them to many people. The most popular plants can help researchers prioritise plants for further investigations to meet farmers' needs. Knowledge of traditional products is a trade which some people depend on for their livelihood and it is very likely that such people are reluctant to divulge and share the information. This could explain why some plants could be less popular. Some of the species in the list have since been confirmed through experimentation to be acaricidal for example, *L. javanica* (Madzimure et al., 2011), *S. spinosa* and *S. incanum* (Madzimure et al., 2013). In

other studies, *A. ferox* was reported as a tick control remedy in some South African villages (Moyo and Masika, 2009). *Aloe chabaudii* and *N. mitis* are also some examples of plants reported in another survey in Nhema village in Zimbabwe to be acaricidal (Maroyi, 2012).

Other plant species reported in this survey, however, do not have known acaricidal or pesticidal properties yet but have other uses in ethnoveterinary medicine. *Aloe* sp. are effective anti-microbials and mostly used in the treatment of poultry diseases and other internal parasites (Mwale and Chimonyo, 2005; Masimba et al., 2011; Moreki et al., 2012). Most recorded uses of *C. quadrangularis* are of a medicinal nature than pesticidal (Mishra et al., 2010; Rao and Annamalai, 2011; Eswaran et al., 2012). However respondents highly regarded this plant and in Chiredzi it was called "*Chiololo"* in Shangani which can be literally translated to "highly effective" in English. *Psydrax livida* belongs to a very important group of plants locally known as "*Muvengahonye*" with many uses in ethnoveterinary medicine in tropical Africa but mainly famed for their antiseptic properties and treatment of wounds (Chamboko et al., 1999; Mukandiwa et al., 2012; Maroyi, 2012). Other plants in the group include *Canthium huillense* and *Clausena anisata.* There have not been reports of these plants against ticks elsewhere but some farmers indicated that the leaves are excellent insect and tick repellents.

3.4.6 Other issues

The use of water and leaves is almost a standard practice in most traditional remedies. Leaves are ideal because they ensure sustainability of the plant and water is a cheap universal solvent. In further studies, there may be need to use organic solvents to fully optimise the extraction process because water has its polarity limitations (Belmain et al., 2012; Grzywacz et al., 2014). In all the districts, through the focus group discussions, most farmers were not comfortable sharing their ethno-knowledge for fear of exploitation but later softened after much persuasion and assurance that the research was not for commercial gain. This just shows some of the challenges that need to be addressed to fully develop the science of ethnoveterinary practices in pest control.

3.5 Conclusion and recommendations

The study showed that there is a reasonably strong use of plant-based materials in controlling cattle ticks and other domestic animals parasites in drier parts of the country. A total of 51 plant species were identified that can be used to control ticks in semi-arid areas of Zimbabwe. There is need, however, to go a step further and conduct safety, efficacy and optimisation trials to verify farmers' claims that the plants are safe and effective for promotion of adoption and development. It is also of paramount importance to start engaging the Government to explore ways of creating an enabling environment for the formal development and use of acaricidal plants and other ethnoveterinary practices in the primary health care of livestock. This should be a critical step in the development of a robust alternative system for managing cattle ticks, especially where veterinary services are limited. Intellectual property rights are an area of concern that need to be investigated and addressed. Since the current type of work is still not yet fully developed locally, there may be need to examine what models other countries which are strong in the use of indigenous knowledge systems are using and determine if such models can also be adopted locally.

Postscript

Several plant species reported to be effective against ticks were identified from the survey even though some of the farmers were not using them. There is need for the efficacy of the species to be determined under controlled laboratory conditions. In the next chapter selected species were examined to determine if reported activity could be replicated under controlled conditions.

CHAPTER 4

4 *In vitro* efficacy of farmer reported anti-tick plant extracts against *Rhipicephalus (Boophilus) decoloratus* ticks

Preface

The plant species used in this study were the most popular with farmers according to frequency of mention from the survey. The efficacy results of extracts prepared using different extractants (water, water and surfactants, acetone, solvent-solvent fractions of *Maerua edulis*) against tick larvae were published in an article in BMC Veterinary Research Journal. The article co authored by Nyahangare Emmanuel T, Mvumi Brighton, McGaw Lyndy J and Eloff Jacobus N is entitled "*Addition of a surfactant to water increases the acaricidal activity of extracts of some plant species used to control ticks by Zimbabwean smallholder farmers*" and is copied below.

Abstract

Many studies have indicated that bioactive compounds for different indications are not extracted from plants using water, the only extractant practically available to rural people. In plants containing saponins, the non-polar compounds may be extracted because the saponins act like soaps in solubilising non-polar compounds. The effects of adding a soap to improve the extraction of biologically active compounds and the use of organic solvents of different polarity were determined *in vitro*. As a model, the efficacy of extracts prepared using different extractants was determined on 13 selected plant species used traditionally in southern Africa to control ticks. The adapted Shaw Larval Immersion Test (SLIT) method was used to determine the activity of the crude water extracts with liquid soap against the tick larvae. The activities of solvent-solvent fractions of crude acetone extracts of different polarity of the most active plant species, *Maerua edulis* tuber and leaf, were also compared to identify the most active fraction. Water extracts were not active, but addition of liquid soap as a surfactant increased mortality of the *R. (B) decoloratus* larvae. With the *M. edulis* tuber extract, the efficacy was comparable to that of the commercial synthetic acaricide. The acetone extracts increased tick mortality in all the plant species with the *M. edulis* (tuber and leaf) and *Monadenium lugardae* and the *Kleinia* sp. extracts as active as the positive control (a commercially available amitraz-based synthetic acaricide). The chloroform and hexane fractions from the leaf and tuber extracts caused up to 100% mortality. This indicates that non-polar to intermediate polarity compounds are responsible for the biological activity. It

can be concluded that chloroform and hexane fractions from *M. edulis* (leaf and tuber) are as effective as the commercially available acaricide. Adding commonly available dishwashing soap to water increased the activity of the extracts. In some cases, it was as active as nonpolar extracts and the positive control. This approach makes it possible for the rural farmers, and traditional healers to extract biologically active compounds from plants by using extractants prepared from widely available materials.

Key words: water extracts, surfactant, acetone, solvent-solvent fractionation, cattle tick, SLIT bioassay, mortality, biological activity

4.1 Introduction

Ticks cause major problems to optimal livestock productivity and also cause human diseases especially in tropical parts of the world by the transfer of pathogens (George et al., 2004). They also affect animals through tick-worry and hide damage (Moyo and Masika, 2009). The cost of managing and controlling ticks and tick borne diseases is estimated to be several billions of United States dollars globally (Jongejan and Uilenberg, 2004). For many years, chemical control using synthetic acaricides has been the preferred control strategy but there are now several legitimate issues against their extensive use. This requires attention and possible alternative control plans. This is because synthetic acaricides are expensive, active environmental pollutants, and have been found as residues in animal products and even more worrying is that their use leads to development of acaricide resistance of ticks (Adenubi et al., 2016). These are the issues that scientists are trying to address by investigating alternative or complementary products, especially for resource-constrained farmers. One of the routes pursued and believed to be the way forward by scientists is the use of botanical pesticides to make effective, safe and environmentally benign products that are effective against ticks (Isman, 2006). Historically, plants played pivotal roles in agricultural pest management, but the advent of synthetic compounds have since reduced this role. There is, however, need to identify and verify plants that are useful from the many potential plant species in the world. This is already a budding industry with potential to alleviate the challenges of tick control using synthetic products. There are several recent review papers that address aspects such as plants that have been used traditionally to combat ticks (Fouche et al., 2016); methodologies used to test efficacy of these plants (Marume et al., 2018), the use of essential oils as deterrents (Benelli, 2018), and a meta-analysis of compounds with activity against ticks (Fouche et al., 2016).

Systematic and standardised determinations of *in vitro* efficacy are crucial steps in the development of ethnobotanical acaricides. This is because they separate truly effective plants as reported by farmers from totally non-effective plants and those in between thus allowing resources for product development to be channelled towards plants with potential. One bioprospecting strategy has been to start by testing farmer practices as reported and determine if the farmers' observations or experiences are replicable *in vitro* and subsequently *in vivo* under controlled conditions. Most traditional practices revolve around the use of water as an extractant because this is the only freely available and low-cost extractant. However, it has since been established that water alone is a limited solvent to use for plant-based products because most active compounds are not soluble in water (Eloff, 1998; Kotze and Eloff, 2002; Sharma et al., 2012; Fouche et al., 2016). Some low-cost measures that can be used include the use of a surfactant and hot water for extraction purposes (Belmain et al., 2012; Grzywacz et al., 2014; Sola et al., 2014; Nyahangare et al., 2016). Apart from surfactants, organic solvents have produced better results than water with many plants, particularly acetone, because of their ability to extract both polar and non-polar compounds and their low toxicity to target test organisms such as ticks (Eloff, 1998; Sharma et al., 2012). Solvent-solvent fractionation into different polarity fractions has also increased efficacy of plant extracts (Eloff et al., 2017). In the current study, we examined the influence of the extractant used on different plant species and parts of the plants that rural farmers in Zimbabwe use to protect their cattle against ticks. We used *Rhipicephalus (Boophilus) decoloratus*, a very important cattle tick in southern Africa as a test organism in the *in vitro* assays. We also investigated whether solvent-solvent fractionation could increase the activity of an extract with high activity to facilitate the isolation and characterization of the active compound(s) in the fractions.

The overall objective of the study was to determine the efficacy of selected plant extracts against cattle ticks using different methods of extract preparation. The specific objectives were to: (1) determine the mortality of *R. (B) decoloratus* tick larvae exposed to crude water extracts of selected plant species compared to that of larvae exposed to crude acetone extracts; (2) determine the organic solvent fraction causing the highest mortality of *R. (B) decoloratus* tick larvae exposed to *M. edulis*. Interest in *M. edulis* developed largely because preliminary screening of the crude organic solvent extracts showed good activity and there was motivation to test fractions produced using solvent-solvent fractionation. In addition, preliminary *in vivo* trials at Henderson Research Station in Zimbabwe showed that the water extracts of the *M. edulis* tuber were effective against cattle ticks (Nyahangare et al., 2017). It was therefore necessary to do further efficacy tests on the plant using different extractants and fractionation of the extract to perhaps optimise its activity.

4.2 Materials and Methods

4.2.1 Study site

The *in-vitro* studies were done partly in the Phytomedicine Laboratory, Faculty of Veterinary Science, University of Pretoria in South Africa and at the Central Veterinary Laboratories, Harare, Zimbabwe.

4.2.2 Plant material collection

The test plant species were collected from the wild around communities who reported use of the species in several parts of Zimbabwe including Muzarabani, Chiredzi, Sanyati and Matobo districts during an ethnobotanical survey reported by Nyahangare et al. (2015). In Zimbabwe, no special permit is required for collection of plant material for research locally. However, before the survey and subsequent collection of the plant samples, the traditional leadership of the communities were apprised of the intent of the research and the need for collection of samples for further scientific investigations. The plant parts harvested were based on information received from the farmers in those areas during the ethnobotanical survey. Samples of the plants were taken to the National Herbarium and Botanic Gardens of Zimbabwe for identification and preparation of herbarium voucher specimens. Mr Christopher Chapano, the resident botanist, identified the plant species and prepared the voucher specimens. The full information on where all the plant species were collected and the voucher specimens are shown in Table 4.1.

Table 4.1: Plant species used and voucher specimen details

Plant species	Collection site(Zimbabwe)	Voucher specimen Number
Albizia amara	Muzarabani	Nyahangare E38
Aloe excelsa	Muzarabani	Nyahangare E29
Carissa edulis	Sanyati	Nyahangare E39
Cassia abbreviata	Sanyati	Nyahangare E72
Cissus quadrangularis	Chiredzi	Nyahangare E6
Croton gratissimus	Matobo	Nyahangare E48
Datura stramonium	Matobo	Nyahangare E51
Kleinia sp.	Matobo	Nyahangare E50
Maerua edulis	Chiredzi	Nyahangare E5
Monadenium lurgadae	Chiredzi	Nyahangare E15
Osyris lanceolata	Chiredzi	Nyahangare E49
Ornithogalum sp.	Chiredzi	Nyahangare E59
Ricinus communis	Matobo	Nyahangare E42
Terminalia sericea	Matobo	Nyahangare E36

4.2.3 Preparation of plant treatments

4.2.3.1 Preparation of crude water extracts

Fresh leaves of *M. edulis* Gilg and Ben Dewolf. (Capparaceae), *C. abbreviata* Oliv. (Fabaceae), *D. stramonium* L. (Solanaceae), *M. lugardae* N.E.Br (Euphorbiaceae), fleshy stems of *C. quadrangularis* L. (Vitaceae), *A. excelsa* L. Burm.f. (Aloaceae) and root tubers of *M. edulis* were crushed separately in a laboratory pestle and mortar and mixed with distilled water at 10% *w/v*. A separate set of treatments was prepared where 1% *v/v* surfactant (Sunlight liquid soap produced by Unilever Pty Ltd) was added to optimise the extraction and the extract's spreading effects (Amoabeng et al., 2014). The mixtures were left for 24 h after which they were filtered using a Whatman No 1 filter paper to remove the plant residues.

4.2.3.2 Preparation of acetone extracts

A total of 13 plant species (*M. lurgadae, C. abbreviata, Kleinia* sp. (Asteraceae), *M. edulis* (leaves and tuber), *C. quadrangularis, A. excelsa, O. lanceolata* Hochst. and Steud. ex A. DC. (Santalaceae), *A. amara* (Roxb.) Boivin (Fabaceae), *R. communis* L. (Euphorbiaceae), *C. edulis* Vahl (Apocynaceae), *T.*

sericea Burch.ex DC. (Combretaceae), *C. gratissimus* Burch. var. *gratissimus* (Euphorbiaceae), *Ornithogalum* sp L. (Liliaceae)) were selected. Dried ground plant material (5 g) was mixed with acetone (50 ml) and shaken vigorously for 20 min and then separated in a centrifuge at 1700 rpm for 10 min. The supernatant was filtered through Whatman No. 1 filter paper into pre-weighed glass jars. The extraction procedure was repeated three times for each aliquot of plant material. The solvent was dried under vacuum using a rotary evaporator. After drying, 0.1 g of the residue was dissolved in 1 ml of acetone to make a 10% w/v (or 100 mg/ml) concentration used in the tick bioassays. Previous studies showed that acetone alone is not toxic or has very low toxicity to ticks and other microorganisms and therefore can be used in bioassays (Adamu et al., 2013).

4.2.3.3 Preparation of fractions of acetone extracts

The dry acetone extracts of the leaves and tuber were each dissolved in hexane and the blend put in an ultrasonic water bath for 30 min. An equal volume of distilled water was then added and a separating funnel used to separate hexane and the aqueous layers. The water fraction was returned to the separatory funnel and chloroform and more distilled water added. The chloroform fraction was separated and the water fraction returned to the funnel. Butanol was added last and the butanol and water fractions were collected separately. This yielded a series of fractions with different polarities. All the extracts were dried under a stream of cold air at room temperature. The dry extracts were kept in a cold room at 4°C. A day before incubation with the tick larvae, approximately 0.2 g of the extract was diluted in approximately 20 ml of double distilled water containing 0.02 % Triton X-100 and 1 % acetone (diluent). The solution was vortexed for up to 10 minutes and then put in a sonicator at 37°C for 10 min to dissolve the extract in the diluent (Mekonnen et al., 2003). Undissolved or partially dissolved extracts after these procedures were used with no further treatment. The concentrations of the plant extracts (10 mg/ml) were not corrected for incomplete dissolution in the diluent.

4.2.4 Tick collection and rearing

Adult fully engorged ticks with no history of acaricidal resistance were collected from cattle in Mutorashanga (17.1488S, 30.6761E), in Makonde district and Mashonaland West province in Zimbabwe. The area is in Natural farming region II, characterized by annual rainfall of values between 700 and 1050 mm and a mean maximum temperature range of 16–19°C. The ticks were prepared as described by Madzimure et al. (2013) by experts at the Central Veterinary Laboratory (Department of Veterinary Services in the Ministry of Agriculture, Mechanisation and Irrigation Development,

Zimbabwe). Initially, the ticks were cleaned of all possible eggs laid during transportation from the field to the laboratory using distilled water. Subsequently, the ticks were put in plastic rearing tubes firmly closed with a ventilated stopper for egg laying in an incubator set 27-28°C and 85–95% relative humidity. All eggs laid were collected within 7 days from commencement of incubation and hatched. Tick larvae between 17 and 21 days were used for the larval immersion test.

4.2.5 Adapted Shaw Larval Immersion Test (SLIT)

The SLIT method described by Shaw in (Mekonnen et al., 2003) was used to determine the efficacy of plant extracts against ticks. The method was modified by increasing the larval incubation post treatment to 72 h (Wellington et al., 2017). Tick larvae approximately 17-21 d old were used in the experiments. Using a soft small paint brush, approximately 200 larvae were placed between two round Whatman No. 1 filter papers (diameter 120 mm) to form a larvae sandwich which was placed in a pie plate (diameter 140 mm). About 10 ml of the test solutions from the plant extracts at a concentration of 10 mg/mL was then poured carefully over the sandwich to expose the larvae to the plant extract for 30 min. After the 30 min, any excess solution was drained off using paper towels and the sandwich transferred to a clean dry filter paper (diameter 250 mm). The sandwich was then opened and each half placed on the dry filter paper. Approximately 100 larvae were brushed off the filter paper to a clean filter paper envelope which was crimped and closed and finally put in an incubator set at temperature 26 ^{0}C ±2 and relative humidity (RH) 70 - 90%) (Wellington et al., 2017). The experiment for each plant extract was duplicated. The diluent and Triatix® (12.5% EC amitraz-based compound manufactured by Ecomed Manufacturing, Belmont, Zimbabwe for Coopers Zimbabwe Pty Ltd) applied at the prescribed label dilution rate of 0.002% v/v, were used as the negative and positive controls, respectively. The number of dead larvae was determined after 72 h. The efficacy of each extract was determined by comparing mortality in the test extracts against the mortality in the negative control from which a corrected mortality (CM) was eventually calculated using Abbott's formula (Abbasi et al., 2013):

$$\boldsymbol{CM = \left[i - \frac{c}{100} - c \right] * 100}$$

Where i = % mortality in test extract; c = % mortality in negative solvent control (Diluent); CM = % corrected mortality.

4.3 Results

i. Activities of crude water extracts

There were no tick mortalities recorded in all the aqueous plant extract treatments and the negative control. However, the addition of a surfactant during extraction with water caused significant mortality ($P < 0.05$) in some of the treatments. The most significant mortality was recorded in the *M. edulis* tuber treatment which was as effective as the amitraz-based positive control ($P>0.05$). The extracts of *M. lugardae* and *M. edulis* leaves accounted for mortalities which were well below 50% (Table 4.2). There was no significant difference ($P > 0.05$) between the negative control and the *C. quadrangularis*, *A. vera* and *C. abbreviata* treatments, although they were applied at different dosages. The corrected mortalities of all the different plants species extracted with water and a surfactant are as shown in Table 4.2.

ii. Activities of crude acetone extracts

In general the acetone extracts had much higher activity against the tick larvae (Table 4.3). The most effective acetone plant extracts were of the *M. edulis* treatments (leaf and tuber) and the *Kleinia* sp. (Table 4.3). There was no significant difference in activity between these treatments and the amitraz based positive control at the dosage tested. The *M. lugardae* extract also had high activity with an 83% corrected mortality. The activities of the other plant extracts are presented in Table 4.3.

iii. Solvent - solvent extraction

The chloroform fractions of the *M. edulis* tuber and leaf and the hexane and butanol fractions of *M. edulis* tuber only were the most effective fractions against tick larvae with no significant difference from the positive control (amitraz). The other fractions were not as effective (Table 4.4).

Table 4.2: Corrected mortality (CM) of tick larvae exposed to crude water with surfactant extracts (100 mg/ml) of seven plant species and Amitraz (2 ml/L) (N=4)

Plant species	Mean Mortality (%)	CM ± SEM (%)
Maerua edulis (tuber)	97.5	97.4 ± 0.96 [a]
Monadenium lugardae (stems)	32.3	30.6 ± 0.96 [b]
Datura stramonium (leaves)	19.2	17.5 ± 4.84 [c]
Cassia abbreviata (leaves)	17.7	15.6 ± 10.16 [c]
Maerua edulis (leaves)	15.4	13.3 ± 3.30 [c]
Aloe vera (stems)	5.0	2.6 ± 3.10 [d]
Cissus quadrangularis (stems)	4.2	1.7 ± 2.34 [d]
Water with surfactant (negative control)	2.49	0 ± 0.87[d]
Amitraz (positive control @ 0.002% v/v)	100	100[a]

Superscripts with different letters in a column are statistically different ($P < 0.05$). Water without surfactant had no significant effect on the tick mortality

Table 4.3: Corrected mortality (%) of tick larvae caused by acetone plant extracts (10% w/v) of different species (N=4)

Plant species	Mortality (%)	CM ± SEM (%)
Maerua edulis (leaf)	97	97 ± 3.3 [a]
Maerua edulis (tuber)	93	93 ± 6.7 [a]
Kleinia sp.	90	90 ± 5.8 [a]
Monadenium lurgadae	83	83 ± 3.3 [b]
Cassia abbreviata	77	77 ± 6.7 [b]
Cissus quadrangularis	57	57 ± 6.7 [c]
Aloe excelsa	53	53 ± 16.7 [c]
Osyris lanceolata	53	53 ± 12.2 [c]
Albizia amara	43	43 ± 13.3 [d]
Ricinus communis	43	43 ± 14.5 [d]
Carissa edulis	37	37 ± 14.5 [d]

Terminalia sericea	27	27 ± 12.0 [e]
Croton gratissimus	23	23 ± 8.8 [e]
Ornithogalum sp.	20	20 ± 20 [e]
Amitraz (+ve control @ 0.002% v/v)	100	100[a]
Acetone (-ve control)	0	0[f]

CM values with different superscripts [abcdef] letters are significantly different within the column (P <0.05).

Table 4.4 Corrected mortality (CM) of tick larvae under different organic solvent fractions (10 mg/ml) and Amitraz (0.002 v/v) (N=4)

Plant extract	Mean mortality (%)	CM ±SEM (%)
M. edulis (L) Water	32.5	27.2 ± 8.54 [c]
M. edulis (L) Butanol	2.0	-5.7 ± 0.41
M. edulis (L) Chloroform	100.0	100.0 ± 0 [a]
M. edulis (L) Hexane	51.5	47.7 ± 18.53 [b]
M. edulis (T) Butanol	96.3	96.0 ± 2.39 [a]
M. edulis (T) Chloroform	100.0	100.0 ± 0 [a]
M. edulis (T) Hexane	100.0	100.0 ± 0 [a]
Amitraz (positive control)	100.0	100.0 ± 0 [a]
Diluent (negative control)	7.2	-

CM values with different superscript letters [abc] are significantly different within the column (P<0.05).

* L (leaf); T (tuber)

4.4 Discussion

Despite being highly rated by farmers (Nyahangare et al. 2015), no water extracts were effective against the ticks, reinforcing a widely accepted point that water alone does not effectively extract less polar antimicrobial or biopesticidal compounds from plant extracts because of its high polarity (Eloff, 1998; Kotzé et al., 2002; Gonçalves et al., 2007). Perhaps, the high activity reported by farmers, if confirmed, may be associated with repellence of volatile emissions from the plant. In this study the poor

activity of water extracts is disappointing because, practically, water is the only extractant available for most rural farmers (Chemat et al., 2012). The use of organic extractants is beyond the scope of many farmers, as they require money and scientific laboratory exposure on how to use the solvents. The results were in contradiction with the widely accepted notion that plant species under study like *C. quadrangularis* have good anti-tick properties from several authors (Eswaran et al., 2012; Santhoshkumar et al., 2012; Nyahangare et al., 2015). The same can be said for *Aloe* species and the other plants. *Cissus quadrangularis* particularly, is also known as "Chiololo" in one of the districts in Zimbabwe because of its perceived "potency". Loosely translated, Chiololo means "highly effective" (Nyahangare et al., 2015). The high activity shown by the treatments with a surfactant are very significant to smallholder rural farmers because the soap used is a realistic low cost intervention that is easily available to farmers.

It is unclear why farmers adamantly report excellent activity with just using ordinary water though. The most likely compounds to be extracted by water include proteins, sugars and salts which ordinarily do not cause toxicity to parasites (Chemat et al., 2012). Given this background, it is possible that toxins develop during the processing and preparation of the extracts before spraying (Adamu et al., 2013). Another possibility is that using water under laboratory conditions does not approximate field conditions where the water may contain microorganisms. If these micro-organisms grow on sugars and proteins in the water extracts, they may lead to solubilizing the intermediate polarity compounds in the plant. It is also possible that some plants contain saponins that act as soap and solubilise intermediate polarity compounds. It is difficult though to discard water as a useful solvent because most ethnoveterinary medicines are still based on water extracts (Talib and Mahasneh, 2010). There is no doubt that farmers get value from the use of water as an extractant across the world. It will be interesting to see whether traditionally prepared extracts can produce the same results with laboratory preparations. It is possible that residual plant products on the cattle may have contact toxicity effects on ticks, or they may emit volatiles which could have fumigant toxicity or have repelling effects on the ticks. There must be a reason why they are reported to be effective.

The marked increase in the mortality of ticks after inclusion of a liquid soap (surfactant) is an indication that the surfactant helps to extract the non-polar bioactive compounds. It may also affect the spreading properties of the extract. It can also weaken the cuticle layer of the insects allowing the extracts to cause toxicity (Sharma et al., 2012; Amoabeng et al., 2014; Céspedes et al., 2014).

Similar high acaricidal activity was reported with the soapy tuber extracts *in vivo* where the *M. edulis* tuber performed just as well as Amitraz, the positive control, under field conditions (Nyahangare et al., 2017). It is rather unfortunate that the tuber had high activity instead of the leaves because this has implications on sustainable harvesting and usage. Other low cost measures that may possibly increase activity include using boiling water for extraction and increasing the extraction period (Nyahangare et al., 2016).

The general improved efficacy of acetone crude extracts for most of the plant species is a clear indication that this solvent has excellent ability to extract plant-based active compounds in plants and has been used by many researchers (Eloff, 1998; Kotzé et al., 2002; Adenubi et al., 2016; Wellington et al., 2017). In the current study, plants with poor activity when water was used showed a marked increase in effectiveness, for example *M. edulis* leaf, *C. abbreviata* and other plant water extracts. Acetone extracts of *C. quadrangularis* gave 53% CM but the same plant showed a 100% CM in a different study by Wellington et al. (2017). The differences may possibly be attributed to differences in stage of growth, time and site of collections. This is one of the biggest challenges to development of botanical acaricides where changes in the soil, environmental and plant conditions may result in changes in the phytochemical composition of the plants (Ramakrishna and Ravishankar, 2011; Sarasan et al., 2011; Belmain et al., 2012; Miresmailli and Isman, 2014).

The use of acetone as the organic extractant of choice is a result of several years of comparisons with other solvents with similar indications. Acetone dissolves a wide range of compounds and has the distinct advantage of being less toxic to most test organisms (Eloff, 1998; Kotze and Eloff, 2002, Sharma et al., 2012). Some solvents like butanol are highly toxic to ticks and ethanol was found to inhibit oviposition and therefore not favourable as solvents of choice (Sharma et al., 2012).

High mortality in the chloroform, hexane and butanol fractions of the tuber and leaf extracts is evidence that there are multiple active compounds in this plant. Hexane extracts of the roots have been previously reported to have antimycobacterial activity when tested against *Mycobacterium smegmatis* and preliminary phytochemical investigation showed that the plant contains linear chain unsaturated fatty acids (Luo et al., 2011). There is therefore a high possibility of antibacterial and anti-tick properties in these fractions. Because not a lot of chemical characterisation has been done on *M. edulis*, it remains to be established what compounds are in the chloroform and butanol fractions.

Many plant extracts that have been determined to be acaricidal or pesticidal are active owing to the presence of essential oils and other toxic compounds (Isman and Machial, 2006; Magano et al., 2011; Ellse and Wall, 2014; Hüe et al., 2015; Adenubi et al., 2016). Essential oils are known to cause a number of effects against ecto-parasites, pests and insects which include mortality, anti-feedant, repellent and oviposition effects (Koul et al., 2008; Pirali-Kheirabadi et al., 2009; Khater, 2012; Wanzala et al., 2014). Some of the essential oils that have been used against ticks as repellents include clove, eucalyptus, lavender, lemon, geranium, palmarosa, pennyroyal, rose and sweet myrrh. Because essential oils are volatile, they are able to act as deterrents against several ectoparasites but the problem is that they evaporate within a relatively short period. To combat ticks effectively in animal production, it appears to be much better to search for compounds with a long lasting effect that are toxic to the ticks and are safe to animals and humans. The success obtained with the pyrethroids proves that searching for plant-based solutions to protect animals against ticks is not wishful thinking. Attempts were made to isolate the active compounds present in the chloroform fraction using open column silica gel chromatography and standard methods. It was not possible to determine the activity of the different fractions on tick larvae because no larvae were available at that stage.

The efforts to determine the structure of a small quantity (5 mg) of compound that gave only one spot in TLC by Liquid Chromatography-Nuclear Magnetic Resonance was frustrated by problems with our apparatus. Prof Vinesh Maharaj, head of the Department of Chemistry, University of Pretoria stated: "There is a large peak at 1.2 ppm indicative of a long chain fatty acid or oily type of compound. This is typical of the use of low quality solvents such as hexane which carry such impurities. There is also a closely related minor compound also present so it is not absolutely pure." Because there was not sufficient material to determine the acaricidal activity and there was not sufficient plant material available at that stage no further work was done.

The active chloroform fraction was analysed by Gas Chromatography-Mass Spectroscopy GC-MS but this did not give much insight either. The GC-MS data for compounds that matched the library by at least 80% are presented in the appendix. It is probably worthwhile to isolate the active compounds in future studies.

4.5 Conclusion

From the results it is clear that intermediate polarity compounds play an important role in acaricidal activities. Water extracts had very low activity but adding a surfactant to the water made it possible to extract some intermediate polarity compounds leading to an increased acaricidal activity of *M. edulis* tuber extracts. The acetone extracts of *M. edulis* (leaf and tuber), *Kleinia* sp. and *M. lugardae* showed

high activity against the tick larvae. The reason why farmers get good results using only water may be due to secondary mobilization of intermediate polarity compounds by physical aspects such as photo-oxidation or temperature effects or by the effect of microorganisms growing in the aqueous extract possibly containing some water-soluble nutrients. It appears that searching for compounds with acaricidal activity combined with safety to animals has a better chance of success than searching for tick repellents in animal production. However, acaricidal and repellent activities of extracts have also been found to play complementary roles to each other in some cases. The fact that water with a surfactant such as soap has high activity has important implications for rural smallholder farmers because soap is not expensive and water is freely available to them. The attempt to isolate the active compound from the cholorform fraction was not successful and in future studies there is need for bioassay-guided isolation of the active compounds (and blends) and their structural characterization by different spectroscopic techniques so that the picture is complete.

Postscript

Acetone extracts and less polar fractions of *Maerua edulis* consistently showed good activity from the in vitro screening. It is hypothesised that other low cost practices like use of hot water, use of a surfactant and other organic solvents can also increase acaricidal activity of this plant species. The toxicity of the extracts may be low since this is a natural product. These aspects on this plant species will be investigated in the next chapter.

CHAPTER 5

5 The effect of different extractants on acaricidal efficacy of *Maerua edulis* (Gilg and Ben) De Wolf against *Rhipicephalus (Boophilus) decoloratus* tick larvae and acute oral mammalian toxicity

Preface

In the previous chapter crude acetone extracts and non-polar fraction plant extracts of *M. edulis* had very high activity against ticks compared to water extracts. In this chapter other extraction alternatives that can be used to extract *M. edulis* were investigated. Widening the alternative extractants base is an adavantage for users who may have challenges in availability of a single extractant. The toxicity of the plant extracts was also determined against Balb/C mice. The results of this study have been published and are copied below: Nyahangare, E. T., B. M. Mvumi, and T. Maramba. 2016. Acute Oral Mammalian Toxicity and Effect of Solvents on Efficacy of *Maerua edulis* (Gilg and Ben) De Wolf against *Rhipicephalus (Boophilus) decoloratus* Koch, 1844 (Acarina : Ixodidae), Tick Larvae. Biomed Research International, https://doi.org/10.1155/2016/7078029

Abstract

The toxicity of aqueous and organic solvent extracts of *Maerua edulis* against ticks and mice were determined. Ground leaves were extracted separately using cold water, cold water plus surfactant (1% v/v Sunlight liquid soap (Triclosan (0.10%)), hot water plus surfactant, hexane, or methanol to make 25% w/v stock solutions from which serial dilutions of 5, 10, 20, and 25% were made. For each concentration, 20 *Rhipicephalus decoloratus* tick larvae were put in filter papers impregnated with extracts and incubated for 48 h at 27°C and 85–90% RH for mortality observation after 24 h and 48 h. In the toxicity experiment, hot water plus surfactant treatments of 5, 10, 20, and 25% (w/v) *M. edulis* were administered in suspension *per os* to sexually mature Balb/C mice and observed for clinical signs and mortality for 72 h. Larvae mortality was highest (>98%) in methanol-extracted *M. edulis* treatments (20 and 25%), which was not different from Tickbuster® the positive control (12.5% EC amitraz-based compound manufactured by Ecomed Manufacturing, Belmont, Zimbabwe for Coopers Zimbabwe Private Ltd and applied at the prescribed (label) dilution rate of 0.2% v/v). Mortality was also higher with the hot water extracts than with cold water plus surfactant treatments ($p < 0.05$). No post administration

adverse health effects were observed in the mice. These results suggest that *M. edulis* is an effective and safe tick remedy best extracted using methanol or hot water plus surfactant.

Keywords: *Maerua edulis*, *in vitro* efficacy, hot water, surfactant, ticks

5.1 Introduction

From several other potentially useful plants investigated, previous experiments showed that *M. edulis* has acaricidal properties that can help reduce tick infestations on animals. However, just like other botanicals, there are still many inconsistencies in the appropriate extraction and application methods and knowledge of toxicity to non-target organisms such that no one method has been prescribed to apply to many plants (Wanzala et al., 2005; Marandure, 2016). The previous experiments used a well-defined laboratory protocol to prepare and administer the treatments on tick larvae. Farmers have no defined standard procedures that they use and it is not clear how much plant material they use to prepare the treatments. In trying to optimise efficacy of plant extracts it is important to appraise all the available approaches and come up with recommendations. As stated earlier, available extraction methods vary in complexity from using simple water to different types of organic solvents. The use of water as a solvent is characteristic of most traditional practices but it is known that it has a limited ability to fully extract active plant compounds (Talib and Mahasneh, 2010; Chemat et al., 2012).

We investigated if there are other unsophisticated methods that may be used to enhance the extractive and wetting properties of the *M. edulis* extracts such as using hot instead of cold water, or addition of soap to enhance the extraction and spreading effects of the extracts (Waka et al., 2004; Amoabeng et al., 2014). Organic solvents on the other extreme end are also known to be excellent extractants of active compounds of many plants but no one solvent can be used for all the plants because efficiency of extraction is determined by the nature of the active ingredients in the plant.

In this study, *M. edulis* plant (leaves) recommended by farmers and confirmed through *in vitro* (water and acetone extracts) and *in vivo* (water extracts with 1% surfactant) experiments to be effective against ticks (Kaposhi, 1992; Stathers et al., 2002; Nyahangare et al., 2015; Fouche et al., 2016; Nyahangare et al., 2017) was extracted using methods familiar to the farmers to assess the best method to use by resource constrained farmers. Despite acetone being the preferred solvent generally, other known effective extractants like methanol and hexane were also evaluated. Safety was determined in a single dose acute oral toxicity experiment against Balb/C mice.

5.2 Materials and methods

Laboratory experiments were conducted to determine the effect of different extractants on efficacy of *M. edulis* extracts against *R. (B.) decoloratus* tick larvae (Study I) and to determine the relative safety of *M. edulis* on Balb/c mice (Study II).

A. The effect of different extractants on the acaricidal activity of *M. edulis* on *R. (B.) decoloratus* tick larvae

5.2.1 Study site

The experiment was conducted at the Central Veterinary Laboratory (CVL) of Zimbabwe, located about 5 km from the Central Business District (CBD), along Borrowdale – Domboshava road in Harare.

5.2.2 Tick collection and rearing

Ticks were collected and prepared as described in section 4.3.4 (Chapter 4)

5.2.3 Plant material collection and preparation

The *M. edulis* plant (leaves and tubers) (Fig 5.1) material was collected from Chiredzi district (21° 2' 20" S, 31° 40' 40" E, altitude 508) in agro-ecological zone five in Zimbabwe. Collected fresh *M. edulis* leaves were shade dried in a room with mean temperatures ranging from 25°C - 32°C. The tubers were washed clean with water and then chopped to very small pieces for ease of drying. After drying, the leaves and the tubers were ground to powder and stored in a cold room at 4°C.

Figure 5.1 Flowering *M. edulis* (A) and *M. edulis* tubers (B)

5.2.4 Treatments

For all the treatments, *M. edulis* powder was weighed and soaked separately in each of the following solvents; cold water, hot water (at about 70^0C), hexane and methanol for 24 h to produce a stock solution of 25% w/v in a ratio of 1:4 solid to liquid. The mixture was filtered through a muslin cloth and serial dilutions of 5, 10, 20 and 25% w/v made with each solvent. Where the effect of adding a surfactant was to be determined, 1% v/v of liquid soap was added during extraction and mixed thoroughly. All aqueous extraction experiments used distilled water as the negative control but organic solvents used the extracting solvent as the negative control (hexane or methanol). A commercial amitraz based acaricide (Tickbuster®) was prepared as per manufacturer's specifications at 0.2% v/v and used as the positive control. A summary of all the treatments is shown in Table 5.1.

Table 5.1: Summary of the experimental treatments used in the study

Treatment	Description
1	5, 10, 20, 25% w/v serial dilutions of cold water extracted *M. edulis*
2	5, 10, 20, 25% w/v serial dilutions of cold water extracted *M. edulis* + 1% v/v surfactant
3	5, 10, 20, 25 and 100% w/v serial dilutions of hot water (70^0C) extracted *M. edulis* + 1% v/v surfactant
4	5, 10, 20, 25 and 100% w/v serial dilutions of hexane extracted *M. edulis*
5	5, 10, 20, 25% serial dilutions of methanol extracted *M. edulis*
6	Tickbuster® prepared at 0.2% *v/v* with water (Positive control, amitraz)
7	Distilled water, methanol, hexane (Negative controls)

5.2.5 Experimental procedure

The *M. edulis* and the negative and positive control treatments were tested on approximately 20, 3 week-old tick larvae, in an adaptation of the Soberanes bioassay technique as explained by Miller *et al* (2007). For each treatment 10 ml of the solution was placed into a 10 cm diameter Petri dish containing a 9 cm diameter Whatman no. 1 filter paper for 5 minutes. The tick larvae were then placed onto the wet filter paper which was then folded and the open end sealed with steel paper clips. The packets were put in an incubator set at 27°C, 85 - 90% relative humidity (RH), and at photoperiod of 12:12 light to dark ratio (L:D). Larval mortality was recorded after 24 and 48h post-incubation. Each treatment was replicated five times.

5.2.6 Statistical Analysis

Mortality data for the tick bioassays was converted to ranks through the Proc. Frequency and Proc. Rank procedures of SAS (2004), version 9 and analysed using the Proc. GLM procedures of SAS.

B. Single dose acute oral toxicity of *M. edulis* on Balb/C mice

5.2.7 Study site and plant material preparation

The acute oral toxicity experiment was carried out at the University of Zimbabwe, Department of Animal Science – Bioassay Laboratory. Hot water plus surfactant extracted *M. edulis* prepared as in the efficacy experiments were used in this experiment.

5.2.8 Animals and experimental design

A total of 30 *BALB/c* mice reared in individual cages from time of weaning until sexual maturity at six weeks were used in a completely randomized design experiment. The mice were randomly allocated to the six treatments where each mouse was an experimental unit and replicated five times. The animals were fed commercial mouse comproids procured from National Foods (Pvty Ltd, Harare) and water *ad libitum* until a day before administration of plant extracts when all feed and water was removed and reintroduced shortly after treatment administration.

5.2.9 Administration of plant extracts and measurements and observations

The *M .edulis* plant extracts (5, 10, 20 and 25% v/v) and the control (distilled water) were orally administered *per os* using a 10 ml plastic syringe with a 16 mm long 22-gavage needle connected to it as described in Nyahangare et al (2012) where the needle was held horizontally, in a position parallel with the head of the mouse and inserted at the back of the animal's throat. A single dose of 4 ml of the test material was carefully introduced into the oesophageal opening. For normal physiological functions, the minimum water requirements of a mouse are on average 4 ml/day depending on mass (Fox et al., 2007) and this was used as the basis for the quantity administered to the mice. The mice were observed on a daily basis for 3 d checking for mortality, behavioural changes and development of clinical signs.

The mice husbandry and the toxicity tests were adapted from the Organization for Economic Cooperation and Materials (OECD) guidelines for assessing acute oral toxicity. The Department of Animal Science (University of Zimbabwe) is licensed by the Veterinary Services Unit in the Ministry of Agriculture, Mechanisation and Irrigation Development in Zimbabwe under the Scientific Animal Experiments Act (License Number L624) to carry out such experiments with mice.

5.3 Results

i. Cold water only extraction

The plant extracts caused mortality of the larvae overall (P<0.05). However, the mortality of ticks was generally low at both recording times (< 50%) with no significant differences between the cold water only extracted *M. edulis* and the untreated negative control after 24h. There was however a significant difference between the plant extract treatments and the negative control after 48h (P<0.05). Mortality was highest in the only positive control (Tickbuster®) for both recording times (Fig 5.2)

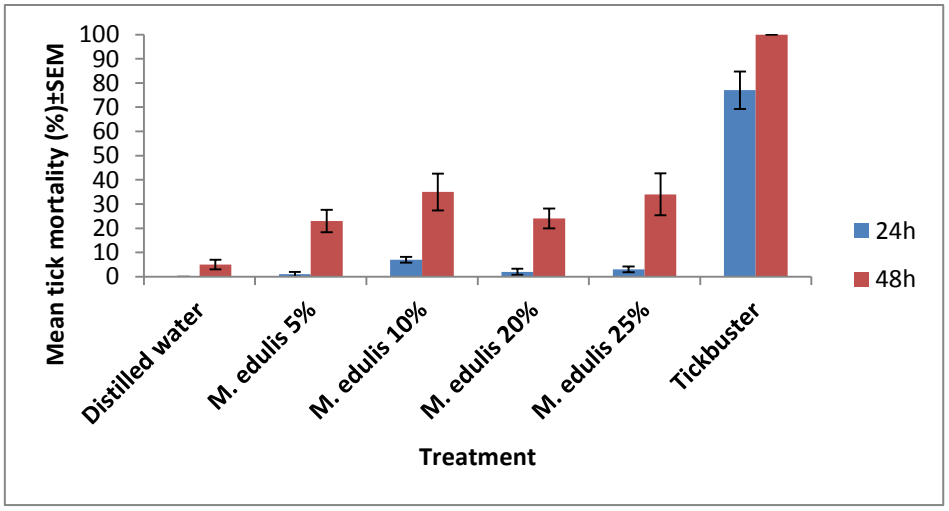

Figure 5.2: Mortality of *Rhipicephalus (Boophilus) decoloratus* tick larvae after incubation with cold water extracted *Maerua edulis* leaves

ii. Cold water plus surfactant

In the cold water plus surfactant treatments, overall tick larvae mortality was also generally low and below the recommended 80% for both sampling periods (Fig 5.3). The least square mean (LSM)

mortalities were all significantly different to the Tickbuster® positive control after 24h and 48h (p=0.0001).

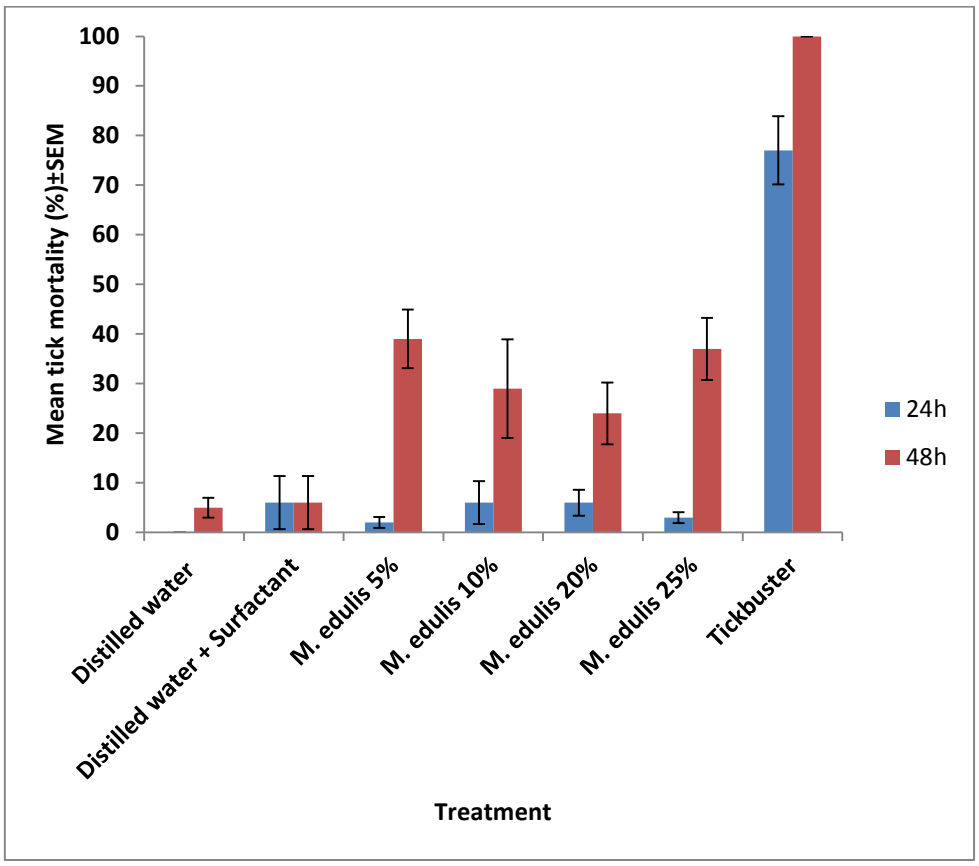

Figure 5.3: Mortality of *Rhipicephalus (Boophilus) decoloratus* tick larvae after incubation with cold water + surfactant extracted *Maerua edulis*.

iii. Hot water plus surfactant extraction

Larval mortalities in the *M. edulis* extracted using hot water and surfactant were lower after 24h but increased significantly after 48h (Fig 5.). There was no significant difference between the positive control and the plant extracts and between mortalities in the different plant concentrations (5, 10, 20 and 25%) (P > 0.05). Overall mortality in the plant based treatments was between 80-90%. Hot water extraction and addition of liquid soap (surfactant) had a significant effect causing higher mortalities compared to cold water extraction (Table 5.2).

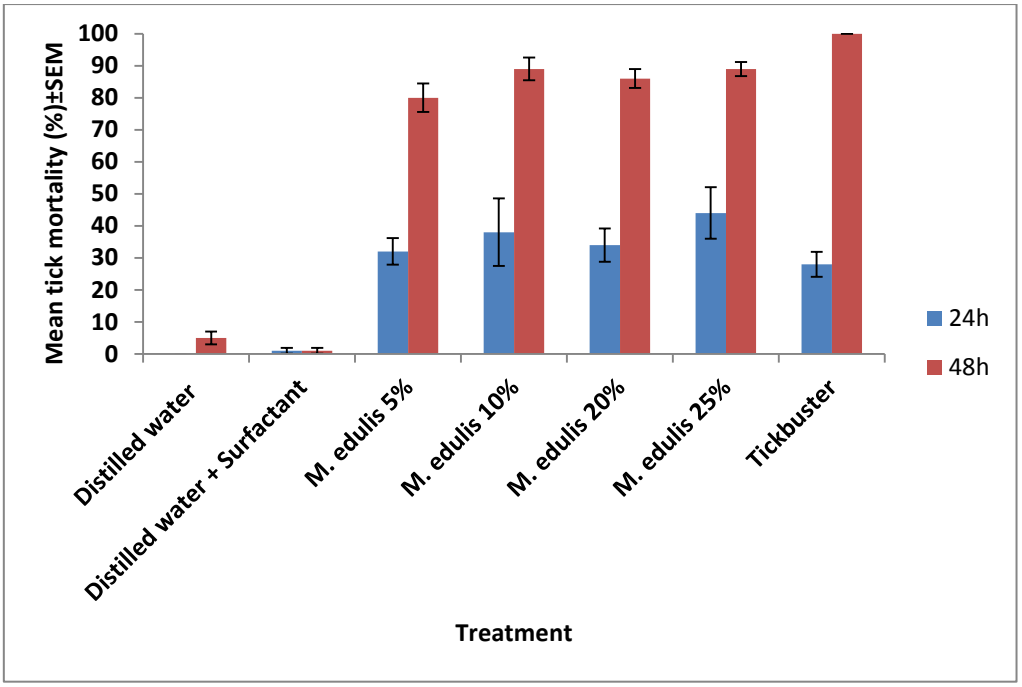

Figure 5.4: Mortality of *Rhipicephalus (Boophilus) decoloratus* tick larvae after incubation with hot water plus surfactant extracted *M. edulis*

Table 5.2: Comparison of Least Square Mean (LSM) mortalities of tick larvae from cold water plus surfactant and hot water +surfactant extracted *M. edulis* treatments

		Mortality ranks	
Treatment	**Concentration (%)**	**24h**	**48h**
Cold water + surfactant	5	54.1[a]	73.1[a]
	10	62.0[a]	46.7[a]
	20	73.9[a]	47.0[a]
	25	63.9[a]	72.1[a]
Hot water + surfactant	5	128.9[b]	107.4[b]
	10	132.7[b]	120.8[b]
	20	132.0[b]	113.5[b]
	25	138.9[b]	113.0[b]

Note: Within a column, means with different superscripts differ significantly ($P < 0.05$)

Hexane and methanol organic solvents extraction

There was no difference in mortality ± 40% between the hexane control treatment and the plant based treatments at 5, 10 and 20% treatments after 48h (P>0.05) (Fig 5.5). There was a significant difference between the *M. edulis* treatments at all concentrations and the positive control (Tickbuster®) (P<0.005). In the methanol treatment, there was a dose dependent effect from 5, 10 and 20% concentrations with mortality increasing from 45% to 100% after 48h (Fig 5.6). Thereafter there was no difference between the 20, 25 and the positive control mortality pegged at 100%. After 48h, all *M. edulis* treatments except the 5% treatment, showed significant mortality differences compared to the negative methanol control (p<0.05) but it was only the 20 and 25% *w/v* treatments which did not differ significantly from the Tickbuster® positive control.

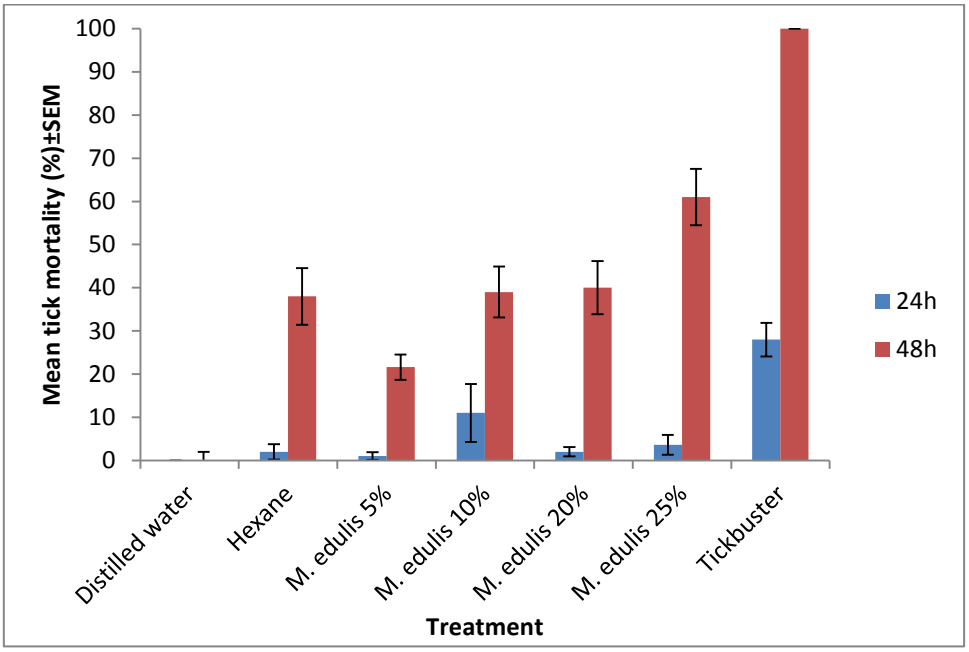

Figure 5.5: Mortality of *Rhipicephalus (Boophilus) decoloratus* tick larvae after incubation with hexane extracted *M. edulis*

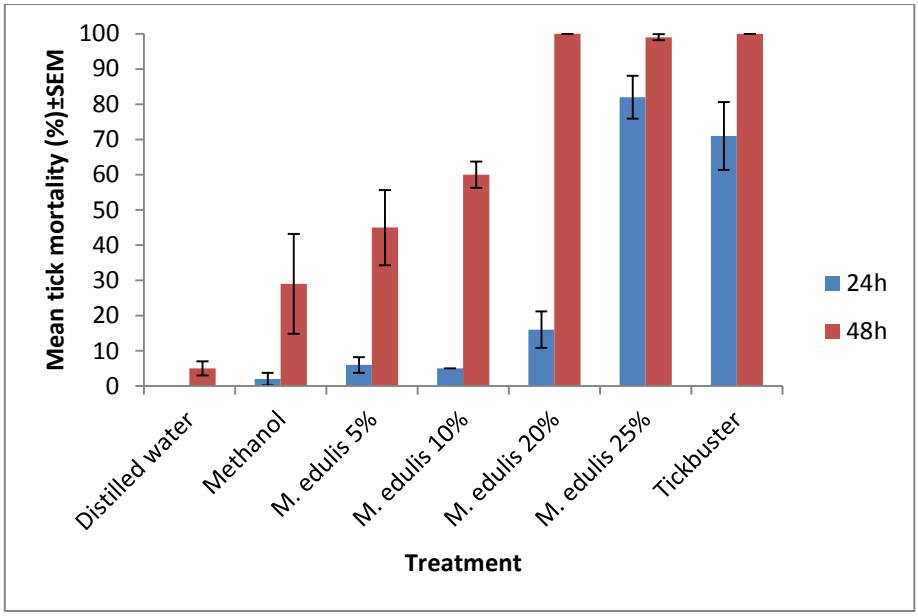

Figure 5.6: Mortality of *Rhipicephalus (Boophilus) decoloratus* tick larvae after incubation with methanol *Maerua edulis* leaf extracts

Acute oral toxicity of *M. edulis* against mice

Both the test and control mice appeared weakened and lethargic soon after oral administration, but regained their normal activity after 30 - 60 minutes. There were no obvious clinical signs observed neither was there any mortality recorded during the observation period. In the absence of dead mice, no tissue pathology analysis was done.

5.4 Discussion

The confirmation of acaricidal activity of *M. edulis* under controlled conditions is important because prior to these studies there was some unconfirmed indications of pesticidal activity in post-harvest stored grain (Stathers et al., 2002). Plant compounds may have multiple activities and other properties are frequently discovered. The tubers of *M. edulis* have been reported to have antihelminithic properties against livestock nematodes and were used successfully against faecal worm (*Heligmosomoides polygyrus*) eggs in mice (Gakuya et al, 2007). *M. edulis* and *B. grandiflora* leaves were used in Zambia to treat some poultry diseases (Komwhangilo et al., 1995). In another study by Hexane extracts of *M. edulis* have notable activity against *Mycobacterium bovis* and *M. tuberculosis* (Luo et al., 2011). While

the medicinal properties of the plant have dominated in literature, the recent past has seen a number or articles focusing on pesticidal properties of the plant (Nyahangare et al., 2017; Stevenson et al., 2018).

The difference between mortality of ticks exposed to cold water extracts and without a surfactant and those from hot water and surfactant treatments show that the acaricidal properties are more prominent with the use of hot water and a surfactant during extraction. Hot water has been used with better results in the preparation of ethnomedicines in countries like China and India (Martin et al., 2001; Huie, 2002). In South Africa and many other countries, *Lippia javanica* leaves are immersed in hot water for the efficient extraction of essential oils in the preparation of a herbal tea for the treatment of colds (Van Wyk, 2011). The arguments on the benefits of using a surfactant have already been made before (Gonçalves et al., 2007; Ssenyongo and Kyaterekera, 2009; Czarnota and Thomas, 2010; Sharma et al., 2012; Amoabeng et al., 2014; Nyahangare et al., 2017).

After 48 h post incubation, hot water plant extracts performed as well as methanol extracts. This is an indication that the yield of bioactive compounds from the plant material extracted using hot water could be as high as that obtained from use of methanol. Afify *et al.* (2011) extracted *Syzygium cumini* using methanol, hexane and ethyl acetate, in experiments to determine acaricidal activity of the plant against the two-spotted spider mite *Tetranychus urticae* Koch. The methanol extracts had the highest acaricidal activity with 95.5% mortality, whilst hexane and ethyl acetate had 94% and 90% mortalities respectively. In this study, hexane extracts of *M. edulis* had low mortalities, but high numbers of lethargic ticks at the end of the 48h period. This suggests investigation for longer incubation periods to test the effects of the material after 48 h.

Organic solvents are probably the best for extraction of bioactive compounds (Eloff, 1998; Bizimenyera et al., 2005) but in the search for least cost animal health products for smallholder marginalised farmers; their use is limited by factors of unaffordability and unavailability. There are also environmental and human health concerns worldwide over their use (Graf et al., 2004).

The absence of adverse clinical signs and behavioural changes in the mice dosed with the leaf extracts may be an indication that it is potentially a relatively safe plant to use without negative health effects in

the short term at concentrations used in the study. It does not however rule out health problems after repeated exposure and misuse. Findings by Gakuya et al. (2007) reported five mortalities, out of eight sampled mice in toxicity experiments that investigated the effect of aqueous *M. edulis* root-tuber extracts on nematodes in livestock using a 20 g/kg concentration. The other treatments in this experiment were 5 g/kg body mass and 10 g/kg body mass, which both had no mortalities in all sample units. Differences in this experiment and the one conducted by Gakuya et al. (2007) may be attributed to the different plant parts used in the experiments. Toxic compounds could be more concentrated in the roots than in the leaves.

5.5 Conclusion

Apart from using organic solvents, it is confirmed that preparing the *M. edulis* plant material by soaking in hot water with a surfactant is an effective and easy way of optimizing activity of this plant in the control of ticks for resource - poor farmers. The plant extracts are less likely to cause health problems for the host animals on accidental exposure at concentrations used in the study.

CHAPTER 6

6 *In vitro* cytotoxicity of non-polar extracts of *M. edulis* against African green monkey kidney and bovine dermal cells

Preface

Hexane and chloroform provided the most active extracts from *M. edulis* against the tick larvae in Chapter 4. Now in this chapter, the widely accepted assumption that natural products from plants are safe and has often been observed to be untrue is tested with the toxicity of the non-polar fractions against kidney and skin cells. Acute oral toxicity results obtained earlier (Chapter 5) show that there is no apparent toxicity after exposure to the extracts. However contact toxicity after topical application is more indicative of the likely toxicity and hence bovine dermal cell lines were used in this study. The choice for Vero cells was made because they are often used in cytotoxicity studies.

6.1 Introduction

From previous experiments and published information, there is convergence in the appreciation that the *Maerua edulis* shrub is a plant with acaricidal and pesticidal properties (Stathers et al., 2002; Nyahangare et al., 2016; Nyahangare et al., 2017; Stevenson et al., 2018). The prospects of developing a botanical product out of it are promising. However, efficacy data alone is not good enough to inform the development of such a product. It is crucial to determine the active ingredients responsible for the observed activity and also establish whether there are toxicity issues to the treated animals and non-target organisms (Nyahangare et al.,2012; El-Wakeil, 2013). There is often a lingering misconception that natural products are safe but this has not always been the case in many circumstances. Some of the most potent toxins today are from plants and unfortunately people and animals have died from their intake.

For most animals, acaricide poisoning is mostly accidental, perhaps because of the ignorance of the users when wrong formulations are sprayed on animals leading to skin irritations. Sometimes drinking from contaminated water sources after improper disposition of acaricides leads to development of contra indications (Sankhla, 2018). In the more unfortunate cases, there can be deliberate poisoning in cases of suicide. Many people across the globe have died from pesticide poisoning (Eddleston et al., 2008).

Therefore, toxicity knowledge is key in preparing users on how they can best protect themselves and the animals they are treating. In pesticide manufacturing it is a legal requirement to provide toxicity data

before a product is registered commercially for purposes of protecting the farmers and the animals during preparation and application (Sola et al., 2014). In some cases, based on the chemical nature of the toxicant, remedies and information on how to deal with poisoning after exposure are only known when its toxicity is established. In this study, highly acaricidal hexane and chloroform fractions of the plant *M. edulis* were tested for cytotoxicity using monkey kidney and bovine dermal cells.

6.2 Materials and methods

6.2.1 Study site

The study was carried out in the Phytomedicine Programme Cell culture Laboratory at the University of Pretoria.

6.2.2 Monkey kidney and bovine dermal cells collection and preparation

The monkey kidney cells were prepared as described in Adenubi et al. (2018) where the cells (ATCC ® CCL-81™) were sourced from Cellonex Company in South Africa and maintained in Minimal Essential Medium (MEM, Whitehead Scientific, South Africa). The media contained 4.5 g/l glucose and 4 mM l-glutamine supplemented with 1% gentamicin and 5% foetal calf serum (FCS, Highveld Biological, South Africa) (Adamu et al., 2013). Cells were passaged three times weekly by trypsinization with trypsin/ethylenediamine- tetraacetic acid solution (Invitrogen, Cergy-Pontoise, France) into 75 cm^2 culture flasks. The bovine dermis cells were obtained from the cell culture collection of the Department of Veterinary Tropical Diseases, University of Pretoria. They were maintained in a similar manner as the Vero cells.

6.2.3 Plant material harvesting and preparation

The *M. edulis* (tubers and leaves) were collected from Chiredzi (section 4.2.2) and the fractions were prepared as described in section 4.3.4.4. Only the non-polar fractions (chloroform and hexane soluble) were tested for toxicity for both the tuber and the leaves.

6.2.4 Experimental design and procedure

Cytotoxicity MTT Assay

The viability of cells after incubation of African green monkey kidney (Vero) cells and bovine dermal cells with the *M. edulis* plant extracts was determined using the tetrazolium-based colorimetric MTT assay [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] described by Mosmann (1983). Subconfluent cultures of the cells were harvested and centrifuged at 200× g for 5 min after which they were resuspended in Minimal Essential Medium (MEM, Whitehead Scientific) which were supplemented with 0.1% gentamicin (Virbac) and 5% foetal calf serum (Highveld Biological). A 200 µl suspension of the cell suspension was added to wells in columns 2 to 11 of a 96 well tissue culture grade microtitre plate. Columns 1 and 12 were filled with medium only as blank control wells. The plates were incubated for 24 h in a 37°C and 5% CO_2 incubator. The growth medium was removed from the cells in triplicate with 200 µl of the plant extracts at different concentrations. Serial dilutions of the test extracts were prepared in serum-free MEM. The microtitre plates were incubated at 37°C in a 5% CO_2 incubator for 48 h with the test extracts. Doxorubicin chloride (positive control) and untreated cells (negative control) were also included.

After the 48 h incubation period, the media was aspirated and the cells washed with 200 µl phosphate buffered saline (Whitehead Scientific). One hundred µl of the media together with 30 µl of MTT (Sigma), stock solution of 5 mg/ml in phosphate buffered saline (PBS) was added to each of the wells. The plates were further incubated for 4 h at 37°C. After incubation, the medium in each well was carefully removed taking care not to disturb the MTT crystals in the wells. These crystals were dissolved by addition of 50 µl of dimethyl sulphoxide (DMSO) to each well and gently shaken. The amount of MTT reduction was measured by detecting the absorbance in a microplate reader (Biotek Synergy) at of 570 nm and 630 nm reference wavelengths. Wells in column 1, containing medium and MTT but without cells were used to blank the plate reader. The LC_{50} values were calculated using the linear regression equation after plotting absorbance values against concentration. The average absorbance given by the untreated cells was made equivalent to 100% viability so that the concentration leading to 50% viability could be calculated.

The experiment was replicated 3 times and the LC_{50} results are expressed as the mean ± standard deviation (SD) of the three replicates. Plants extract having an LC_{50} value > 20 µg/mL have an acceptable level of toxicity, whilst a value < 20 µg/mL is regarded as toxic (Kuete and Efferth 2010).

6.2.5 Data analysis

The LC_{50} was determined using the linear regression model and presented as the arithmetic mean values ± standard error of mean. Significance was analysed using one-way analysis of variance followed by Tukey's multiple comparison tests on GraphPad Prism 7.02 (GraphPad Software, San Diego, CA, USA). Values were considered to differ statistically when $p \leq 0.05$.

6.3 Results

The MTT assay results on the kidney Vero cells show that the hexane fraction of the *M. edulis* tuber extract had the highest LC_{50} value (lowest toxicity) of 168.12 µg/mL. The LC_{50} values for the hexane and chloroform leaf extracts of the same plant were lower (higher toxicity) at 49.11 and 57.54 µg/mL respectively (Table 6.1). Doxorubicin, the positive control, had the highest toxicity signified by a very low LC_{50} value of 10.2 µg/mL.

Table 6.1 Mean viability of kidney cells post exposure to different organic fractions of *M. edulis* extracts

	Mean cell viability (%) ± SEM			
Concentration (mg/mL)	***M. edulis* L. (Chloroform)**	***M. edulis* L. (Hexane)**	***M. edulis* T. (Hexane)**	**Doxorubicin**
0.0075	105.24 ±5.6	85.85±4.9	90.58±4.6	86.53±1.8
0.0100	89.82 ±4.9	85.41±1.1	84.04±6.7	71.48±4.6
0.0250	68.26±12.7	71.60±5.4	81.41±6.6	56.10±6.6
0.0500	34.40±8.3	48.73±9.9	81.74±3.4	42.70±1.4
0.0750	19.29±5.7	38.48±11.8	76.22±2.3	37.38±2.0
0.1000	10.24±1.6	23.46±8.5	62.08±17.6	36.98±2.2
***LC_{50} (µg/mL)**	49.11±0.021	57.54±0.022	168.12±0.032	10.20±0.003

**LC_{50} values < 20 µg/mL are considered toxic*

Cytotoxicity results on the bovine dermal cells show the least toxic extract was the hexane fraction of the *M. edulis* tuber with the highest LC_{50} value of 471.40 µg/mL. The chloroform and hexane fractions of the *M. edulis* leaf crude extracts both had low LC_{50} values of 33.0 and 25.9 µg/mL respectively (Table 6.2). The negative control doxorubicin had the highest toxicity with LC_{50} = 0.54 µg/mL compared to the plant based treatments.

Table 6.2: Mean viability of bovine dermal cells post exposure to hexane and chloroform fractions of *M. edulis* extracts

	Mean cell viability (%) ± SEM			
Concentration (mg/mL)	***M. edulis* (L) (Chloroform)**	***M. edulis* (L) (Hexane)**	***M. edulis* (T) (Hexane)**	**Doxorubicin**
0.0075	101.94 ± 2.8	83.34 ± 1.5	87.08 ± 3.7	74.82
0.01	88.65 ± 1.1	77.92 ± 1.6	87.43 ± 2.7	71.80
0.025	54.44 ±3.1	46.43 ± 4.0	85.75 ± 2.7	47.62
0.05	36.94 ± 2.3	32.51 ± 9.7	85.47 ± 2.1	35.24
0.075	23.06 ± 3.1	22.60 ± 5.3	83.80 ± 2.6	22.62
0.1	15.02 ± 1.8	14.17 ± 4.4	78.6 ± 2.4	13.68
LC_{50} µg/mL	**33.00 ± 0.000**	**25.90 ± 0.006**	**471.40 ± 0.014**	**0.540±0.003**

*****LC_{50} *values < 20 µg/mL are considered toxic*

6.4 Discussion

The cytotoxicity experiments using kidney and skin cell lines showed that both the leaf and tuber non-polar fractions of *M. edulis* extracts can be considered relatively non-toxic because they all have high LC_{50} values greater than 20 µg/mL (Kuete and Efferth 2010). Higher LC_{50} values indicate lower toxicity and therefore indicate that the extracts are likely to be relatively safe to use topically on the animals (Adamu et al., 2013). This is a significant finding because it is possible to get plant extracts with very good activity but fail the toxicity test because the extracts are detrimental to the animal cells or potentially harmful to users. In the case of medicinal products, highly toxic material worsens the condition of the animals by killing the body cells. Kidneys are excretory organs and they have a high

metabolic activity and good blood supply (Adamu et al., 2013). Their cells can therefore be used to show toxicity potential arising from accidental oral exposure to the animals or accidental ingestion by users, and have often been used as the first line for testing cellular toxicity (Fernandes Freire et al., 2009). There is little danger of such toxicity since ticks are normally found on the exterior of the animal unless there is accidental ingestion. The tuber had the lowest toxicity with LC_{50} of 168 µg/mL and this probably explains why animals have been found eating these tubers especially in extremely dry conditions with scarce sources of drinking water. The moisture content of the tubers may be very high, perhaps at the level similar to that of *Neorautanenia brachypus,* which has been observed to be eaten by cattle in very dry conditions in some parts of Zimbabwe (Murungweni et al., 2012). Dermal toxicity provides details on the potential effect the extracts have on the skins of the animals during and after spraying. Some products may lead to skin irritation, development of lesions or even loss of hair. It is therefore important to ascertain or anticipate what will likely happen when a new product is sprayed on animals for the control of ticks.

Stevenson et al (2018) isolated two new isomeric sets of active components, E and Z isomers of cinnamoyl-4-aminobutylguanidine and the E and Z isomers of 4-hydroxycinnamoyl-4-aminobutylguanidine from the leaves of *M. edulis*. A further 11 closely related structures were also identified including stachydrine and 3-hydroxystachydrine which were present in the leaf extract at very high concentrations (Stevenson et al., 2018). Some of these compounds were tested on the cowpea bruchid *Callosobruchus maculatus* and the results showed that the cinnamoylamides together with the stachydrine compounds were as effective as the crude extracts after 72 h even though this was not as effective as the rotenone positive control. After 6 h, there was no significant difference between the positive control and the negative control. These compounds also were able to inhibit oviposition in female beetles (Stevenson et al., 2018). If these compounds also show acaricidal effects against ticks, then this plant would be a good target for downstream exploitation for tick control.

6.5 Conclusions and Recommendations

The active extracted blend of compounds in the non-polar fractions of *M. edulis* leaves and tuber had significantly lower levels of cellular toxicity compared to the positive control (doxorubicin). They may therefore be potentially safe to be used in anti-tick formulations with no adverse effects to the animals on the tested doses. This has to be tested *in vivo* too, to obtain more information under animal operating conditions.

CHAPTER 7

7 *In vivo* efficacy of selected acaricidal plants against cattle ticks

Preface

In vivo studies are important in acaricidal plant studies. In this study, crude aqueous extracts of plants were screened for efficacy. The result has been published and copied below. *Nyahangare, E. T., B. M. Mvumi, C. Magona, and J. N. Eloff. 2017. An aqueous extract of Maerua edulis (Gilg and Ben) DeWolf tuber is as effective as a commercial synthetic acaricide in controlling ticks on cattle in vivo. Industrial Crops and Products. 110:88–93*

Abstract

On-station experiments were conducted at Henderson Research Station, Zimbabwe to determine the *in vivo* efficacy of crude aqueous (with 1% detergent and 2% w/v cooking oil) extracts of *Cissus quadrangularis* (succulent stems), *Aloe vera* (succulent leaves) and *Maerua edulis* (leaves and tuber separately) at concentrations of 15%, 15% and 10% w/v respectively, against cattle ticks. An amitraz-based acaricide and water were used as positive and negative controls, respectively. Thirty Mashona steers were allocated to the six treatments in a completely randomised design experiment where each animal was an experimental unit replicated five times. The animals were each sprayed weekly with 5 L of the test or control solutions using a knapsack sprayer after which full body tick counts were recorded every other day for seven weeks. The experiments were conducted between January and February when conditions are optimal for tick development. The *M. edulis* tuber extract was as effective as the amitraz-based commercial acaricide. The other three plants extracts were, however, as ineffective as the negative control (water). *Maerua edulis* tuber plus soapy water-oil extract is effective against cattle ticks and have potential to be developed into an acaricidal product and thus benefit mostly resource challenged smallholder farmers who cannot afford commercial synthetic acaricides. *In vivo* studies using acaricidal plants are rare.

Keywords: Acaricidal plants, Cattle ticks, Indigenous knowledge, Smallholder farmers

7.1 Introduction

Ethnoveterinary studies all over the world through surveys and literature reviews show that many plant species with reported acaricidal properties have been used since time immemorial by farmers in the management of cattle ectoparasites (Maroyi, 2012; Ndhlovu and Masika, 2012; Nyahangare et al., 2015; Adenubi et al., 2016; Marandure, 2016). In the recent past there have been serious considerations of pursuing use of traditional plants in the primary health care of animals. This in turn has led to many scientific screening studies to verify credibility of claims of efficacy by farmers since most of the activity is orally reported activity. In the cases where efforts have been made to validate the effectiveness of acaricidal plants, research has been mainly limited to *in vitro* laboratory bioassays and not so much on *in vivo* trials (Adenubi et al., 2016). This is largely because *in vivo* experiments are very expensive and a bit more complicated to carry out and there are not many institutions that have the facilities for these kind of experiments (Moyo et al., 2009; Santillán-Velazquez et al., 2013). However, live animal *in vivo* data are crucial because they provide proof of the efficacy of the plant extracts under field conditions on the animal. It may not be very useful to have excellent *in vitro* data that cannot be replicated under actual operating field conditions. Furthermore, it is a legal requirement to have proof of *in vivo* activity of a particular product before it can be produced for the formal market. This explains why there are not many plant-based acaricidal products on the formal markets but a lot of concoctions and various types of preparations in the unregulated informal market. In this study, some plant species with positive acaricidal properties from *in vitro* trials (Chapter 4) and ranked highly by farmers in the survey reported in Chapter 3 were tested *in vivo* to confirm the acaricidal properties exhibited in laboratory trials and what was reported by the farmers.

7.2 Materials and methods

7.2.1 Study site

The study was carried out at Henderson Research Station (17° 35' S, 30° 58' E) in Mazowe district about 32 km north east of Harare, the capital City of Zimbabwe. The station is in natural farming region II which receives an average annual rainfall of 750 to 1000 mm. Peak tick infestation occurs during the wet summer months between January and March and the trial was conducted in January and February of 2016. The most common tick species found in the area include *Rhipicephalus (Boophilus) microplus* (Family; Ixodidae; Order; Parasitoformes, Authority: Canestrini), *Rhipicephalus evertsi evertsi* (Family: Ixodidae; Order: Parasitoformes, Authority: Neuman 1897)., *Rhipicephalus appendiculatus* (Family:

Ixodidae; Order; Parasitoformes; Authority; Neuman, 1901), *Hyalomma* sp. and *Amblyomma* sp. (Madzimure et al., 2011).

7.2.2 Plant collection and preparation of treatments

Cissus quadrangularis (Family: Vitaceae; Order: Vitales; Authority: (L)) stems and *Maerua edulis* (Family: Capparaceae; Order: Brassiacales; Authority: Gilg and Ben) DeWolf leaves and root tubers were collected from Chiredzi district while *Aloe vera* (Family: Xanthorrhoeaceae, Order: Asparagales; Authority: (L.) Burm. F.) succulent leaves were collected at Henderson Research Station. The plants were positively identified and voucher specimens deposited at the National Herbarium and Botanic Gardens of Zimbabwe by a qualified botanist, Mr Christopher Chapano. The voucher specimen numbers are: *C. quadrangularis* (Nyahangare E6), *M. edulis* (Nyahangare E5) and *A. vera* (Nyahangare E37). The leaves and root tubers of *M. edulis* and fleshy stems of *C. quadrangularis* and *A. vera,* were separately crushed and mixed with water containing a 1% w/v detergent (green bar soap) for 24 h to create a 25% g/100 ml stock solution. The green bar soap (Sunlight produced by Lever Brothers Pvt Ltd) widely available in shops in southern Africa was used. The soap was first pulverized and dissolved in 1 L of the stock solution and then added back to the parent solution. After 24 h, each mixture was filtered through a mutton cloth (Fig 9.1) and sufficient water added to yield 10% extracts v/v of *M. edulis* leaves and tubers and 15 % v/v of *C. quadrangularis* and *A. vera*. Vegetable cooking oil (Olivine cooking oil produced in Zimbabwe by Olivine Industries Pvt Ltd) was added to each preparation at 2 % w/v. The cooking oil was used as a low cost measure of dissolving the active compounds and preserving their acaricidal properties. Olive oil is a better preservative but is of higher cost and may not be applicable to the intended consumers of these technologies. The concentrations (10% and 15%) were optimal recommendations from previous laboratory bioassays (Chereni, 2014). Table 9.1 summarizes the treatments made from the plant extracts.

7.2.3 Experimental animals and design

Thirty Mashona steers of the same age (approximately 2 years) and raised under the same environmental conditions at Henderson Research Station were used. The steers were randomly allocated to the six treatments with each steer acting as an experimental unit and replicated 5 times in a complete randomized design experiment. Treatments were applied weekly in accordance with the Government of Zimbabwe regulations for summer dipping as opposed to fortnightly for winter dipping (Ndhlovu et al., 2009; Masuku et al., 2015).

Table 7.1: Summary of experimental treatments

Treatment	Description
1	10% w/v *Maerua edulis* leaves water extract + 1% w/v detergent + 2% w/v cooking oil
2	10% w/v *Maerua edulis* tubers water extract + 1% w/v detergent + 2% w/v cooking oil
3	15% w/v *Aloe vera* water extract + 1% w/v detergent + 2% w/v cooking oil
4	15% w/v *Cissus quadrangularis* water extract + 1% w/v detergent + 2% w/v cooking oil
5	Water with 1% w/v detergent and 2% w/v cooking oil (Negative control)
6	Tickbuster® (Positive control)

7.2.4 Experimental procedure

Prior to commencement of the experiment, the cattle were exposed to natural tick infestation for 14 days (adaptation period) after which they were subjected to the treatments. During treatments, each animal was restrained in a cattle crush and a full body tick count for different species made and recorded. After counting, each animal was sprayed with 5 L test extract using a knapsack sprayer (Fig 9.1). The ticks on the animals were counted every other day thereafter for seven weeks from February to March (duration of the trial). The treatments were applied weekly after counting the ticks. Animals in different treatments were kept in separate paddocks throughout the experiment to avoid cross contamination. All animals were closely monitored throughout the experiment for any signs of tick-borne and other diseases by a veterinary practitioner at Mazowe Veterinary College, located approximately 1 km from the Henderson Research Station, Zimbabwe.

Figure 7.1: Heavy ear tick infestation (A); Sieving plant extracts with a mutton cloth (B&C); Cattle spraying with plant extract (D)

7.2.5 Data analysis

The efficacy ratio per animal was calculated from the daily tick counts using the formula adapted from O'Neill (2006).

$$\text{Acaricidal efficacy} = 1 - \left(\frac{\text{Treatment tick count}}{\text{Untreated control tick count}}\right)$$

The repeated measures analysis of variance of the mean efficacy ratios of the treatments were conducted using the GLM procedures of SAS version 9. 3.1 (SAS, 2006) using the following statistical model:

$Y_{ijk} = \mu + T_i + W_j + T_i \times W_j + e_{ijk}$

Where:

- Y_{ijk} is the response variable (tick count efficacy ratio)
- μ is the overall population mean
- T_i is the fixed effect of the i^{th} treatment (i=treatment (1,...5)
- W_j is the fixed effect of time post-treatment application (j=weeks 1,...7)
- $T_i \times W_j$ is the interaction between time post-treatment application and treatment
- e_{ijk} is the residual error

7.3 Results

7.3.1 Acaricidal efficacy ratios

Maerua edulis tuber aqueous extract was the only plant-based treatment with high efficacy ratios against cattle ticks with a mean overall efficacy ratio of 80% over the seven weeks of study (Figure 7.2). There was no statistically significant difference (P=0.05) in efficacy between the tuber extract and the positive control (Triatix®) throughout the experimental period ($p > 0.05$). In the seven weeks, the tuber extracts had efficacy ratios of greater than 50 % (Fig. 7.2). *Maerua edulis* leaves, *A. vera* and *C. quadrangularis* treatments were not effective in reducing tick loads and were not significantly different from the negative control (water) ($p > 0.05$).

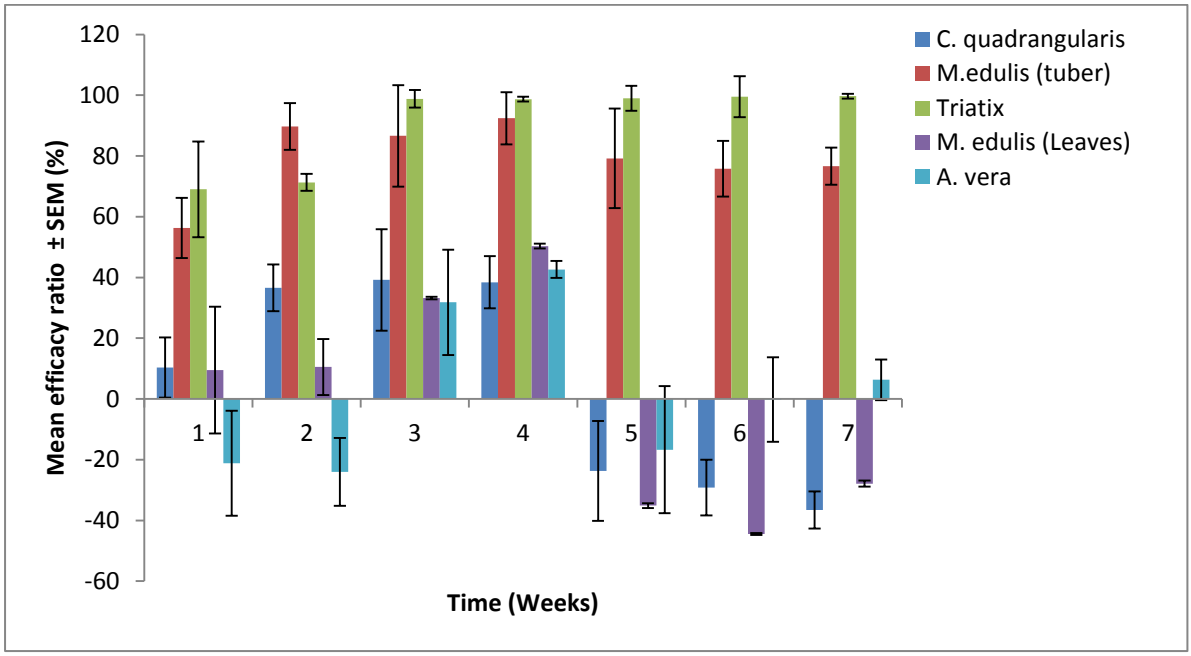

Figure 7.2: Mean weekly efficacy ratios of extracts against ticks over 7 weeks (February-March 2015) (n=5)

The weekly overall mean efficacy ratios of treatments between sprayings and after spraying are presented in Table 7.2. *M. edulis* tuber was the only plant based treatment that was significantly effective against cattle ticks.

Table 7.2: Mean % mortality ratios for treatments against counting time within weeks (n= 28)

Treatment	Count 1	Count 2	Count 3	Count 4
C. quadrangularis	-14.0[a]	-25.5[a]	-39.0[a]	-15.5[a]
M. edulis tuber	71.5[b]	76.7[b]	71.5[b]	69.4[b]
Triatix	82.5[b]	94.7[b]	89.1[b]	91.2[b]
M. edulis leaves	-40.4[a]	-6.4[a]	-36.1[a]	-13.4[a]
A. vera	-43.9[a]	-27.1[a]	-39.5[a]	-30.1[a]
±SEM	17.7	11.4	14.9	11.6

Within the columns and rows, means with different superscripts letter are significantly different *(P<0.05).*

7.3.2 The variation of total tick populations over time

There was both a time and time x treatment effect on the total tick population ($p<0.05$). In the first week there was a general increase in the number of ticks for both treatments although the degree of increase differed among treatments. The least increase was on Triatix® (positive control), followed by *M. edulis* and *C. quadrangularis* respectively (Fig 7.3). The negative control of water led to the highest increase. However, over time, the total tick population across the treatments was reduced significantly.

7.3.3 Engorged and tick species identified

In the *M. edulis* leaves, *C. quadrangularis*, *A. vera* extracts and water treatments, there was a high number of engorged ticks. Only a few engorged ticks were found in the *M. edulis* tuber treatment and the positive control Triatix® (Fig 7.4). The largest population of ticks for all treatments was of brown ear tick (*Rhipicephalus appendiculatus*) followed by blue ticks (*Boophilus decoloratus*). There were no Bont ticks (*Ambylomma* species) recorded throughout the experiments and very few red legged ticks (*Rhipicephalus evertsi evertsi*) as well as bont legged ticks (*Hyalomma*) that were recorded including a species which was classified under 'other ticks'. There was a slight increase in the numbers of red legged ticks in contrast to brown ear ticks towards the end of the experiments although the numbers were still lower than for brown ear ticks.

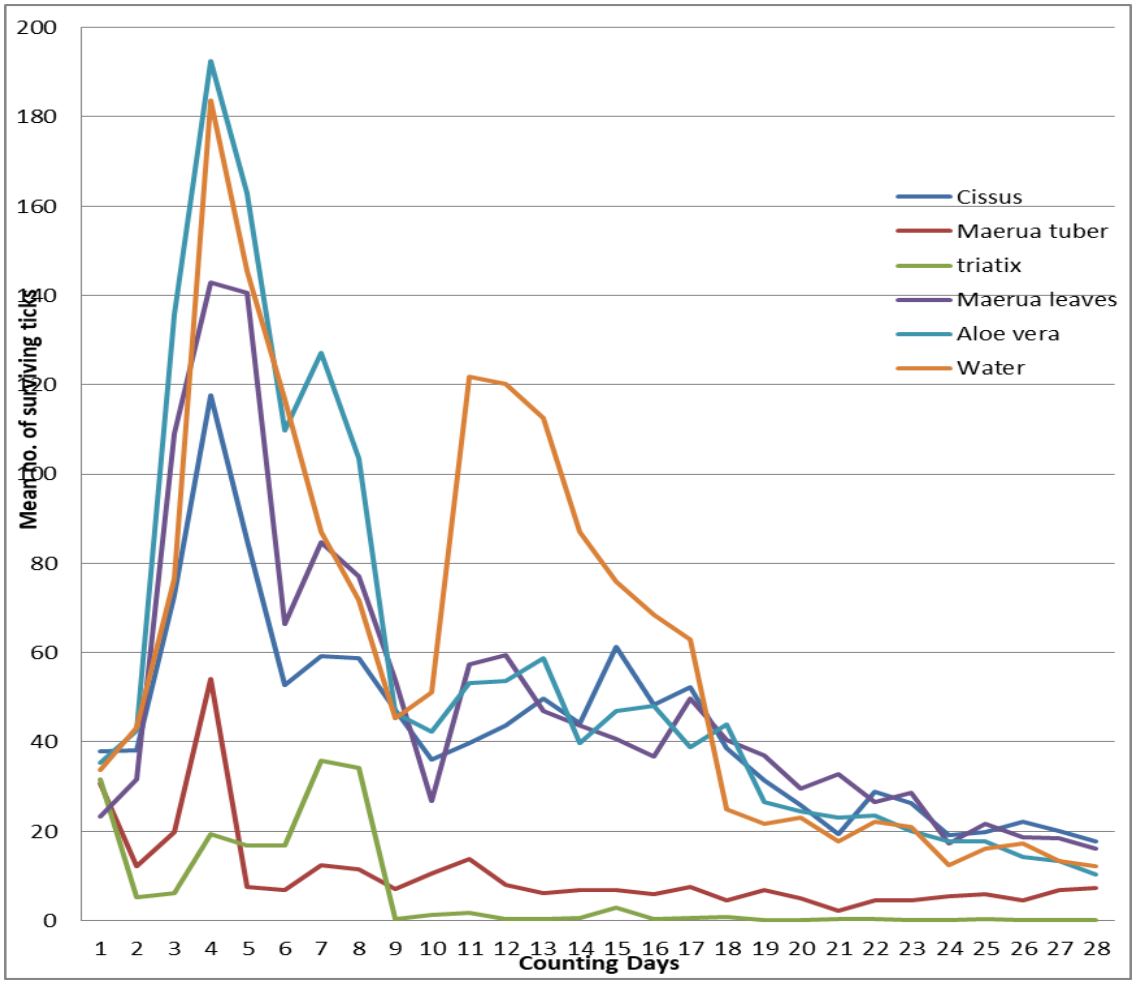

Figure 7.3: Mean overall ticks surviving for all treatments over 7 weeks from February to March 2016 (n =5)

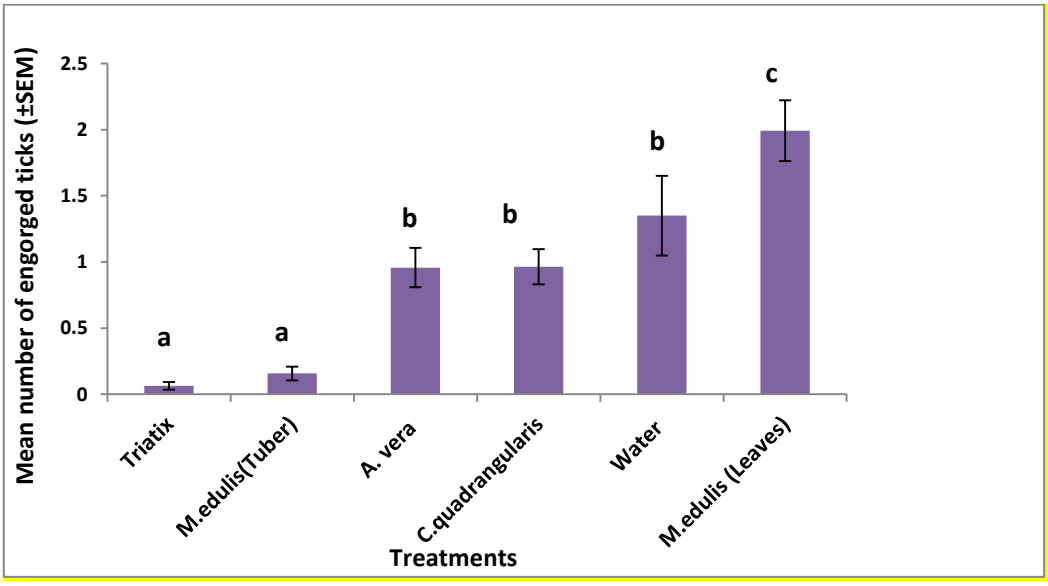

Figure 7.4: Average number of engorged ticks in the different treatments over 7 weeks (n =5)

7.4 Discussion

The *M. edulis* tuber treatments showed high efficacy comparable to the synthetic acaricide (Triatix®). Similar results were reported for the same extract against tick larvae in the laboratory (Simuunza et al., 2011). The mean tick count for the tuber extract treatment was 9.8 ticks per animal which implies that the animals were not tick infested according to Zimbabwe Veterinary standards which stipulate that when more than 10% of the animals have 10 or more live ticks, they are considered tick infested (Madzimure et al., 2011).

The acaricidal efficacy observed in the *M. edulis tuber* extracts could have been caused by the presence of linear chain unsaturated fatty acids which were found to be the main active ingredients upon preliminary phytochemical analysis (Luo et al., 2011). In the current study, the water extract of *M. edulis* leaves was not effective in reducing tick numbers which contradicts preliminary *in vitro* bioassays results on tick larvae (Chereni, 2014) and strong perceptions expressed by farmers on the effectiveness of the leaf extracts against pests (Stathers et al., 2002; Nyahangare et al., 2015).

Water is a very poor extractant for antimicrobial and antiparasitic compounds from plants compared to acetone and other intermediate polarity solvents. In these experiments we focused on using an extractant that could be available to rural farmers and could deliver active extracts. To increase the solubility of relatively non-polar compounds soap and oil were added to the water. Perhaps using organic solvents can increase the efficacy of the leaves because it is known that water has limited extraction properties. Solvents like acetone which extract a wide array of active compounds and are less toxic to the ticks may possibly lead to better results (Eloff, 1998; Zorloni et al., 2010).

The increase in efficacy in the tuber extracts from week 1 to week 5 could be due to residual effects. It is an indication that apart from the dose-dependency characteristics evident with most conventional acaricides, residual effects may also influence efficacy with increase in application over time. Such a phenomenon is very desirable in the use of acaricidal plants and has been previously reported in another acaricidal plant, *Lippia javanica.* At the 5% w/v concentration, there was a significant increase in efficacy over time with subsequent applications (Madzimure et al., 2011).

Aloe vera, *M. edulis* leaves and *C. quadrangularis* extracts had no significant acaricidal activities against cattle ticks. This is despite the fact that these plants had been ranked very highly by rural farmers in Zimbabwe in the survey reported by Nyahangare et al. (2015). In Chiredzi district of Zimbabwe, respondents call *C. quadrangularis* “Chiololo” in their language of Kalanga which loosely translates to “deadly effective” in English. The variance in activity may possibly be explained after a

phytochemical analyses to identify the different active components. The poor activity of water extracts of *C. quadrangularis* was also observed *in vitro* when silver nanoparticles made from its stem showed positive results against *Rhipicephalus (B) microplus* tick larvae and *Hippobosca maculata* (Family: Hippoboscidae; Order: Diptera, Authority: Linnaeus, 1758) compared to water extracts (Santhoshkumar et al., 2012). The activity may be caused by the silver nanoparticles and not by a compound from the plant. On the other hand, *A. vera* was moderately acaricidal against ticks, fleas and mosquitos in *in vitro* experiments in addition to its anti-coccidiosis activity (Urch, 1999; Chereni, 2014). The negative results obtained with *M. edulis leaves, A. vera* and *C. quadrangularis* extracts in this study are contrary to the results from laboratory trials by Chereni (2014). This questions the validity of laboratory results without confirming the activity in animal studies. According to Lim (2011), factors such as temperature, sunlight, as well as air properties could contribute to plant loss of pesticidal efficacy. If the compounds responsible for activity in *in vitro* studies with *A. vera* and *C. quadrangularis* extracts are thermolabile or susceptible by photo-oxidation and the compounds in *M. edulis* bark extracts are not sensitive, it could explain the results obtained. Therefore, further research needs to be conducted to establish the exact cause for loss of the acaricidal properties.

It is disappointing that the *M. edulis* leaf water extract had no activity against the ticks because the sustainable use of *M. edulis* tuber extracts to protect animals against ticks is questionable. Water is a poor solvent compared to other extractants, especially acetone in determining antimicrobial and antiparasitic activities (Eloff, 1998; Kotzé et al., 2002). Therefore, there is potential to optimise both the extraction of *M. edulis* leaf and tuber using organic solvents.

The general decline in total tick count over time is not unexpected because tick populations naturally decline as the summer season progresses and rainfall and temperatures decrease because ticks prefer warm and wet conditions (Muchenje et al., 2008). This is why in Zimbabwe dipping is done on a weekly basis in summer and fortnightly in winter in the normal tick management systems. This balance may be changing because of the changing climatic and weather patterns (Collier et al., 2008; Estrada-Peña and Salman, 2013).

There was no change in tick species that are commonly found at the research station with *R.* (*B) decoloratus* (blue tick), *R. appendiculatus* (brown ear tick), *Hyalomma* species and *R. evertsi evertsi* (red legged tick) found during the trial. All previous trials at the station recorded the same tick species (Madzimure et al., 2011; Madzimure et al., 2013).

The high number of engorged ticks which were present on *A. vera*, *M. edulis* leaves and *C. quadrangularis* treatments and the negative control, is an indication that ticks were not adversely

affected and thus would drop off naturally after being fully engorged so that they would lay eggs (Madzimure et al., 2011). On the contrary, in the positive control and the *M. edulis* tuber treatment there were fewer engorged ticks recorded on the cattle showing a possible negative effect on the reproductive activities of the ticks.

7.5 Conclusion

This study showed that extraction of *M. edulis* tuber with water containing a surfactant and oil can effectively reduce tick populations on cattle and can be used as an alternative to synthetic acaricides in controlling cattle ticks. *Cissus quadrangularis, A. vera* and *M. edulis* leaf water extracts were not significantly effective against cattle ticks compared to the negative control.

Further investigations are required to compare the *in vivo* efficacy of different concentrations of the tuber extracts. Identification of the anti-tick compounds in the tuber extract could provide useful information and may lead to the identification of new acaricidal compounds or blends. Issues such as optimisation of the extraction, health and safety, propagation and sustainable harvesting should also be investigated. It may be very interesting to investigate why our results differed from the traditional use and from laboratory results. If water used by rural farmers had microbial contaminants that could solubilize non-polar compounds or if other chemical changes due to e.g. photooxidation took place, it may explain the differences. There is also need to determine the possible effects of the harvesting timing on the chemical composition and acaricidal activity of both the *M. edulis* leaves and tuber. It could be one of the reasons causing the differential acaricidal activity that was observed.

Postscript

In the next section all the results will be discussed and recommendations for future research made.

CHAPTER 8

8 General Discussion, Conclusions and Recommendations

8.1 Discussion

The aim of this research project was to confirm acaricidal activities of selected plants used by farmers for tick control and explore downstream development of a product for potential use by resource poor smallholder farmers. The specific objectives outlined in Chapter 1 can be summarised as follows:

- Identification of plants used for tick control in semi arid and arid areas in Zimbabwe through an ethnobotanical survey
- *In vitro* screening for efficacy of selected plant extracts against ticks
- Further evaluation of other extraction methods to optimise efficacy of crude *M. edulis* plant extracts and safety against non target organisms
- Cytotoxicity of non-polar fractions of *M. edulis* extracts against kidney and dermal cells
- Perfomance of selected plant extracts against ticks on station

In the coming discussion, the extent to which these objectives have been met is analysed.

8.1.1 To identify ethnoveterinary plants used for tick control

It was clear from the survey that communities have knowledge on traditional plant species used for the management of cattle ticks as indicated by the more than 51 different plant species identified. This is justification for more ethnobotanical surveys to be done in other areas so as to exploit the rich flora and cultural diversity in the southern African region. Identification of more plants increases the database of useful plants from which bioprospecting may be done. The finding that indigenous knowledge is mainly found in the older generations means that there is need to document these practices before they are lost forever when the elderly die or shift to urbanization and modernization which are real threats to survival of traditional practices (Wanzala et al., 2005). Traditional practices by oral tradition is not exactly a secure way of preserving knowledge because there is always the risk of possible alterations in the narratives leading to recording of ineffective plants or those with low efficacy (Maroyi, 2012). The most common method of preparation of the extracts was soaking the ground leaves in water after which the extracts were applied to the animals. The use of leaves is commendable and shows a good understanding of the farmers on aspects of sustainable use of finite resources. Something of concern though from the present findings is that despite farmers being knowledgeable about acaricidal plants it

seems that plant-based technologies are only used in the absence of conventional synthetic acaricides. There is a need to investigate this lack of confidence in the locally-available resources which they claim work effectively and identify strategies to influence policy to recognise and institutionalise traditional practices at national government level.

8.1.2 To determine the acaricidal efficacy of extracts of ethnoveterinary plants ranked superiorly by farmers against ticks *in vitro*

The *in vitro* screening results showed that despite being ranked very highly by the farmers, not all plant species proved to be effective under laboratory conditions. The actual efficacy in most cases was way below expected efficacy especially for the water only extracted plants, such as *C. quadrangularis, Aloe* sp. and *C. abbreviata*. There are several possible reasons for these variances which include exaggerations/overestimation of potency by farmers during surveys, poor choice of extractant for example in the use of water, inefficiency of transmission of knowledge from one generation to the other, incorrect identification of plants and methodological variations in the preparation and application of the extracts (Wanzala et al., 2005). It is also possible that water extracts in the laboratory are not as effective as farmer practice because of the differences in the extraction and application environments. On the other hand, it is also known that time of harvesting, location and stage of growth of the plant may contribute to the phytochemical status of any particular plant and hence different results (Sarasan et al., 2011).

Considering the results, the importance of science in increasing the efficacy of plant extracts using novel practices like using hot water, surfactants and organic solvents cannot be underplayed (Djilani et al., 2006; Sharma et al., 2012; Nyahangare et al., 2016). The efficacy of water extracts of many plants were increased remarkably with these modifications. It remains unclear though why there are reports of excellent activity yet experimental evidence shows that water alone as an extractant does not improve activity under laboratory conditions .Water fails to extract most plant-based intermediate polarity active ingredients (Eloff, 1998; Kotzé et al., 2002). There is an argument though that the use of water in the laboratory is different with how traditional practitioners use water for extraction. Often times the water may be impure or contaminated with microorganisms and is boiled and then stored for some time unlike in the laboratory (Adamu et al., 2013). Until laboratory practices approximate what happens in traditional use, then dismissing water extractions would be misdirected.

8.1.3 To evaluate alternative low cost methods of optimising efficacy of crude *M. edulis* plant extracts and *in vivo* toxicity of the extracts against Balb/C mice

Nonetheless, while it may not apply for all plant species, it is noteworthy that the use of low-cost locally-available materials like soap and hot water can markedly increase the activity of *M. edulis* leaf extracts. Initially use of surfactant increased efficacy of the *M. edulis* tuber extracts but not the leaves. High activity in the leaves is more desirable for sustainable use considerations. This becomes very practical and useful especially for poorly-resourced farmers without access to modern veterinary remedies and who might not have access to better extractants like organic solvents to use to prepare acaricidal plant products. According to Isman (2008) these kind of farmers are the most likely to benefit from these pro poor technologies.

8.1.4 To evaluate *in vitro* cytotoxicity of non-polar fractions of *M. edulis* extracts against Vero monkey kidney cells and bovine dermal cells

The chloroform and hexane leaf and tuber extracts did not cause high levels of toxicity to kidney Vero and bovine dermal cells (LC_{50} values above 20 µg/mL). This provides a preliminary indication that this plant may be incorporated into tick control programmes at different levels of sophistication from the farm to perhaps a commercially packaged product without negative health implications to the animals although *in vivo* studies are necessary to confirm this.

8.1.5 To determine efficacy of superiorly ranked plants against cattle ticks in on-station trials

Farmers were of the opinion that *Maerua edulis* was a multipurpose pesticidal plant protecting not only cattle against ticks, but also post-harvest grains against pests (Stathers et al., 2002; Nyahangare et al., 2015). The plant also demonstrated good efficacy against vegetable pests in on-station experiments (Mazhawidza and Mvumi, 2017). Laboratory screening showed that adding a surfactant in water increased activity of the tuber extract remarkably. Crude acetone extracts of the leaves were highly effective against ticks *in vitro* while solvent – solvent extraction showed that the activity was more concentrated in the chloroform and hexane fractions. *Maerua edulis* tuber extracts were not only highly effective *in vitro* but also under practical field conditions when extracted using water, a surfactant and

cooking oil. The downside of using the tubers is that this has negative implications on sustainable use due to destructive harvesting and it may be counter productive to encourage farmers to use tubers for tick control. There is a risk of making the plants extinct because of over-harvesting. There is not much information on the propagation properties of this plant including for example how long it takes for the leaves to grow and the tubers to be fully developed. Plant biologists may assist in this kind of work. In the absence of that information however, leaves are preferable because new shoots can always grow to replace those harvested. Acetone extracts of the leaves of the same plant, however, managed to show very good activity *in vitro* even though it was not as good as the tuber and it remains to be seen if the same results can be replicated *in vivo* as the latter tests were not done in the current study. A product that is able to demonstrate high activity under field conditions is a candidate for product development. One of the reasons why there have not been many products on the market is perhaps because not many extracts have shown adequate efficacy *in vivo* that they can reduce tick populations. A lot more work is still to be done in this regard with many plant extracts.

8.2 Conclusions

It can be concluded from this study that there are plants used and reported by farmers that have clear anti-tick efficacies in both *in vitro* and *in vivo* experiments. It was also demonstrated that while water maybe the most easily available extractant for most farmers, better activity of plant extracts can only be realized using hot water, surfactants and organic solvents. With organic extractants, solvent–solvent extraction increases further the efficacy of plant extracts. There is great potential to develop products from the *M. edulis* plant because of its promising efficacy and low toxicity. However there is still more that can be done to fully comprehend other properties of the plants used in ethnoveterinary medicine, and associated phytochemicals that can facilitate the development of safe, effective and sustainable products.

8.3 Recommendations

Development of acaricidal plant products is in its very nature complex with many things required before a product is made available. The following are some aspects of future research that could be prioritised following this study:

- Bioassay guided isolation and identification of the active chemical compounds in the *M. edulis* leaf and roots and testing them against ticks
- Developing and testing crude formulations of *M. edulis* extracts *in vivo* that can be used by farmers
- Comprehensive analysis of the other plants which showed good activity *in vitro* which can lead to the identification of new products
- Conducting antimicrobial tests on the acaricidal plants to address the problem of tick abscesses and lesions
- Propagation of *M. edulis* and other acaricidal plants
- Carry out more ethno surveys in other areas to build a comprehensive acaricidal plant database
- Compare using traditional on-host deployment of aqueous products of ethnoveterinary plant products on target ticks with in vitro experimental results

There is also need for policy makers in the agricultural sector, particularly in countries that do not support traditional acaricidal plant technologies, to reconsider and facilitate promotion and integration of traditional practices that can be used in tick control. Zimbabwe, for example, has serious challenges with availability of commercial synthetic acaricides and has already lost thousands of cattle. Formalisation and promotion of known remedies can help ease this challenge. Industrialists should also take advantage of this generated body of knowledge and facilitate in designing acaricidal plant packages that can be used by different types of farmers.

One of the concerns that communities raised during surveys was that scientists never get back to communities with results of how the plants that they provided performed under scientific scrutiny. It is recommended that the findings that can benefit smallholder farmers should be communicated back to them in training sessions and the information also transferred to extension workers. Use of a surfactant and boiling water are easy ways of increasing activity of local remedies and this information should be made available to beneficiaries of these plant based technologies. Users should also be trained in methods of sustainable harvesting of plant materials.

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Appendix

Table 0.1: Compounds identified from the chloroform fraction of *M. edulis* leaf with similarity >80%

Name of compound	% Area	Formula	Similarity (%)	1st dimension
2,4,6 Trimethyldecane	0.172449	$C_{13}H_{28}$	87.1	1527.7
3-Hexadecene, (Z)	0.152149	$C_{16}H_{32}$	90.4	1103.5
Hexadecane	0.071266	$C_{16}H_{34}$	93.4	1112.3
4-Ethyltetradecane	0.087172	$C_{16}H_{34}$	88.4	1238.2
2-Methyloctadecane	0.06174	$C_{19}H_{40}$	87.5	1389.5
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	0.172487	$C_{17}H_{24}O_3$	90.3	1335.2
5-Eicosene, (E)	0.880857	$C_{20}H_{40}$	94.3	1268
Eicosane	0.103813	$C_{20}H_{42}$	91.8	1423.8
2,6,10,14 Tetramethylheptadecane	0.085143	$C_{21}H_{44}$	89.9	1275.6
1-Docosene	1.010035	$C_{22}H_{44}$	94.8	1417.2
Bumetrizole	0.126153	$C_{17}H_{18}ClN_{3O}$	82.3	1750.9
1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	0.05747	$C_{20}H_{30}O_4$	95.7	1366.1
Sulphurous acid, pentadecyl pentyl ester	3.792396	$C_{20}H_{42}O_3S$	85.9	2001.6
Hexanedioic acid, bis(2-ethylhexyl) ester	0.232749	$C_{22}H_{42}O_4$	90.0	1666.4
Octadecane, 1-iodo-	0.054685	$C_{18}H_{37I}$	88.4	1493
Heptacosane	0.21586	$C_{27}H_{56}$	93.4	1559.1
Di-isooctyl phthalate	0.356488	$C_{24}H_{38}O_4$	90.7	1746.5
Hentriacontane	1.041533	$C_{31}H_{64}$	93.1	1690.8
Dodecamethylcyclohexasilocane	0.937986	$C_{12}H_{36}O_6Si_6$	80.4	866.4